ANNUAL REVIEWS

Annual Review of Virology

Lessons from Acquired Natural Immunity and Clinical Trials to Inform Next-Generation Human Cytomegalovirus Vaccine Development

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Annu. Rev. Virol. 2022. 9:491–520

First published as a Review in Advance on June 15, 2022

The *Annual Review of Virology* is online at virology.annualreviews.org

https://doi.org/10.1146/annurev-virology-100220-010653

Keywords

human cytomegalovirus, acquired natural immunity, clinical trial, animal model, immune correlates of protection, next-generation vaccine

Abstract

Human cytomegalovirus (HCMV) infection, the most common cause of congenital disease globally, affecting an estimated 1 million newborns annually, can result in lifelong sequelae in infants, such as sensorineural hearing loss and brain damage. HCMV infection also leads to a significant disease burden in immunocompromised individuals. Hence, an effective HCMV vaccine is urgently needed to prevent infection and HCMV-associated diseases. Unfortunately, despite more than five decades of vaccine development, no successful HCMV vaccine is available. This review summarizes what we have learned from acquired natural immunity, including innate and adaptive immunity; the successes and failures of HCMV vaccine human clinical trials; the progress in related animal models; and the analysis of protective immune responses during natural infection and vaccination settings. Finally, we propose novel vaccine strategies that will harness the knowledge of protective immunity and employ new technology and vaccine concepts to inform next-generation HCMV vaccine development.

1. INTRODUCTION

Human cytomegalovirus (HCMV), a double-stranded DNA virus with the largest genome (around 235 kbp) of known human viruses, belongs to the human betaherpesvirus family (1). HCMV infection causes persistent, life-long infection in broad types of host cells mediated by four major envelope glycoprotein complexes (GCs): glycoprotein B (gB) oligomer, gM/gN dimer, gH/gL/gO trimer, and gH/gL/UL128/UL130/UL131A pentamer complex (PC) through distinct entry mechanisms (1, 2).

Although primary HCMV infection in immunocompetent individuals is typically asymptomatic (3), congenital HCMV infection often causes long-lasting sequelae in newborns worldwide such as brain damage, sensorineural hearing loss, and neurodevelopmental delay (4, 5). Congenital HCMV affects 0.5–2% of all live-born infants globally (6). In the United States alone, approximately 30,000 congenital HCMV infections occur annually, of which more than 5,000 infections lead to permanent disabilities (7). Additionally, HCMV infection leads to life-threatening diseases in immunocompromised individuals, such as solid organ transplant recipients and acquired immunodeficiency syndrome (AIDS) patients (8, 9). Furthermore, reinfection or reactivation can often occur when seropositive individuals contract new HCMV strains or a latent virus in seropositive subjects is activated, leading to viral replication (10). Altogether, HCMV infection in these populations is an immense public health and economic burden (11). Therefore, an effective HCMV vaccine is urgently needed to prevent infection and HCMV-associated diseases in those affected populations and to reduce associated healthcare costs (7).

Unfortunately, no successful HCMV vaccine is yet available despite more than 50 years of vaccine development (12). The challenges that hinder effective HCMV vaccine development include the following: (*a*) HCMV effectively evades the host immune responses (12); (*b*) immune correlates of protection (CoPs) remain elusive (13, 14); (*c*) relevant animal models to assess vaccine efficacy need further development (15); and (*d*) broadly and potent neutralizing antibodies (NAbs) have proven difficult to elicit (12). Yet, recent advances in understanding natural immunity, the lessons learned in HCMV vaccine clinical trials, and immune correlates analyses in both natural infection and vaccination settings are encouraging for the hope for an effective HCMV vaccine (13, 14, 16). New findings from related animal models and new technology advances from other vaccine pursuits set the stage for novel vaccine strategies and rational design of a next-generation HCMV vaccine, potentially accelerating the licensing of a successful HCMV vaccine by breaking current barriers to vaccine development (**Figure 1**).

2. NATURALLY ACQUIRED IMMUNITY FROM HCMV INFECTION

A comprehensive understanding of immune responses mounted during natural HCMV infection and transmission helps identify the components of protective immunity (17), offering opportunities to mimic these immune responses through immunization (17). Meanwhile, HCMV infection is able to evade the immune responses, and dissection of these evasion mechanisms may provide useful information for effective vaccine strategies (18, 19). To maximize the protective efficacy, we should consider harnessing both innate and adaptive immunity (12), further described below.

2.1. Natural Killer Cells: The First Line of Defense Against HCMV Infection

Innate immune responses serve as the first line of defense against viral infection and allow the host to rapidly exert antiviral functions (20). Among them, natural killer (NK) cell responses are the most well-documented innate memory response to HCMV infection (20). Following HCMV primary infection, NK cells are rapidly activated to kill the infected cells and prevent severe disease

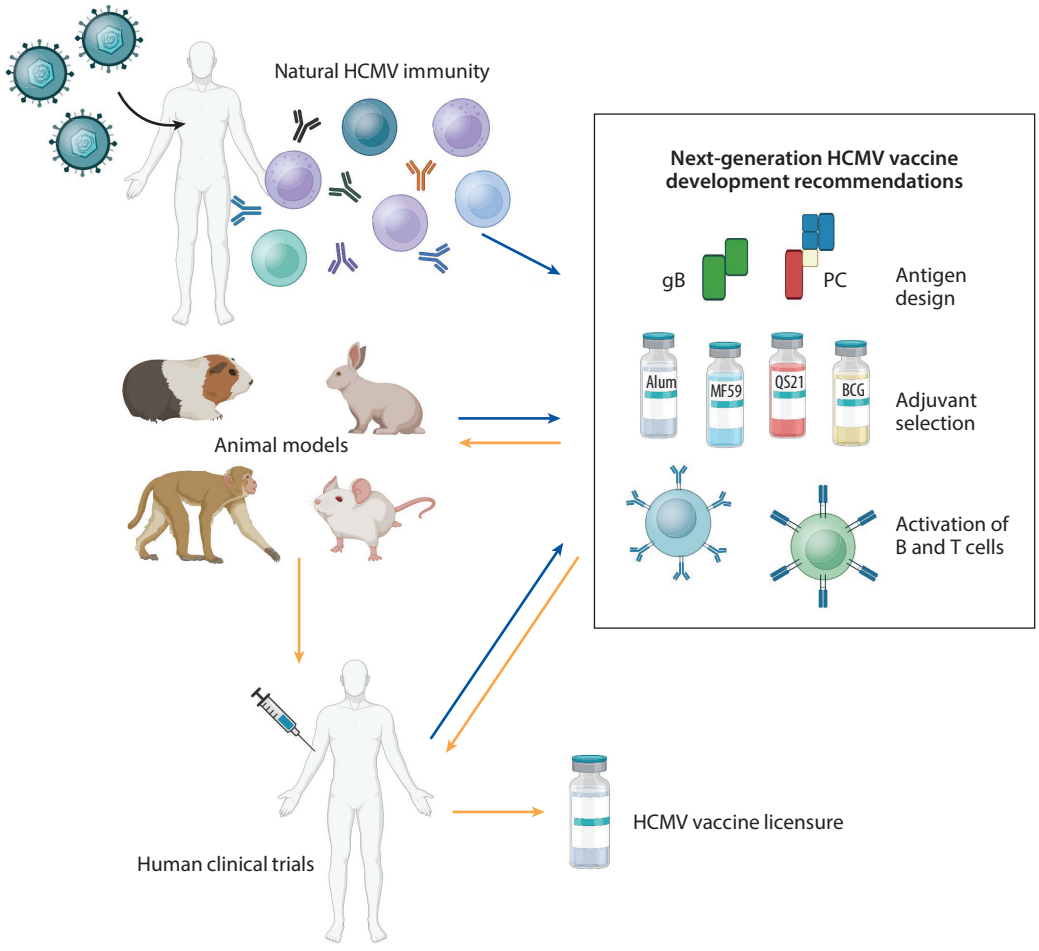

Figure 1

Schematic of next-generation HCMV vaccine development strategies. Dissection of protective immune responses during natural HCMV infection, the development of animal models, and lessons learned from human clinical trials led to our next-generation HCMV vaccine development proposal. These findings from iterative studies will further facilitate the highly relevant animal model development and a deep understanding of the protective immune component from both infection and vaccination settings to guide future HCMV vaccine development. Stepwise, hopefully, an effective HCMV vaccine will be licensed to the clinic by employing these strategies. Abbreviations: gB, glycoprotein B; HCMV, human cytomegalovirus; PC, pentamer complex. Figure adapted from images created with BioRender.com.

by releasing inflammatory cytokines and chemokines (21). Furthermore, it is well documented that severe cytomegalovirus (CMV) disease can develop in individuals who lack NK cells, suggesting the importance of NK cells to mitigate consequences of HCMV infection (22, 23).

