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MAJOR ARTICLE

Association of Cytomegalovirus Serostatus With Severe Acute Respiratory Syndrome Coronavirus 2 Vaccine Responsiveness in Nursing Home Residents and Healthcare Workers

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Background. Latent cytomegalovirus (CMV) infection is immunomodulatory and could affect mRNA vaccine responsiveness. We sought to determine the association of CMV serostatus and prior severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection with antibody (Ab) titers after primary and booster BNT162b2 mRNA vaccinations in healthcare workers (HCWs) and nursing home (NH) residents.

Methods. Nursing home residents ($N = 143$) and HCWs ($N = 107$) were vaccinated and serological responses monitored by serum neutralization activity against Wuhan and Omicron (BA.1) strain spike proteins, and by bead-multiplex immunoglobulin G immunoassay to Wuhan spike protein and its receptor-binding domain (RBD). Cytomegalovirus serology and levels of inflammatory biomarkers were also measured.

Results. Severe acute respiratory syndrome coronavirus 2-naive CMV seropositive (CMV^+) HCWs had significantly reduced Wuhan-neutralizing Ab ($P = .013$), anti-spike ($P = .017$), and anti-RBD ($P = .011$) responses 2 weeks after primary vaccination series compared with responses among CMV seronegative (CMV^-) HCWs, adjusting for age, sex, and race. Among NH residents without prior SARS-CoV-2 infection, Wuhan-neutralizing Ab titers were similar 2 weeks after primary series but were reduced 6 months later ($P = .012$) between CMV^+ and CMV^- subjects. Wuhan-neutralizing Ab titers from CMV^+ NH residents who had prior SARS-CoV-2 infection consistently trended lower than titers from SARS-CoV-2 experienced CMV^- donors. These impaired Ab responses in CMV^+ versus CMV^- individuals were not observed after booster vaccination or with prior SARS-CoV-2 infection.

Conclusions. Latent CMV infection adversely affects vaccine-induced responsiveness to SARS-CoV-2 spike protein, a neoantigen not previously encountered, in both HCWs and NH residents. Multiple antigenic challenges may be required for optimal mRNA vaccine immunogenicity in CMV^+ adults.

Keywords. antibodies; COVID-19; cytomegalovirus; SARS-CoV-2; vaccination.

Coronavirus disease 2019 (COVID-19) is an ongoing, worldwide pandemic caused by severe acute respiratory disease coronavirus 2 (SARS-CoV-2) infection. Because advanced age and multiple comorbidities increase susceptibility to more severe COVID-19 morbidity and mortality, nursing home (NH) residents represent an especially vulnerable population [1]. Emerging evidence suggests that latent herpesvirus infections may predispose people to worse COVID-19 outcomes [2]. For example, individuals who are β-herpesvirus cytomegalovirus (CMV) and α-herpesvirus herpes simplex virus-1 seropositive have disproportionate overrepresentation among patients hospitalized with COVID-19 compared to those with mild disease [3]. In addition, reactivation of the γ-herpesvirus Epstein-Barr virus (EBV) during acute SARS-CoV-2 infection has recently been shown to be associated with worse disease as well as with the onset, persistence, and severity of long-term sequelae [4–8].

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On the other hand, evidence from humans and mouse models suggests that latent CMV infection may improve T-cell and antibody (Ab) responses to antigenic challenge. This includes responses to vaccines and infectious agents, possibly via elevated myeloid cell activation and increased soluble interferon levels observed during CMV infection [9, 10]. However, older individuals who have often harbored infection for a longer time may have lost such improvement or even have suppressed responses [9, 11, 12].

One success of the COVID-19 pandemic response has been the unprecedented rapid development and rollout of highly efficacious vaccines. The mRNA-based vaccines have shown remarkable efficacy at preventing severe disease, even from highly evolved SARS-CoV-2 variants, such as the Delta and Omicron variants [13]. However, vaccine-induced total and neutralizing Ab titers wane, particularly in NH populations, 35%–69% of whom have undetectable Wuhan strain neutralizing Ab titers by 6 months after vaccination, suggesting vaccine-mediated protection may lessen over time [14]. This is particularly notable for neutralizing titers to the Omicron variant, which do not readily develop in either healthcare workers (HCWs) or NH residents until after a boost [15]. More importantly, not all factors that govern the generation, peak, and durability of Ab responses after COVID-19 vaccination have been elucidated.

In this report, we assessed the association of latent CMV and EBV infections with Ab responses (neutralizing, anti-spike, anti-receptor binding domain [RBD]) to the BNT162b2 mRNA vaccine in ambulatory HCWs and NH residents before and after the initial vaccination series and boost. For each population, we analyzed the vaccine responses among those who had prior SARS-CoV-2 infection (SARS-CoV-2-naive), in whom vaccine-elicited SARS-CoV-2 spike protein would be a neoantigen never previously encountered, separately from the vaccine responses among those who had prior SARS-CoV-2 infection (SARS-CoV-2-experienced), in whom the pre-existing anti-SARS-CoV-2 immunity could affect vaccine immunogenicity. Our goals were to determine whether latent CMV and/or EBV infections affected vaccine-elicited Ab responses, whether this was associated with age, and whether prior SARS-CoV-2 infection further mediated the response.

