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Clinical Infectious Diseases

MAJOR ARTICLE

In Utero Human Cytomegalovirus Infection Is Associated With Increased Levels of Putatively Protective Maternal Antibodies in Nonprimary Infection: Evidence for Boosting but Not Protection

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Background. Although primary maternal cytomegalovirus infections are associated with higher risk of in utero transmission, most fetal infections worldwide result from nonprimary maternal infections. Antibodies directed at glycoprotein B (gB) and the gH/gL/pUL128–130–131 pentamer can neutralize virus, and higher levels of antibody directed at several particular pentamer epitopes defined by monoclonal antibodies (mAbs) are associated with reduced risk of fetal cytomegalovirus (CMV) transmission during primary maternal infection. This had not been explored in maternal nonprimary infection.

Methods. In a setting where most maternal CMV infections are nonprimary, 42 mothers of infants with congenital CMV infections (transmitters) were compared to 75 CMV-seropositive mothers whose infants were CMV-uninfected (nontransmitters). Control infants were matched by sex, maternal human immunodeficiency virus (HIV) status, and gestational age. We measured the ability of maternal antibodies to block 3 key pentameric epitopes: one in the gH subunit, another straddling UL130/UL131, and the third straddling gH/gL/UL128/UL130. We tested if levels of antibodies directed at these epitopes were higher in nontransmitters compared to transmitters.

Results. Levels of all 3 putatively protective pentamer-directed antibodies were significantly higher in transmitters compared to nontransmitters. In contrast, antibodies targeting an epitope on gB were not different. Total antibody specific for pentamer and for gB were also higher in transmitters.

Conclusions. We found no evidence that higher levels of any CMV-specific antibodies were associated with reduced risk of congenital CMV infection in nonprimary maternal infection. Instead, we found higher maternal antibody targeting epitopes on CMV pentamer in transmitters than nontransmitters, providing evidence for antibody boosting but not protection.

Keywords. congenital infection; cytomegalovirus; antibody.

Congenital cytomegalovirus (cCMV) infection is the most common infectious cause of birth defects worldwide, with sequelae including sensorineural hearing loss, psychomotor impairments, mental retardation, cerebral palsy, and seizures [1]. CMV infection, once acquired, is not eradicated by the host [2], and reinfections and reactivations are common [2]. Antiviral treatment generally does not reverse negative sequelae in the newborn [2–5], and many maternal nonprimary infections are difficult to identify [6]; therefore, efforts have focused on preventing transmission to the fetus. In many low- and middle-income countries, CMV seroprevalence in the general population is very high, and most women acquire CMV before they reach childbearing age [7–9]. Thus, most cCMV infections worldwide likely occur in the context of nonprimary maternal CMV infection [1, 10]. Maternal human immunodeficiency virus type 1 (HIV-1) infection also drives the incidence of cCMV higher in high HIV-1 prevalence areas; mothers living with HIV-1 have 2.9–3.5 times the odds of transmitting CMV in utero to their children [11, 12].

Because of the neurological sequelae of cCMV, prevention is a high priority public health goal [1]. The risk of CMV transmission to a fetus is generally considered to be lower in maternal nonprimary infection than maternal primary CMV infection, suggesting that maternal immunity plays a role, and that a maternal CMV vaccine could reduce the risk of fetal transmission [1, 13–15].

CMV vaccine design has focused upon gB and gH/gL/pUL128–130–131 pentamer ("pentamer" herein) to induce antibodies [16] because both are involved in cell entry [17] and are targets of neutralizing antibodies [18–20]. A gB vaccine was

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only moderately efficacious in preventing primary maternal infection in women of child-bearing age (50%, $P = .02$) [21] or teenage girls (43%, $P = .08$) [22], with the former study not powered to measure efficacy in preventing congenital CMV infections. Similar studies for nonprimary infection with infection as an endpoint would be challenging because identification of active CMV infections in previously exposed individuals by serological means and other methods is of uncertain reliability [16]. Vaccines containing pentamer elicit neutralizing antibodies in mice [23, 24] and have undergone phase I safety/immunogenicity studies [25], but no efficacy studies have been reported.