However, HCMV has developed strategies to effectively escape recognition by NK cells and prevent NK cell killing through virus-encoded gene products (18, 19, 24), such as UL18, UL40, UL16, UL141, UL142, and UL83, which inhibit the CD155 ligand presentation for the NK cell activating receptor NKG2D on infected cells (25, 26). Therefore, when live-attenuated or disabled viral vaccine platforms are employed, deleting these genes that inhibit NK cell killing on CMV-infected cells could be relevant in controlling infection (27).

Recent studies indicated that NK cells may have a memory phenotype, despite historically being considered innate immune cells (28–31). One relevant piece of evidence regarding NK memory came from the mouse model of CMV infection: Liver-resident NK cells can control murine cytomegalovirus (MCMV) infection at initial infection sites in an interleukin (IL)-12-dependent manner. Moreover, NK recall responses were rapidly mounted upon the MCMV rechallenge and further enhanced the protection (32). Further analysis indicated these NK memory responses were related to the MCMV-encoded glycoprotein m12 (32). Following acute HCMV infection, NKG2C^{+} NK cells, one unique subset of NK cells, were rapidly expanded, became NKG2C^{hi}, and acquired CD57, a carbohydrate antigen expressed on highly mature cells within the CD56^{dim}CD16^{+} NK cell compartment (33, 34). This specialized NK cell population (NKG2C^{+}) was persistent in HCMV seroconverted individuals (35–37), indicating the adaptive features of this subset of NK cells (33, 38, 39).

2.2. Adaptive Immune Responses

Adaptive immune responses induced by natural HCMV infection include humoral and cellular responses mediated by B and T lymphocytes, respectively, which serve as key components induced by conventional vaccination to provide protection (12, 14). A comparative study demonstrated that maternal immunity prior to conception could partially protect the infant from severe congenital diseases caused by HCMV infection (40). In this study, infected infants born to mothers who acquired primary HCMV infection during pregnancy showed higher risk of HCMV-associated symptoms and long-term sequelae compared to those born to HCMV-seropositive mothers (40), indicating that maternal adaptive immunity plays a protective role in preventing severe congenital HCMV disease.

2.2.1. Humoral immune responses to HCMV after infection. Primary HCMV infection induces robust humoral responses targeting multiple HCMV antigens, including structural protein phosphoprotein 65 (pp65), nonstructural immediately early protein IE1, and the envelope GC (gB, gM/gN, gH/gL/gO, PC) (41, 42) (**Figure 2*a***). These envelope glycoproteins play essential roles in viral entry into fibroblast, epithelial, and endothelial cells and for viral replication (43). gB, composed of an ectodomain subunit (gp116) and a polypeptide resembling a type I membrane protein (gp58), is essential for HCMV viral fusion and is required for entry into all permissive cell types and for cell-to-cell spread (2). PC is essential for cell entry into epithelial, endothelial, dendritic, and monocytic cells (44–46), whereas the gH/gL/gO trimer promotes the gB fusion in all cell types (47). HCMV enters fibroblast cells in a pH-independent manner via fusion at the cell surface through the gB and gH/gL/gO GC (48). In contrast, the virus enters the epithelial, endothelial, dendritic, and monocytic cells in a pH-dependent fusion mechanism via endocytosis or micropinocytosis through the gB, gH/gL/gO, and PC (44–47, 49). Of note, placental trophoblasts are specialized epithelial cells that are an important cell type to block the virus from entering for prevention of congenital CMV infection (50).

Recent evidence indicates that NAbs play an important role in preventing HCMV infection and the progression of diseases (51–53); thus, NAb induction is a major goal for vaccine development (54). NAb responses mounted during natural infection may block infection of multiple different cell types (42). NAbs blocking infection of endothelial/epithelial cells arise in serum earlier and with higher magnitude compared to NAbs blocking fibroblast infection (54).

gB-specific antibodies mounted during infection mediate both NAb and non-NAb responses (55, 56). Recently, structural biology studies revealed insight into the gB prefusion (**Figure 2*b***) and postfusion (**Figure 2*c***) structure during virus entry into the host (57, 58). There are five antigenic domains (ADs) observed in the gB (58) (**Figure 2*d***). After infection, AD-1 elicits the most

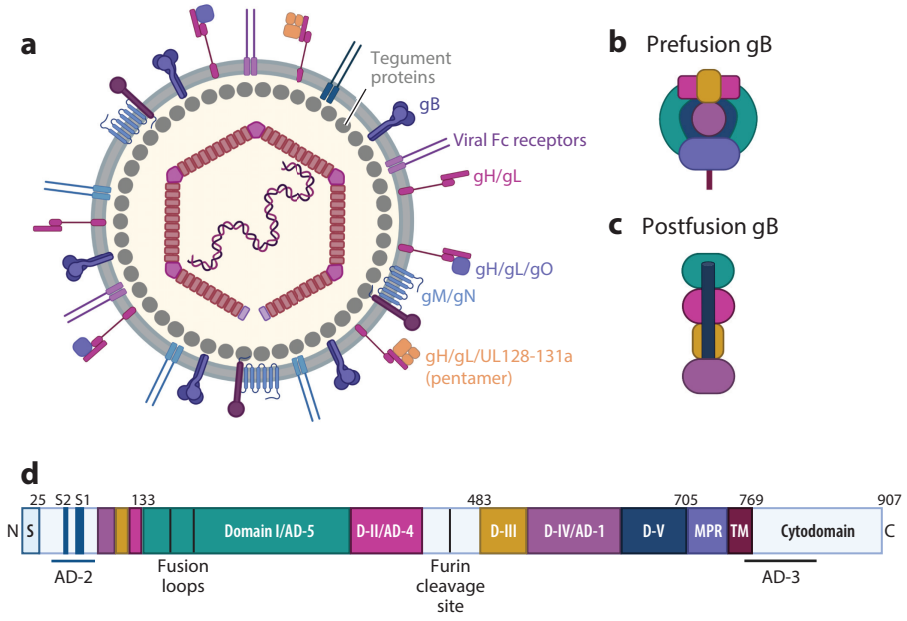

Figure 2

Schematic structure of HCMV and structural and antigenic domain mapping in the gB. (*a*) A simplified structural diagram of the HCMV virion. HCMV is a double-stranded DNA virus, with the genome encapsulated by capsid proteins. Many glycoprotein complexes are displayed on the surface of the virion, including gM/gN, and gH/gL heterodimers, the gH/gL/gO trimer, and the gH/gL/UL128/UL130/UL131A PC, viral Fc receptors, and the gB homotrimer. In addition, there are various tegument proteins between the capsid and viral envelope glycoproteins, including pp65. Focusing on an important immunogen and essential fusion protein, the prefusion (*b*) and postfusion (*c*) gB structures are shown (modeled after Reference 57) with domains colored to match each structural and antigenic domain shown in panel *d*. The domain map is based on the Towne strain. gB includes five structural domains (D-I through D-V) and five antigenic domains (AD-1 through AD-5). The signal sequence (S) is located at the N terminus before AD-2, which is composed of site 1 (S1) and site 2 (S2). Domains II, III, and IV are expressed nonlinearly, with a component of each domain expressed before Domain I and its two incorporated fusions loops. The furin cleavage site located near the middle of the amino acid sequence is noted with a black line. The MPR (*blue*), TM (*maroon*), and cytosolic domain are located toward the C terminus. Abbreviations: gB, glycoprotein B; HCMV, human cytomegalovirus; MPR, membrane-proximal region; PC, pentamer complex; TM, transmembrane. Figure adapted from images created with BioRender.com.

potent antibody response but predominantly mediates non-NAb responses. AD-2 triggers mild antibody responses and comprises two subantigenic sites: Site 1 is the target for potent NAb induction, while site 2 stimulates only non-NAb responses. AD-3 is located in the cytosolic domain and does not induce NAbs. AD-4 (Domain II) and AD-5 (Domain I) elicit strong immune responses and induce NAbs conserved across different strains (58). Hence, AD-2 site 1 (AD2S1), AD-4, and AD-5 represent the most promising vaccine target candidates in inducing potent and broadly NAb responses. NAbs targeting PC are able to potently neutralize the infection of endothelial/epithelial cells (42), and antibodies against the UL128 component, but not UL130 or UL131A, were reported to play a protective role against congenital infection (52).