METHODS

Study Design and Participants

We collected blood and froze the serum for later analysis from a total of 107 HCWs (aged 26–78, median 48 years) and 143 NH residents (aged 48–99, median 76 years) in northeast Ohio (Table 1). Nursing home residents were administered the BNT162b2 mRNA vaccine (Pfizer/BioNTech) from December 2020 to February 2021 at 4 NHs and vaccinated concurrently with HCWs, by a second dose 3 weeks later during the emergency use authorization period, and then a booster dose with the same vaccine 7 to 10 months after primary series. The mean time from second dose to booster was 273 days, and >80% of the population was vaccinated within 10 days of this mean interval. The minimum interval was 208 days and the maximum interval was 335 days. We did not acquire samples from all participants at every time point, nor did we perform every assay on every sample. We sampled both HCWs and NH residents (1) before they received their first BNT162b2 mRNA vaccine ($N_{NH} = 143$; $N_{HCW} = 74$) immunization and (2) again 2 weeks after the second dose ($N_{NH} = 138$; $N_{HCW} = 107$), (3) 6 months after the second dose ($N_{NH} = 123$; $N_{HCW} = 93$), and (4) before ($N_{NH} = 86$; $N_{HCW} = 54$) and (5) 2 weeks after ($N_{NH} = 77$; $N_{HCW} = 47$) a third dose (boost). All participants received 2 doses of vaccine in their primary series regardless of prior SARS-CoV-2 infection. Participants received their third dose approximately 9 months after completion of the initial immunization series. We deemed participants to have had prior SARS-CoV-2 infection ("SARS-CoV-2-experienced") if they had a diagnostic polymerase chain reaction or antigen test that confirmed acute SARS-CoV-2 infection and/or elevated antibody levels to the SARS-CoV-2 spike, RBD, or nucleocapsid before immunization and deemed "SARS-CoV-2-naive" if none of these conditions were met. During this period, laboratory-confirmed SARS-CoV-2 infection in an individual employee or resident triggered contact tracing among HCWs, and within residents' individual NHs, more frequent testing. We removed the few participants who acquired SARS-CoV-2 infection after vaccination ($N = 5$) from subsequent analyses.

Anti-Spike and Anti-Receptor-Binding Domain Assay

We assessed immune responses to BNT162b2 by measuring immunoglobulin G (IgG) to spike protein and its RBD by bead-multiplex immunoassay targeting the Wuhan strain, as we have previously described [14–16]. In short, stabilized full-length Wuhan spike protein (amino acid [aa] 16–1230, with furin site mutated) and RBD (aa 319–541) were conjugated to magnetic microbeads (Luminex, Austin, TX). Antigen-specific IgG was detected in serum/plasma using phycoerythrin-conjugated donkey F(ab)2 antihuman IgG, with Fcγ (Jackson ImmunoResearch, West Grove, PA) added. Using the Magpix assay system (Bio-Rad, Hercules, CA), the mean fluorescent index was recorded. To provide an internal standard and control for plate-to-plate variation, a pool of convalescent plasma was generated from people with known prior SARS-CoV-2 infection and was run on each plate as a standard curve of half-log dilutions starting at a 1:100 dilution. A secondary standard from the Frederick National Laboratory calibrated to the World Health Organization standard 20/136 was used to quantify

Table 1. Participant Characteristics

Characteristics	HCW	NH	*P* Value[a]
N	107	143	…
Age (years), median (IQR)	48 (29–56)	76 (70–87)	**<.001**
Female, *N* (%)	56 (52.3)	54 (37.8)	**.028**
Race	…	…	**…**
African American	9 (8.4)	16 (11.2)	.672
Asian	5 (4.7)	1 (0.7)	.087
Hispanic	4 (3.7)	1 (0.7)	.167
White	89 (83.2)	125 (87.4)	.367
CMV$^+$, *N* (%)	54 (50.5)	89 (62.2)	.071
EBV$^+$, *N* (%)	98 (91.6)	137 (95.8)	.186
SARS-CoV-2-experienced, *N* (%)	39 (36.4)	59 (41.3)	.513
IL-6 (pg/mL), median (IQR)	3.01 (2.10–4.31)	6.42 (4.57–9.86)	**<.001**
CRP (ng/mL), median (IQR)	4617 (1785–11 182)	10 779 (3777–28 184)	**<.001**
sTNFR-I (pg/mL), median (IQR)	1307 (1122–1588)	2463 (1982–3410)	**<.001**
sTNFR-II (pg/mL), median (IQR)	2257 (1931–2746)	4305 (3404–5716)	**<.001**

[a]Abbreviations: CMV, cytomegalovirus; CMV$^+$, seropositive; CRP, C-reactive protein; EBV, Epstein-Barr virus; HCW, healthcare workers; IL-6, interleukin 6; IQR, interquartile range; NH, nursing home residents; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sTNFR, soluble tumor necrosis factor receptor.

[a]*P* values less than .05 are considered statistically significant and are depicted in bold font. Categorical variables were analyzed using Fisher's exact test; continuous variables were analyzed using nonparametric Mann-Whitney *U* test.

antibodies to the spike protein in binding Ab units (BAU) per milliliter.