Key epitopes within the CMV pentamer entry receptor have been identified and characterized [26]. Antibodies target a range of epitopes displayed on the pentamer, including epitopes shared by UL130 and UL131, an epitope on gH, one on UL128, as well as conformational epitopes or those with surfaces shared more widely among the pentamer subunits [20, 26]. Antibodies to these pentamer epitopes were measured in serum samples by their ability to block binding by the monoclonal antibodies that define the epitopes. Among mothers with primary CMV infections, greater blocking activity for several epitopes was observed in serum samples from mothers who did not transmit compared to those who did transmit, including 3 that are the focus of this study [20]. This suggested that maternal antibodies to these key epitopes may be important for protection from fetal infection.

We investigated the association of antibodies targeting these 3 putatively protective pentamer epitopes in serum from peripartum mothers, in a population with predominantly nonprimary CMV infections, between mothers who transmitted and did not transmit CMV to their newborns in utero. Second, we compared levels of these antibodies in mothers living with human immunodeficiency virus (HIV) with those who were HIV-1 negative because women living with HIV-1 have an increased risk of transmitting CMV to their fetus [11, 12]. These 3 epitopes were chosen because antibodies targeting them were observed to be higher in transmitting mothers with primary infection until at least 60 days following onset of CMV infection [20]. The chosen epitopes targeted UL130/UL131 (10P3), the gH subunit (11B12), and an epitope dependent upon 4 of the pentamer subunits (8I21) [20, 26]. For comparison, an epitope on glycoprotein B (gB) (6B4) was also tested [26]. Additionally, total antibodies targeting pentamer or gB were also tested.

METHODS

Study Design

We analyzed maternal blood samples collected from a cross-sectional study that identified congenital CMV infections in newborn infants at the Chris Hani-Baragwanath Academic Hospital from May 2016 to December 2016 [12, 27] (ClinicalTrials.gov: NCT03722615). The study was nested within a larger cohort, in which mother-newborn dyads were enrolled from June 2014 to investigate the serologic correlates of protection against invasive Group B streptococcal disease (ClinicalTrials.gov: NCT02215226). The nested CMV cross-sectional study [12, 27] identified congenital CMV (cCMV) cases, who were defined as neonates who were CMV polymerase chain reaction (PCR) positive in saliva within 3 days of birth and positive in a confirmatory PCR of saliva and/or urine at up to 3 weeks of age, and controls who tested negative for CMV within 3 days and again within 21 days and had no history of hospitalization within the first 3 weeks of life. Forty-six cCMV cases were identified and were matched to a target of 2 controls. Controls were matched to cases by maternal HIV-1 status, child gender, and gestational age at birth (within 2 weeks) [12, 27]. We compared results from mothers of cases ("transmitters") to mothers of matched cCMV negative controls ("nontransmitters") [12, 27]. In total, we analyzed 45 maternal serum samples collected from transmitters (28 living with HIV-1; 17 HIV-1 negative) because there was one set of twins among the cCMV cases and 75 maternal serum samples of nontransmitters (42 living with HIV-1; 33 HIV-1 negative). Maternal samples were collected between admission and 24 hours following delivery.

Reagents

Purified his-tagged gB was obtained from SinoBiologicals (Beijing, China), and purified CMV pentamer was obtained from the Native Antigen Company (Oxford, England).

Monoclonal antibodies 8I21, 10P3, and 11B12 are directed against different sites on the CMV gH/gL/pUL128–130–131 pentamer [20]. Monoclonal antibody 6B4 [26] is directed against a neutralizing epitope on gB and has moderately potent neutralizing activity against CMV invading monocyte-derived dendritic cells [26]. Monoclonal antibodies were produced using DNA constructs synthesized (Genscript, Piscataway, New Jersey, USA) from publicly available sequences, as previously described [28]. Monoclonal antibodies were biotinylated using an EZ-Link Sulfo-NHS-LC-Biotinylation Kit (Thermo Fisher, Waltham, Massachusetts, USA). Horseradish peroxidase-conjugated goat polyclonal anti-biotin and 1-step Ultra TMB-ELISA substrate were obtained from Thermo Fisher. Horseradish peroxidase conjugated anti-human immunoglobulin G (IgG) (Fc specific) was obtained from Sigma Aldrich (Schnelldorf, Germany).

