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BRIEF REPORT

Passively Acquired Constant Region 5–Specific Antibodies Associated With Improved Survival in Infants Who Acquire Human Immunodeficiency Virus

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Studying vertical human immunodeficiency virus (HIV) transmission enables the impact of passively transferred antibodies on HIV transmission and pathogenesis to be examined. Using phage display of HIV envelope peptides and peptide enzyme-linked immunosorbent assay (ELISA), we found that, in infants who acquired HIV, passive antibody responses to constant region 5 (C5) were associated with improved survival in 2 cohorts. In a combined analysis, C5 peptide ELISA activity was correlated directly with survival and estimated infection time and inversely with set point viral load. These results suggest that preexisting C5-specific antibodies may be correlated with the survival of infants living with HIV, motivating additional research into their protective potential.

Keywords. antibodies; HIV envelope; HIV vertical transmission; phage display; placental antibody transfer.

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As the search for an effective human immunodeficiency virus (HIV) vaccine continues, there remains a need to identify immune correlates of protection from HIV acquisition and/or pathogenesis. While animal models have been fruitful for proof-of-concept studies, measurement of protection from HIV infection requires human studies. The setting of HIV vertical transmission is unique in this regard because HIV-specific antibodies are passively transferred to infants during gestation and remain in circulation after birth [1]. For this reason, numerous studies of vertical transmission have examined whether the quantity and/or qualities of maternal and passive antibodies are correlated with infant outcomes. Some studies have suggested that binding antibodies targeting variable loop 3 (V3), the CD4 binding site, and/or the glycoprotein (gp) 41 ectodomain are correlated with vertical transmission risk, although these studies are based primarily on antibodies present in the mother, and results have been variable [2–5].

The role of passively transferred HIV-specific antibodies in infant outcomes can best be evaluated in cases where infants are exposed through breastfeeding and test HIV negative at birth, so that passive antibodies can be measured around the time of HIV exposure. Here, we examined epitopes beyond the well-studied and immunodominant antigenic sites identified in previous studies to characterize antibody responses to linear epitopes outside these domains in 2 Kenyan cohorts of breastfeeding mother-infant pairs [6]. Using a high-throughput screen of plasma from 1 cohort, we identified passively acquired responses to variable loop 1 and 2 (V1/V2) and constant region 5 (C5) as correlates of improved survival in infants who acquired HIV during the study (infants living with HIV [ILWH]). In an analysis combining binding results from 2 cohorts, C5 peptide enzyme-linked immunosorbent assay (ELISA) activity was correlated with improved ILWH survival, delayed HIV acquisition, and lower set point viral load.

METHODS

Study Design

In both the Nairobi Breastfeeding Trial (NBT) and Cytotoxic T Lymphocyte (CTL) cohorts, a subcohort was selected for inclusion in this study based on infant criteria, which included an HIV-negative DNA/RNA test result at birth, breastfeeding history ≥3 months or until the time of transmission, and availability of an infant sample from the first week of life to measure passive antibody. In all, 72 and 86 mother-infant pairs from NBT and CTL, respectively. For the infants who acquired HIV during the follow-up period, the estimated time of infection was defined as the midpoint between the last negative and first positive HIV-1 DNA/RNA polymerase chain reaction test

result. Study participants provided written informed consent before enrollment and for use of their data and samples for future studies Approval to conduct this study was provided by the Kenyatta National Hospital–University of Nairobi Ethics and Research Committee, the Fred Hutchinson Cancer Center Institutional Review Board, and/or the University of Washington Institutional Review Board.

Phage Display of HIV Envelope Peptides

Phage display immunoprecipitation sequencing was performed as described elsewhere (see Supplementary Methods). An oligonucleotide pool encoding 1369 peptides was generated for the *env* ectodomain and transmembrane domain (HXB2; amino acids 30–704) from 6 HIV-1 strains (further defined in Supplementary Methods): B41, BF520, BG505, BL035, QA013, and ZA1197. Phages displaying envelope (Env) peptides were incubated with heat-inactivated plasma samples. Antibody-bound phages were immunoprecipitated and samples were then prepared for multiplexed sequencing.

