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BRIEF REPORT

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Specificity and functional humoral immune responses induced by the VBI-1501A eVLP HCMV gB vaccine compared to the gB/MF59 vaccine

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ABSTRACT

Human cytomegalovirus (HCMV) is the leading infectious cause of birth defects globally yet has no licensed vaccine. Though some HCMV glycoprotein B (gB)-based vaccine candidates, such as the MF59-adjuvanted gB vaccine (gB/MF59) elicited limited virus neutralization, enveloped virus-like particle (eVLP) expression of gB induced robust CMV-neutralizing antibodies in a phase I trial in CMV-seronegative participants. Here, we further characterize the anti-gB binding and functional antibody responses induced by the VBI1501A gB eVLP vaccine in comparison to gB/MF59, the leading clinically tested vaccine to date. VBI1501A vaccination induced higher IgG binding to antigenic domains (AD) that are neutralizing antibody targets, AD-4 and AD-4+5, compared to the gB/MF59 vaccine, but elicited lower IgG binding to gB full-length and ectodomain. VBI1501A-elicited IgG responses showed no binding to the linear neutralizing domain AD-2 and elicited minimal binding to the AD-6 domain associated with viral cell-associated spread. While VBI1501A did not elicit IgG that bound to the clade-matched strain nor antibody-dependent cellular cytotoxicity responses, plasma IgG binding to cell associated gB and antibody dependent cellular phagocytosis responses were higher compared to the gB/MF59 vaccine. This study offers insight into strategies to improve on vaccine design of partially successful gB-containing HCMV vaccines.

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Introduction

Human cytomegalovirus (HCMV) is a pervasive herpesvirus with >60% incidence rates globally in adult populations.[1,2] HCMV remains dormant and asymptomatic in healthy adults but is particularly devastating in organ transplant patients and immunocompromised individuals.[3] HCMV is also the most common congenital infection worldwide and approximately 1 in 200 babies are born in the U.S. annually with congenital CMV (cCMV), and 1 in 5 of them will show clinical signs and/or suffer long-term health complications, such as hearing loss and neurodevelopmental delays.[2,4–6]

Vaccine efforts have been underway for the past five decades to prevent HCMV acquisition and congenital transmission.[7–11] The most efficacious vaccine candidate to date is the MF59-adjuvanted gB subunit vaccine (gB/MF59), developed by Sanofi and Novartis, which demonstrated a 43–50% efficacy in prevention of HCMV transmission in multiple phase II clinical trials (NCT00133497).[8,9] The protection observed from the gB/MF59 vaccine was previously linked to non-neutralizing antibody functions and has recently also been associated with a newly identified region on gB, antigenic domain 6 (AD-6).[12–14] Due to the complexity of HCMV and its immune escape mechanisms, neutralization on its own is likely not enough to deem a vaccine successful.[13,15] Vaccines targeting only gB have failed to induce potent neutralizing antibody responses in Phase II adolescent and postpartum participants.[10,13]

VBI Vaccines funded the development of a prophylactic CMV vaccine (VBI-1501A) that uses enveloped virus-like particle (eVLP) technology to present a modified gB ectodomain fused to domains of the vesicular stomatitis virus (VSV) G protein (CMV gB-G) in a lipid bilayer with an alum adjuvant.[7]

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Studies thus far have demonstrated the vaccine's ability to neutralize epithelial and fibroblast cells infected with HCMV.[7] In this paper, we assessed the specificity of the gB-specific IgG responses elicited by the VBI vaccine against antigenic domains of gB, and non-neutralizing functions such as antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) responses. We compared the immunogenicity of this vaccine to that of the partially efficacious gB/MF59 vaccine as a strategy to guide decisions on late phase clinical trials for gB-based CMV vaccine candidates.

Results

VBI1501A elicited strong IgG binding to soluble and cell-associated HCMV gB but not to the vaccine-strain matched whole virion

This study evaluated the immunogenicity of a three-dose eVLP CMV gB-G vaccine from a randomized, observer-blind, phase I clinical trial performed by VBI (NCT02826798).[7] A total of 128 participants enrolled at three Canadian Immunization Research Network study centers were randomly allocated to 5 different formulation groups, with VBI1501A one of the four versions of the prophylactic CMV eVLP vaccine tested in the trial, along with one placebo group (randomized 4:1). The participant cohort was made up of 26 CMV seronegative adults of mixed gender, ages 21 to 40 years. None of the participants previously received any form of a CMV vaccine and all participants of childbearing potential had a negative pregnancy test for the entirety of the study and agreed to use investigator approved contraception. Participants were made privy to the purpose and procedures required for this study. Exclusionary criteria included history of immune compromise or clinically significant illnesses. Participants received three 2.0 *ug* intramuscular injections at months 0, 2, and 6. Serum collected prior to injection on day 0 and after dose three (day 196) were used to evaluate the protective functions elicited by the vaccine.[7] The vaccine is an enveloped virus-like particle (eVLP) gB-G based on Towne HCMV, referred to as VBI-1501A, administered intramuscularly in a liquid state and adjuvanted with alum. Further details regarding the design and the development of the vaccine can be found in Kirchmerier et al.[16]

