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Novel trimeric human cytomegalovirus glycoprotein B elicits a high-titer neutralizing antibody response ☆,☆☆

Xinle Cui [a,*], Zhouhong Cao [a], Shuishu Wang [b], Ronzo B. Lee [c,1], Xiao Wang [d], Haruhiko Murata [d], Stuart P. Adler [e], Michael A. McVoy [c], Clifford M. Snapper [a]

[a] Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, United States
[b] Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, United States
[c] Department of Pediatrics, Virginia Commonwealth University, Richmond, VA 23298, United States
[d] Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD 20993, United States
[e] CMV Research Foundation, Richmond VA 23229, United States

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ABSTRACT

Human cytomegalovirus (HCMV) is a major cause of disability in congenitally infected infants and in the immunosuppressed. There is currently no licensed prophylactic HCMV vaccine. The HCMV envelope glycoprotein B (gB) is considered a major vaccine target antigen based on its critical role in mediating viral-host cell fusion and thus viral entry. The natural conformation of HCMV gB within the viral envelope is a trimer, but there has been no reported success in producing a recombinant trimeric gB suitable for vaccine use. Phase II clinical trials of a monomeric recombinant gB protein demonstrated 50% efficacy in preventing HCMV infection in seronegative women of reproductive age, and in reducing viremia in solid organ transplantation recipients. We now report the production of a uniformly trimeric recombinant HCMV gB protein in Chinese ovary cells, as demonstrated by Western blot analysis under modified non-reducing conditions and size exclusion chromatography with multi-angle scattering. Immunization of mice with trimeric HCMV gB induced up to 11-fold higher serum titers of total gB-specific IgG relative to monomeric HCMV gB using Alum + CpG as adjuvants. Further, trimeric HCMV gB elicited 50-fold higher complement-independent and 20-fold higher complement-dependent HCMV neutralizing titers compared to monomeric HCMV gB using the fibroblast cell line, MRC-5, and up to 6-fold higher complement-independent and –dependent HCMV neutralizing titers using the epithelial cell line, ARPE-19. The markedly enhanced HCMV neutralizing activity in response to trimeric HCMV gB was also observed using an additional four distinct clinical HCMV isolates. These data support a role for trimeric HCMV gB as an important component for clinical testing of a prophylactic HCMV vaccine.

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1. Introduction

Human cytomegalovirus (HCMV) is an enveloped, double-stranded DNA β-herpesvirus of the Herpesviridae family. HCMV is the leading non-genetic cause of sensorineural hearing loss in childhood and a significant cause of neurodevelopmental delay, including mental retardation [1–3]. Additional congenital sequelae include microcephaly, seizures, intracranial calcifications, cerebral palsy, hepatitis, and chorioretinitis resulting in vision loss [4–7]. In addition to congenital infections, HCMV produces significant clinical disease in the immunosuppressed, including transplant recipients and patients with AIDS [8–11]. Although HCMV infection in immunocompetent individuals is generally asymptomatic, it may produce a mononucleosis syndrome in 10% of primary infections of older children and adults [12]. In 2001, the Institute of Medicine of the U.S. National Academy of Sciences stated that a vaccine to prevent congenital HCMV infection is among the highest U.S. priorities [13].

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* Corresponding author at: Department of Pathology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, United States.

E-mail address: xinle.cui@usuhs.edu (X. Cui).

[1] John Tyler Community College, Midlothian, VA 23114, United States.

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HCMV is spread mainly via saliva and urine to seronegative children and adults, and via transplacental transmission to the fetus [14,15]. The target cells of HCMV include fibroblasts, epithelial cells, endothelial cells, monocyte-macrophages, hepatocytes and neurons [14,16,17]. The mechanism of HCMV fusion and entry into mammalian cells is analogous to that employed by other members of the herpesvirus family [16,17]. HCMV enters cells by fusing its envelope with either the plasma membrane or endosomal membrane [18,19]. HCMV envelope proteins, glycoprotein B (gB), gH, gL, gO and UL128/UL130/UL131A proteins have collectively been identified as the envelope proteins that play critical roles in HCMV fusion and entry into host cells [reviewed in [21]]. The gB protein is the direct mediator of HCMV fusion with all target host cell membranes. The activation of HCMV gB for fusogenic activity requires its association with the gH/gL/gO protein complex. However, the gH/gL/UL128/UL130/UL131A protein complex (pentameric complex) is further required for efficient targeting of HCMV to epithelial and endothelial cells [19,22–24]. HCMV gB or gH/gL proteins have been shown to elicit serum HCMV neutralizing antibodies that block entry into both fibroblasts and epithelial cells. However, the pentameric complex induces the highest serum neutralizing titers for epithelial and endothelial cells [24–26].

