

Maternal Envelope gp41 Ectodomain-Specific Antibodies Are Associated With Increased Mother-to-Child Transmission of Human Immunodeficiency Virus-1

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Mother-to-child transmission of human immunodeficiency virus (HIV) occurs in the setting of maternal and passively acquired antibodies, providing a unique window into immune correlates of HIV risk. We compared plasma antibody binding to HIV antigens between 51 nontransmitting mother-infant pairs and 21 transmitting mother-infant pairs. Plasma antibody binding to a variety of gp41 ectodomain-containing antigens was associated with increased odds of transmission. Understanding the reasons why gp41 ectodomain-targeting antibodies are associated with transmission risk will be important in determining whether they can directly enhance infection or whether their presence reflects a redirecting of the humoral response away from targeting more protective epitopes.

Keywords. Mother-to-child transmission; HIV; antibodies; gp41.

Mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) is a natural setting in which the effect of HIV-specific antibodies on HIV infection risk can be studied. Antibodies circulating in the mother and passively acquired maternal antibodies circulating in the infant may both influence MTCT risk. In the case of breastfeeding transmissions,

infants who are uninfected at birth have pre-existing passively acquired HIV-specific antibodies present before breastfeeding HIV exposure. Studies have suggested both enhancing and protective effects of maternal HIV antibodies on MTCT risk [1–8]. Some studies of binding antibodies have shown that antibodies specific for the CD4 binding site (CD4bs), V3, gp120, p24, or gp41 are associated with reduced MTCT, whereas others have shown an association with increased MTCT or no association [1–6]. It is unclear which, if any, of these epitopes are targeted by protective binding antibodies in MTCT. The majority of these studies included in utero and peripartum transmissions where it is impossible to sample infant antibody responses to investigate pre-existing passively acquired antibodies present at the time of HIV exposure before infection [1–6]. Most studies included maternal samples but did not investigate passively acquired antibodies in infants [1–4]. Measuring both maternal and passively acquired antibody responses from mother-infant pairs where transmissions are due to breastfeeding could identify protective characteristics of pre-existing antibodies and help clarify results from prior studies.

We screened plasma from a cohort of antiretroviral therapy (ART)-naive breastfeeding mother-infant pairs for binding against a panel of HIV antigens to determine whether maternal and/or passively acquired antibodies targeting specific epitopes were associated with MTCT risk.

MATERIALS AND METHODS

Study Design and Plasma Samples

This study used samples from ART-naive mother-infant pairs from the Nairobi Breastfeeding Clinical Trial conducted from 1992 to 1998. All pairs meeting the selection criteria defined by Milligan et al [9] were included. Infants tested HIV-negative at birth, were breastfed for at least 3 months, and infant samples were collected from the first week of life, before the estimated time of infection (Supplementary Table S1). Paired maternal samples were also tested. The cohort included 70 paired samples (50 nontransmitting, 20 transmitting), 1 unpaired transmitting maternal sample, and 1 unpaired HIV-exposed uninfected infant sample. Cohort characteristics are listed in Supplementary Table S1. This research has been approved by the Kenyatta National Hospital Ethics and Research Committee and the Institutional Review Boards of the University of Nairobi, University of Washington, and the Fred Hutchinson Cancer Research Center.

Binding Antibody Multiplex Assay

The binding antibody multiplex assay (BAMA) was performed on maternal and infant plasma as described previously using

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a panel of 20 HIV antigens (Supplementary Table S2) [10]. Median fluorescence intensity (MFI) of plasma antibody binding to each antigen was measured and averaged across duplicate wells. Plasma antibody binding to an appropriate negative control antigen (described in Supplemental Methods) was considered background and subtracted from the plasma MFI for each HIV antigen. To average data from biological replicates, we normalized the background subtracted MFI to that of the positive control HIV immunoglobulin (HIVIG) and converted it to a percent: percent binding = $(\text{plasma MFI}_{\text{HIV antigen}} - \text{plasma MFI}_{\text{negative control antigen}}) / (\text{HIVIG MFI}_{\text{HIV antigen}} - \text{HIVIG MFI}_{\text{negative control antigen}}) \times 100\%$.