An HCMV gB-transfected cell binding assay was used to assess the proportion of gB-transfected cells that are bound by serum IgG from VBI1501A vaccinees. VBI1501A elicited potent IgG binding to cell-associated Merlin gB (Figure 1A, 57% of responders). We also tested IgG binding to cell-associated gB of the clade-matched strain, Towne, and saw an IgG binding response in 97% of participants post vaccination (Figure 1B). While there was significant difference in the magnitude of IgG binding to Towne whole virions as assessed by a whole virion enzyme-linked immunosorbent assay (ELISA) after vaccination, this response was overall very low and none of the vaccinees developed a response above the positivity cut off (median AUC = 0.93, range AUC = 1.0, 0% responders (Figure 1C).

Serum IgG binding to multiple conformations and individual antigenic domains of gB were assessed using the binding antibody multiplex assay (BAMA). VBI1501A induced a significant increase in IgG binding to prefusion-like gB ectodomain (P = <.0001, 1:250 dilution, Figure 1D, 79% responders), full-length post-fusion gB (P = <.0001, 1:2000 dilution, 92% responders, Figure 1E), and postfusion gB ectodomain (P = <.0001, 1:2000 dilution, Figure 1F, 92% responders), all derived from the Towne strain. IgG binding to AD-4 (Merlin), located on domain II of gB and a target of neutralizing antibodies, was elicited in 92% vaccinees (P = <.0001, 1:250 dilution, Figure 1G). The eVLP vaccine also induced IgG binding to the AD-4+5 region (Merlin), located on domains I and II, in the majority of vaccinees (P = <.0001, 1:50 dilution, 95% responders, Figure 1H). Compared to AD-4 and AD-4+5, there was only a modest increase from baseline in IgG binding to AD-5 (Merlin, Domain I), also a known target of neutralizing antibodies, but this binding was still significantly higher compared to baseline (P = <.0001, 1:50 dilution, 27% responders, Figure 1I). There were no significant changes in IgG binding to AD-1 (Merlin) after vaccination based on the high baseline responses to this region (P = .8361, 1:50 dilution, 0% responders, Figure 1J).

IgG binding to AD-2 site 1 (AD169) and AD-6 (Towne) were assessed via ELISA. VBI1501A vaccination did not induce detectable IgG binding significantly above baseline to the linear epitope