ELISA Testing for Total Antibody Levels Directed at gB or Pentamer

Enzyme-linked immunosorbent assay (ELISA) plates were coated with a pretitrated amount of gB or pentamer in sodium carbonate pH 9.6 overnight, washed with phosphate buffered saline + 0.05% Tween-20 (PBST), and blocked with PBST + 5% fetal bovine serum (PBST-S). Serum samples diluted in PBST-S to 2 pretitrated levels, each in duplicate were added and incubated, then washed.

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Horseradish peroxidase conjugated anti-human IgG diluted in PBST-S was added and incubated, then washed. Plates were developed with 1-step Ultra TMB-ELISA substrate. Known CMV seronegative and seropositive serum samples were run in every experiment. One sample was run as a standard and assigned an antibody level of 1 arbitrary unit per mL; units of antibodies targeting gB and pentamer were thus unrelated.

ELISA Testing for Epitope-Specific Antibody Levels

Epitope-specific ELISAs were run largely as described previously [20]. Plates were prepared as above for total antigen-specific ELISAs. Four 4-fold dilutions, starting at 1/10, in duplicate of serum samples were incubated, and then the plates were washed with PBST. The monoclonal antibody conjugated to biotin was incubated in PBST-S at a pretitrated concentration, 0.3 µg/mL, and the plate was washed. Bound monoclonal antibody was detected with horseradish peroxidase-conjugated anti-biotin in PBST-S, as above. The level of antibodies directed against each epitope was defined by the dilution of serum required to inhibit 50% of binding of that monoclonal antibody to pentamer protein (50% inhibitory dilution [ID_{50}]).

Statistical Analysis

Total antigen-specific antibody levels were normalized to a sample run in every assay, as described above. For epitope-specific antibody levels, inhibition of binding of the monoclonal antibody for that epitope were calculated using a log inhibitory graph fit model in GraphPad Prism (San Diego, California, USA). ID_{50} values below 1 were presumed to be negative [20] and set to 1 for statistical analysis. Results from transmitters versus nontransmitters were compared using multilevel mixed effects linear regression (Stata 13.1, College Station, Texas, USA) with a random effect added for each group of one transmitter (mother of a case) and the mother(s) of that case's matched control(s) (nontransmitters(s)). Thus, in the statistical analyses, transmitters were directly compared only to nontransmitters who were mothers of matched controls of that transmitter's child. Logged arbitrary units (total antigen-specific antibody levels) or logged ID_{50} values (epitope-specific antibody levels) were the input values. Differences in log values from the models were converted back to fold differences of unlogged values to describe fold differences in antibody levels. Values from mothers living with HIV-1 compared to HIV-1 negative mothers were compared using a *t* test (Stata 13.1) on logged output values. Number of previous pregnancies were compared between the groups using multilevel mixed effects ordered logistic regression. Other demographic parameters were compared using multilevel mixed effects linear regression. Box plots depict median values, with 25th- and 75th-percentile values represented by the edges of boxes. Error bar locations were calculated using the Tukey-style display method [29].

Study Approval

The Human Research Ethics Committee of the University of Witwatersrand, Johannesburg, South Africa (HREC M151161), approved this study. Mothers provided written informed consent for screening their neonates for CMV, and a separate written consent was obtained for infant participation in this study and a longitudinal study of sequelae in the children [12, 27].

RESULTS

Transmitters Have Higher Levels of Antibodies Directed Against gB and Pentamer

We compared levels of antibodies directed against gB and gH/gL/pUL128–130–131 pentamer between transmitting and nontransmitting mothers. We found that transmitting mothers had 1.81 times higher levels of anti-pentamer antibodies (95% confidence interval [CI]: 1.32–2.49; $P = .0003$) and 1.59 times higher levels of anti-gB antibodies (95% CI: 1.15–2.21; $P = .0053$) compared to nontransmitting mothers at delivery (Figure 1).

We also found that transmitters were significantly younger and had significantly fewer previous pregnancies (Table 1).