A large cohort study of 3,461 multiparous women in the southern United States demonstrated that the congenital HCMV infection rate in infants born to seronegative mothers is 3% compared with a 1% rate from those born to seropositive mothers. This study indicated preexisting

natural maternal immunity, likely humoral responses, was associated with a 69% reduction in the risk of vertical transmission in future pregnancies (59). Furthermore, longer intervals between pregnancies increased the protection against congenital HCMV infection (60). The maturation of HCMV-specific antibody avidity potentially contributed to this effect (61). Hence, it is important that new vaccines induce high-avidity protective antibody responses similar to the naturally acquired protective immunity. However, which HCMV antigen-specific antibody responses mediate protection still requires exploration and is highly relevant to the vaccine immunogen selection.

A recent paper showed that the maternal monocyte-mediated antibody-dependent cellular phagocytosis (ADCP) and a high-avidity immunoglobulin G (IgG) binding to HCMV envelope glycoproteins were associated with a decreased risk of congenital HCMV infection (62). Moreover, HCMV-specific IgG engagement of FcγRI and FcγRIIA, which mediate non-NAb responses, was enhanced in nontransmitting mother-infant dyads and strongly correlated with ADCP responses (62). These findings suggest that Fc-mediated effector functions including ADCP protect against placental HCMV transmission. Similarly, other Fc-mediated IgG functionalities such as antibody-dependent cellular cytotoxicity (ADCC) (63) and antibody-dependent complement deposition (63) correlated with the vaccine-induced protection in other viral vaccine studies (63–65). It is worthwhile to further explore these antibody functionalities in HCMV infection and vaccine design.

Nevertheless, HCMV envelope glycoproteins utilize multiple strategies to escape humoral immune responses. These immune evasion mechanisms include (*a*) strain polymorphism (particularly in gH and gN antigens) to contribute to humoral response evasion, (*b*) epitope competition (e.g., non-neutralizing epitopes compete with neutralizing epitopes in gB antigen) to mislead the humoral responses, (*c*) Fc receptor-mediated endocytosis (e.g., specific domain of gH), (*d*) glycan shielding to mask vulnerable epitopes (highly glycosylated envelope proteins such as gB and gO antigens), and (*e*) cell-to-cell spread to avoid antibody-mediated inhibition (reviewed in 2). These humoral immunity evasion strategies represent challenges for the envelope glycoprotein-based vaccine immunogen design, which should be addressed in next-generation vaccine development through iterative antigen design.

2.2.2. T lymphocyte–mediated cellular immune responses during HCMV infection. Upon CMV infection, the T lymphocyte–mediated cellular responses including $CD4^+$ and $CD8^+$ T cell–mediated antiviral functionalities can potently suppress viral replication and protect from severe disease. In fact, it is estimated that at least 10% of the total T cell repertoire is committed to HCMV specificity in seropositive individuals (66). T cell responses mounted during primary CMV infection mainly target conserved viral functional antigens such as IE1 and matrix protein pp65 (67–69). Several dominant T cell epitopes were identified in pp65 (70–73) and IE1 antigens (74, 75).

Although the protective role of CMV-specific $CD8^+$ cytolytic T cell responses against disease has been repeatedly documented in immunocompromised individuals, such as solid organ transplant recipients and AIDS patients (76–79), some reports also support that CMV-specific $CD8^+$ T cells contribute to protection from congenital HCMV infection (80). Likely, the breadth but not the magnitude of the antigen-specific $CD8^+$ T cell and effector memory $CD8^+$ T cell population plays a major protective role (76–78, 80).

Recent human studies also demonstrated the maternal pp65-specific memory $CD4^+$ T cellular responses, but not IE1- or IE2-specific $CD4^+$ T cell responses, correlated with the protection from vertical transmission in congenital CMV infection (81, 82). Moreover, the IgG avidity index also correlated with the protection in the same cohort, suggesting $CD4^+$ T cells may cooperate with mature antibody responses to mediate this protection. The nonhuman primate (NHP) model

of congenital HCMV infection has also indicated the importance of maternal $CD4^+$ T cells in protection from congenital HCMV transmission and disease (83).

Similar to the NK cell responses, primary HCMV infection evades HCMV-specific $CD8^+$ T cell responses by downregulating class I major histocompatibility complex expression in virus-infected cells using several different viral proteins [unique short (US) 2, US3, US6, and US11] (84). Fully understanding the cellular response escape mechanisms of HCMV will benefit future vaccine design (85).

3. ANIMAL MODELS FOR HCMV VACCINE EVALUATION TO DE-RISK VACCINE HUMAN CLINICAL DEVELOPMENT

While some HCMV vaccines have begun phase 1 clinical trials without in vivo preclinical testing, animal models can be instrumental in assessing the immunogenicity, efficacy, and optimization of vaccine regimens and predicting success of clinical trials. To date, the vaccines trialed to prevent HCMV acquisition have focused primarily on eliciting NAbs, which can be assessed using many different animal models, including mice, rabbits, guinea pigs, or NHPs. For example, enveloped virus-like particles (eVLPs) expressing the ectodomain of Towne strain gB fused with the transmembrane and cytosolic domains of vesicular stomatitis virus (VSV) G protein were more immunogenic than eVLPs expressing native gB when tested in mice (86). The outcome from a phase 1 clinical trial (NCT0286798) (87) for this eVLP vaccine by VBI Vaccines Inc. was promising, but phase 2 has yet to begin despite the design of a phase 2 trial that was expected to begin by the end of 2019 (88). More recently, the gB and PC messenger RNA (mRNA) vaccine (mRNA-1647) from Moderna elicited potent and durable NAbs responses in mice and cynomolgus macaques (89).

Due to the high seroprevalence of HCMV and ease of reinfection in humans, vaccine development should shift away from prevention of viral acquisition to focus more on viral containment and prevention of disease, including immunocompromised patients, who are the most at risk for HCMV-related complications. This includes prevention of vertical HCMV transmission in pregnant individuals and prevention of viremia in transplant recipients (90). Thus far, CoPs against transmission and disease in these settings have not been fully defined, but evidence suggests that multiple antigen targets will be needed and that NAbs alone are not sufficient for protection (14, 16, 62, 76, 83, 91–95). Thus, defining preclinical efficacy by any specific vaccine-elicited immune response is likely not sufficient to predict efficacy in human trials and may explain the moderate efficacy of vaccines tested in late clinical trials thus far. For example, early studies of a Pfizer vaccine tested in rhesus macaques including rhesus macaque CMV (RhCMV) PC, gB, and a pp65 expression plasmid formulated with QS21 (saponin adjuvant) induced NAbs similar to those elicited by natural infection, which has historically been considered the gold standard for vaccine immunogenicity (96). Despite vaccine-induced NAb and T cell responses as measured by gamma interferon ELISPOT greater than that observed following natural infection, animals were not protected from infection or viral dissemination following oral RhCMV challenge. The efficacy of this vaccine in preventing vertical transmission has yet to be assessed, but this does demonstrate that NAbs and T cell responses meeting previously set standards may not be sufficient to prevent viral acquisition. It is also important to note that immune CoPs may be different for prevention of viral acquisition in seronegative individuals, vertical transmission during pregnancy, and control of viremia following solid organ transplant. Thus, until definitive immune CoPs have been defined for each high-risk population, more informative approaches are necessary to better evaluate vaccines at the preclinical stage of development.