Peptide ELISA

Plasma samples were added to plates coated overnight with NeutrAvidin Protein and then a biotinylated C5 peptide (Biotin-SELYKYKVVKIEPLGIAPTAAKRRVVQREKR; HXB2; amino acids 481–511). Immunoglobulin G was detected using goat anti-human immunoglobulin G–horseradish peroxidase. The secondary antibody was detected using 1-Step TMB-Ultra substrate. The reaction was stopped using 1N sulfuric acid and absorbance (optical density at 450 nm) was measured. The background absorbance of uncoated wells was subtracted from the absorbance of all wells.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism v9 or RStudio software v1.4.1106. All data represent the mean of 2 biological replicates. Principal components analysis was performed using enrichment data from all infants in the NBT cohort to identify regions explaining high variance with the entire data set, defined as the regions with the greatest loading vectors in the first 2 principal components, as described elsewhere (see Supplementary Methods). For ELISAs, the area under the curve was divided by 1000 for use in subsequent analyses.

RESULTS

We first tested infant samples and matched maternal plasma samples from the NBT to measure responses to HIV Env peptides, using a high-throughput phage display approach. All 72 infants were defined as HIV negative at birth, of whom 21 infants acquired HIV during the study (ILWH) and 51 remained HIV exposed and uninfected. Infant samples were obtained in first week of life to focus on passively acquired antibody responses circulating when infants were exposed to HIV through breastfeeding. Using an Env library lacking V3 and immunodominant epitope (IDE) peptides, epitopes targeted by passively transferred antibodies in infant plasma included C1, the C terminus of V1 and beginning of V2 (V1/V2), and C5 within the gp120 subunit, as well as peptides within the C-heptad repeat (CHR) (Figure 1). Nearly all individuals showed responses to C5, whereas responses to other epitopes were less common. Maternal plasma samples from the third trimester of pregnancy showed similar responses to infants (Supplementary Figure 1).

Principal components analysis was then used to identify regions of high variance across samples. Three regions contributed high variance in responses among infant samples: V1/V2, C5, and the gp41 CHR (Supplementary Figure 2). To compare differences in the aggregate responses to these regions between individuals while also limiting multiple hypothesis testing, we summed the enrichment values for strain-specific peptides spanning each region and averaged the enrichment values from each library strain. We next examined whether summed enrichment for any of these regions was associated with infant infection status or clinical outcome. Maternal and passively acquired infant responses to any region were not associated with HIV acquisition risk in binomial logistic regression analysis, adjusted for maternal viral load. By contrast, there was an association between improved survival of ILWH and aggregate infant responses to V1/V2 (hazard ratio [HR], 0.84; $P = .046$) and C5 (0.95; $P = .048$), but not to CHR, in Cox proportional hazards models of infant survival, adjusted for maternal viral load. There was a trend between maternal plasma responses and infant survival for C5 but not for V1/V2 (HR, 0.95 for C5 [$P = .06$] and 0.97 for V1/V2 [$P = .18$]).

To further address whether C5-specific antibodies were correlated with infant survival, we synthesized a peptide spanning the C-terminal end of C5 and tested plasma antibody binding to this peptide via ELISA. Compared with nonsurviving infants, surviving ILWH showed a trend for higher C5-specific binding activity measured by ELISA , although this difference did not reach statistical significance ($P = .07$) (Figure 2*A*). In a Cox proportional hazards model of infant survival adjusted for maternal viral load, C5-specific ELISA activity was associated with improved ILWH survival (HR, 0.90; $P = .03$). C5-specific ELISA activity was also correlated with C5 enrichment (Spearman $r = 0.59$; $P = .006$) (Supplementary Figure 3), suggesting that the peptide ELISA measured responses similar to those detected via phage display.

To further validate the correlation between C5-specific antibody responses and ILWH survival, we repeated the C5 peptide ELISA using week 1 plasma from a second cohort (CTL) of infants who acquired HIV during breastfeeding (n = 14). There was also a trend for higher C5-specific ELISA activity in surviving compared with nonsurviving infants ($P = .06$) (Figure 2*B*). In