Transmitters Have Higher Levels of Antibodies Directed Against Three Epitopes in Pentamer but Not an Epitope in gB

We compared levels of antibodies targeting 3 epitopes on pentamer (defined by monoclonal antibodies 8I21 (site 7 on gH, gL/UL128/UL130), 10P3 (site 4 on UL130/UL131), 11B12 (site 9 on gH) [20, 26] and one epitope on gB (defined by monoclonal antibody 6B4 [26]) in maternal serum antibodies and compared transmitting versus nontransmitting mothers. Transmitters had higher levels of antibodies directed against the 3 pentamer epitopes: 1.62-fold higher against the 8I21 epitope (95% CI: 1.10–2.40; $P = .0146$, Figure 2A), 1.59-fold higher against the 10P3 epitope (95% CI: 1.13–2.25; $P = .0080$, Figure 2B), and 2.53-fold higher against the 11B12 epitope (95% CI: 9 1.00–6.40; $P = .0492$, Figure 2C) compared to nontransmitters. Antibodies directed against the 6B4 epitope on gB (0.95-fold higher, 95% CI: .62–1.48; $P = .8365$, Figure 2D) did not differ between transmitter and nontransmitters. This suggests that the enhanced responses to pentameric epitopes described above is epitope-specific and not just a reflection of a generally stronger immune response in transmitting mothers. We considered the possibility that our results might be skewed by the high HIV-1 prevalence in our population. However, a subanalysis of only HIV-1 negative mothers, suggested our findings are likely applicable in areas with lower HIV-1 prevalence. Levels of 10P3 specific antibodies were higher in transmitters compared to nontransmitters among the HIV-1 negative mothers (1.70-fold, 95% CI: 1.05–2.75, $P = .0316$), as were total pentamer-specific antibody levels (2.04-fold, 95% CI: 1.29–3.23, $P = .0024$) and total gB-specific antibody levels (1.90-fold, 95% CI: 1.20–3.00,

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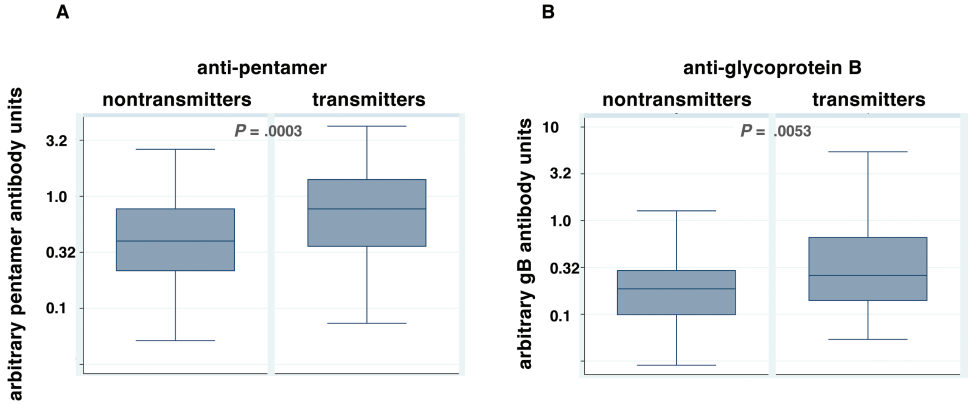

Figure 1. A comparison between transmitters and nontransmitters of the levels of serum antibody directed against CMV pentamer (*A*) and glycoprotein B (*B*). Levels are displayed in arbitrary units, and the arbitrary units of the 2 antigens are not comparable. Abbreviation: CMV, cytomegalovirus.

P = .0060). Levels of 8I21-specific antibodies (1.51-fold, 95% CI: .79–2.89, *P* = .2098) and 11B12-specific antibodies (1.97-fold, 95% CI: .64–6.16, *P* = .2386) trended in the same direction, although were not statistically significant, perhaps due to the smaller sample size.

Some of the Antibody Measures Were Higher in Mothers Living With HIV-1

A comparison of the anti-CMV antibody levels between women living with and without HIV-1 indicated that, among the different antibody types measured, only 11B12 blocking levels and total gB levels were higher in those living with HIV-1 (Table 2). A stratified analysis performed separately in transmitters and nontransmitters indicated that total antibody levels specific for gB were higher in women living with HIV when analyzing the nontransmitters and not when analyzing the transmitting mothers (Figure 3).

DISCUSSION

In a setting with mostly nonprimary CMV infections, antibodies directed against key sites on the gH/gL/pUL128–130–131 pentamer were significantly higher in CMV transmitting than nontransmitting women. This is the opposite association compared to a previous study of women with primary CMV infection [20]. This was also evident for total antibodies directed against pentamer and against glycoprotein B. Antibodies that block the 6B4 epitope on gB were not different between the groups, suggesting that this effect is specific to certain epitopes.

