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REVIEW ARTICLE

CYTOMEGALOVIRUS VACCINES IN THE PIPELINE

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SUMMARY

Human cytomegalovirus (HCMV) is the most common cause of congenital viral infection in the developed world and is a major cause of birth defects in newborns. Preconception immunity to HCMV lessens the risk of congenital infection and its attendant neurodevelopmental sequelae. This observation has driven interest in the development of vaccines, with a particular emphasis on women of childbearing age, toward the goal of lessening the burden imposed by congenital HCMV infection. Such vaccines may also have a role in the prevention of HCMV-associated disease in immunocompromised individuals, particularly solid organ and hematopoietic stem cell transplant patients. Although there is currently no licensed HCMV vaccine available, significant progress has been made in recent years. This review summarizes some of the newer HCMV vaccine approaches "in the pipeline", including vaccine strategies currently in various stages of preclinical development, as well as those vaccines that are currently in clinical trials.

INTRODUCTION: THE RATIONALE FOR A VACCINE AGAINST HUMAN CYTOMEGALOVIRUS

Human cytomegalovirus (HCMV) is a ubiquitous betaherpesvirus that primarily causes disease in immunocompromised transplant patients, individuals with advanced HIV disease and newborns. Of the clinical manifestations of HCMV disease, the most significant is the problem of congenital HCMV infection. HCMV is transmitted to the fetus in up to 2% of all pregnancies, and has the potential to cause serious sequelae in the infected infant, including sensorineural hearing loss, mental retardation and developmental disabilities (1-4). Among congenitally infected infants, approximately 10% have signs and symptoms of disease at birth, and such symptomatic infants have the greatest long-term risk of permanent disability. However, all congenitally infected infants are at risk for sequelae. The public health impact of congenital HCMV infection is substantial and under-recognized. Congenital HCMV infection extracts a major economic burden on society, since severe, symptomatic infants often require a lifetime of care and have extensive disabilities. A major unmet challenge in dealing with the problem of congenital HCMV infection is the lack of awareness about the condition among women of childbearing age. More children suffer from long-term neurodevelopmental handicaps as a result of congenital HCMV infection than either Down syndrome or fetal alcohol syndrome (5). In spite of the common and disabling nature of congenital HCMV infection, most women are unaware of this risk. In a survey exploring awareness among women of the common causes of birth defects, women's awareness of CMV ranked last (6). This lack of awareness complicates the development of prevention strategies.

An attractive prevention strategy for congenital HCMV infection would be the development of an effective vaccine. However, in this context, it is noteworthy that transmission of HCMV to the fetus can occur in the context of a primary maternal infection, or secondary to reinfection with a novel strain of the virus (7-10). The fact that reinfection can lead to congenital HCMV transmission even in the setting of preconception maternal immunity complicates vaccine development. Despite this concern, several lines of evidence indicate that preconception immunity to HCMV provides some degree of protection against transmission of the virus to the fetus, and some degree of protection against the development of neurodevelopmental sequelae if infection occurs. The risk of transmission of HCMV to the fetus in the setting of primary maternal infection was reported to be approximately 30% in a meta-analysis of published studies (11). By comparison, the overall risk of HCMV transmission to the fetus after a recurrent maternal infection during pregnancy is in the range of 0.15-2% (12). The risk of congenital HCMV transmission appears to vary among racial, ethnic and geographic lines, and the biological basis for this is unclear. A recent multicenter newborn screening study found an overall incidence of congenital HCMV infection of 0.45% across several geographically and racially diverse populations in the U.S. (13).

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The observations regarding the protective effect of preconception maternal immunity on the incidence of transmission provide support for the development of a preconception vaccine that would target women of childbearing age. The impact of preconception immunity on both the incidence of infection and the magnitude of injury in infected babies has been demonstrated in several studies. In one study, a 69% reduction in the risk of congenital HCMV infection was identified among women seropositive for HCMV when compared to seronegative women (14); thus, this 69% efficacy of natural preconception immunity serves as a useful yardstick of comparison for potential future clinical trials of vaccines targeted at preventing transmission. Even if congenital infection occurs in the context of maternal immunity, some studies suggest a decreased severity of disease in these infants compared to infants born to women with primary HCMV infections documented in pregnancy. In a study of the outcome of congenital HCMV infection in relation to maternal antibody status, approximately 25% of congenitally infected infants whose mothers had primary HCMV infection during pregnancy had at least one neurological or neurodevelopmental sequelae, compared with 8% of infants born to women with preexisting immunity, who were presumably reinfected with a new strain of virus during their affected pregnancy (12).

The potential theoretical benefit of an HCMV vaccine is not limited to prevention of congenital HCMV infection and/or disease. A vaccine could potentially benefit other patient populations, particularly recipients of solid organ or hematopoietic stem cell transplants. Women of childbearing age, however, represent the dominant target population for which the greatest cost–benefit of an HCMV vaccine could be anticipated. The Institute of Medicine, when commissioned to develop a report outlining vaccine priorities for the 21st century, characterized an HCMV vaccine as having the highest-level (Level 1) priority for the new millennium (15, 16). This review summarizes current clinical trials for HCMV vaccines, and outlines preclinical vaccine development strategies currently under investigation.