Severe Acute Respiratory Syndrome Coronavirus 2 Pseudovirus Neutralization Assay

The neutralization activity of participant sera against lentiviral particles pseudotyped with Wuhan and Omicron (BA.1) strain spike proteins was assessed as described previously [15, 17, 18]. In short, neutralization assays were performed using a Fluent 780 liquid handler (Tecan, Männedorf, Switzerland) in 384-well plates (Grenier Bio-One, Monroe, NC). Three-fold serial dilutions ranging from 1:12 to 1:8748 were performed and added to 50–250 infectious units of pseudovirus for 1 hour. We calculated the pNT50 values by taking the inverse of the 50% inhibitory concentration value for all samples with a pseudovirus neutralization value of 80% or higher at the highest concentration of serum. The lower limit of detection of this assay is 1:12 dilution.

Cytomegalovirus and Epstein-Barr Virus Serologies and Serum Cytokine Measurements

Serum anti-CMV and anti-EBV IgG serologies were measured by standard enzyme-linked immunosorbent assays following manufacturer's instructions (both kits from Abcam, Cambridge, UK). Serum levels of interleukin (IL)-6, C-reactive protein (CRP), soluble tumor necrosis factor (TNF) receptor (sTNFR)-I, and sTNFR-II were measured by ELLA assay (ProteinSimple, San Jose, CA), per manufacturer's instructions.

Statistical Analyses

For analyses of the association of CMV serostatus on vaccine-induced antibody titers, log-transformed assay values (ie, neutralizing Ab, anti-spike IgG, anti-RBD IgG titers) were compared by least squares multiple linear regression analysis, with adjustment for age, sex, and race. Comparisons of Ab titers between NH and HCW groups, and all comparisons of soluble cytokine levels, were performed without adjustment by nonparametric Mann-Whitney *U* test. All categorical demographic value differences between groups were analyzed using Fisher's Exact test (eg, sex, race, and CMV and EBV serostatus). All analyses were performed in Prism version 9.5.0 (GraphPad, San Diego, CA), and *P* values less than .05 were considered statistically significant. Due to the low number of subjects who were EBV seronegative, we did not perform detailed analyses of the association of EBV serostatus on vaccine-induced antibody titers.

Patient Consent Statement

This study was approved by the WCG institutional review board (IRB), and reliant review accepted by Case Western Reserve University local IRB. The WCG IRB approved use of verbal consent and assent for subjects, and documentation of informed consent and assent was obtained from all subjects or their legally authorized representatives.

RESULTS

Demographics and Seroprevalence of Cytomegalovirus and Epstein-Barr Virus Among Healthcare Workers and Nursing Home Residents

As shown in Table 1, the overall seroprevalence of CMV was 50.5% among HCWs and 62.2% among NH residents ($P = .071$), and the seroprevalence of EBV was uniformly high —91.6% among HCWs and 95.8% among NH residents ($P = .186$). A greater proportion of NH residents than HCWs

Table 2. Participant Characteristics by CMV Serostatus

Characteristics	HCW			NH		
	CMV⁻	CMV⁺	*P* Value[a]	CMV⁻	CMV⁺	*P* Value
N	53	54	…	54	89	…
Age (years), median (IQR)	48 (38.5–56.5)	49.5 (38.8–56.3)	.402	72.5 (67.0–81.3)	79.0 (71.3–89.0)	**.003**
Female, *N* (%)	29 (54.7)	27 (50.0)	.700	15 (27.8)	39 (43.8)	.075
Race						
African American	2 (3.8)	7 (13.0)	.161	2 (3.7)	14 (15.7)	**.030**
Asian	1 (1.9)	4 (7.4)	.363	0 (0.0)	1 (1.1)	>.999
Hispanic	1 (1.9)	3 (5.6)	.618	0 (0.0)	1 (1.1)	>.999
White	49 (92.5)	40 (74.1)	**.018**	52 (96.3)	73 (82.0)	**.017**
EBV⁺, *N* (%)	46 (86.8)	52 (96.3)	.093	51 (94.4)	86 (96.6)	.673
SARS-CoV-2-experienced, *N* (%)	19 (35.8)	20 (37.0)	>.999	16 (29.6)	43 (48.3)	**.036**
IL-6 (pg/mL), median (IQR)	2.85 (1.82–4.26)	3.23 (2.46–4.61)	.256	6.30 (4.41–10.63)	6.81 (4.97–9.71)	.641
CRP (ng/mL), median (IQR)	3404 (879–7557)	5251 (2833–15 492)	.076	13 948 (6395–30 600)	8018 (2520–23 356)	**.038**
sTNFR-I (pg/mL), median (IQR)	1268 (1114–1528)	1373 (1141–1662)	.431	2485 (1982–3468)	2441 (1975–3388)	.580
sTNFR-II (pg/mL), median (IQR)	2190 (1920–2677)	2534 (1944–2857)	.347	4153 (3335–5504)	4305 (3450–5755)	.838

Abbreviations: CMV, cytomegalovirus; CMV⁻, CMV seronegative; CMV⁺, seropositive; CRP, C-reactive protein; EBV, Epstein-Barr virus; HCW, healthcare workers; IL-6, interleukin 6; IQR, interquartile range; NH, nursing home residents; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sTNFR, soluble tumor necrosis factor receptor.