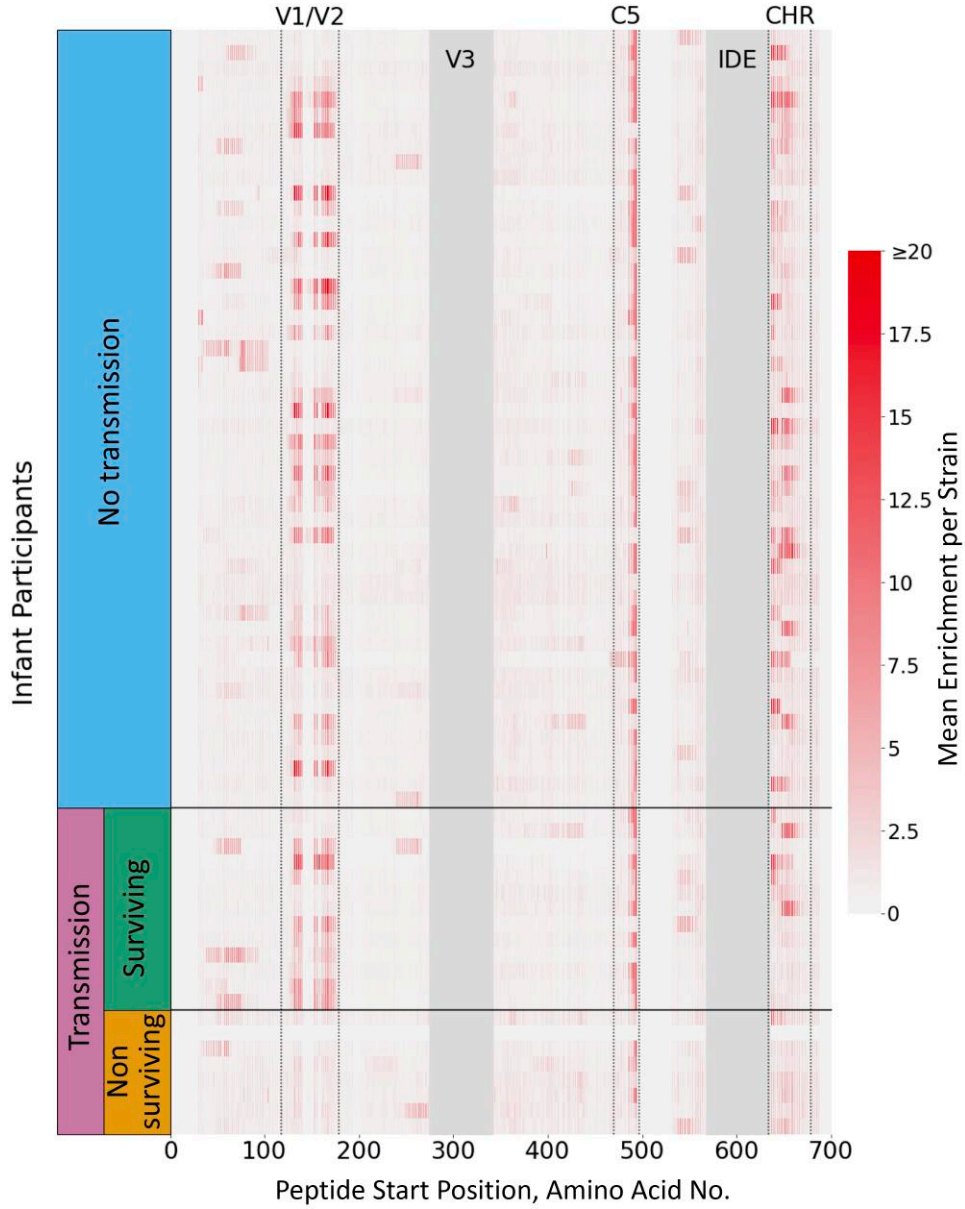

Figure 1. Envelope protein phage display responses in the Nairobi Breastfeeding Trial (NBT) cohort of infants. Heat map shows the average enrichment for peptides at each position across the envelope protein. Enriched peptides are shown, with darker intensity indicating higher enrichment (binding), as indicated by the scale on the right, for infants in the NBT cohort (n = 72). Peptides are ordered by the start position of the N-terminal amino acid, beginning at HXB2 amino acid 31 and spanning amino acid 704. Regions excluded from the library, variable loop 3 (V3) and the glycoprotein (gp) 41 disulfide loop, are indicated in light gray. Rows indicate plasma samples and are grouped by infant human immunodeficiency virus (HIV) acquisition status and survival for infants living with HIV (ILWH; n = 20). Insufficient sample remained for 1 ILWH; this sample is shown in white (no data) to maintain consistency between figures for infant and maternal data (Supplementary Figure 1). Within each group, individuals are ordered by ascending maternal viral load. Dotted vertical lines indicate regions contributing high variance in principal components analysis, with epitopes indicated above: variable loop 1 and 2 (V1/V2; HXB2; amino acids 125–210), constant region 5 (C5; HXB2; amino acids 462–511), and the gp41 C-heptad repeat (CHR; HXB2; amino acids 617–6-96). Data are from 2 replicate phage display immunoprecipitation sequencing experiments. Abbreviation: IDE, Immunodominant epitope.