Total antibody directed at pentamer and glycoprotein B were present at similarly higher levels in transmitters. Notably, for all measures in which there was a significant difference between transmitters and nontransmitters in antibody levels, the direction was uniform: higher levels in transmitting mothers. Thus, in this study of mothers with nonprimary CMV infection, we found no association that suggested a maternal immune response that might protect from in utero CMV transmission.

Based on our observations, we speculate that the higher antibody levels we observed in transmitters may have been due to boosting from CMV reinfections or reactivations. Such reactivations and reinfections may have been more common in the transmitters compared to the nontransmitters because these events were likely necessary for transmission to the fetus.

We analyzed a population in which most people are exposed to CMV prior to adulthood [7, 11] because most congenital CMV infections worldwide arise from maternal nonprimary infections [10]. We considered that such a study might reveal a critical immunity gap that permits transmission in the face of an already existing immune response. If so, we might have been able to distinguish a directly protective antibody response from among the other responses found in individuals previously exposed to CMV.

Table 1. Demographic Information for Mothers

Attribute	Transmitters[a], n = 45	Nontransmitters[b], n = 75	*P* value
Age at delivery, y median (IQR)	25 (22–29) (19–40)	28 (24–33) (19–40)	.0030
Previous pregnancies median (IQR)	1 (0–2) (0–4)	1 (1–2) (0–4)	.0326
Time from childbirth to sample collection, h[c] median (IQR)	2.9 (1.7–18.2) (−3.3–31.8)[d]	9.5 (1.4–20.0) (−9.2–54.6)[d]	.4461

Abbreviation: IQR, interquartile range.

[a] Mothers of congenital cytomegalovirus (CMV) cases.

[b] Mothers of matched CMV negative control infants.

[c] Negative value indicates sample collected before birth

[d] Data missing for one study participant in each group (n = 44 and n = 74 shown).

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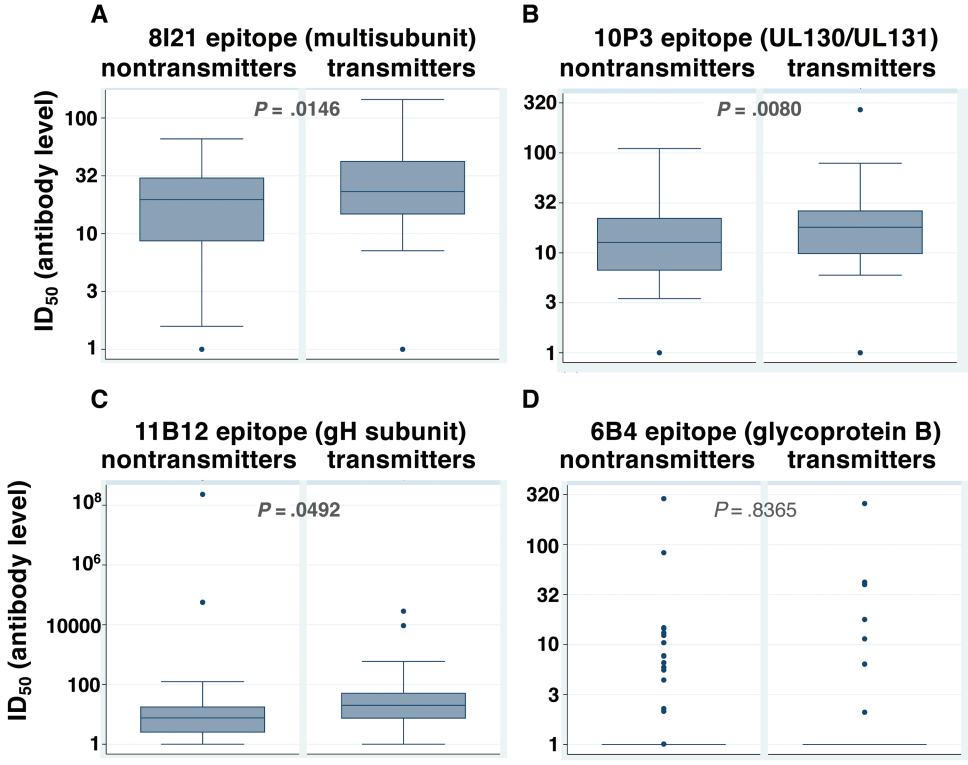