[a] *P* values less than .05 are considered statistically significant and are depicted in bold font. Categorical variables were analyzed using Fisher's exact test; continuous variables were analyzed using nonparametric Mann-Whitney *U* test.

were male ($P = .028$), due to the recruitment of NH residents from the largely male population at a Veterans' nursing home. For HCWs, CMV seronegative (CMV⁻) and seropositive (CMV⁺) persons were similarly aged (48 vs 49.5 years, respectively), had a similar sex distribution (54.7% vs 50% female sex, respectively), similar proportion of EBV seropositivity (86.8% vs 96.3%, respectively), and similar frequencies of prior SARS-CoV-2 infection (35.8% vs 37%, respectively) (Table 2). A greater proportion of CMV⁻ HCWs were White compared with CMV⁺ HCWs (92.5% vs 74.1%, respectively; $P = .018$).

As shown in Table 2, among NH residents, CMV⁻ donors were significantly younger than CMV⁺ donors (72.5 vs 79 years, respectively; $P = .003$). Female donors tended to be enriched within CMV⁺ donors (43.8% vs 27.8% among CMV⁻ donors; $P = .076$). The proportion of EBV seropositivity was similar between CMV⁻ and CMV⁺ donors (94.4% vs 96.6%, respectively), and a lower proportion of CMV⁻ donors were SARS-CoV-2-experienced than the proportion among CMV⁺ donors (29.6% vs 48.3%, respectively; $P = .036$). As observed with the HCWs, a greater proportion of CMV⁻ NH residents were White compared with CMV⁺ NH residents (96.3% vs 82%, respectively; $P = .017$).

There were no statistically significant differences in any of the demographic or serological indices measured when HCWs and NH residents were stratified by their EBV serostatus (Supplementary Table 1). As shown in Supplementary Table 2, when stratified by their SARS-CoV-2 infection history, a lower proportion of SARS-CoV-2-naive HCWs were female than SARS-CoV-2-experienced HCWs (44.1% vs 66.7%, respectively; $P = .028$), and a lower proportion of SARS-CoV-2-naive NH residents were CMV⁺ than SARS-CoV-2-experienced NH residents (54.8% vs 72.9%, respectively; $P = .036$). All other comparisons were statistically similar among the groups.

Effect of Cytomegalovirus Serostatus on Antibody Responses

To measure differences in Ab responses between CMV⁻ and CMV⁺ donors (Supplementary Table 3), we performed multivariable least squares linear regression adjusting for the potential confounders age, sex, and race. After adjustment, we found that among COVID-19-naive HCWs receiving their primary vaccine series, the neutralizing Ab titers to the Wuhan strain for CMV⁺ donors were significantly reduced at 2 weeks after vaccination ($P = .013$) compared with CMV⁻ donors (Figure 1A). Neutralizing Ab titers were similar among SARS-CoV-2-experienced donors at 2 weeks after completing the primary series. No significant differences remained in neutralizing Ab titers between CMV⁺ and CMV⁻ HCWs 0–14 days before their booster (which generally occurred 7–10 months after the primary series). There was also no difference in neutralizing Ab titers between CMV⁺ and CMV⁻ HCWs 2 weeks after the booster dose, regardless of SARS-CoV-2 infection history (Figure 1B).

Similar to our previous reports [14, 16], SARS-CoV-2-naive NH residents had substantially reduced Wuhan strain neutralizing Ab titers compared with HCWs before vaccination and 2 weeks and 6 months after vaccination, regardless of their CMV serostatus (Supplementary Table 4). We did not observe these differences when comparing SARS-CoV-2-experienced NH residents or HCWs. Cytomegalovirus seropositivity was associated with significantly reduced Wuhan strain neutralizing Ab titers 6 months after the primary vaccine in SARS-CoV-2-naive