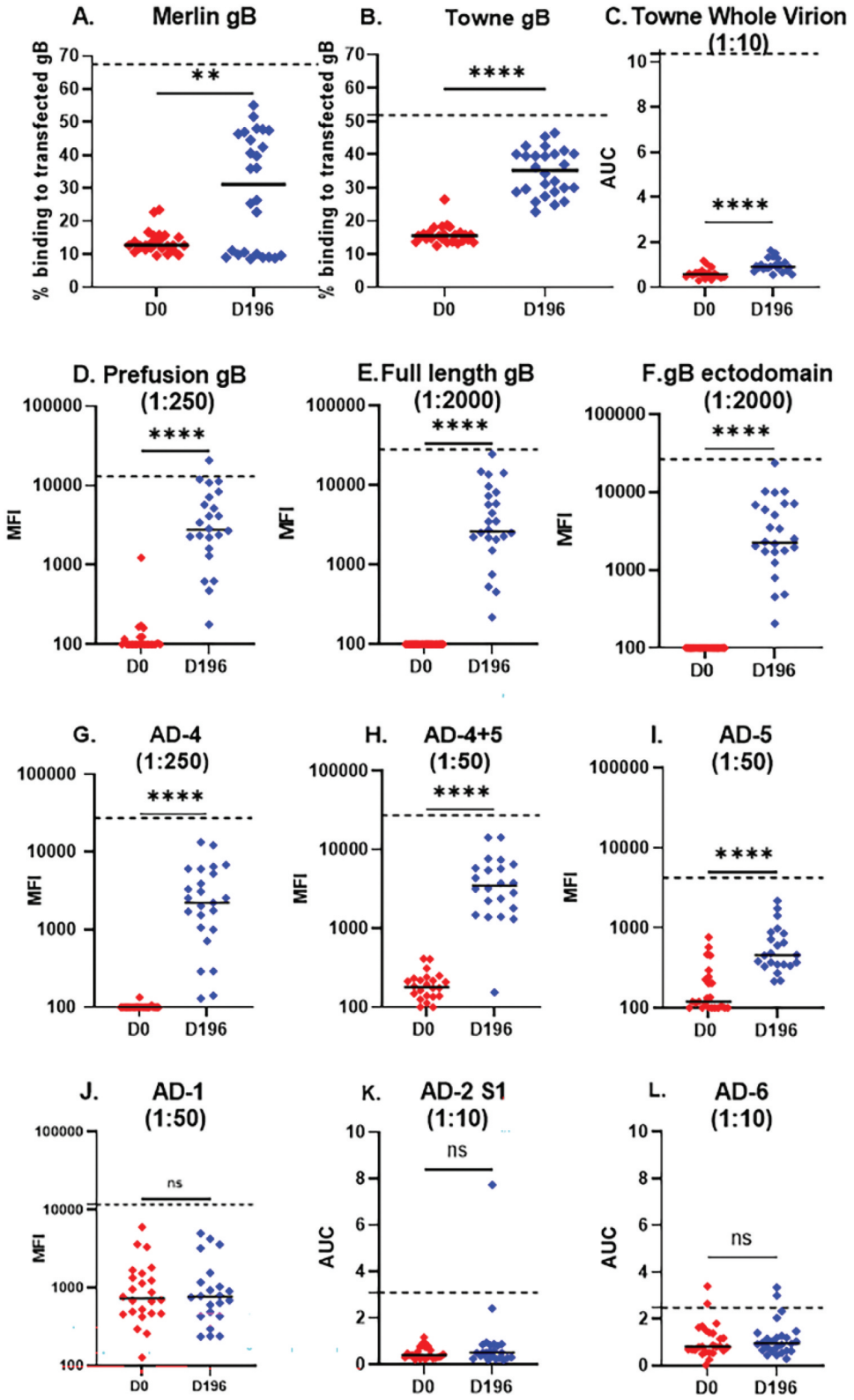

Figure 1. VBI1501A vaccine induces strong binding to neutralizing domains AD-4 and AD-5 of gB. Red and blue diamonds represent trial participants on day 0, pre-vaccination and day 196, after the 3rd dose of VBI1501A, respectively. The bold dashed line represents maximum Cytogam binding at 1 mg/mL. The bold solid line represents median binding for each group. Data was analyzed through GraphPad Prism version 10.4.1 (GraphPad Software, Inc, La Jolla, CA). The transfected cell binding (TCB) assay assessed IgG binding to HCMV gB (A,B). The Y-axis represents the percentage of IgG from serum binding to the cells transfected with (A) Merlin and (B) Towne gB. (C) Binding to Towne whole virion was measured via whole virion ELISA (WVE) at a 1:10 starting dilution. WVE data was graphed using area under the curve (AUC) calculated on GraphPad

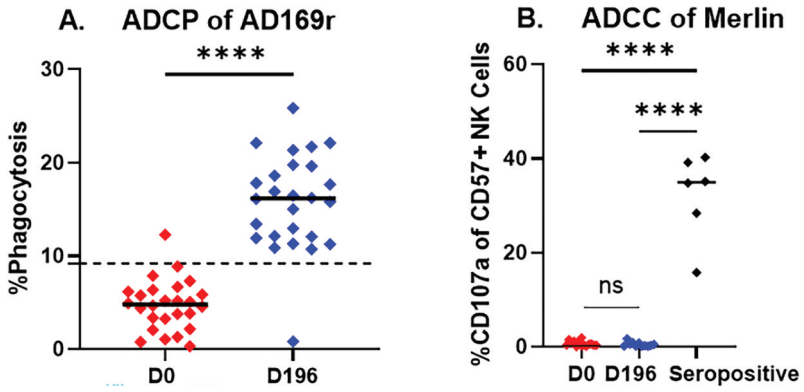

Figure 2. VBI1501A vaccine induces strong ADCP responses against AD169r but no detectable ADCC activity. Red and blue diamonds represent trial participants at day 0, pre-vaccination and day 196, after the 3rd dose of VBI1501A, respectively. Black diamonds represent seropositive controls. Data was analyzed using GraphPad Prism version 10.4.1 (GraphPad Software, Inc, La Jolla, CA). (A) % phagocytosis induced with VBI1501A compared to that induced without. *P* values were calculated using a Wilcoxon test on GraphPad Prism. *P* <.05 is considered nonsignificant (ns). The Y-axis for the ADCP data is %phagocytosis (B) VBI1501A did not induce antibody dependent cellular cytotoxicity (ADCC) with Merlin. Statistical significance was assessed via one-way ANOVA. *p* <.05 was considered significant.

AD2 site 1 ($P = .2247$, 8% responder, Figure 1J). Similarly, there was no detectable induction of IgG binding above baseline to AD-6 post vaccination ($P = .326$, 3.8% responder, Figure 1K).

VBI150A induced IgG capable of inducing ADCP but not ADCC and did not significantly reduce the spread of HCMV from cell to cell

VBI150A vaccination elicited a robust ADCP response against the Towne strain of HCMV ($P = <.0001$, Figure 2A, 96% responders). Yet, VBI1501A vaccination did not induce detectable ADCC to Merlin strain HCMV-infected cells ($P = .19$, 0% responders, Figure 2B). Sera from HCMV seropositive individuals were able to mediate ADCC that was significantly higher than both unvaccinated seronegative samples ($P = <.0001$) and vaccinated seropositive samples ($P = <.0001$), demonstrating ADCC responses are effectively induced by natural infection.