Another challenge to effective preclinical vaccine testing is that animal models of HCMV infection are limited by virus species specificity. Each species has its own CMV species, which differs

significantly from that of other species due to millennia of divergent coevolution (97, 98). Thus, any vaccine platform that relies on replication of live-attenuated or replication-defective (single cycle) HCMV will not yield meaningful immunogenicity results when tested in preclinical models. Furthermore, as a result of these animal model limitations, CMV challenge studies must be performed with vaccine targets and challenge viruses adapted to the model organism. Additionally, if the goal of the vaccine is to prevent vertical HCMV transmission, the translational application of animal models is further limited to guinea pigs and NHPs by model species' placental biology and the established ability of their respective species-specific CMV to cross the placenta (99–102).

Preclinical models have been used for proof-of-concept studies for some HCMV vaccine strategies, but thus far, the concepts investigated have yet to translate effectively to the clinic. A DNA vaccine encoding guinea pig CMV (GPCMV) gB administered before conception was protective against congenital CMV infection in the guinea pig model (103). However, clinical trials of ASP0113 (NCT02103426, NCT01974206, NCT01877655), a DNA vaccine expressing HCMV gB and pp65, were primarily assessed in the setting of organ transplantation but did not meet efficacy end points for reduction in the need for antivirals and/or prevention of viremia, CMV disease, and end-organ disease (104–106). Deletion of key viral proteins involved in immune evasion mechanisms has been assessed as a way to produce safer live-attenuated vaccine strains. GP83 is a major tegument protein, homolog of HCMV pp65, expressed by GPCMV. This protein has been considered a key target of host T cell immunity and has also been implicated in innate immune evasion mechanisms (107). Preconception vaccination with an attenuated GPCMV with a GP83 deletion was effective in preventing placental infection and fetal mortality (10% in vaccinated dams versus 70% in placebo control dams), providing support for pp65 deletion as an approach for producing a live-attenuated HCMV vaccine (108). Similarly, deletion of a viral chemokine homolog closely related to the macrophage inflammatory protein 1α gene family in GPCMV resulted in attenuation in vivo but elicited protective immune responses, resulting in reduced maternal viremia, congenital pup infection, and pup mortality (109). Together with several other studies, these results suggest that deletion of immune evasion genes is an effective means of viral attenuation in animal models. However, live-attenuated CMV vaccines have not been the focus of HCMV clinical trials in the last two decades, likely because they pose more potential risks than most other vaccine platforms because the virus may still be able to establish latency depending on the manner of attenuation. Furthermore, live-attenuated vaccines are generally not recommended for pregnant individuals, a key target population for HCMV vaccination, due to the potential for the virus to cross the placenta and harm the fetus.

4. HUMAN CLINICAL TRIALS OF HCMV VACCINE CANDIDATES

The global need for an HCMV vaccine has made it an attractive target for vaccine development, and thus several vaccine platforms have been trialed in humans. Outside of live-attenuated or disabled HCMV vaccine candidates, several HCMV proteins are commonly integrated into the vaccine design, including gB, PC, pp65, IE1, and IE2. In clinical trials, healthy adults are usually involved in phase 1 studies to investigate the safety and the immunogenicity of the HCMV vaccine. In phase 2 or 3 studies, study participants are either healthy adults or high-risk patients for HCMV infection. These patients include women of childbearing age (congenital HCMV infection) and transplant and AIDS patients. Because the immune CoP for HCMV vaccine remains elusive, besides safety, the outcome measures of clinical trials have varied. The common immune end points are antibody binding to HCMV antigens, HCMV-specific NAb, and T cell responses.

Below we outline the HCMV vaccine strategies that have been completed or are currently ongoing from ClinicalTrials.gov (**https://clinicaltrials.gov/**). Specifically, we review the details

of HCMV vaccine clinical trials related to congenital CMV infection, including vaccine design (vaccine modalities and encoded antigens), study participants, and outcome measures (summarized in **Table 1**).

4.1. Live-Attenuated and Disabled-Infectious Single Cycle Vaccine

Live-attenuated vaccine and disabled-infectious single cycle vaccine contain replication-impaired or replication-deficient HCMV virions. The HCMV strains AD169 (110), Towne (111–113), and Toledo (114–117) have been proposed in live-attenuated vaccine design. The live-attenuated Towne vaccine was given to HCMV-seronegative women of childbearing age (average maternal age: 33 years). However, the infection rates between the Towne vaccinees and the placebo group were similar, and the HCMV-specific binding antibodies and NAb response was tenfold lower than with HCMV-seropositive women. These results suggest the Towne vaccine failed to provide protection against HCMV infection (112). The Towne vaccine was later investigated in HCMV-seronegative adults in phase 1 clinical trials (NCT00370006, NCT00373412) with no updated results posted. Because the Towne strain might be too attenuated to provide protection, four Towne-Toledo strain chimera vaccines have been proposed and tested in HCMV-seropositive individuals (117) and HCMV-seronegative male adults (NCT01195571) (116). Both phase 1 studies showed that the Towne-Toledo strain chimera vaccine was well tolerated, and chimera 4 appeared to be the most immunogenic.

Although no safety issues have emerged from these early phase trials, the replication and latency of live-attenuated viruses remain concerns. Therefore, replication-defective HCMV vaccines, such as the V160 vaccine based on the AD169 strain, were developed (118, 119). The safety profile, route of administration, and immunogenicity of V160 were tested in phase 1 clinical trials (NCT03840174, NCT01986010), and it was shown to be well tolerated in healthy adults and capable of eliciting humoral and cellular response comparable to natural immunity (120–122). A detailed monoclonal antibody (mAb) characterization showed that V160 elicited a broad range of potent antibody responses, including PC- and gHgL-specific NAbs, as well as gB-, pp65-, and pp28-specific mAbs with minimal or no neutralizing activity (122). Additionally, V160 was shown to induce polyfunctional pp65- and IE1-specific $CD4^+$ and $CD8^+$ T cell responses with clonotype diversity (121). These promising results led to this vaccine entering a phase 2 clinical trial (NCT03486834) to investigate the vaccine efficacy by two-dose or three-dose regimen in healthy HCMV-seronegative female adolescents and women of childbearing age. Although V160 was well tolerated and immunogenic, vaccine efficacy reached only 42.4% in the three-dose group and 32.0% in the two-dose group (123), which may be seen as too low to move into phase 3 trials. Yet, continuing to hold promising vaccines to such a high bar for efficacy against HCMV acquisition may cause the field to halt clinical development on vaccines that could provide reduction of congenital transmission and disease in immunocompromised patients.

Additionally, live-attenuated RhCMV strain 68-1 (RhCMV68-1) as a vaccine vector expressing simian immunodeficiency virus (SIV) antigens [human immunodeficiency virus (HIV) counterpart in the rhesus macaque model] significantly broadened the immune response compared to the conventional vaccine vectors and protected 55% of vaccinated rhesus macaques via nonclassical HLA-E-restricted $CD8^+$ T cells upon SIV challenge (124). Furthermore, the same RhCMV68-1 vaccine vector expressing *Mycobacterium tuberculosis* proteins elicited protective immunity to efficiently control tuberculosis-associated disease in the rhesus macaque challenge model. Yet, the protection was not mediated by nonclassical $CD8^+$ T cells (125). Similarly, rhesus macaques were protected against malaria challenge by a highly durable T cell response upon immunization with four *Plasmodium knowlesi* antigens delivered in the RhCMV68-1 vector (126). These studies have revealed the high potential of CMV as a vaccine vector that may broadly protect across diseases (127).