a Cox proportional hazards model, C5-specific ELISA activity trended with improved survival of ILWH (HR, 0.63; $P = .07$). Because the selection criteria for infants included from each study were similar (as discussed in Supplementary Methods), we combined the ELISA results from both cohorts to determine whether C5-specific ELISA activity was correlated with infant survival in the combined cohort (n = 34). In this combined analysis of a larger sample size, C5 ELISA activity was also associated with improved survival of ILWH in a Cox proportional hazards model (HR, 0.91; $P = .01$).

We next examined whether C5-specific responses were correlated with other measures of protection, including estimated

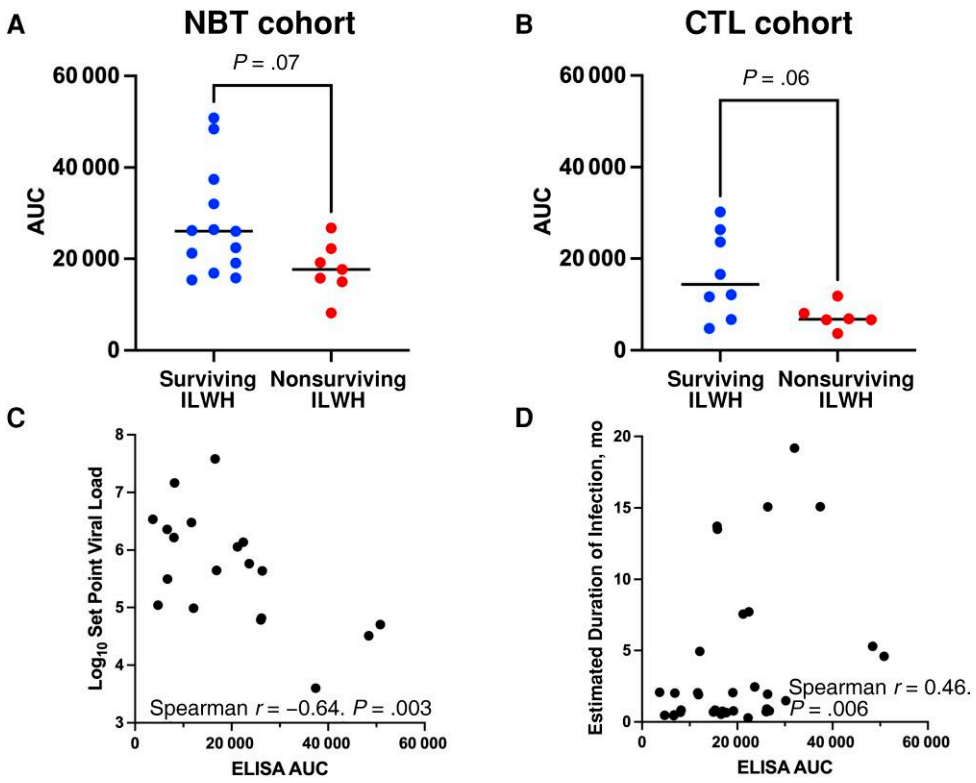

Figure 2. Correlation of constant region 5 (C5) peptide enzyme-linked immunosorbent assay (ELISA) activity with clinical measures in infants living with human immunodeficiency virus (HIV) (ILWH). *A,* C5 peptide ELISA activity among surviving (*blue*; n = 13) and nonsurviving (*red*; n = 7) ILWH in the Nairobi Breastfeeding Trial (NBT) cohort. *B,* C5 peptide ELISA activity among surviving (*blue*; n = 8) and nonsurviving (*red*; n = 6) ILWH in the Cytotoxic T Lymphocyte (CTL) cohort. *A, B,* Median activity was compared between groups using the Mann-Whitney *U* test. Data are from 2 technical and biological replicates. *C,* Plot of combined cohort C5 peptide ELISA activity versus $\log_{10}$ set point viral load for infants with viral load data available (n = 20). *D,* Plot of combined cohort C5 peptide ELISA activity versus estimated duration of HIV infection for ILWH (n = 34), as defined in Methods. Abbreviation: AUC, area under the receiver operating characteristic curve.