Earlier clinical trials using live attenuated Towne or AD169 HCMV viral vaccines, both of which lacked expression of the pentameric complex, proved to be ineffective in preventing HCMV infection in either healthy volunteers or renal transplant recipients, although some efficacy was demonstrated in overt HCMV disease in high risk Recipient-Donor + renal transplant recipients [14,27]. New HCMV viral strains engineered to express the pentameric complex are currently being evaluated, but safety concerns persist using this approach. In contrast, recombinant protein subunit vaccines have in general shown excellent safety and efficacy profiles in humans. Of note, a phase II clinical trial using a recombinant HCMV gB protein (Chiron gB) derived from the Towne strain of HCMV and adjuvanted with MF59 [28] demonstrated 50% efficacy in preventing HCMV infection in HCMV seronegative women [15,29]. Similar results were obtained in adolescent girls, though the efficacy did not reach conventional levels of statistical significance [20]. In another phase II study, patients awaiting solid organ transplantation who were HCMV seronegative, were vaccinated with the same recombinant HCMV gB protein as described above, and the vaccine was effective in preventing viremia in 5 out of 11 subjects, compared to 0 out of 5 subjects in the placebo group [30]. The recombinant HCMV gB used in these phase II clinical trials was originally developed at Chiron, whereby the wild-type gB was modified to remove the furin cleavage site, since retention of this site interfered with recombinant protein production. The resulting Chiron gB protein, however, did not assume its native trimeric conformation that is postulated to be the ideal form for vaccine use [31]. Indeed there have subsequently been no reported successes in producing a fully trimeric HCMV gB protein suitable for vaccine use. Another promising HCMV vaccine candidate, the pentameric complex has been extensively studied in recent years, and is likely to provide protection against HCMV infection of epithelial cells, endothelial cells and monocytes, but not fibroblasts [24,25,27].

The clinical trials with Chiron gB have encouraged further evaluation of gB as a prophylactic HCMV vaccine, and also suggested that a more effective HCMV vaccine might be achieved by vaccinating with a fully trimeric, native HCMV gB. Using a novel approach, we now describe the efficient production of a fully trimeric recombinant HCMV gB protein. We demonstrate that immunization with this protein elicits markedly higher titers of serum HCMV neutralizing antibodies in mice relative to its monomeric counterpart using Alum + CpG as adjuvants. The data generated by this study provides critical new information for the future design of a prophylactic HCMV vaccine for clinical use.

2. Material and methods

2.1. Cell lines, HCMV strains, and reagents

The Chinese hamster ovary (CHO) cell line, DG44 was purchased from Invitrogen and maintained in CD DG44 medium. MRC-5 and ARPE-19 cell lines were purchased from ATCC, and cultured using EMEM or DMEM/F-12K medium, respectively, both supplemented with 10% fetal bovine serum. Purified monomeric recombinant HCMV gB protein was purchased from Sino Biologicals, Inc. (Beijing, P. R. China). HCMV strain AD169^{WT131} was provided by Drs. Xiao Wang and Haruhiko Murata (Food and Drug Administration) and strain AD169 was purchased from ATCC. HCMV clinical isolates UXC, CSL5001, 38532 and 39621 were provided by Dr. Michael McVoy (Virginia Commonwealth University). HCMV strain AD169WT^{131} was propagated in ARPE-19 cells, HCMV strain AD169 and clinical isolates were propagated in MRC-5 cells. CytoGam was a gift from CSL Behring to Dr. Michael McVoy. Rabbit complement was purchased from Sigma-Aldrich. Monoclonal mouse IgG1 anti-gB antibody 2F12 was purchased from Virusys corporation (Taneytown, MD), and monoclonal mouse IgG1 anti-gB antibody LS-C64457 was purchased from LifeSpan BioSciences, Inc. (Seattle, WA).

2.2. Expression and purification of trimeric HCMV gB protein

The coding sequence for HCMV gB was downloaded from NCBI, reference sequence # NC_006273.2, strain Merlin. The DNA sequence encoding for amino acids 23–750 was used. The signal peptide (amino acids 1–22) was replaced with an IgG κ leader sequence, and the coding sequence for the cleavage site, RTKRS between amino acids 456 (N) and 462 (T) was replaced with a 15 aa $(Gly_4Ser)_3$ linker sequence (Fig. 1A). A His_6 sequence was added to the 3′ end for protein purification. The DNA coding for the gB protein was synthesized by Blue Heron, cloned into pOptiVEC (Invitrogen), and verified by sequencing. CHO cells were transfected with pOptiVEC-gB using Free-style Max reagent (Invitrogen), and selected with increasing concentrations of methotrexate up to 4 uM for optimal protein expression, followed by limited dilution cloning. CHO cells were then cultured in a FiberCell bioreactor (FiberCell Systems, Frederick, MD), the supernatants were concentrated for affinity purification using a cobalt column (Thermo Scientific), and further purified using size exclusion chromatography on a Superose 6 column (GE Lifesciences, Pittsburgh, PA).

2.3. Western blot analysis

Purified proteins were analyzed by electrophoresis on 3–8% NuPAGE Tris-Acetate Mini-Gels, under both reducing condition and modified non-reducing conditions as previously described [64]. For reducing conditions, purified HCMV gB was boiled for 10 min in lithium dodecyl sulfate (LDS) loading buffer containing 50 mM DTT, resolved on 3–8% PAGE in SDS running buffer, and blotted with an anti-gB monoclonal antibody 2F12. For modified non-reducing conditions, protein samples were mixed with LDS loading buffer without DTT, resolved on 3–8% PAGE in Tris-glycine native running buffer (Invitrogen), and blotted with anti-gB monoclonal antibody LS-C64457. Membranes were then incubated with polyclonal HRP-goat anti-mouse IgG (Thermo Fisher Scientific), followed by incubation with SuperSignal West Pico chemiluminescent substrate, with signal captured on X-ray film.

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