V1V2 Enzyme-Linked Immunosorbent Assay

Plasma was added to plates coated with clade B V1V2 caseA2-mulvvp70. Immunoglobulin (Ig)G was detected with goat antihuman IgG conjugated to horseradish peroxidase (HRP). Horseradish peroxidase was detected with 3,3',5,5'-tetramethylbenzidine substrate. The reaction was stopped with 1 N H₂SO₄, and the absorbance (optical density [OD]) was read at 450 nm. The background absorbance (OD without plasma present) was subtracted from the absorbance of each well. The background-subtracted OD was normalized to that of HIVIG and converted to a percentage.

Soluble CD4 Blocking Assay

The soluble CD4 (sCD4) blocking assay was performed similar to that described previously [3]. Clade A BG505.W6M.C2.T332N.L111A or clade D C2-94UG114 gp120 were used as antigens. Plasma samples were added to the plates followed by sCD4. Soluble CD4 binding was detected with biotinylated anti-OKT4 antibody followed by incubation with streptavidin-HRP. Horseradish peroxidase was detected as described above. Background absorbance (without sCD4) at 450 nm was subtracted from the OD of each well. Percent inhibition of sCD4 was calculated as follows: $100\% - [(\text{background-subtracted OD}_{\text{plasma}} / \text{background-subtracted OD}_{\text{no plasma}}) \times 100\%]$.

Statistical Analysis

Statistical analyses were performed with Stata v15 SE (StataCorp, College Station, TX). To measure the association of plasma antibody binding to each HIV antigen with MTCT risk, we performed a logistic regression analysis on the percentage of plasma antibody binding or sCD4 inhibition adjusted for maternal plasma ribonucleic acid viral load. Statistical significance was prespecified as $P < .05$. P values between .05 and .1 were considered nonsignificant statistical trends. The Holm-Bonferroni method was used to account for multiple comparisons.

RESULTS

Infant plasma collected before estimated infection and paired maternal plasma were screened for binding to HIV antigens via BAMA (Supplementary Table S2). Maternal and infant

samples showed similar binding patterns across antigens (Supplementary Figure S1). Logistic regression analysis showed that maternal plasma antibody binding to the clade C gp41 ectodomain antigen, clade A1 gp140, and clade B gp41 protein were significantly associated with increased odds of MTCT (ectodomain adjusted odds ratio [aOR] = 1.04, $P = .006$; gp140 aOR = 1.03, $P = .023$; gp41 aOR = 1.03, $P = .047$). Passively acquired IgG binding to these antigens likewise increased risk of transmission but failed to reach statistical significance (ectodomain aOR = 1.03, $P = .058$; gp140 aOR = 1.02, $P = .071$; gp41 aOR = 1.04, $P = .08$). Maternal plasma antibody binding to 6-Helix (provided by Peter Kim and Nitya Ramadoss), in which all 6 helices forming the gp41 trimer-of-hairpins have been linked into a single polypeptide [11], was also associated with a trend towards increased odds of MTCT (aOR = 1.03, $P = .075$). Plasma antibody binding to the remaining antigens showed no association with odds of MTCT. After correcting for multiple comparisons, plasma antibody binding showed no significant or trending associations with odds of MTCT for any antigen.

For most BAMA antigens, there was a wide dynamic range of plasma antibody binding above the limit of detection (100 MFI after background subtraction). However, for 2 V1V2 antigens (clade C ZM109 and clade B case A2), consensus D V3 peptide (conDV3), and the CD4bs core, over 40% percent of samples had binding below the limit of detection in multiple biological replicates, making it difficult to compare binding across transmission groups. To measure plasma antibody binding to these regions, we performed a V1V2 enzyme-linked immunosorbent assay (ELISA) and sCD4 blocking assays. We used V1V2 caseA2 as the antigen in the V1V2 ELISA because IgG binding to this antigen was correlated with protection in the RV144 trial [12]. Because the BAMA antigen panel contained 3 other V3 peptides, we did not use an alternative assay to measure plasma antibody binding to conDV3. Plasma antibody binding to V1V2caseA2 and the CD4bs as measured by ELISA and sCD4 blocking assays, respectively, showed a wide dynamic range (data not shown) but were not associated with odds of MTCT (Figure 1).

Figure 2 illustrates common features of the gp41 antigens. All 4 antigens that showed significant or trending associations with increased odds of MTCT contain the gp41 ectodomain. The common feature is the presence of the N heptad repeat (NHR) and C-terminal heptad repeat (CHR) that form the 6-Helix peptide.