infection time, set point viral load, or peak viral load, all of which have previously been associated with decreased survival and/or increased HIV pathogenesis in infants [7, 8]. Set point and peak viral load estimates were available for 9 of 20 infants in the NBT cohort and 11 (set point) or 14 (peak) of 14 infants in the CTL cohort. Among this subset of 20 or 23 infants, C5 ELISA activity was inversely correlated with set point viral load (Spearman $r = -0.64$; $P = .003$ (Figure 2*C*) and trended inversely with peak viral load (Spearman $r = -0.38$; $P = .08$) (Supplementary Figure 4). Furthermore, C5 ELISA activity was correlated with the estimated time of HIV acquisition for ILWH (Spearman $r = 0.46$; $P = .006$) (Figure 2*D*).

DISCUSSION

In the current study, we used phage display of Env ectodomain peptides to assess whether maternal or passively acquired infant antibody responses to specific peptides were associated with HIV vertical transmission or survival of ILWH in 2 Kenyan cohorts (see STROBE [Strengthening the Reporting of Observational studies in Epidemiology] diagrams in Supplementary Figure 5. Plasma samples showed consistent responses to peptides spanning the V1/V2, C5, and CHR regions of Env. Passively acquired responses to both V1/V2 and C5 were associated with improved survival of ILWH. Further interrogation of the C5 responses showed that levels of C5-specific antibodies are also associated with various measures of protection.

Our conclusion that passively acquired C5-specific antibody responses are associated with improved survival in ILWH is supported by results from 2 cohorts and the use of orthogonal methods. In addition to the association with survival in combined analyses, C5-specific ELISA activity was correlated inversely with set point viral load and directly with estimated infection time. Because both of these measures are known correlates of infant survival, the correlation of C5-specific antibody activity with these measures may explain how such passively transferred antibodies might directly affect infant survival. It is noteworthy that several early studies of human cohorts support a role of C5-specific antibodies in slowed disease progression [9, 10]. In 1 study, rapid progressors exhibited higher responses to a C5 peptide than nonprogressors at early time

points, but this response waned in rapid progressors and was maintained or gained in nonprogressors [9]. In another study, slow-progressing individuals showed higher early antibody responses to C5 than rapid progressors [10]. The consistency of the results presented here provides support for a role of C5-specific antibodies in improved clinical outcome.

This study does have several limitations. The limited length of peptides in phage display does not allow for capture of responses to conformational epitopes. The phage display library used in this study also did not capture responses to V3 or the gp41 disulfide loop, but this was by design to improve detection of other, less-dominant responses. V1/V2 responses were correlated with improved survival in the NBT cohort, although these results are limited to only 1 cohort and phage display of V1/V2 peptides. Given previous findings that V1/V2-specific antibody levels were correlated with vaccine efficacy in the RV144 trial and that vaccine efficacy was dependent on specific residues in V1/V2, the results of the present study could be pursued further [11, 12]. Finally, viral load data were not available for all ILWH, and the statistical power of these analyses was therefore limited within each single cohort, though C5 ELISA activity was correlated with set point viral load in the combined cohort, even with this small sample size.

Overall, these findings raise the question of how C5-specific antibodies directly affect HIV pathogenesis. Several described C5-specific monoclonal antibodies are capable of mediating antibody-dependent cellular cytotoxicity [13], which is also a correlate of infant survival [14], but the functional properties of C5-specific plasma antibodies have not been thoroughly evaluated in human cohorts. One proposed alternative hypothesis is that C5-specific antibodies reduce immune activation stemming from the homology between the C5 and human HLA proteins, though this hypothesis has not been widely tested [15]. In either case, further cohort and molecular studies of C5-specific antibodies are merited to determine whether such antibodies can be leveraged to inform immune responses that could contribute to vaccine protection.

Supplementary Data

Supplementary materials are available at *Open Forum 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.

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Potential conflicts of interest. All authors: No reported conflicts.

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