Figure 2. Comparison between transmitters and nontransmitters of the levels of serum antibodies specific for key epitopes on CMV pentamer (*A–C*) and glycoprotein B (*D*). Levels are displayed as 50% inhibitory dilution (ID_{50}) values corresponding to the dilution of serum that inhibits 50% of binding of the applicable biotinylated monoclonal antibody to the pentamer antigen coated on the bottom of the ELISA well. Abbreviations: CMV, cytomegalovirus; ELISA, enzyme-linked immunosorbent assay.

To our knowledge, levels of antibodies directed against specific epitopes within pentamer have not been previously investigated in the context of nonprimary CMV infection. Vanarsdall et al measured levels of neutralizing antibody to pentamer in women with nonprimary infection and detected no difference between transmitters and nontransmitters [30]. This study assessed antibody levels during the first trimester, likely before any maternal CMV reactivations/reinfections that could give rise to the child's cCMV infection or a boost in maternal antibody levels. Although our results cannot be directly compared, this study also did not support a role for anti-pentamer antibodies in prevention of vertical transmission of CMV.

Table 2. Comparison of Antibody Levels Between Mothers Living With HIV-1 and HIV-1 Negative Mothers

Measure	Ratio PLWH/HIV neg (95% CI)	*P* value
8I21 blocking, ID_{50}	1.34 (0.91–1.99)	.1388
10P3 blocking, ID_{50}	1.29 (0.89–1.88)	.1770
11B12 blocking, ID_{50}	2.94 (1.18–7.35)	**.0212**
6B4 blocking, ID_{50} (anti-gB)	1.02 (0.66–1.58)	.9236
Total anti-pentamer	1.34 (0.95–1.90)	.0946
Total anti-gB	1.50 (1.07–2.10)	**.0176**

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; PLWH, people living with HIV; ID50: 50% inhibitory dilution.

A limitation of our study compared to the equivalent studies in primary infection is our inability to pinpoint the timing of the maternal CMV infection(s) that gave rise to the transmission to the fetus. However, we chose 3 epitopes for which the higher levels in nontransmitters with primary CMV infection persisted until 60 days following onset of symptoms, and longer for one epitope [20], to maximize our potential to detect such associations. It is also challenging to identify nonprimary CMV infections in pregnant women because they are often be transient and/or restricted anatomically [6]. We did not attempt to identify such infections in this study. Because of this, it is likely that we preferentially included women who did not have a CMV reactivation or reinfection during pregnancy among the nontransmitters. Although this may have diluted a correlation showing higher antibody levels in transmitters, it cannot explain why we observed the opposite effect. We are also unable to rule out maternal age or pregnancy history as potential confounders.

The risk of fetal CMV transmission is elevated in mothers living with HIV-1 [10, 12]. We thus considered that a defect in CMV control might lead to this increased risk, and that such increased risk might be reflected in reduced levels of protective antibodies. However, only 2 measures significantly differed between the groups (total antigen-specific gB antibodies and 11B12 epitope-specific antibodies), and these differences were

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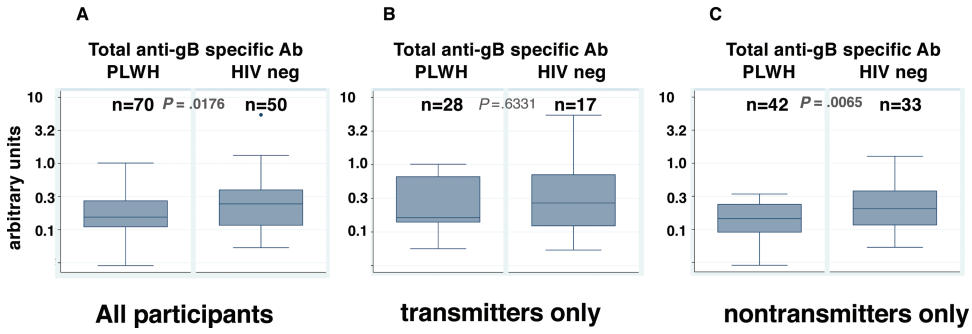

Figure 3. Comparison between mothers living with HIV-1 (PLWH) and HIV-1 negative mothers of the levels of serum antibodies directed against glycoprotein B in all study participants (*A*) and stratified by CMV transmitter status (*B, C*). Abbreviations: CMV, cytomegalovirus; HIV-1, human immunodeficiency virus type 1.

in the direction that did not support the hypothesis that these antibodies were protective.