DISCUSSION

This study used the unique setting of breastfeeding MTCT, in which maternal antibodies and infant pre-existing HIV-specific antibodies present at the time of breastfeeding HIV exposure can be measured, to assess whether antibodies targeting certain HIV epitopes are associated with reduced risk of transmission. Antibody binding to V3, the CD4bs, gp41, gp120, and p24 have

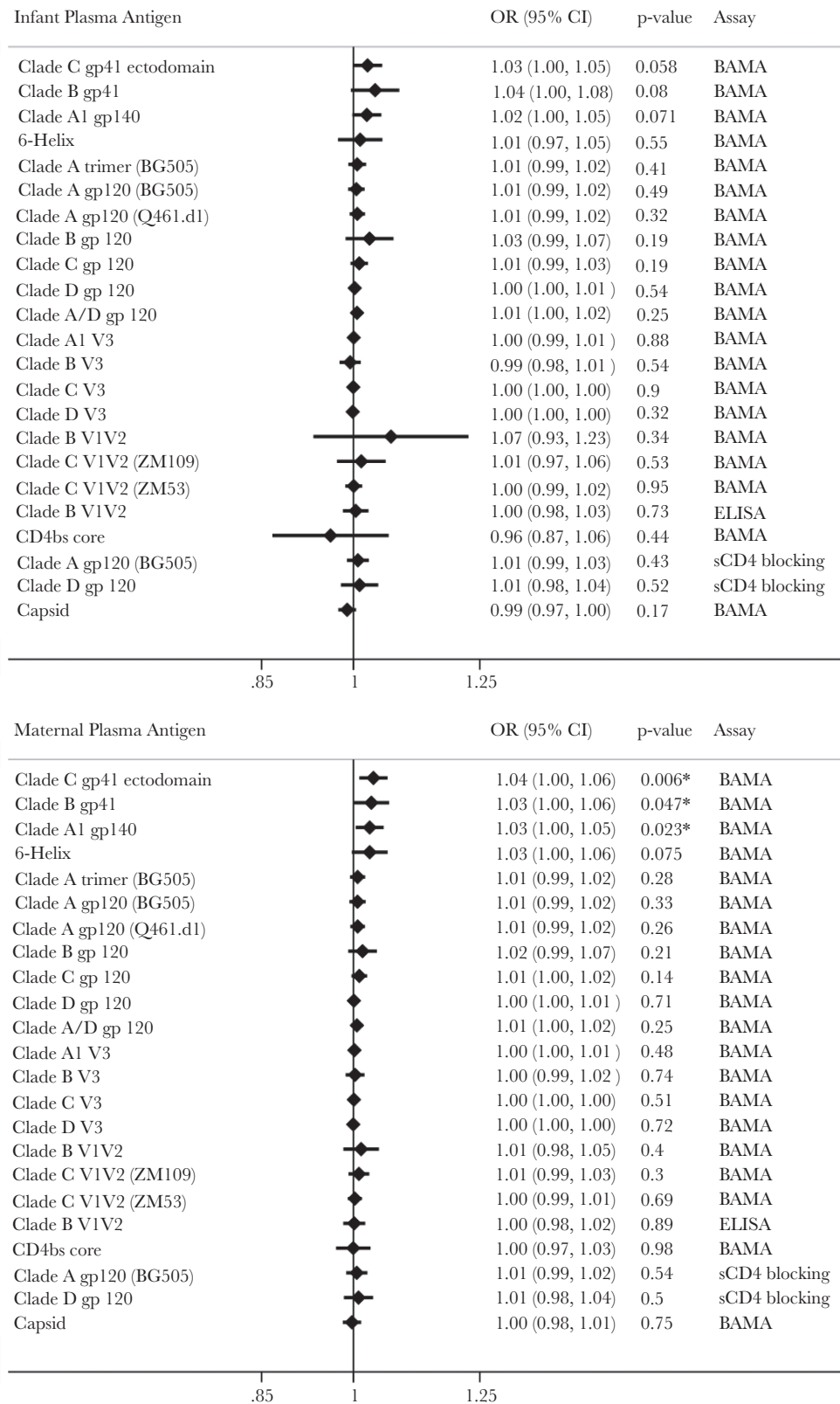


Figure 1. Association of plasma binding with odds of mother-to-child transmission (MTCT). The association of plasma binding to each antigen with odds of MTCT was measured using a logistic regression analysis adjusted for maternal plasma ribonucleic acid viral load. Results are shown as forest plots. Adjusted odds ratios ([OR] diamonds), 95% confidence intervals ([CI] horizontal lines), and *P* values are shown for infant samples (left) and maternal samples (right). Statistical significance was defined as *P* < .05 (*). Shown in the rightmost column is the assay used for each antigen: binding antibody multiplex assay (BAMA), enzyme-linked immunosorbent assay (ELISA), or soluble CD4 (sCD4) blocking assay.