Table 1 Human clinicals for HCMV vaccine candidates

Vaccine name(s)	Vaccine	Sponsor	NCT number	Phase	Study cohort	Route(s)	Reference(s)
Live-attenuated vaccine and disabled-infectious single cycle vaccine							
Towne vaccine	Attenuated Towne strain	University of California, San Francisco	NCT00370006 NCT00373412	1	Healthy CMV (−) adults aged 18–45 years who received VCL-CT02 vaccine	IM, ID	NA
Towne-Toledo chimera vaccine	Recombined genome of Towne and nonattenuated Toledo strains	CMV Research Foundation	NCT01195571	1	Healthy CMV (−) male adults aged 30–50 years with CMV (+) partner	SC	116
V160 (+APA)	Replication-defective AD169 HCMV strain	Merck Sharp & Dohme Corp.	NCT03840174	1	Healthy Japanese males aged 20–64 years	IM	120–123
V160 (±APA)			NCT01986010	1	Healthy adults age ≥18 years	IM, ID	
V160 (+APA)			NCT03486834	2	Healthy CMV (−) female adolescents and adults aged 16–35 years	IM	
Adjuvanted recombinant protein vaccine							
gB/MF59 vaccine	MF59-adjuvanted recombinant soluble Towne strain gB	Robert Pass, MD	NCT00125502	2	Healthy CMV (−) postpartum females aged 14–40 years	IM	128–131, 146
		NIAID	NCT00133497	2	Healthy CMV (−) female adolescents aged 12–17 years	IM	
			NCT00815165	NA	NCT00133497 participants	NA	
		University College, London	NCT00299260	2	Patients aged ≥18 years awaiting a kidney or liver transplant	NA	
GSK1492903A	Adjuvanted AD169 strain gB subunit vaccine	GlaxoSmithKline	NCT00435396	1	Healthy CMV (−) adults aged 18–40 years	IM	NA
			NCT01357915	NA	NCT00435396 participants	NA	NA

(*Continued*)

Table 1 (*Continued*)

Vaccine name(s)	Vaccine	Sponsor	NCT number	Phase	Study cohort	Route(s)	Reference(s)
1. Pentamer(low)/gB(low)/adjuvant vaccine 2. Pentamer(med)/gB(low)/adjuvant vaccine 3. Pentamer(med)/gB(med)/adjuvant vaccine 4. Pentamer(high)/gB(med)/Adjuvant vaccine	Adjuvanted protein subunit vaccine encoding low/medium/high HCMV PC and low/medium gB	GlaxoSmithKline	NCT05089630	1/2	Healthy adults aged 18–50 years	IM	NA
DNA vaccine							
CyMVectin (withdrawn)		Virginia Commonwealth University	NCT02594566	1	Healthy female adults aged 18–35 years	IM	NA
VCL-CT02 (VCL-CB01) pDNA vaccine (VCL-6365, VCL-6368)	Poloxamer-formulated, bivalent DNA vaccine expressing HCMV pp65 and gB	Astellas Pharma Inc.	NCT00285259	2	HSCT donor-recipient pairs and unpaired recipients; recipients are CMV (+), aged 18–65 years	IM	104–106, 132, 133, 175–177
ASP0113 (VCL-6365, VCL-6368)	Poloxamer-formulated, bivalent DNA vaccine expressing HCMV pp65 and gB	Astellas Pharma Global Development, Inc.	NCT02103426	1	Healthy adults aged 18–70 years; CMV (−) dialysis patients	IM	
			NCT01903928	2	CMV (+) HSCT patients aged 20 years or older	IM	NA
			NCT01974206	2	CMV (−) recipients aged 18 years or older who received CMV (+) kidney	IM	NA
			NCT01877655	3	CMV-seropositive HSCT recipients	IM	NA

(*Continued*)

Table 1 (*Continued*)

Vaccine name(s)	Vaccine	Sponsor	NCT number	Phase	Study cohort	Route(s)	Reference(s)
mRNA vaccine							
1. mRNA-1647 2. mRNA-1443	1. mRNA-1647: HCMV gB+ PC mRNA 2. mRNA-1443: HCMV pp65 mRNA	ModernaTX, Inc.	NCT03382405	1	Healthy adults aged 18–49 years	IM	NA
mRNA-1647	HCMV gB+ PC mRNA	ModernaTX, Inc.	NCT05105048	1	Healthy Japanese adults aged 18–40 years	IM	NA
			NCT04232280	2	Part 1: healthy adults aged 18–40 years Part 2: healthy female adults aged 18–40 years	IM	NA
			NCT04975893	2	NCT04232280 participants	NA	NA
			NCT05085366	3	Healthy female adolescents and adults aged 16–40 years	IM	NA
VLP vaccine							
VBI-1501 (±APA)	VLP expression of gB(HCMV)-G protein (VSV)	VBI Vaccines Inc.	NCT02826798	1	Healthy CMV (−) adults aged 18–40 years	IM	86
Viral vectored vaccine							
ALVAC-CMV (vCP260)	Canarypox vector–expressing HCMV pp65	National Heart, Lung, and Blood Institute	NCT00353977	2	Healthy adults and HSCT donor-recipient pairs	IM	136, 138
AVX601	Bivalent alphavirus replicon vaccine expressing CMV gB and pp65/IE1 fusion protein	AlphaVax, Inc.	NCT00439803	1	Healthy CMV (−) adults aged 18–45 years	IM, SC	178

(*Continued*)

Table 1 (*Continued*)

Vaccine name(s)	Vaccine	Sponsor	NCT number	Phase	Study cohort	Route(s)	Reference(s)
CMV-MVA triplex vaccine	Attenuated poxvirus modified vaccinia Ankara encoding CMV pp65, IE1-exon 4, and IE2-exon5	Masonic Cancer Center, University of Minnesota	NCT03383055	1	Lymphoma or multiple myeloma patients aged 18 years and older	NA	137
		City of Hope Medical Center	NCT01941056	1	Healthy adults aged 18–60 years	IM	NA
			NCT03354728	1/2	Pediatric HSCT patients, CMV (+)	IM	NA
			NCT03560752	2	HSCT donors aged 18–80 years; CMV (+) recipients	NA	NA
CMV-MVA triplex vaccine	Attenuated poxvirus modified vaccinia Ankara encoding CMV pp65, IE1-exon 4, and IE2-exon5	City of Hope Medical Center	NCT02506933	2	HSCT donors aged 18–75 years; CMV (+) recipients	IM	NA
			NCT04060277	2	CMV (+) recipients aged 18 years and older who stop letermovir prophylaxis treatment	IM	NA
		NIAID	NCT05099965	2	HIV-infected, CMV (+) adults aged 18–65 years	IM	NA
HB-101	Bivalent vaccine containing 2 replication-deficient lymphocytic choriomeningitis viruses expressing CMV pp65 and gB	Hookipa Biotech GmbH	NCT02798692	1	Healthy CMV (−) adults aged 18–45 years	IM	NA
			NCT03629080	2	CMV (−) adults awaiting a kidney transplant from CMV (+) donors	NA	NA

(*Continued*)

Table 1 (*Continued*)

