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Brief Report AIDS/HIV

Despite the wide availability of antiretroviral drugs, more than 250,000 infants are vertically infected with HIV-1 annually, emphasizing the need for additional interventions to eliminate pediatric HIV-1 infections. Here, we aimed to define humoral immune correlates of risk of mother-to-child transmission (MTCT) of HIV-1, including responses associated with protection in the RV144 vaccine trial. Eighty-three untreated, HIV-1–transmitting mothers and 165 propensity score–matched nontransmitting mothers were selected from the Women and Infants Transmission Study (WITS) of US nonbreastfeeding, HIV-1–infected mothers. In a multivariable logistic regression model, the magnitude of the maternal IgG responses specific for the third variable loop (V3) of the HIV-1 envelope was predictive of a reduced risk of MTCT. Neutralizing Ab responses against easy-to-neutralize (tier 1) HIV-1 strains also predicted a reduced risk of peripartum transmission in secondary analyses. Moreover, recombinant maternal V3–specific IgG mAbs mediated neutralization of autologous HIV-1 isolates. Thus, common V3-specific Ab responses in maternal plasma predicted a reduced risk of MTCT and mediated autologous virus neutralization, suggesting that boosting these maternal Ab responses may further reduce HIV-1 MTCT.

Maternal HIV-1 envelope–specific antibody responses and reduced risk of perinatal transmission

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Despite the wide availability of antiretroviral drugs, more than 250,000 infants are vertically infected with HIV-1 annually, emphasizing the need for additional interventions to eliminate pediatric HIV-1 infections. Here, we aimed to define humoral immune correlates of risk of mother-to-child transmission (MTCT) of HIV-1, including responses associated with protection in the RV144 vaccine trial. Eighty-three untreated, HIV-1–transmitting mothers and 165 propensity score–matched nontransmitting mothers were selected from the Women and Infants Transmission Study (WITS) of US nonbreastfeeding, HIV-1–infected mothers. In a multivariable logistic regression model, the magnitude of the maternal IgG responses specific for the third variable loop (V3) of the HIV-1 envelope was predictive of a reduced risk of MTCT. Neutralizing Ab responses against easy-to-neutralize (tier 1) HIV-1 strains also predicted a reduced risk of peripartum transmission in secondary analyses. Moreover, recombinant maternal V3–specific IgG mAbs mediated neutralization of autologous HIV-1 isolates. Thus, common V3-specific Ab responses in maternal plasma predicted a reduced risk of MTCT and mediated autologous virus neutralization, suggesting that boosting these maternal Ab responses may further reduce HIV-1 MTCT.

Introduction

Antiretroviral (ARV) treatment can significantly reduce the risk of mother-to-child transmission (MTCT), yet implementation barriers, adherence challenges, infant toxicities, and ARV-resistant HIV-1 strains will impede the achievement of an HIV-1–free generation. MTCT is a unique setting in which to investigate humoral immune correlates of transmission, whereby recipients are passively immunized with maternal Abs. High levels of maternal IgG Abs directed against the envelope (Env) gp120 protein, including the variable region 3 (V3) loop, have been correlated with protection against MTCT (1). However, not all studies established this association (2, 3). Several studies reported more potent HIV-1–neutralizing Ab responses in nontransmitting mothers compared with those in transmitting mothers (4) as well as transmission of neutralization escape variants (5–8), yet other studies did not (9–11). These disparate results may be due to small cohort sizes, distinct timing of infant HIV-1 diagnosis, and inadequate control of major nonimmune risk modifiers of transmission. It is also of interest to determine whether the humoral immune correlates of risk of HIV-1 acquisition identified in vaccinees in the RV144 adult HIV-1 vaccine trial (12, 13) play a role in MTCT.

We studied a cohort of U.S. nonbreastfeeding, HIV-1–infected mother–infant pairs enrolled in the pre–ARV era Women and Infants Transmission Study (WITS) (14), removing the impact of 2 important modifiers of MTCT risk: ARV prophylaxis and breastfeeding. Other unique aspects of this study include the cohort size, propensity score matching on clinical factors known to impact transmission, and combined assessment of a number of HIV-1 Env binding and functional Ab responses to determine which responses best predict reduced MTCT risk.