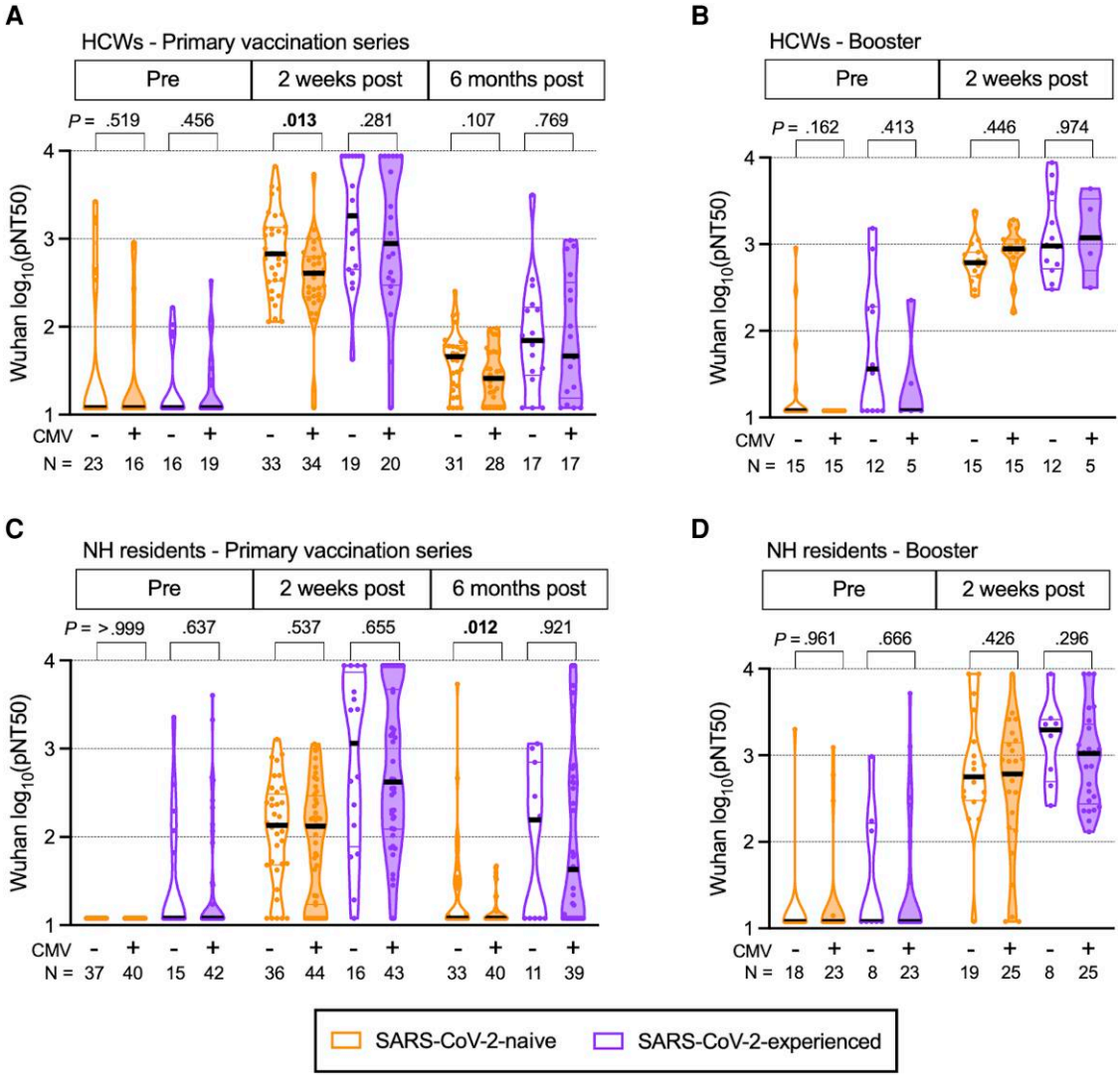

Figure 1. Wuhan strain neutralization titers in primary series and booster series with BNT162b2 mRNA vaccination in healthcare workers (HCW) and nursing home (NH) residents, with and without prior severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Violin plots show Wuhan (vaccine) strain pseudovirus neutralization (pNT50) values for (A) HCWs during primary series, (B) HCWs during booster series, (C) NH residents during primary series, and (D) NH residents during booster series. The upper limit of detection of the assay is 1:8748 and the lower limit of detection is 1:12. Participants who were SARS-CoV-2 naive (SARS-CoV-2-naive) are shown in orange, participants with prior SARS-CoV-2 infection (SARS-CoV-2-experienced) are shown in purple, open plots are from cytomegalovirus (CMV) seronegative (CMV^-) donors, and filled plots are from CMV seropositive (CMV^+) donors. *N* values indicate the number of participants measured in each group. *P* values are between CMV^- and CMV^+, determined by least squares multiple linear regression, adjusted for age, sex, and race. *P* values less than .05 are considered statistically significant and are depicted in bold font.

NH residents ($P = .012$) (Figure 1C). No other differences in Wuhan-neutralizing Ab titers after the primary series or booster were seen according to CMV serostatus among SARS-CoV-2-naive NH residents. Of note, neutralizing Ab titers trended lower among CMV^+ SARS-CoV-2-experienced NH residents at all postvaccine timepoints, but these differences were not statistically significant (Figure 1C and D).

We next measured the titers of anti-spike Abs, expressed as binding Ab units (BAU) per milliliter and anti-RBD titers, in AU (Supplementary Table 3). As we observed with Wuhan-neutralizing Ab titers, we found a significant reduction in anti-spike Ab titers ($P = .017$) (Figure 2A) and anti-RBD titers ($P = .011$) (Supplementary Figure 1A) among CMV^+ SARS-CoV-2-naive HCWs compared to titers among CMV^- HCWs at 2 weeks after primary vaccination and in anti-RBD titers prebooster ($P = .042$) (Supplementary Figure 1B), after adjustment for age, sex, and race. It is interesting to note that we found a small but significant increase in anti-spike Ab titers among SARS-CoV-2-experienced CMV^+ HCWs compared with CMV^- donors ($P = .006$) (Figure 2B). As with the Wuhan-neutralizing titers, we did not observe any differences in anti-spike or anti-RBD Ab titers between CMV^+ and CMV^-