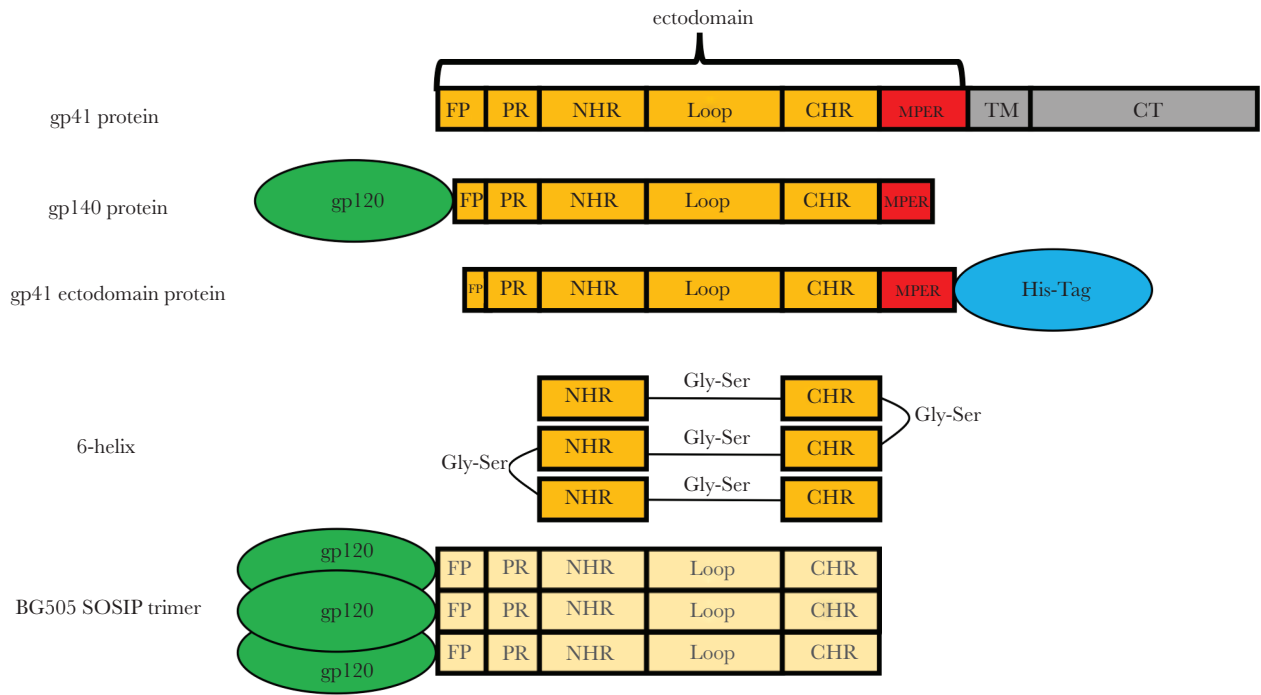


Figure 2. gp41-containing binding antibody multiplex assay (BAMA) antigens. Schematics of the gp41 domains present in BAMA antigens. The BG505 SOSIP trimer and 6-helix are both trimeric. 6-helix is a continuous trimer of heterodimers of N heptad repeat (NHR) and C-terminal heptad repeat (CHR) regions connected with glycine-serine linkers. The gp41 ectodomain antigen contains a C-terminal His-tag. The gp41 ectodomain is occluded in the BG505 SOSIP trimer as denoted by the light orange and gray text [13]. CT, cytoplasmic tail; FP, fusion peptide; loop, immunodominant C-C loop; MPER, membrane proximal external region; PR, proximal region; TM, transmembrane domain.

been identified as correlates of reduced risk of MTCT [1–6]. We were surprised to find that there were no correlates of reduced risk of MTCT for plasma antibody binding to our antigen panel; rather, we found an association with increased MTCT for plasma antibody binding to 4 antigens. Even though the odds ratios were small (possibly because the unit of percentage binding is small, and there is a wide dynamic range of percentage binding among samples), not all of these associations were statistically significant, and none were significant after correcting for multiple comparisons. What is remarkable is that all of these associations, whether maternal or infant, were with gp41-containing antigens (Figure 2). Furthermore, all of the associations were with increased odds of MTCT. The consistency of these results, along with multiple statistically significant associations, supports a biologically relevant association rather than random chance. Studies that included in utero and peripartum transmissions have also shown gp41-specific binding antibodies to be associated with increased risk of MTCT [2, 8]. Although one of these studies was conducted before methods to define the timing of infant infection were available [2], they nonetheless provide additional support for a role for gp41 antibodies in MTCT.