Results and Discussion

Primary maternal Env-specific humoral immune responses and MTCT risk. The primary correlate analysis revealed that MTCT risk was not predicted by Env V1V2 IgG binding score, clade B Env IgA binding score, IgG avidity, or ADCC responses (Table 1 and Supplemental Figure 1). The composite maternal plasma neutralization score was inversely associated with MTCT risk (0.76 per SD), however, this association did not reach significance ($P = 0.1$, $Q = 0.48$). Yet, in a prespecified second humoral response model that included responses previously implicated as important in

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Table 1. ORs of perinatal HIV-1 transmission in multivariable analyses of the immune correlate models

Humoral immune variables	Multivariate logistic regression		
	OR (95% CI)	P^A value	Q^A value
RV144-clade B–modified model			
IgG binding to B.case A2 V1V2	1.06 (0.80–1.42)	0.67	0.76
Env IgA binding (score)	0.96 (0.72–1.27)	0.76	0.76
Neutralizing Abs (clade B, tiers 1 and 2)	0.76 (0.55–1.05)	0.10	0.48
Avidity (B.6240)	1.12 (0.83–1.51)	0.45	0.76
ADCC (B.SF162)	0.94 (0.71–1.24)	0.65	0.76
MTCT model			
IgG binding to B.MN gp120	1.18 (0.69–2.03)	0.55	0.91
IgG binding to B.MN gp41	1.09 (0.72–1.65)	0.69	0.91
IgA binding to B.MN gp41	1.02 (0.76–1.36)	0.91	0.91
IgG binding to B.V3 (score)	**0.64 (0.42–0.97)**	**0.04**	**0.15**

[A]Immune variable interactions with $P < 0.05$ and $Q < 0.2$ are in bold font.

MTCT, IgG binding to both linear and scaffolded V3 antigens (combined IgG V3 binding score) predicted reduced MTCT risk (odds ratio [OR]: 0.64 per SD; $P = 0.04$, $Q = 0.15$; Table 1 and Figure 1, A–D). A single-predictor, change point logistic model (15) indicated that a threshold at the tenth percentile of the IgG V3 binding score was associated with transmission ($P = 0.04$). The transmission rate of mothers with IgG V3 binding responses below this threshold was 56% (14 of 25) compared with 31% (69 of 223) for mothers with responses above the threshold.

Secondary analysis of individual maternal humoral responses and MTCT risk. In a secondary analysis of each measured Ab response (Supplemental Table 3), neutralization of easy-to-neutralize (tier 1A) HIV-1 variants B.SF162 (OR: 0.67 per SD; $P = 0.006$) and B.MN.3 (OR: 0.71 per SD; $P = 0.02$; Figure 1E) best predicted reduced MTCT risk (FWER, 0.13), yet the FDRs did not fall below the preset criterion of less than 0.2 ($Q = 0.25$ and 0.4, respectively; Supplemental Table 3). Because of the differences in the biology of in utero and peripartum transmission, we analyzed the immune responses in only peripartum transmitters and matched controls in whom the neutralization response against these tier 1A HIV-1 strains predicted reduced peripartum transmission (OR: 0.54 per SD; $P = 0.005$, $Q = 0.1$ for both; Supplemental Table 4). Interactions between the humoral response variables were also explored, revealing an interaction between avidity and V3 binding (Supplemental Table 5).

In a post-hoc secondary analysis of responses against another common target of weakly neutralizing Abs, the CD4 binding site, represented by maternal plasma blocking of soluble CD4 (sCD4) binding to clade B HIV Env proteins (Figure 1F and Supplemental Table 6), a SD increase in sCD4 blocking against 2 of 3 clade B Envs was a significant predictor of MTCT risk (Supplemental Table 6; B.63521: OR = 0.7, $P = 0.014$; B.JFRL: OR = 0.04, $P = 0.036$; B.6240: OR = 0.75, $P = 0.058$). In fact, measures of sCD4 blocking, tier 1A virus neutralization, and IgG V3 binding in maternal plasma were highly correlated (Figure 1G and Supplemental Table 7) and colinear in the logistic regression model, indicating that they account for the same variance in MTCT risk.