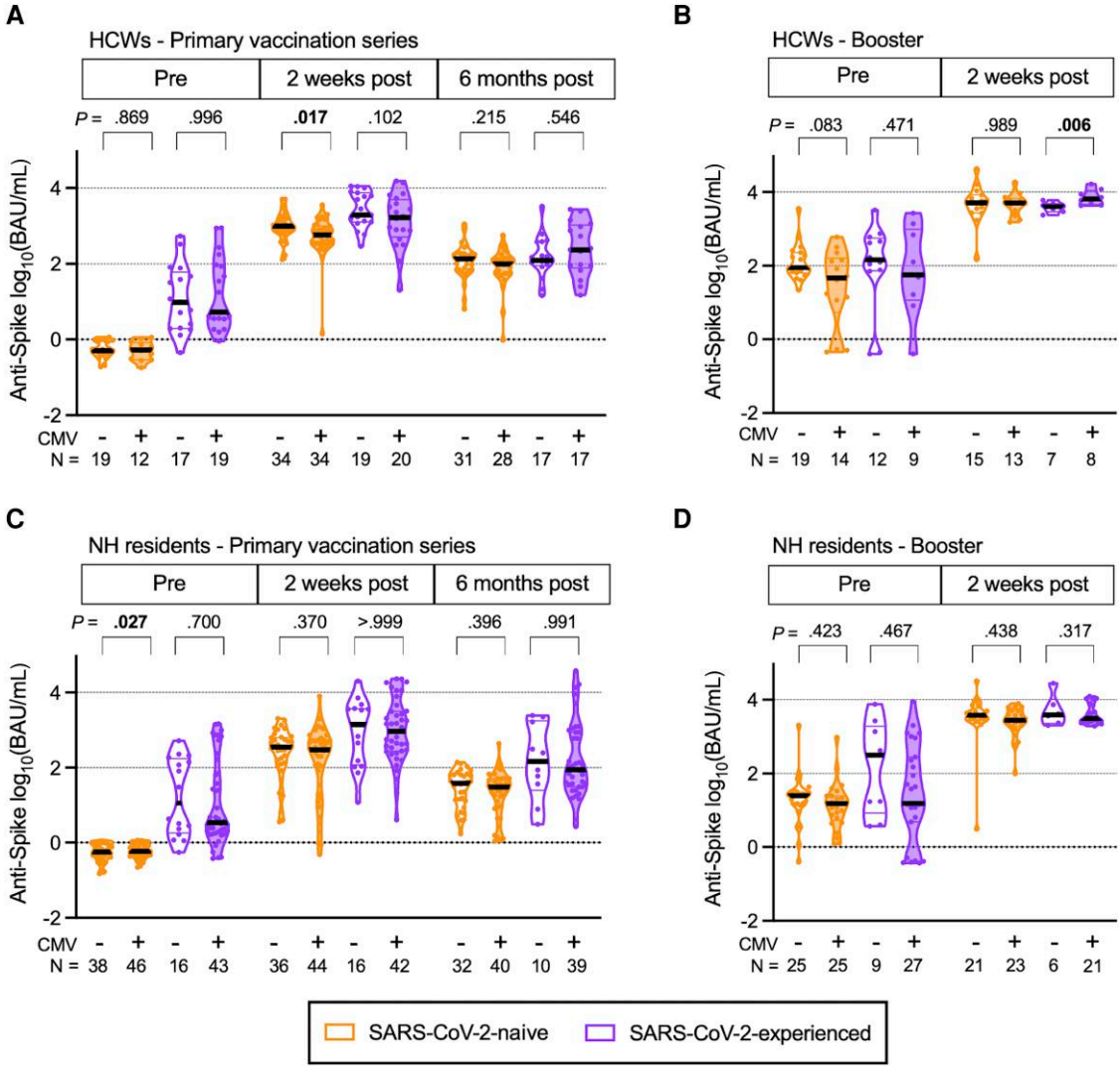

Figure 2. Anti-spike antibody (Ab) titers in primary series and booster series with BNT162b2 mRNA vaccination in healthcare workers (HCW) and nursing home (NH) residents, with and without prior severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Violin plots show anti-spike Ab values depicted in the binding arbitrary units/milliliter (BAU/mL) based on the World Health Organization standard for (A) HCWs during primary series, (B) HCWs during booster series, (C) NH residents during primary series, and (D) NH residents during booster series. The cutoff for a positive anti-spike response over prepandemic controls is 3.8 BAU/mL. Participants who were SARS-CoV-2 naive (SARS-CoV-2-naive) are shown in orange, participants with prior SARS-CoV-2 infection (SARS-CoV-2-experienced) are shown in purple, open plots are from cytomegalovirus (CMV) seronegative (CMV^-) donors, and filled plots are from CMV seropositive (CMV^+) donors. *N* values indicate the number of participants measured in each group. *P* values are between CMV^- and CMV^+, determined by least squares multiple linear regression, adjusted for age, sex, and race. *P* values less than .05 are considered statistically significant and are depicted in bold font.

donors after the primary series (Figure 2C, Supplementary Figure 1C) or booster (Figure 2D, Supplementary Figure 1D) among NH residents, regardless of SARS-CoV-2 infection history.