A spread reduction assay was performed on ARPE-19 cells infected with the Ts15nR strain, a modified version of Towne-BAC-GFP with an intact pentamer complex. There was a significant reduction in viral spread of Ts15nr compared to day 0 ($P = .006$, Figure 3A). *In vivo*, clinical HCMV spreads by direct cell-cell transmission, and this is more resistant to antibody inhibition than cell-free spread.[17] To test the capacity of samples to inhibit cell-cell spread, the assay was repeated using a HCMV Merlin strain that encodes a wildtype genome and therefore exhibits almost exclusive cell-cell spread, along with a version of the same virus in which the genes RL13 and UL128 have been mutated in order to promote both cell-cell and cell-free spread.[18] As with Ts15nR, which is not exclusively a cell-cell disseminating virus, there was also reduction of spread with the cell-free disseminating virus after vaccination ($P = .001$, 3B). There was no significant reduction in viral spread against a cell-cell disseminating version of Merlin ($P = .6$, Figure 3C) induced by VBI1501A vaccination.

Prism. IgG binding to antigenic domains (AD) -1, -4, -5, and -4 + 5 and conformations of gB were measured via BAMA. (E,F) IgG binding to full-length gB and gB ectodomain Towne was run at a 1:2000 dilution. (D,G) IgG binding to AD-4 and prefusion gB was run at a 1:250 dilution. (H,I,J) IgG binding to AD-4 + 5, AD-5, and AD-1 was run at a dilution of 1:50. The Y-axis for the BAMA data is MFI, log10. BAMA binding values below an MFI of 100 MFI were set to 100. A Mann-Whitney test was run to determine statistical significance with the *P* value reported on the graph. "ns" means the *P*-value was not significant. (K,L) IgG binding to AD-2 and AD-6 were run via ELISA at a 1:10 dilution and was reported as AUC. The *p*-values for the ELISAs and TCB were calculated using a Wilcoxon test. $p < .05$ was considered significant.

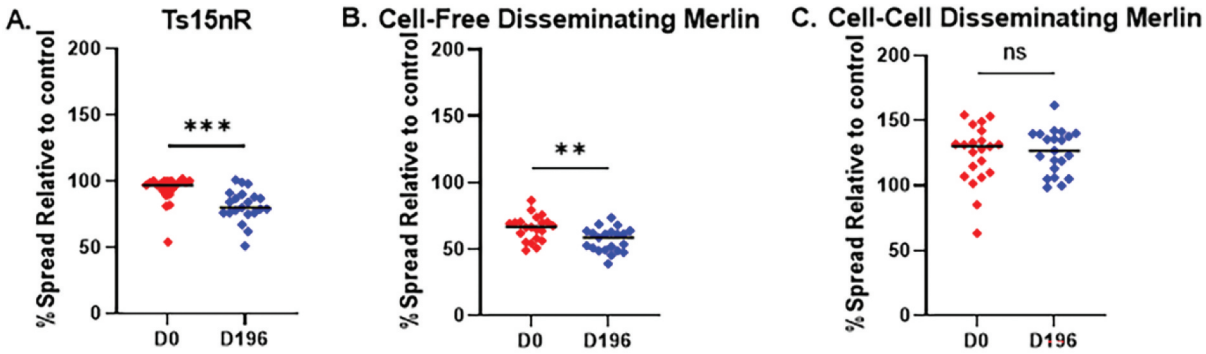

Figure 3. VBI1501A induced minimal reduction in cell-to-cell spread of HCMV. Red diamonds represent day 0 samples, blue diamonds represent day 196 samples. Bold, dashed line represents maximum Cytogam binding, 1ug/mL. Data was run through GraphPad Prism version 10.4.1 (GraphPad Software, Inc, La Jolla, CA). (A) Serum vaccinated with VBI1501A reduced cellular spread of Ts15nR significantly more than unvaccinated serum (B) Vaccinated serum reduced viral spread of a cell-free disseminating Merlin variant HCMV strain more than unvaccinated serum, relative to the seropositive control. (C) There was not a significant difference in percentage of viral cell-cell disseminating Merlin variant spread relative to seropositive controls between vaccinated and unvaccinated serum. Paired t-test on GraphPad Prism was used to determine statistical significance. $p < .05$ was considered significant.

VBI1501A elicited better IgG binding to antigenic domains of gB and cell associated gB than gB/MF59

To investigate how the presentation of gB in a VLP might alter epitope-specific immune responses compared to soluble protein, we compared immune responses between VBI1501A and gB/MF59. Interestingly, the VBI 1501A vaccine elicited higher magnitude IgG binding to AD-4 and AD-4 + 5 than the gB/MF59 vaccine ($P =$ <.0001, Figure 4A), but gB/MF59 elicited approximately 3-fold higher magnitude IgG binding to both full length gB and gB ectodomain ($P =$ <.0001, Figure 4A). VBI1501A elicited stronger IgG binding to both Merlin and Towne strain gB-transfected cells than gB/MF59 ($P =$ <.0001, Figure 4B,C). Finally, the VBI1501A vaccine induced lower magnitude serum ADCP ($P = .0004$, Figure 4D) than the gB/MF59 vaccine.