Autologous virus neutralization by maternal V3-specific IgG Abs. Given the association of weakly neutralizing Abs with MTCT risk, we produced 10 recombinant V3-specific IgG mAbs from blood memory B cells of a nontransmitting, HIV-1–infected mother (Supplemental Tables 8 and 9 and Supplemental Figure 2) and determined their ability to neutralize 38 autologous HIV-1 *env* pseudoviruses (Supplemental Figure 3). While recombinant V3-specific IgG mAbs only neutralized easy-to-neutralize heterologous HIV-1 strains, they neutralized the majority of autologous maternal HIV-1 strains (average autologous viruses neutralized by each V3-specific mAb: 26 of 38, 67.4%; mean IC_{50}: 26.4, range: 3.1–49.6 μg/ml; Figure 2). Neutralization sensitivity of all 38 autologous viruses was classified as intermediate (tier 1B) on the basis of sensitivity to heterologous IgG and serum of HIV-1–infected individuals (Supplemental Figure 4 and ref. 16). Yet, the neutralization sensitivity of the 38 viruses to autologous V3-specific Abs fell into 2 groups (Figure 2), despite uniform V3 loop sequences. Interestingly, the viruses most sensitive to neutralization by the anti-V3 Abs (Figure 2A) shared 2 unique amino acids: Ser188 in the V2 loop, which moves a potential N-linked glycosylation site, and Ile200 in the C2 region (Supplemental Figure 5). In fact, introduction of *env* mutations Ser and Ile at positions 188 and 200, respectively, conferred neutralization sensitivity of a resistant variant to autologous V3-specific mAbs, demonstrating in vivo selection pressure exerted by these Abs (Figure 2B).

As commonly induced, weakly neutralizing Abs — such as those against V3 — do not protect against heterologous HIV-1 transmission (17), it was initially surprising that our results implicated these Abs in decreased MTCT risk. However, it is highly relevant to MTCT that V3-specific Abs can neutralize concomitant autologous virus strains (ref. 18 and Moody, MA, et al., in review). In fact, maternal V3-specific IgG Abs neutralized and exerted selection pressure on circulating autologous maternal viruses at inhibitory concentrations compatible with that associated with decreased MTCT risk. Nonetheless, it is important to caution that measuring maternal IgG V3 binding and tier 1 virus neutralization responses may be a surrogate for a yet-unmeasured antiviral function.

The WITS study offered a large historical cohort of HIV-1–infected pregnant women, yet was limited by case and specimen availability. Propensity score matching was used to maximize the power. Moreover, a pilot study of humoral responses in women with 2 samples available during the study window (25 weeks' gestation to 2 months postpartum) found limited variation in the magnitude of maternal Ab responses over this period (Supplemental Table 2). Finally, as maternal Ab transfer occurs in late pregnancy, the logistic regression model controlled for infant gestational age, in addition to other key risk factors of MTCT (maternal viral load and $CD4^+$ T cell count). Interestingly, in a secondary analysis, tier 1 virus neutralization significantly (OR: 0.54, $P = 0.005$, $Q = 0.1$) predicted transmission risk in the peripartum transmission cohort, but not in the entire cohort, indicating differences in the role of maternal Abs in distinct modes of infant transmission.