HCMV VACCINES CURRENTLY IN CLINICAL TRIALS

A number of HCMV vaccines are currently being evaluated in clinical trials, and there have been recent substantial advances in the field of HCMV vaccine development that have provided momentum for accelerating these efforts. One central challenge in the field is the issue of whether an HCMV vaccine should be a purified protein subunit vaccine, or a live, attenuated vaccine: both approaches are currently being evaluated. Another challenge in the development of HCMV vaccines is the continued uncertainty about the precise correlates of immunity for the protection of the fetus. Both humoral immunity (antibody) and cellular (T cell) immunity appear important in protection against HCMV, and many of the key viral proteins involved in the host response to HCMV infection have been well defined (17). Accordingly, vaccine strategies have evolved that induce antibody to the major envelope glycoprotein, glycoprotein B (gB), as well as vaccines that evoke T-cell responses to the major cellular immune targets, such as the pp65 (ppUL83) protein. These vaccines are summarized below and outlined in Table I.

Glycoprotein B (gB) adjuvanted recombinant protein vaccine

The most extensively studied HCMV subunit vaccine is based on the viral glycoprotein designated gpUL55, also known as gB. The gB protein is the major target of virus-neutralizing antibodies following HCMV infection. All HCMV seropositive individuals studied to date have been shown to have antibody to gB, and up to 70% of the virus-neutralizing capacity of serum from seropositives targets this single protein (18). A number of recombinant expression strategies are being examined as candidates for gB vaccines, but the most advanced studies have been performed using a form of gB expressed in Chinese hamster ovary (CHO) cells, purified chromatographically from cell supernatants prior to admixture with a novel adjuvant, a squalene-in-oil emulsion known as MF59 (19). The efficacy of this vaccine in preventing primary HCMV infection was recently demonstrated in a randomized, double-blind, placebo-controlled phase II clinical trial in seronegative women of childbearing age (20). The primary study endpoint was the time to acquisition of a primary HCMV infection, as documented by seroconversion using an ELISA assay, in which the study participants' sera were depleted of anti-gB antibodies (21, 22). Thus, the development of antibodies to proteins other than gB provided evidence for acquisition of a primary HCMV infection. HCMV infection was confirmed in 18 of 225 (8%) in the vaccine group compared to 31 of 216 (14%) in the placebo group, with an overall vaccine efficacy of 50% (95% confidence interval [CI]: 7-73%).

Only limited information was available from this study about the potential impact of gB vaccine on congenital HCMV infection. Congenital HCMV infection occurred in 1 of 81 (1%) and 3 of 97 (3%) infants born to HCMV vaccine and placebo recipients, respectively. One infant in the placebo group was reported to have severe symptomatic HCMV disease. The sample size for the study was too small to support any conclusions about efficacy against congenital infection. Therefore, although the study demonstrated that the vaccine could significantly reduce the risk of acquiring primary HCMV infection in young women, the study was not designed to address whether vaccine-induced HCMV immunity is equivalent to natural immunity in modulating either infection rate or sequelae for the fetus (23). Future studies, such as a phase III clinical trial using congenital infection as the primary study endpoint, would be required to determine the possibility of protecting women of childbearing age (and, more importantly, their newborns) through universal immunization with the gB vaccine. The efficacy demonstrated in this study, coupled with the highly favorable safety profile of gB/MF59 vaccine, supports continued clinical trial evaluation.

Polynucleotide (DNA) vaccines against HCMV

An active vaccine program has been developed using DNA vaccines targeting immunodominant HCMV proteins. DNA vaccines offer a number of potential advantages over other vaccine technologies, including a favorable safety profile, exquisite specificity, an absence of lot-to-lot variability and ease of storage and administration. Additionally, DNA vaccines elicit both robust $CD4^+$ and $CD8^+$ T-cell and strong neutralizing antibody responses (24). DNA vaccines for HCMV have focused on immunogens gB, pp65 and another important target of the T-cell response to infection, IE1 (25). A vaccine designated VCL-CB01, a bivalent vaccine consisting of two plasmids encoding HCMV pp65 and gB adjuvanted with poloxamer CRL1005 and benzalkonium chloride, was recently evaluated in an open-label, dose-escalating phase I trial in healthy HCMV-seropositive and HCMV-seronegative adults (26). Both antibody responses to gB

Table I. *Human cytomegalovirus vaccines undergoing active evaluation in clinical trials.*