Methods

Cell culture

Human retinal pigment epithelial (ARPE-19), Human epithelial kidney (HEK293T), and Human foreskin fibroblast (HFF-1) cells were obtained from ATCC and cultured at 37°C in media detailed in Karthigeyan et al.[19]

HCMV production

The HCMV strains used in these assays are derived from bacterial artificial chromosomes (BACs) gifted from Tom Shenk (AD169) or generated in house (Merlin).[18] Some Merlin variants expressed GFP from a P2A-GFP cassette after UL36.[20] Virus production is further detailed in Karthigeyan et al.[19] AD169 revertant virus (AD169r-BAC-GFP) was produced by transfection of HFF-1 cells using Lipofectamine-3000 (ThermoFisher Scientific), Merlin BACs were transfected into HFFF-Terts by Amaxa Nucleofector. Ts15nR was propagated on ARPE-19 cells and Towne-BAC-GFP was propagated on HFF-1 cells.

HCMV gB specific IgG binding

IgG binding to gB-related antigens was measured using a Luminex-based binding antibody multiplex assay as described in Karthigeyan et al.[19] Four gB antigenic domain-related antigens were included in one panel, gB AD-1 (MyBioSource), gB AD-4, AD-5, and AD-4+5 (produced in house, based on Merlin strain).

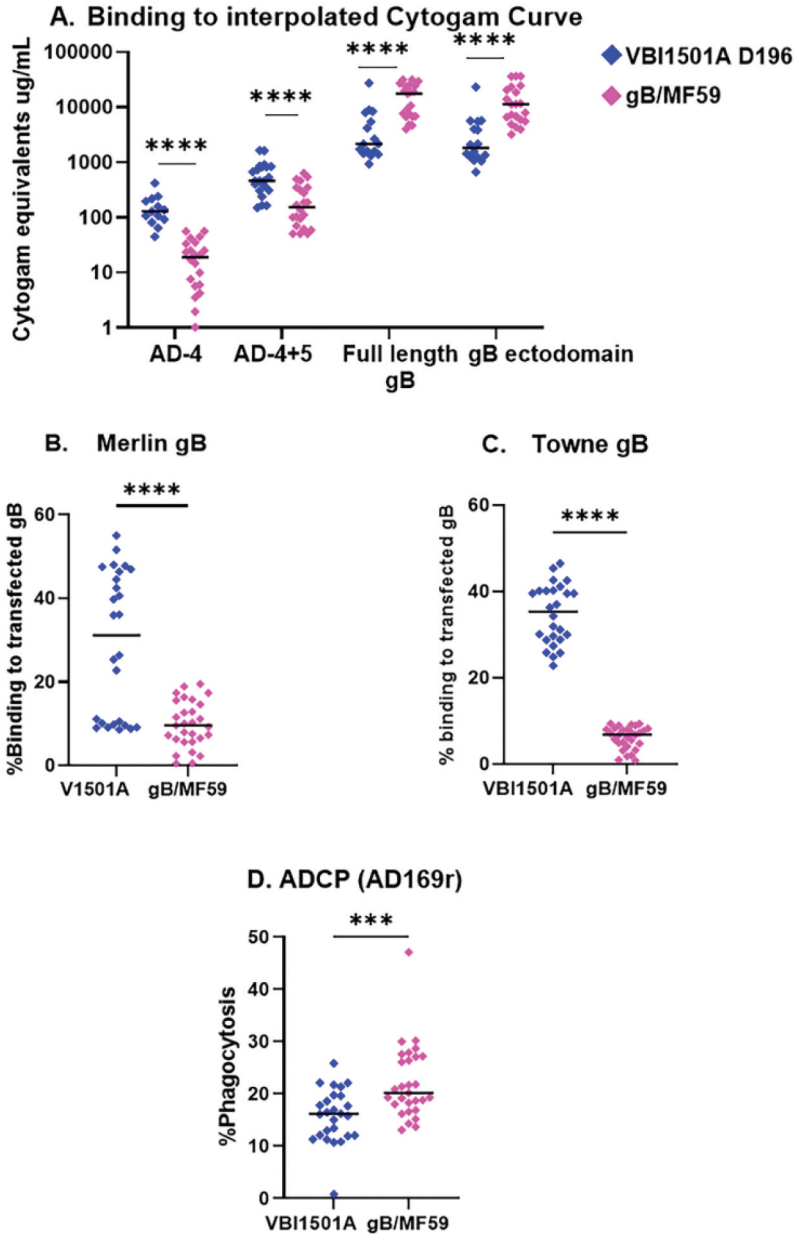

Figure 4. VBI1501A had better binding to antigenic domains 4 and 5 and cellular gB, while gBMF59 induced stronger ADCP and stronger binding to AD6 and gB. Data was run through GraphPad Prism version 10.4.1 (GraphPad Software, Inc, La Jolla, CA). Data from the gB/MF59 vaccine was collected from previous studies run in 2022 on an adolescent cohort. The gB/MF59 (phase II, NCT00133497) vaccine trial was conducted by administering 20ug of the vaccine via intramuscular injection at months 0, 2 and 6Serum from participants after their 3rd dose of the gB/F59 vaccine (pink diamonds) was compared to serum from participants after their 3rd dose of VBI-1501A (blue diamonds). To compare IgG binding to gB and antigenic domains of gB for both vaccine cohorts, BAMA data was interpolated to Cytogam curves run with each antigen panel. A custom script generates a standard curve using the Cytogam control run with each assay and plots IgG binding to respective antigens run in that panel. Samples outside of the curve were then separated from that data set. Values within the curve were then multiplied by the dilution factor used for that antigen. Only raw values that passed the raw positivity cutoff (baseline average plus 3xSD) were interpolated and then included in the interpolation graph (A). An unpaired t-test was used to calculate statistical significance between BAMA outputs for the two vaccine cohorts. Four stars represent $P < .001$. (B,C) IgG binding to Merlin and Towne gB transfected cells was compared Via TCB. An unpaired t-test was used to calculate statistical significance. (D) ADCP of AD169r for both cohorts was compared with a Mann-Whitney test. $p < .05$ was considered significant.