Interestingly, when we considered the effect of HIV-1 status upon total antigen-specific gB antibody levels, stratified by CMV transmission status, we observed that the higher antibody levels in mothers living with HIV was apparent only in nontransmitters. This is consistent with the possibility of a boosting effect from greater, more frequent, and/or more recent exposure to CMV reinfections or reactivations in women living with HIV. The difference was not observed among the transmitters, possibly because this group is selected for those with reactivations or reinfections that could be transmitted to a fetus, irrespective of the mother's HIV status. The design of this study, with control infants matched by maternal HIV status, could be expected to partially obscure effects of HIV-1 status that are not independent of transmitter status. Nonetheless, our observations are not easily consistent with the absence of a protective antibody response in mothers living with HIV-1 that could explain their higher risk of transmitting CMV in utero.

Our findings have significant implications for CMV vaccine development. None of the antibody measures we tested were higher in nontransmitters or higher in HIV-uninfected mothers who are at lower risk of in utero CMV transmission to their fetuses. Indeed, all of the associations we observed were in the opposite direction. This suggests that antibodies elicited by a CMV vaccine including CMV pentamer or gB are unlikely to reduce in utero transmission of CMV in settings with a high prevalence of prior CMV exposure in pregnant women. Studies of $CD4^+$ T-cell responses [31] may be more enlightening in identifying suitable vaccine targets.

Notes

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References

1. Permar SR, Schleiss MR, Plotkin SA. Advancing our understanding of protective maternal immunity as a guide for development of vaccines to reduce congenital cytomegalovirus infections. J Virol **2018**; 92:e00030-18.
2. Pass RF. Epidemiology and transmission of cytomegalovirus. J Infect Dis **1985**; 152:243–8.
3. Bale JF, Miner L, Petheram SJ. Congenital cytomegalovirus infection. Curr Treat Options Neurol **2002**; 4:225–30.
4. Sharland M, Luck S, Griffiths P, Cotton M. Antiviral therapy of CMV disease in children. Adv Exp Med Biol **2011**; 697:243–60.
5. Rawlinson WD, Boppana SB, Fowler KB, et al. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. Lancet Infect Dis **2017**; 17:e177–88.
6. Barbosa NG, Yamamoto AY, Duarte G, et al. Cytomegalovirus shedding in seropositive pregnant women from a high-seroprevalence population: The Brazilian Cytomegalovirus Hearing and Maternal Secondary Infection Study. Clin Infect Dis **2018**; 67:743–50.
7. Schoub BD, Johnson S, McAnerney JM, et al. Is antenatal screening for rubella and cytomegalovirus justified? S Afr Med J **1993**; 83:108–10.
8. Miles DJ, van der Sande M, Jeffries D, et al. Cytomegalovirus infection in Gambian infants leads to profound CD8 T-cell differentiation. J Virol **2007**; 81:5766–76.
9. Mussi-Pinhata MM, Yamamoto AY, Aragon DC, et al. Seroconversion for cytomegalovirus infection during pregnancy and fetal infection in a highly seropositive population: "The BraCHS Study." J Infect Dis **2018**; 218:1200–4.
10. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev **2013**; 26:86–102.
11. Manicklal S, van Niekerk AM, Kroon SM, et al. Birth prevalence of congenital cytomegalovirus among infants of HIV-infected women on prenatal antiretroviral prophylaxis in South Africa. Clin Infect Dis **2014**; 58:1467–72.
12. Pathirana J, Groome M, Dorfman J, et al. Prevalence of congenital cytomegalovirus infection and associated risk of in utero human immunodeficiency virus (HIV) acquisition in a high-HIV prevalence setting, South Africa. Clin Infect Dis **2019**; 69:1789–96.
13. Plotkin SA. Seroconversion for cytomegalovirus infection during pregnancy and fetal infection in a highly seropositive population: "The BraCHS Study," by Mussi-Pinhata *et al*. J Infect Dis **2018**; 218:1188–90.
14. Gugliesi F, Coscia A, Griffante G, et al. Where do we stand after decades of studying human cytomegalovirus? Microorganisms **2020**; 8:685–714.
15. Britt WJ. Congenital human cytomegalovirus infection and the enigma of maternal immunity. J Virol **2017**; 91:e02392–16.