Vaccine name(s)	Vaccine	Sponsor	NCT number	Phase	Study cohort	Route(s)	Reference(s)
Peptide vaccine							
CMVpp65-A*0201 peptide vaccine (CMVPepVax)	HLA-A*0201-specific pp65 CD8 T cell epitope +P2 epitope of tetanus toxin or pan-DR epitope +PF03512676 adjuvant	City of Hope Medical Center	NCT00712634	1	Healthy adults aged 18–65 years	SC	179–181
1. PADRE-CMV fusion peptide vaccine (±PF03512676) 2. Tetanus-CMV fusion peptide vaccine (±PF03512676)	1. PADRE-CMV: HLA-A*0201 specific pp65 CD8 T-cell epitope +pan-DR epitope ±PF03512676 adjuvant 2. Tetanus-CMV: HLA-A*0201-specific pp65 CD8 T cell epitope +P2 epitope of tetanus toxin ±PF03512676 adjuvant		NCT00722839	1	Healthy adults aged 18–55 years, HLA-A*0201 subtype	SC	
Tet-CMV + PF03512675	HLA-A*0201-specific pp65 CD8 T cell epitope +P2 epitope of tetanus toxin +PF03512676 adjuvant		NCT01588015	1b	HSCT patients aged 18–75 years, CMV (+), HLA-A*0201 subtype	SC	
CMVpp65-A*0201 peptide vaccine (CMVPepVax)	HLA-A*0201-specific pp65 CD8 T cell epitope +P2 epitope of tetanus toxin or pan-DR epitope +PF03512676 adjuvant		NCT02396134	2	HSCT patients aged 18–75 years, CMV (+), HLA-A*0201 subtype	SC	

Abbreviations: APA, aluminum phosphate adjuvant; CMV, cytomegalovirus; gB, glycoprotein B; HCMV, human cytomegalovirus; HSCT, hematopoietic stem cell transplantation; HIV, human immunodeficiency virus; ID, intradermal; IM, intramuscular; mRNA, messenger RNA; NA, not available; NCT, National Clinical Trial; NIAID, National Institute of Allergy and Infectious Diseases; PC, pentamer complex; SC, subcutaneous; VLP, virus-like particle; VSV, vesicular stomatitis virus.

4.2. Adjuvanted Recombinant Protein Vaccine

HCMV gB has been identified as one of the major vaccine targets due to its essential role in virion fusion with the target cells and ability to elicit both NAb (55) and non-NAb responses (56, 91). MF59-adjuvanted gB (gB/MF59) vaccine is composed of the squalene adjuvant MF59 and monomer gB expressed in recombinant Chinese hamster ovary cells. The immunization schedule (0, 1, 6 months) and dosage (20-μg gB) were determined in adults after comparing the data from HCMV gB ELISA and plaque-reduction assays (128). Another phase 1 study of the gB/MF59 vaccine was conducted in toddlers aged 12 to 35 months. Interestingly, not only was the vaccine well tolerated in toddlers, but also the gB-specific antibody response was shown to be significantly higher than that of adults (129), suggesting that HCMV vaccination in childhood could be a feasible solution to reduce HCMV infection. Later, the gB/MF59 vaccine was tested in phase 2 clinical trials in healthy HCMV-seronegative postpartum (NCT00125502) and adolescent females (NCT00133497, NCT00815165) as a strategy to prevent congenital infection. The gB/MF59 vaccine demonstrated 50% and 45% efficacy against HCMV acquisition in postpartum women and adolescents, respectively (130, 131), yet it did not proceed to the next stage due to this moderate vaccine efficacy.

Besides the gB/MF59 vaccine, another adjuvanted gB subunit vaccine, GSK1492903A, has been developed by GlaxoSmithKline, and adjuvanted recombinant PC and gB were tested in healthy adults (NCT05089630).

4.3. DNA Vaccine

DNA vaccine is composed of plasmids encoding vaccine antigens. Two DNA vaccines have been studied in clinical trials, CyMVectin (withdrawn) and VCL-CT02. The VCL-CT02 vaccine is a poloxamer-formulated bivalent DNA vaccine composed of two plasmids, encoding the AD169 strain gB ectodomain, and mutated AD169 strain pp65 (132). The phase 1 clinical trials for the VCL-CT02 vaccine showed it was well tolerated and elicited more effective antigen-specific T cell responses than the gB-specific antibody responses (133). The VCL-CT02 vaccine was later renamed as ASP0113 and was assessed in healthy adults and HCMV-seronegative dialysis patients in a phase 1 trial (NCT02103426). Consistent with previous results, the ASP0113 vaccine elicited memory T cell response but minimal humoral response in HCMV-seronegative vaccinees. These results have concluded that the HCMV DNA vaccine appears to be most effective in eliciting antigen-specific cellular immunity. The ASP0113 vaccine is now being investigated in hematopoietic stem cell transplantation (HSCT) donors and recipients in phase 2 and 3 clinical trials (NCT00285259, NCT01903928, NCT01974206, NCT01877655).

4.4. Messenger RNA Vaccine

mRNA vaccine is vaccine antigens in modified mRNA form encapsulated by lipid nanoparticles (LNPs) or liposomes. ModernaTX, Inc. tested two mRNA vaccines, with the mRNA-1647 vaccine composed of one mRNA from gB and five mRNAs from PC and the mRNA-1443 candidate composed of pp65. Both vaccines were evaluated in healthy adults in phase 1 clinical trials (NCT03382405, NCT05105048). The mRNA-1647 vaccine showed a persistent immune response 6 months after the third dose and high NAb titer specifically on epithelial cells. The NAb titers of HCMV-seronegative vaccinees showed a 2.8- to 17.0-fold increase in epithelial cells and 0.8- to 5.0-fold increase in fibroblasts compared to HCMV-seropositive individuals. Additionally, the NAb titer of HCMV-seropositive vaccinees demonstrated a 4.0- to 7.1-fold increase in fibroblasts and a 13.4- to 40.8-fold increase on epithelial cells and robust epithelial cell neutralization responses achieved by inclusion of PC into vaccine design.

Several changes were made before the mRNA-1647 vaccine entered phase 2 clinical trials, including altering the ratio of mRNA components, optimizing the manufacturing process, and transitioning from liquid to the lyophilized power (commercial formulation). The safety and immunogenicity of the modified mRNA-1647 vaccine were then dose optimized in phase 2 clinical trials (NCT04232280, NCT04975893), and now healthy female adults aged 18 to 40 are being recruited into a phase 3 clinical trial employing a 100-μg dose. The vaccine efficacy of the mRNA-1647 vaccine (NCT05085366) will be primarily determined by monitoring seroconversion of HCMV antigens that are not gB or PC as a marker of HCMV infection.

4.5. Virus-Like Particle Vaccine

Virus-like particle (VLP) vaccine, a nanostructure vaccine composed of viral structural capsid proteins, lacks the viral genetic materials and is thus noninfectious. The eVLP structure consists of an outer mosaic lipid membrane embedded by viral glycoproteins and an internal matrix protein layer (134). The VBI-1501 vaccine is an eVLP vaccine produced by cotransfecting the plasmid DNA encoding structural protein and the protein of interest: the group antigen (Gag) gene of murine leukemia virus and the plasmid DNA expressing fusion gB-G protein (VSV), respectively, in human embryonic kidney 293T cells. Unlike the conventional gB, gB-G protein is a recombinant protein composed of the gB ectodomain from the HCMV Towne strain fused with the transmembrane and cytosolic domain from VSV G protein. Although no direct evidence was demonstrated, the fusion of the VSV G protein domains has been purported to retain the prefusion structure of gB, the conformation necessary to elicit the gB-specific humoral response associated with prevention of viral entry and cell fusion. The VBI-1501 vaccine was tested in a mouse model and elicited more potent NAb responses in fibroblast and epithelial cells but not better proliferative $CD4^+$ T cell responses than the recombinant gB protein vaccine (86). In 2018, the VBI-1501 vaccine with and without aluminum phosphate adjuvant (APA) was tested in HCMV-seronegative adults in a phase 1 clinical trial (NCT02826798). Compared to the placebo group, the vaccine was well tolerated with different vaccine doses, and the 2-μg dose with APA group showed the highest gB-specific antibody and NAb responses. VBI Vaccines Inc. announced in 2019 that they plan to test higher doses of the VBI-1501 vaccine in a phase 2 clinical trial.

4.6. Viral Vectored Vaccine

Viral vectored vaccines are recombinant attenuated viral vectors encoding the vaccine antigens. Because these vectors can elicit robust cytotoxic T lymphocyte (CTL) responses, the viral vectored vaccines have been tested in lymphoma or transplant patients who suffer from HCMV infection (135). The outcome measures of these clinical trials usually target the T cell responses, and HCMV-specific T cell antigens are commonly included in the viral vector vaccine. For example, pp65 is commonly included in viral vector HCMV vaccine because pp65 was discovered as an important target of CTLs. Several HCMV viral vectored vaccines have been tested in early phase clinical studies, including the ALVAC-CMV (vCP260), AVX601, HCMV-MVA triplex, and HB-101 vaccines.