Samples were also run against entry glycoproteins, gB ectodomain (produced in house, based on Towne strain) and full-length post-fusion gB (Sino Biological). Quality control was performed using a custom R-script (https://github.com/SergeyAnanyev-PermarLab/Bioplex-BAMA-Primary-QC-Script).

Serum binding to AD-2 and AD-6 was measured via ELISA as detailed in Karthigeyan et al.[19] EC_{50} and ED_{50} were reported as the area under the curve (AUC) using GraphPad Prism.

Whole virion ELISA

Serum Binding to HCMV Towne, the autologous vaccine strain, was measured via whole virion ELISA, as described in *Jenks* et al.[21] 384-well plates were coated overnight with HCMV Towne, 1000 PFU per well. The same protocol as the standard ELISA, described above, was followed.

gB-transfected cell IgG binding

Transfected cell binding (TCB), to gB expressed on the cell surface as described in Karthigeyan et al. was measured by co-transfecting HEK 293T cells with plasmids expressing Towne or Merlin HCMV, along with GFP.[19] The frequency of PE+ cells was reported for each sample based on the live, singlet, GFP+ population. Positivity cut off was the average + 3SD of baseline (Week 0) samples.

Antibody-dependent cellular phagocytosis (ADCP)

ADCP was evaluated as previously described.[10] Briefly, an optimized amount of AD169r virions (0.25×10^3 PFU/well) was conjugated to AF647 NHS ester prior to incubation with sera samples diluted 1:20 and centrifuged with 50,000 THP-1. Cells were stained with Aqua Live/Dead stain, fixed with 10% formalin, and washed prior to acquisition on the flow cytometer (Fortessa; BD) using the HTS as previously described. The percentage of AF647+ cells was reported for each sample based on the live, singlet population.

Antibody dependent cellular cytotoxicity (ADCC)

ADCC assays were carried out as previously described.[22] In brief, HFFF were infected with HCMV strain Merlin for 48 h at MOI = 5, then detached with trypLE and co-cultured with PBMC that had been separated by density gradient centrifugation, along with Golgi-stop and anti-CD107a-FITC antibody (biolegend). After 5 h cells were stained with antibodies against CD56-BV605, CD3-PECy7, CD57-APC (biolegend) and live/dead aqua (Thermofisher) then fixed in 4% paraformaldehyde. Data was acquired on an Attune NxT Flow Cytometer (Thermofisher).

Inhibition of cell-associated virus spread

Inhibition of cell-associated virus spread was performed as previously described.[19] 384-well plates were seeded with 5000 ARPE-19 cells and infected with Ts15nR (MOI 0.05) and incubated overnight at 37°C. Alternatively, 96-well plates were infected with Merlin-GFP at MOI 0.1–0.05, then cultured for 5–7 days in the presence of 10% heat inactivated serum. Virus dissemination was assessed by live cell imaging on an incucyte or the Pico automated cell imager and plotted relative to virus dissemination in the presence of serum from HCMV seronegative individuals.

Statistical analysis

All data included are part of a post-hoc analysis, and thus, not included in the original protocol. Statistical analysis of all data was performed on GraphPad Prism version 10.4.1. Assay quality control (QC) was performed by assessing the %CV of samples run in duplicate. Positivity cutoff for assays was calculated by determining the average +3x standard deviation of baseline samples. % responders was calculated by dividing the number of day 196 participants that had binding above the positivity cutoff by the total

number of participants included in the data set. All figures were generated using Graphpad Prism and assembled using Adobe Photoshop.

Discussion

The findings of this study suggest that the eVLP gB vaccine, VBI-1501A, elicits some non-neutralizing antibody functions in addition to the previously reported neutralizing functions.[7] While it is likely that an effective CMV gB vaccine will require induction of antibodies that neutralize viral entry into host cells, several studies have indicated that neutralization alone may not be sufficient in mediating protection against transmission.[12,13,15,23,24] The gB/MF59 vaccine, in particular, induced epithelial cell neutralization titers 15-fold lower than those observed during natural infection, and it was recently shown that the partial protection observed with the gB/MF59 vaccine could be attributed to non-neutralizing antibody functions and inhibition of cell-cell spread of CMV.[14,15,25] In fact, several studies have shown that Fc-mediated effector functions are associated with decreased risk of congenital transmission of CMV.[12,13,23,26] Cell-cell spread, ADCP, and ADCC are mechanistic antibody functions that future CMV vaccine studies should evaluate, expanding the immunologic endpoints of CMV vaccine immunogenicity beyond neutralization alone.