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16. Gerna G, Lilleri D. Human cytomegalovirus (HCMV) infection/re-infection: development of a protective HCMV vaccine. New Microbiol **2019**; 42:1–20.
17. Ryckman BJ, Rainish BL, Chase MC, et al. Characterization of the human cytomegalovirus gH/gL/UL128-131 complex that mediates entry into epithelial and endothelial cells. J Virol **2008**; 82:60–70.
18. Cranage MP, Kouzarides T, Bankier AT, et al. Identification of the human cytomegalovirus glycoprotein B gene and induction of neutralizing antibodies via its expression in recombinant vaccinia virus. EMBO J **1986**; 5:3057–63.
19. Fouts AE, Chan P, Stephan JP, Vandlen R, Feierbach B. Antibodies against the gH/gL/UL128/UL130/UL131 complex comprise the majority of the anti-cytomegalovirus (anti-CMV) neutralizing antibody response in CMV hyperimmune globulin. J Virol **2012**; 86:7444–7.
20. Lilleri D, Kabanova A, Revello MG, et al. Fetal human cytomegalovirus transmission correlates with delayed maternal antibodies to gH/gL/pUL128-130-131 complex during primary infection. PLoS One **2013**; 8:e59863.
21. Pass RF, Zhang C, Evans A, et al. Vaccine prevention of maternal cytomegalovirus infection. N Engl J Med **2009**; 360:1191–9.
22. Bernstein DI, Munoz FM, Callahan ST, et al. Safety and efficacy of a cytomegalovirus glycoprotein B (gB) vaccine in adolescent girls: a randomized clinical trial. Vaccine **2016**; 34:313–9.
23. Chiuppesi F, Nguyen J, Park S, et al. Multiantigenic modified vaccinia virus ankara vaccine vectors to elicit potent humoral and cellular immune responses against human cytomegalovirus in mice. J Virol **2018**; 92:e01012–18.
24. Kabanova A, Perez L, Lilleri D, et al. Antibody-driven design of a human cytomegalovirus gHgLpUL128L subunit vaccine that selectively elicits potent neutralizing antibodies. Proc Natl Acad Sci U S A **2014**; 111:17965–70.
25. Adler SP, Lewis N, Conlon A, et al; V160-001 Study Group. Phase 1 clinical trial of a conditionally replication-defective human cytomegalovirus (CMV) vaccine in CMV-seronegative subjects. J Infect Dis **2019**; 220:411–9.
26. Macagno A, Bernasconi NL, Vanzetta F, et al. Isolation of human monoclonal antibodies that potently neutralize human cytomegalovirus infection by targeting different epitopes on the gH/gL/UL128-131A complex. J Virol **2010**; 84:1005–13.
27. Pathirana J, Texeira L, Munian H, et al. Neurological and growth outcomes in South African children with congenital cytomegalovirus: a cohort study. PLoS One **2020**; 15:e0238102.
28. Sacks D, Bhiman JN, Wiehe K, et al. Somatic hypermutation to counter a globally rare viral immunotype drove off-track antibodies in the CAP256-VRC26 HIV-1 V2-directed bNAb lineage. PLoS Pathog **2019**; 15:e1008005.
29. Krzywinski M, Altman N. Visualizing samples with box plots. Nat Methods **2014**; 11:119–20.
30. Vanarsdall AL, Chin AL, Liu J, et al. HCMV trimer- and pentamer-specific antibodies synergize for virus neutralization but do not correlate with congenital transmission. Proc Natl Acad Sci U S A **2019**; 116:3728–33.
31. Bialas KM, Tanaka T, Tran D, et al. Maternal CD4+ T cells protect against severe congenital cytomegalovirus disease in a novel nonhuman primate model of placental cytomegalovirus transmission. Proc Natl Acad Sci U S A **2015**; 112:13645–50.

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