The ALVAC-CMV vaccine and HCMV-MVA triplex are made of a highly attenuated replication-deficient vaccinia virus strain from the poxvirus family. Due to the large genome size and capacity to encode the foreign gene insertion, vaccinia virus is commonly applied as a viral vector in vaccine design. The long history of vaccinia-vectored vaccine development also fills the large-scale manufacturing skill gaps, accelerating the vaccine manufacturing process (135). The ALVAC-CMV vaccine is a canarypox vector vaccine expressing HCMV pp65, while the HCMV-MVA triplex vaccine is the modified vaccinia Ankara (MVA) strain encoding HCMV

pp65, IE1-exon 4, and IE2-exon5 (136, 137). The safety profile of both vaccines was tested in healthy adults in phase 1 clinical trials (136, 138; and NCT03383055, respectively). In clinical trials, both vaccines showed that the HCMV-specific T cell response was successfully expanded.

The AVX601 and HB-101 vaccines are both bivalent vaccines encoding slightly different antigens. The AVX601 vaccine is a bivalent alphavirus replicon vaccine expressing HCMV gB and pp65/IE1 fusion protein. The AVX601 vaccine was shown to be well tolerated in healthy adults in a phase 1 clinical trial (NCT00439803) and elicited polyfunctional $CD4^+$ and $CD8^+$ T cell responses. Although the AVX601 vaccine was claimed to elicit NAb responses, this response was measured against virus-like replicon particles instead of directly targeting HCMV strains (137). The HB-101 vaccine is composed of two replication-deficient lymphocytic choriomeningitis viruses expressing HCMV pp65 and gB, respectively. Before entering clinical trials, the HB-101 vaccine was shown to provide protection in the guinea pig model of congenital CMV infection (139). Additionally, the HB-101 vaccine elicited a robust T cell response in a mouse model and a potent NAb response in both mouse and rabbit models (140). The safety profile and immunogenicity of the HB-101 vaccine were examined in HCMV-seronegative healthy adults in a phase 1 clinical trial (NCT02798692). This vaccine specifically elicited a polyfunctional pp65-specific $CD8^+$ T cell response and a dose-dependent gB-binding and NAb response (141). The promising results in both cellular and humoral response led to the HB-101 vaccine entering a phase 2 clinical trial (NCT03629080) to investigate the vaccine efficacy in kidney transplant patients.

4.7. Peptide Vaccine

One of the advantages of the peptide vaccine including viral peptide antigens is to stimulate epitope-specific T cell response. The only HCMV peptide vaccine tested in clinical trials is the HCMVpp65-A*0201 peptide vaccine (CMVPepVax), which includes the HLA-A*0201-specific pp65 CD8 T cell epitope, the P2 epitope of tetanus toxin or pan HLA DR-binding epitope, and the PF03512676 adjuvant. The PF03512676 adjuvant is a CpG DNA adjuvant also known as CpG 7909 and CpG 2006, a synthetic single-stranded phosphorothioate DNA containing CpG motifs. The safety and the immunogenicity profile of the HCMVpp65-A*0201 peptide vaccine delivered subcutaneously in healthy adults has been assessed in phase 1 trials (NCT00712634, NCT00722839, NCT01588015). The function and kinetics of the HCMV-specific T cell immunity of this vaccine were later investigated in a phase 2 clinical trial (NCT02396134).

5. IMMUNE CORRELATES OF PROTECTION AGAINST HCMV

One leading strategy to improve on HCMV vaccines is the identification of immune CoPs, which are immune markers associated with a reduction in the incidence of infection or clinical disease (142, 143). Immune CoPs may be used to identify potential targets for vaccination or to determine appropriate vaccine platforms for antigen presentation, and as immunological end points for measuring vaccine efficacy in preclinical trials. The identification of CoPs is particularly important for vaccine design against HCMV because natural HCMV infection confers only partial protection from superinfection and vertical transmission (144). Accordingly, efficacious HCMV vaccines will need to elicit immune responses even more protective than or different from those elicited by natural infection, and the development of protective HCMV vaccines will likely require rational vaccine design, which may be guided by immune CoPs. Previous studies have identified CoPs against congenital HCMV transmission and viremia in natural infection (reviewed in 14, 94). Indeed, these immune CoPs have been used to direct the design of HCMV vaccines. Follow-up studies to historical HCMV clinical trials have also aimed at identifying vaccine-elicited CoPs. The most efficacious HCMV vaccine to date (gB/MF59) achieved approximately 50% protection in clinical

trials performed in 2011, but clinical trials of newer HCMV vaccine candidates have not yet improved on this vaccine efficacy (145). A better understanding of the multifaceted vaccine-elicited immune responses and CoPs may help guide vaccine design, including the selection of more antigenic vaccine targets, biologically relevant antigen structures, and appropriate vaccine platforms.

Multiple studies aimed at identifying immune CoPs for the gB/MF59 vaccine. In renal transplant recipients vaccinated with the gB/MF59, the magnitude and duration of HCMV viremia were found to be inversely correlated with gB-specific antibody titer, suggesting HCMV gB-specific IgG responses as a vaccine-elicited CoP (146). Follow-up studies revealed that the presence of antibodies specifically against the gB epitope antigenic domain-2 (gB AD-2) was correlated with protection from viremia (147). Yet, surprisingly, serum neutralizing function, which was previously identified as an immune CoP against congenital HCMV transmission in multiple mother-infant cohorts (148, 149), was not associated with protection from viremia (150). Indeed, gB/MF59 vaccination of seronegative transplant recipients (150) and of seronegative adolescent subjects (91) elicited only minimal NAb and ADCC responses. These results suggested that gB/MF59 conferred protection from primary infection via a non-neutralizing, non-ADCC mechanism, such as ADCP. A follow-up correlate study of both the seronegative postpartum and adolescent vaccinee populations found that the presence of serum antibodies against gB as expressed on a cell surface, which represents a mix of gB in the prefusion and postfusion conformations (151), was associated with protection from primary infection (16). These findings suggested that there may be structural differences in cell-associated and soluble gB, as used in the vaccine, relevant to the generation of protective immunity. Supporting this, the authors identified gB-specific mAbs that differentially recognized soluble or cell-associated gB (16). Collectively, these studies revealed that there may be unique gB/MF59 vaccine-induced CoPs relevant to different populations that may include gB-specific antibody responses and the presence of antibodies recognizing cell-associated gB. Thus, the gB-based vaccine efficacy could be improved by increasing the duration of antigen presentation and presenting gB antigens in cell-associated or prefusion forms.

There are several caveats to applying vaccine-elicited CoPs to the design and testing of future HCMV vaccines. Identified CoPs may not indicate the mechanism of protection, in that they may be correlative but not causative and should be interpreted accordingly. CoPs may also be specific to a particular vaccine or platform, limiting their generalizability. Moreover, the end points for HCMV trials remain unclear, and although vaccine-elicited CoPs against viremia or primary infection have been identified, these CoPs may not be associated with protection from congenital HCMV transmission. Further studies are required to identify relevant surrogate markers of infection and disease for HCMV clinical trials and immune CoP studies.

6. NEXT-GENERATION HCMV VACCINE STRATEGIES

The overall immune CoP of HCMV infection is not entirely understood. Yet, harnessing the newly identified protective host immunity, employing new vaccine technologies, and optimizing animal models may help rationally inform next-generation HCMV vaccine design. Therefore, we propose the following next-generation HCMV vaccine development strategies.