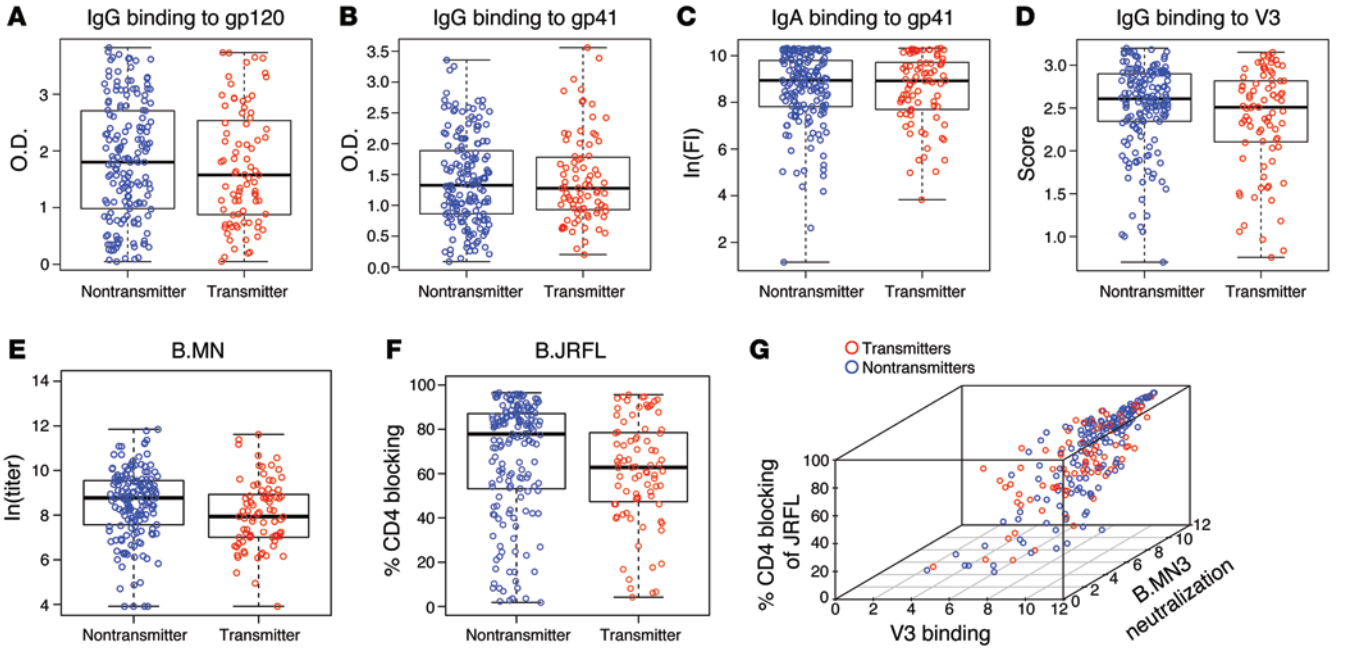


Figure 1. Comparison of humoral immune responses measured in HIV-1–infected transmitting and nontransmitting mothers. MTCT risk was not predicted by maternal MN gp120–specific IgG binding (**A**), MN gp41–specific IgG binding (**B**), or IgA binding (**C**) responses; however, the maternal IgG V3 binding score predicted a reduced risk of MTCT (**D**). The magnitude of the tier 1 neutralization (B.MN, **E**) and plasma sCD4–blocking response (against B.JFRL, **F**) was associated with reduced MTCT risk in exploratory analyses. Maternal plasma sCD4 blocking of B.JFRL Env, neutralization potency of B.SF162, and B.V3 IgG binding were highly correlated (**G**). Nontransmitting women are indicated in blue, and transmitting women are indicated in red. ln(FI), natural log MFI; ln(titer), natural log ID_{50}.

Our study did not implicate an immune correlate of protection identified in the RV144 vaccine study — V1V2-specific IgG response — as a correlate of MTCT risk. Differences in the transmitted viruses (autologous versus heterologous), distinct transmission modes, and vaccine-induced versus infection-induced Ab responses likely contribute to differences in correlates of risk. In contrast to RV144 vaccinees, we found no correlation between the infant infection and maternal Env IgA score, yet infants acquire only maternal IgG in utero.