Because neutralizing Ab titers to the early Omicron subvariant BA.1 do not readily develop among HCWs or NH residents until after a booster dose [15], we tested whether CMV infection was associated with impaired neutralizing Ab titers to the Omicron (BA.1) strain in HCW and NH residents during the booster series. Although CMV^+ SARS-CoV-2-naive HCWs had reduced Omicron (BA.1)-neutralizing Ab titers _before their booster dose ($P = .039$) (Figure 3A), we found no other statistically significant differences in Omicron (BA.1)-neutralizing Ab titers between CMV^+ and CMV^- donors, whether they were HCWs or NH residents, regardless of SARS-CoV-2 infection history.

Prevaccination Levels of Soluble Inflammatory Mediators

We next considered whether CMV infection-associated inflammation at the time of immunization could have contributed to a differential vaccine efficacy in our cohort. Using available prevaccination samples, we measured 4 factors that, although not unique to CMV, have a demonstrated association with CMV infection: IL-6, CRP, sTNFR-I, and sTNFR-II [19–23].

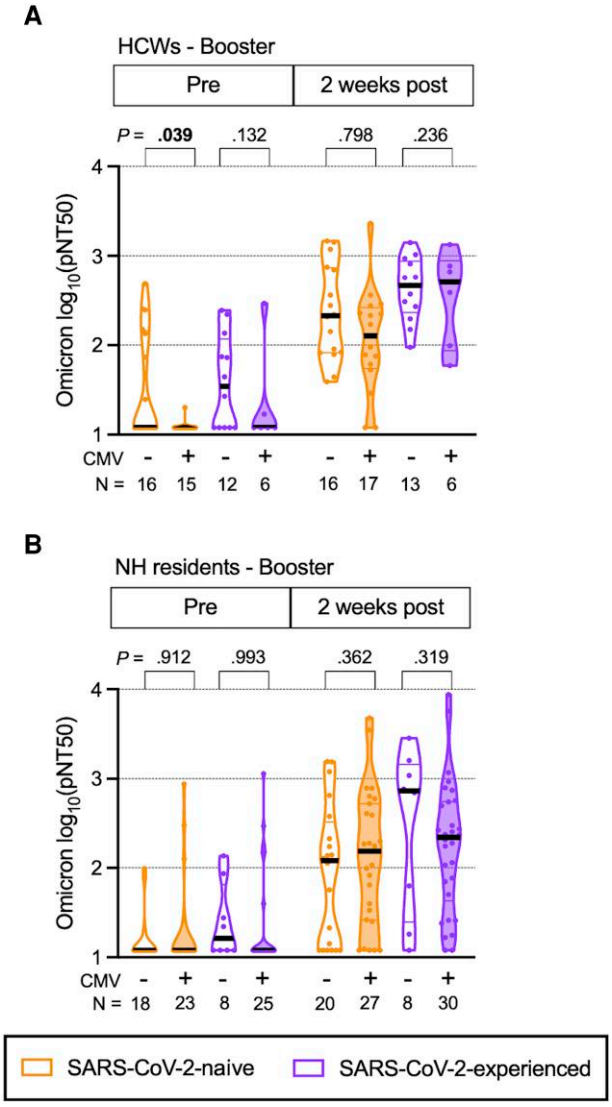

Figure 3. Omicron (BA.1) strain neutralization titers over time in booster series with BNT162b2 mRNA vaccination in healthcare workers (HCW) and nursing home (NH) residents, with and without prior severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Violin plots show Omicron (BA.1) strain pseudovirus neutralization (pNT50) values during the booster series for (A) HCWs and (B) NH residents. The upper limit of detection of the assay is 1:8748 and the lower limit of detection is 1:12. Participants who were SARS-CoV-2 naive (SARS-CoV-2-naive) are shown in orange, participants with prior SARS-CoV-2 infection (SARS-CoV-2-experienced) are shown in purple, open plots are from cytomegalovirus (CMV) seronegative (CMV^-) donors, and filled plots are from CMV seropositive (CMV^+) donors. *N* values indicate the number of participants measured in each group. *P* values are between CMV^- and CMV^+, determined by least squares multiple linear regression, adjusted for age, sex, and race. *P* values less than .05 are considered statistically significant and are depicted in bold font.

Although we found that NH residents had significantly elevated levels of all 4 analytes compared with HCWs (Table 1), the only difference we observed among NH residents was that CMV^- NH residents had significantly higher CRP levels than CMV^+ NH residents (Table 2). We found no differences among HCWs whether we stratified by CMV or EBV serostatus, or prior SARS-CoV-2 experience (Table 2, Supplementary Tables 1 and 2). We also found no correlations among all HCWs or NH residents between any of the analytes and neutralizing Ab titers 2 weeks after the primary series (data not shown).

DISCUSSION

Cytomegalovirus, a highly prevalent pathogen, has infected approximately half the adults in the United States, and approximately 100% of adults elsewhere [24, 25]. Although primary CMV infection is usually mild or asymptomatic, the virus is retained in a latent state and is never fully cleared from the host. Cytomegalovirus reactivation from latency can be a major problem in settings of immune compromise, such as in transplant recipients or in those with untreated human immunodeficiency virus infection. Even in the absence of overt reactivation, carriage of CMV is associated with inflammation and numerous complications and comorbidities, suggesting that long-term effects of CMV can lead to poor health outcomes. Indeed, in older persons, having CMV is a major component of the immune risk phenotype, which is associated with reduced longevity [26]. However, latent CMV infection may also protect the host from subsequent pathogen challenge, as has been observed in mouse models of bacterial and viral infection, but this protection has been shown to wane over time [9, 10].