The differences in the humoral immune responses elicited by the two vaccines compared in this study could be due to the distinct conformation of gB in each vaccine formulation. While the gB/MF59 vaccine presents a full-length, soluble post-fusion conformation gB primarily in its monomeric form,[13] the VBI1501A vaccine is expressed as a membrane-associated trimeric gB.[7] Therefore, the VBI1501A vaccine and its distinct conformation of gB presentation is an important guide in the design of an immunogen that will elicit the most protective responses against HCMV acquisition, reactivation, or congenital transmission.

This study revealed that three doses of the VBI1501A eVLP gB-G vaccine at 2.0 μg induced potent IgG binding to AD-4 and AD-4+5 that is 7-fold higher than that induced by the gB/MF59 vaccine (Figure 4A). Enhanced IgG binding to AD-4 and AD-5 of domains I and II of gB has shown to be associated with enhanced neutralizing antibodies against CMV.[27] VBI1501A also induced IgG binding to post-fusion full length gB, and the ectodomain of Towne gB, but this binding was not as high as that induced by the gB/MF59 vaccine (Figure 4A). This implies that gB/MF59 induced higher responses to epitopes other than AD-4/AD-5, which has been identified to include the AD3 transmembrane domain[28] and the recently identified linear domain of gB involved in the cell-cell spread termed AD-6.[14]

While the neutralizing antibody titers elicited by VBI1501A were still lower than those observed during natural infection, our results indicate that the superior induction of IgG responses to gB AD-4 and the AD-4 +5 region by the VBI1501A vaccine compared to that of the gB/MF59 vaccine may contribute to the higher levels of neutralization observed in phase I VBI1501A participants. However, neither the VBI1501A, nor the gB/MF59 vaccine induced IgG binding to AD-2 site 1, the target of some of the most potently neutralizing antibodies. While AD2 site 1 responses are detected in approximately half of HCMV seropositive individuals, researchers have yet to develop a vaccine capable of eliciting robust responses against this region, which could be a strategy in future vaccine design to induce even stronger neutralization responses in combination with non-neutralizing responses.[29] AD-1, an immunodominant target for HCMV-specific humoral responses, is believed to be the target of neutralizing and non-neutralizing antibodies.[30] While studies suggest that most CMV-seropositive individuals mount an AD-1 response, this response has not been correlated with protection against acquisition or transmission.[30] VBI1501A nor gB/MF59 vaccinees mounted responses against AD-1 that were above baseline levels.[31] An important caveat in our binding study is that while AD-1, 4, 4+5, and 5 were from the heterologous Merlin strain, the other peptides/proteins were based on the vaccine strain Towne. While we wouldn't expect to see major differences in binding to these two strains as they both express gB of the gB1 genotype and gB is highly conserved, variability in the AD-4 region between these two strains could lead to differences in binding.

VBI1501A participants did not have detectable serum IgG binding to AD-6, a response which has been suggested to have contributed to immune protection of transplant vaccinees following the gB/MF59 vaccination, and which is capable of inhibiting cell-cell dissemination of HCMV.[14] Although VBI1501A-elicited IgG was able to reduce spread of a cell-free disseminating version of Merlin, and Ts15nR, an epitheliotropic version of Towne capable of both cell-free and cell-cell transmission (Figure 3), it was

ineffective at preventing spread of a wild-type version of Merlin that is almost completely cell-cell disseminating (Figure 3C), consistent with previous reports that this mode of spread is highly resistant to classical neutralizing antibodies.[17]

The VBI1501A vaccine elicited significantly stronger IgG binding to gB expressed on the surface of cells than the gB/MF59 vaccine (Figure 4B,C), though the difference in binding to heterogenous and vaccine-strain matched gB was noteworthy. While gB is generally thought as a highly conserved protein, these differences in binding could point to an aspect of HCMV evolution to evade humoral immune responses, which should be kept in mind for future vaccine design strategies. Previous studies have suggested that IgG binding to gB-transfected cells is reflective of infected cell binding.[13] VBI1501A induced anti-HCMV Fc-mediated IgG functions previously associated with natural immune protection against cCMV transmission risk. VBI1501A also induced ADCP against AD169r virions, a response previously associated with protection in the gB/MF59 vaccine trial, but the induced response was not as high magnitude as that elicited by the gB/MF59 vaccine (Figure 4D). Future work will be needed to map the specific gB epitopes that dictate the ADCP response to explain these differences. VBI1501A could not induce ADCC responses against Merlin HCMV (Figure 4E), an activity that was recently associated with reducing fetal transmission risk in naturally infected cord blood donors.[23] While gB/MF59 induced detectable ADCC responses in the adolescent cohort, serum from postpartum and SOT patients vaccinated with gB/MF59 did have detectable ADCC responses.[32] This implies that gB antibody responses alone may not be sufficient to promote potent ADCC. Interestingly, another HCMV vaccine that included multiple entry glycoproteins induced ADCC responses, yet the responses were still below the level raised by natural infection, indicating that this function is not restricted to antibody responses against entry glycoproteins. Both studies are consistent with reports that additional early-expressed (non-entry) cell surface antigens may be an important additional component of the anti-HCMV ADCC response,[10,22] and suggest that a vaccine targeting gB and these cell surface antigens in tandem has potential to be successful.