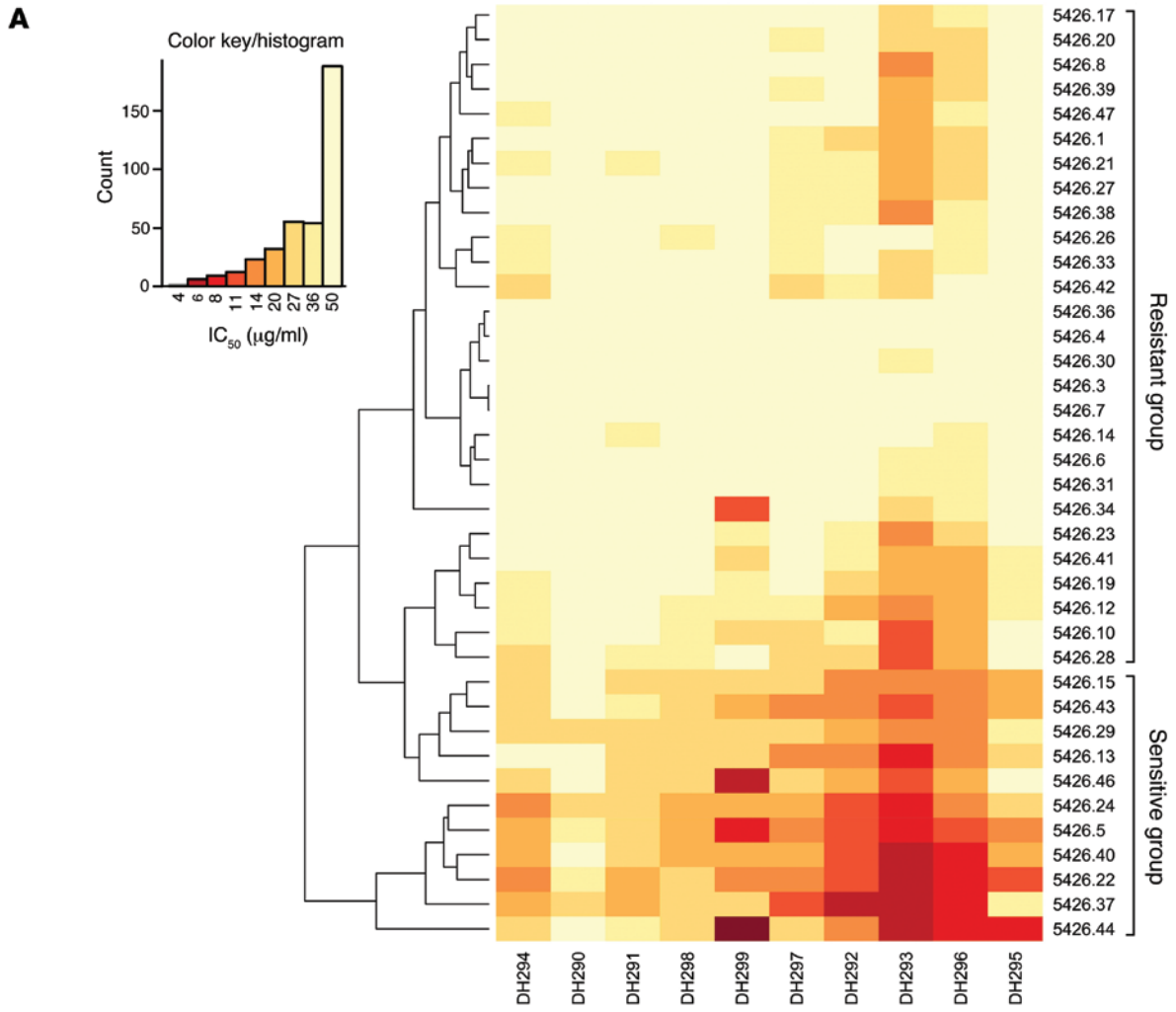
The hypothesis-generating work identifying potentially interrelated maternal humoral correlates of MTCT risk: V3-specific IgG, CD4-blocking, and tier 1A virus–neutralizing Abs are trends that provide a framework for further studies to define a mechanistic immune correlate of MTCT. Yet, the colinearity of these responses and the ability of the autologous V3–specific Abs from a nontransmitting mother to mediate neutralization and exert selection pressure on autologous virus, as well as the findings of previous studies (8), all point to autologous virus neutralization as being important in MTCT. V3 immunization can boost tier 1 virus–neutralizing Abs in infected individuals (19), although the ability of pregnant HIV-1–infected women to respond to Env vaccination remains to be shown (20). Our current study raises the hypothesis that temporary augmentation and placental transfer of V3-specific autologous virus–neutralizing Abs in HIV-1–infected pregnant women may be a plausible strategy to further reduce peripartum transmission of HIV-1.