6.1. Systematic Dissection of Protective Immunity After Natural Infection

Comprehensive dissection of natural HCMV-specific immune responses that are protective against reinfection and congenital infection could reveal the protective immunity at the single-antigen levels, which are relevant to rationally screen immunogens and select vaccine modalities in future vaccines. Meanwhile, a complete understanding of the molecular mechanism concerning the interplay between the virus and host, including the pathogenesis mechanism during vertical

transmission, will also inform HCMV vaccine development, facilitated by other disciplines such as molecular cell biology and bioinformatics, systems biology, and structural biology.

6.2. Optimization of Antigen Design

Antigen design is central to vaccine development. Therefore, we proposed the strategies detailed below to improve next-generation vaccine antigen design.

6.2.1. B cell lineage- and structure-based glycoprotein B antigen design to induce the strong and broadly neutralizing antibodies. The gB/MF59 vaccine achieved partial success in multiple clinical trials, demonstrating that the gB-specific response is essential for vaccine-induced protection. Hence, further efforts are required to improve the gB immunogen design to broaden the coverage of immune responses. However, passive administration of NAbs in human trials has indicated the protective role of NAbs (152–155). These observations provide the rationale for B cell lineage- and structure-based antigen design aimed at inducing potent and broad NAb responses against gB. Yet, antibody responses directed against a crucial gB neutralizing epitope, AD2S1, identified as the target of the most potent gB-specific NAb in naturally infected individuals, were not elicited by this vaccine strategy (16). This lack of response against the AD2S1 antigenic region combined with a narrow variable gene usage of identified gB AD2S1-specific mAbs indicates a genetic restriction in B cell lineage induction. Thus, there is a potential for precursor B cells to be engaged through the antibody-antigen structure-based design of gB immunogens. One preliminary study defined the B cell lineage development path of two well-characterized gB AD2S1-specific potently neutralizing mAbs, TRL345 and mAb3-25, and the V_H A33N mutation was identified as both necessary and sufficient to confer neutralizing function to the non-neutralizing unmutated common ancestor (UCA) (data were not published) (155a). Structure-guided engineering can select gB antigen mutants with enhanced binding to the UCA of potent gB AD2S1-specific mAbs such as TRL345 and test for their ability to elicit NAbs in small animal models using a sequential immunization strategy (156, 157).

6.2.2. Addition of pentamer complex and glycoprotein H/L/O to broaden the neutralizing antibody responses. The HCMV PC is currently being included in the vaccine regimen to broaden the vaccine-induced humoral immunity, particularly NAbs, to block the infection of epithelial and endothelial cells because HCMV PC was shown to be a major NAb target (42). Additionally, HCMV gH/gL/gO trimer was demonstrated to promote gB fusion on all cell types, suggesting gH/gL/gO trimer also plays an essential role in viral entry (47). Hence, gH/gL/gO trimer could be included in the vaccine regimen, potentially preventing HCMV from escaping current clinically tested vaccine-elicited NAbs.

6.2.3. T cell–based antigen selection. The pp65-specific $CD4^+$ T cell response has been shown to potentially contribute to prevention of congenital HCMV infection and thus should be incorporated into the vaccine regimens to evaluate potency and efficacy (82). In addition, IE1-specific T cells appeared important to viral load reduction in immunocompromised HCMV patients (78). Protective responses mediated by IE1-specific immunity could potentially apply to other populations in the vaccination settings. Hence, the inclusion of IE1 in vaccine design may improve HCMV vaccine efficacy.

6.3. New Vaccine Approaches to Elicit Both Humoral and Cellular Responses

A vaccine triggering humoral and cellular responses would likely confer maximum protection against HCMV infection and disease. We propose the following approaches to realize this goal.

6.3.1. Combination of different vaccine modalities. It is well known that the optimized DNA vaccine and the viral vector platform excel in eliciting strong and broad T cell responses (158–160), which could be used as a vehicle to deliver T cell–specific antigens such as pp65 and IE1. In contrast, the adjuvanted subunit vaccine and LNP-encapsulated nucleoside-modified mRNA vaccine are good at stimulating potent humoral responses (161–163). These two platforms are more suitable for encoding GC antigens such as gB and PC to elicit potent functional antibody responses. Combining different vaccine modalities in a prime-boost and/or coadministration manner could maximize both functional cellular and humoral responses (64, 164).

6.3.2. Selection of the appropriate immunization route. Intramuscular (IM) injection is good at inducing cellular and humoral responses, while intradermal (ID) delivery excels in generating potent antibody responses (165). Additionally, the in vivo electroporation following IM/ID delivery of DNA vaccine significantly increased the vaccine-induced immune responses (166, 167). Yet, mucosal delivery of a vaccine may be needed to prevent virus acquisition from the mucosal routes and/or block systemic spread after local virus replication (168).

6.4. Novel Adjuvant to Modulate the Vaccine-Induced Immune Responses

Innate responses triggered by vaccination can be modulated through the novel adjuvants, such as the molecular adjuvant IL-12, to enhance the memory NK responses (39, 169). However, novel adjuvant formulation with vaccine antigens still needs further exploration to harness the desired vaccine-induced responses and enhance the vaccine efficacy and durability (170), key features of a successful vaccine.

6.5. De-Risking Clinical Vaccine Development Through Highly Relevant Animal Models and Standard Assay Development

Highly relevant animal model development and standard assay establishment will facilitate vaccine development. We detail our recommendations below.

6.5.1. Preclinical animal models development. Appropriate preclinical animal models to evaluate the immunogenicity and efficacy of vaccines will improve the likelihood of developing a successful HCMV vaccine in clinical trials. The common congenital HCMV transmission animal models, including the guinea pig or NHP model, allow the examination of preclinical vaccine potency and efficacy for the primary clinical end point and enhance our understanding of viral transmission and pathogenesis. New animal/challenge models may be needed to shorten the vaccine candidates' research and development process. For example, chimeric rhesus-human CMV bearing HCMV gB might help evaluate the vaccine efficacy for all HCMV gB-encoded vaccine antigens/regimens, similar to the simian-human immunodeficiency virus/rhesus macaque model, which is widely applied in the HIV vaccine field (171, 172).

Additionally, the humanized mouse model reconstructing the human immune system could be a relevant animal model instrumental in evaluating the immunogenicity of vaccine candidates and vaccine efficacy upon HCMV challenge. Yet, efforts are still needed to optimize the current humanized mouse model to recapitulate the different stages of HCMV infection and the complex immunological host-pathogen interactions (reviewed in 173), potentially accelerating the clinical translation of HCMV vaccine candidates from the preclinical stage.

6.5.2. Standard assay establishment. Establishing standard assays compatible with good clinical and laboratory practice environment (174), particularly for identified immune CoPs, will facilitate preclinical and early clinical vaccine assessment and cross-compare potency for the

different vaccine platforms/regimens to rationally guide the advanced vaccine human clinical trial development.

Comprehensively employing these next-generation vaccine strategies will de-risk the vaccine candidate development and maximize the success of an effective HCMV vaccine.

7. CLOSING REMARKS

Rapid immunogenicity testing and efficacy evaluation for HCMV vaccine candidates against congenital transmission in the highly relevant animal models in the preclinical stage for new HCMV vaccine concepts/regimens will aid in moving the most promising HCMV vaccine candidates into clinical trials and improving their ultimate chance of success. Depending on the outcome of clinical trials at each stage, vaccine candidates may need to go back to the preclinical stage to optimize the vaccine strategy/immunogen design by enhanced understanding of the natural protective immunity and CoPs. New vaccine adjuvants and technologies could also accelerate the success of the next-generation HCMV vaccine, eventually leading to improved infant and immunocompromised patient health by ending the consequences of HCMV disease through immunization.

DISCLOSURE STATEMENT

S.R.P. is a consultant to Moderna, Merck, Pfizer, GSK, Dynavax, and Hoopika CMV vaccine programs and leads sponsored programs with Moderna and Merck. S.R.P. also serves on the board of the National CMV Foundation and as an educator on CMV for Medscape. The other authors have no financial conflicts of interest.

ACKNOWLEDGMENTS

This work was funded by the P01 AI129859 to S.R.P. The contents of this review article do not necessarily reflect the views or policies of the Department of Health and Human Services/funding agencies, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

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