Overall, while the VBI1501A vaccine elicited lower non-neutralizing IgG responses compared to the gB/MF59 vaccine, it outperformed the gB/MF59 vaccine in binding to the AD-4 and AD-5 neutralizing domains on gB, neutralizing activity, and binding to gB expressed on the surface of a cell. This study also demonstrated that the VBI1501A eVLP vaccine activates ADCP against HCMV but is unable to induce IgG against AD2S1 or sufficiently block cell-associated spread or elicit ADCC, indicating that gB alone may not be sufficient for all desirable anti-CMV antibody functions. It is important to note that the cohorts in these vaccine trials consist of participants of different age groups, with the gB/MF59 vaccine cohort comprising adolescent girls, and the VBI1501A comprising participants aged 21–40. Therefore, the results of these experiments could be skewed by the differences in immune system development observed during adolescence and adulthood.[13] These immunogenicity metrics could warrant further development of the eVLP CMV gB vaccine as it has the potential to outperform the partially successful CMV vaccines in the prevention of HCMV acquisition. In addition, eVLP technology may be a better alternative to adjuvanted protein vaccines because it closely mimics the natural structure of gB without the viral genome, which in theory creates a safer vaccine candidate that displays a “native-like” conformation of gB.[33] We acknowledge that vaccines that only target gB have presented shortcomings in the CMV field, and we suggest that it would be advantageous to explore targeting gB in tandem with other antigens. For example, the mRNA-1647 vaccine which is mRNA encoding gB and pentameric complex (PC), performed better than gB/MF59 in terms of neutralization and ADCC, but gB-specific IgG and ADCP responses were lower (Hu et al.). Other entry glycoprotein complexes have also been suggested as new vaccine targets, such as gH/UL116/UL141, and it is possible that targeting these complexes could induce antibody responses that current gB-based vaccines are incapable of inducing, potentially improving on the potential for vaccine efficacy against virus acquisition and/or congenital transmission.[34] While there is still a gap in vaccine development that needs to be filled by a vaccine capable of robustly eliciting both neutralizing and non-neutralizing functions, the results from immunogenicity comparisons between these two vaccines can be used to guide the next phases of the gB antigen in HCMV vaccine development.

Author contributions

CRediT: **Megan R. Connors:** Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Krithika P. Karthigeyan:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Adelaide S. Fuller:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing; **Libby Mitchell:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing; **Hannah Preston:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing; **Sergey Ananyev:** Methodology, Software, Validation, Writing – review & editing; **Chelsea M. Crooks:** Data curation, Methodology, Software, Supervision, Writing – review & editing; **Richard Stanton:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing; **David E. Anderson:** Conceptualization, Funding acquisition, Resources, Writing – review & editing; **Sallie R. Permar:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Disclosure statement

S. R. P. has served as a consultant to Moderna, Pfizer, GSK, and Dynavax vaccine programs and has led sponsored programs on CMV vaccines with Moderna, Pfizer, and Dynavax. D.E.A. is an inventor on the patent describing the vaccine evaluated in this manuscript and was an executive officer and received financial compensation and stock from the company that owned the vaccine evaluated in this manuscript. All other authors declare no competing interests.

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Notes on contributor

Sallie R. Permar is a physician scientist whose work focuses on the prevention and treatment of neonatal viral infections and the immunology of the maternal-fetal interface. She has a PhD in Microbiology/Immunology from Johns Hopkins Bloomberg School of Public Health in Baltimore, an MD from Harvard Medical School, and completed her clinical training in pediatric infectious diseases at Children's Hospital in Boston. In addition to caring for children with infections, she leads a research laboratory investigating immune protection against vertical transmission of neonatal viral pathogens, including HIV-1, cytomegalovirus (CMV), and Zika. She has made important contributions to the development of vaccines for prevention of vertical HIV, CMV, and Zika transmission utilizing novel nonhuman primate models, and designing pediatric vaccine trials. Dr. Permar has received several prestigious awards, including the Presidential Early Career Award in Science and Engineering (PECASE), the Society for Pediatric Research Mead Johnson Award, and induction into the American Association of Physicians.

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Data availability statement

All data sets generated as part of this study are included in the main text. Datasets and statistical analyses will also be included on Github post publication.

Ethical statement

The samples utilized in this study were part of a Phase I study conducted by VBI Vaccines (ClinicalTrials.gov NCT02826798). The study was undertaken in compliance with Good Clinical Practice guidelines, the Declaration of Helsinki, national regulatory requirements, and was approved by the local institutional review boards of Dalhousie University (Nova Scotia, CN), McGill University (Montreal, CN), University of Montreal (Montreal, CN), and the Children's Hospital Research Institute and the University of British Columbia (Vancouver, CN). An independent Data Safety Monitoring Committee regularly reviewed accumulating data.

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