Methods

Study design. Eighty-three HIV-1–transmitting mothers from the WITS cohort were selected according to the following inclusion criteria: no documented ARV treatment, detectable plasma viral load (>50 copies/ml), and nonheparin plasma or serum samples available between the end of the second trimester (≥25 weeks of gestation) and 2 months postpartum. Nontransmitting women were selected at a 1:2 ratio (n = 165) using propensity score matching (21) for maternal plasma viral load, peripheral $CD4^+$ T cell count at the time point closest to delivery, mode of delivery, and infant gestational age (Supplemental Table 1). Humoral immune assays used in the RV144 HIV-1 vaccine immune correlate analysis (Env V1V2 IgG binding, IgA binding breadth, IgG avidity, ADCC, neutralization)(12) were tailored to focus on clade B Env antigens and viruses. Additional humoral responses previously implicated as playing a role in MTCT were included: gp120 IgG binding, gp41 IgG binding, V3 IgG binding, and gp41 IgA binding (1, 22, 23).

Further details are provided in the Supplemental Methods.

Statistics. All regression analyses were adjusted for delivery mode, gestational age, log maternal plasma viral load, and peripheral $CD4^+$ T cell count. Two predefined multivariable logistic regression models were used (RV144 clade B–modified and MTCT models), with transmission status as the dependent variable and continuous Ab response variables mean centered and scaled to one. Combined immune variable scores (such as IgG V3 binding score) were defined as a weighted combination of immune response variables against related antigens or viruses. Change-point models explored the threshold of identified immune correlates and transmission status (15). In secondary analyses, individual humoral immune responses were analyzed by logistic regression. To correct for multiplicity, we computed both permutation-based FWER (24) and FDR (Q value)(25), applying a threshold of a Q value of less than 0.2 (12, 26) to optimize discovery of immune correlates at the expense of a 20% false positivity rate.



B

	DH290	DH291	DH292	DH293	DH294	DH295	DH296	DH298	DH299	HIVIG-C
5426.31	>50	>50	>50	>50	>50	>50	>50	>50	>50	12.4
5426.31.V200I	>50	>50	>50	>50	>50	>50	>50	>50	>50	9.4
5426.31.N188S	>50	>50	>50	>50	>50	>50	25.2	37.2	>50	6.5
5426.31.V200I+N188S	>50	11	24	6	39.6	40.9	8.8	16.6	>50	9.7

Figure 2. Heatmap of neutralization sensitivity (IC_{50}) to autologous V3-specific IgG mAbs of *env* pseudoviruses isolated from the plasma of a nontransmitting mother. *env* pseudoviruses (5426.1–5426.47) were divided into 2 groups by their neutralization sensitivity to the autologous V3-specific mAbs (DH290-299) on the basis of hierarchical clustering of neutralization sensitivity (**A**). Insertion of mutations V200I and N188S into an *env* pseudovirus from the resistant group (5426.31; Supplemental Figure 5) conferred sensitivity to autologous V3-specific mAbs (DH290–299) (**B**). Darker color indicates greater neutralization sensitivity (lower IC_{50}).

Maternal autologous virus and mAb isolation. HIV-1 *env* sequences were generated from plasma of a nontransmitting mother by single genome amplification at 2 months postpartum and produced as pseudoviruses (27). V3-specific B cells were isolated from autologous peripheral blood mononuclear cells (PBMCs) at 6 months postpartum (first available) by flow cytometric sorting using a ConB V3 peptide tetramer. Overlapping PCR constructed IgG1 and light-chain cassettes for Ab expression (28) for HIV-1 neutralization assays in TZM-bl cells.

Study approval. The IRBs of each study site approved the original protocol, and written informed consent was obtained from all participants (14). Duke University deemed this human subjects research exempt from IRB approval.

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