The 3 antigens associated with MTCT for both infant and maternal plasma antibody binding contained the fusion peptide, fusion peptide proximal region, NHR, the immunodominant

C-C loop, CHR, and membrane proximal external region (MPER). Although MPER is exposed in the clade B gp41 protein and clade A gp140 antigens, MPER is likely occluded in the gp41 ectodomain antigen by a C-terminal His-tag because MPER-positive control antibodies do not bind this antigen (data not shown). Plasma antibody binding to the clade C gp41 ectodomain antigen showed the strongest association with MTCT, suggesting that plasma antibody binding to MPER is not driving the association with increased MTCT. A fourth antigen, 6-Helix, containing NHR and CHR regions linked in trimeric form, showed a trend for an association with MTCT for maternal plasma antibody binding. This suggests that the key target of these antibodies is the heptad repeat regions. The BG505.SOSIP.664.D7342 trimer includes part of the gp41 ectodomain, but the ectodomain is known to be occluded in this antigen (Figure 2, light orange) [13]. Plasma antibody binding to this antigen was not associated with odds of MTCT. None of the gp120-only antigens were associated with odds of MTCT upon plasma antibody binding. This suggests that plasma antibody binding to the gp41 ectodomain, most likely the heptad repeat regions, is driving the association with increased odds of MTCT.

The association of plasma antibody binding to the gp41 ectodomain with MTCT could be direct, by which gp41 ectodomain-specific antibodies are risk-enhancing, perhaps by

binding to gp41 on the virus and promoting infection through Fc-mediated mechanisms [14]. On the other hand, the association may reflect an indirect mechanism, perhaps where the gp41 ectodomain is acting as an immune decoy. In contrast to the native-like trimeric antigen (where the ectodomain is occluded [13]) that was not associated with MTCT upon plasma antibody binding, gp41 antigens associated with increased MTCT have conformations similar to nonnative forms of envelope such as monomers, uncleaved envelope, and gp41 stumps [15], which may have the ectodomain exposed.

A limitation of our study is that 7 of the 21 infants that acquired HIV had an estimated time of infection after 6 months. Passively acquired HIV-specific antibodies wane in the infant over time and are often gone by 6 months [9]; therefore, it is unclear whether passively acquired antibodies were present near the time of transmission. The associations of maternal binding antibodies with odds of transmission were similar, but weaker, when transmissions after 6 months of age were excluded (data not shown). This may suggest a loss of statistical power or that gp41 ectodomain-specific antibodies are a marker of maternal disease progression or other risk factors.

CONCLUSIONS

Ours is one of only a few studies to investigate the role of both maternal binding antibodies and infant passively acquired binding antibodies on risk of breastfeeding MTCT using samples with well timed infant infection data. The inconsistencies between our study and prior studies may be due to differences in infecting clades, availability of maternal and/or infant samples, ART treatment, or mode of transmission. Most of the aforementioned studies included in utero and peripartum transmissions, whereas our study focused on breastfeeding transmissions and cases in which the infants tested HIV negative at birth. This allowed us to assess the impact of passively acquired antibodies in HIV-uninfected infants and to observe their impact on incident infection risk. Our results suggest that antibody binding to the gp41 ectodomain is a correlate of increased risk of MTCT, rather than a correlate of decreased risk. Investigating the specific gp41 epitope(s) correlated with increased risk and the mechanism of this association has the potential to help guide future vaccine and therapeutic design.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Figure S1. Correlation matrix of plasma binding to human immunodeficiency virus (HIV) antigens. Spearman correlation matrix of infant (left) or maternal (right) percentage plasma antibody binding to the panel of HIV antigens. Spearman rank

correlation coefficients are color coded, with red denoting a negative correlation, blue denoting a positive correlation, and light gray denoting no correlation. Statistical significance was defined as $P < .05$ (*). Alternative non-BAMA assays (ELISAs or soluble CD4 blocking assays are noted in parentheses for appropriate antigens).

Notes

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