How CMV infection affects vaccine immunogenicity is unknown. One report suggests that inflammatory signals caused by latent CMV infection may activate the immune system, and thereby improve antibody titers with influenza vaccination [9], but this effect may be temporary. Other studies have shown conflicting results, with CMV being associated with impaired influenza vaccine responses in both older and younger adults [27–33], possibly via reduced B-cell activity [31], or no having no effect [34, 35], and all of these may be confounded by various levels of pre-existing immunity to seasonal influenza strains in the vaccine recipients [36]. Few studies to date have examined CMV and SARS-CoV-2 vaccine immunogenicity, none of which have found a substantial association of CMV serostatus with vaccine-elicited antibody responses in either younger or older adults [37–39].

In this report, we investigated the association of latent CMV infection with Pfizer/BioNTech BNT162b2 mRNA vaccine immunogenicity in NH residents and HCWs who either had or did not have prior SARS-CoV-2 infection. We measured vaccine responses in 3 ways and found lower Ab titers in the serum of CMV^+ HCWs than in serum from CMV^- HCWs 2 weeks after the primary vaccination series, even after adjustment for age, sex, and race. The difference disappeared after the booster dose and was absent among HCWs who had prior SARS-CoV-2 experience. Thus, in this population of adult

HCWs, latent CMV infection is associated with a diminished response to SARS-CoV-2 spike protein, primarily when presented a neoantigen having only been encountered in the context of the vaccine. In SARS-CoV-2-experienced HCWs, who had previously been infected with SARS-CoV-2 and for whom spike is not a neoantigen, CMV infection did not appear to greatly affect vaccine-enhanced spike-specific recall responses. Indeed, for SARS-CoV-2-experienced HCWs who have encountered spike at least 3 times (prior infection, primary vaccination, boost), we actually observed a small but significant increase in anti-spike Ab titers in CMV^+ donors. Unexpectedly, however, NH residents—who have elevated levels of systemic inflammatory mediators compared with HCWs before vaccination—did not exhibit CMV-associated reduced Ab titers 2 weeks after either the primary vaccination series or the booster series but did have a small but significant reduction in neutralizing Ab titers 6 months after primary vaccination. These results suggest that despite their overall elevated inflammatory profile compared with HCWs, CMV carriage in older individuals does not seem to substantially impair mRNA vaccine immunogenicity.

Epstein-Barr virus is another very common herpesvirus associated with comorbidities, including recent mechanistic evidence of a role for EBV in the development of multiple sclerosis [40, 41]. Epstein-Barr virus establishes latent infection in B cells, making it plausible that latent EBV infection could affect vaccine-induced Ab titers. Because so few of our donors were seronegative for EBV ($N_{NH} = 6$; $N_{HCW} = 9$), and because we saw no substantial demographic differences between EBV^- and EBV^+ donors (Supplementary Table 1), we did not further assess the effect of EBV on Ab titers after vaccination.

This report is not without limitations. First, we had a relatively small sample size for each group. This hinders our power to test the association of EBV serostatus with vaccine efficacy because of the high seroprevalence in the study populations. Second, not all subjects are represented at each time point, limiting our ability to make longitudinal assessments of vaccine activity. Third, our NH residents are disproportionately male due to the recruitment from the largely male population at the Veterans' nursing home. Although our analyses were controlled for age, sex, and race, these discrepancies limit the generalizability of our findings. Fourth, SARS-CoV-2-experienced NH residents may have been asymmetrically culled by lethal infection, because prevaccine a substantial proportion of these vulnerable individuals died from infection. This survivor effect might be an explanation for the significantly increased proportion of CMV seropositivity among SARS-CoV-2-experienced NH residents. Fifth, we only measured surrogate immunologic readouts of protection and did not track rates of postvaccination breakthrough infections. Finally, we did not assess T-cell immunity in these assays. Given the profound effect of latent CMV infection on T-cell responses [42, 43], it will be of interest to measure the association of CMV serostatus with vaccine-induced T-cell responses in these donors and in other well characterized vaccine cohorts.

CONCLUSIONS

Our results demonstrate that latent CMV infection has a detrimental effect on BNT162b2 mRNA vaccine responsiveness in a well characterized cohort of HCWs when spike protein is a neoantigen. This may leave CMV^+ adult vaccine recipients more susceptible to SARS-CoV-2 breakthrough infections after the primary series than CMV^- vaccinees. Because CMV seroprevalence tends to increase with age, our findings also may identify a contributor to the lower Ab titers seen in older adult populations in other studies, compared with titers in younger individuals. It is notable that CMV^+ study participants did mount robust Ab responses to a third vaccination, including neutralizing Ab titers to Omicron (BA.1), offering support for primary and booster vaccinations for everyone, including those with latent CMV infection.

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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