Vaccine	Current status	Rationale for continued development
gB/MF59 adjuvant	Phase II study completed	• Highly immunogenic • gB represents major target of neutralizing antibody response to HCMV infection • Acceptable safety for further studies • Evaluated in HCMV-seronegative women • Vaccine efficacy - 50%
gB/pp65/IE1 alphavirus replicon trivalent vaccine	Phase I study recently completed	• Favorable safety profile • Evaluated in healthy, nonpregnant adults • Elicits both humoral and cellular immune responses to key HCMV antigens • Based on novel replication-deficient alphavirus technology
gB/pp65 bivalent DNA vaccine	Ongoing phase II study	• Well tolerated, with no serious adverse events reported in phase I study • Holds promise for eliciting both humoral and cellular immunity • HCMV-seropositive or -seronegative healthy adults evaluated in phase I and HCT transplant recipients in phase II studies • Augmentation of immune response in HCMV-seropositives may be theoretically useful in the vaccine-mediated protection against reinfection for women of childbearing age • Higher frequencies of HCMV-specific pp65 and gB T cells compared to placebo • Suggestion of efficacy in HCT setting (decreased use of antiviral therapy, reduced HCMV replication)
Towne ± rhIL12 ± priming by DNA vaccine encoding pp65, IE1 and gB proteins	Phase I studies completed	• Favorable safety profile; no evidence for viral latency or viral shedding in recipients with Towne vaccine • Evaluated in HCMV-seronegative healthy adults • Augmentation of immunogenicity by inclusion of rhIL12 or DNA vaccine in phase I studies • Augmentation of immune response to Towne vaccine using rhIL12 or DNA vaccine may overcome the intrinsic lack of immunogenicity of highly attenuated Towne strain of HCMV • Safety and regulatory concerns nonetheless persist for live, attenuated HCMV vaccines
Towne/Toledo "chimeric" HCMV live attenuated vaccines	Phase I studies completed	• Mixing genomes of highly attenuated Towne strain and minimally attenuated Toledo strain holds theoretical promise of striking opti mal balance between safety and immunogenicity • Vaccines were safe and well tolerated in phase I studies with no evidence of vaccine shedding among vaccinees • Safety and regulatory concerns nonetheless persist for live, attenuated HCMV vaccines

gB, glycoprotein B; HCMV, human cytomegalovirus; pp65, phosphoprotein 65 (ppUL83); IE1, immediate-early protein with a molecular mass of 72 kDa; HCT, hematopoietic stem cell transplant; rhIL12, recombinant human IL-12.

by ELISA and cellular immune responses, as determined by interferon γ (IFN-γ) ELISPOT assay with pp65 or gB antigens, were documented in both seronegative and seropositive vaccinees. These results suggested that VCL-CB01 has the ability to prime antigen-specific T cells with the capacity to proliferate and secrete IFN-γ on restimulation with antigen (27). Although the vaccine boosted the existing pp65-specific T-cell response in seropositive vaccine recipients, the gB antibody response was not augmented.

Clinical trials of VCL-CB01 have focused on individuals undergoing allogeneic hematopoietic stem cell transplantation (28-30). In preliminary immunogenicity analyses, higher frequencies of HCMV-specific pp65 and gB T cells were observed in HCMV-seropositive transplant recipients, immunized 3-5 days prior to and 3-6, 12 and 28 weeks after transplantation, compared to placebo (28, 29). There were no differences in anti-gB antibody levels after transplantation. VCL-CB01 vaccine had an impact on HCMV disease in this population. In interim results from a phase II study in 80 HCT recipients, VCL-CB01-vaccinated recipients demonstrated a 24-70% reduction in the occurrence of HCMV infection, recurrence of HCMV infection, duration of DNAemia and peak magnitude of DNAemia compared with recipients receiving placebo. In addition, vaccine recipients required a shorter duration of antiviral therapy and were less likely to initiate antiviral therapy compared with placebo

recipients (29, 30). Although the preliminary efficacy data and favorable safety profile of DNA vaccines support the transplantation/oncology patient setting as a logical target population for evaluation and possible licensure of an HCMV DNA vaccine, efficacy in this patient population could lead to future studies of DNA vaccines toward the goal of prevention of HCMV infection and disease in mothers and infants.

Alphavirus replicon-vectored HCMV vaccine

Alphavirus replicon vector systems represent a potentially useful platform for the development of a number of prophylactic and therapeutic vaccines for infectious diseases and cancer (31). Preclinical evaluation of an alphavirus-vectored vaccine demonstrated protection against congenital transmission in a guinea pig model, using the guinea pig cytomegalovirus (GPCMV) homolog of the *pp65 (UL83)* gene product (32). The alphavirus platform has been extended to HCMV vaccines. A randomized, double-blind phase I trial of an alphavirus replicon vaccine for HCMV in healthy HCMV-seronegative adult volunteers has been recently reported (33). This vaccine, designated AVX601, is a two-component alphavirus replicon particle vaccine expressing both gB and a pp65/IE1 fusion protein (34). In the initial clinical trial, vaccine (low-dose or high-dose groups) or placebo was administered by i.m. or s.c. injection in a three-dose regimen at weeks 0, 8 and 24 (33). Four weeks after the third dose, 93% of subjects in the low-dose group and 100% of subjects in the high-dose group developed neutralizing antibody to gB. IFN-γ ELISPOT detected responses in 90-97% of vaccine recipients after the second dose, with similar rates of response in the lower and higher dose groups. Flow cytometry demonstrated that HCMV antigen-specific $CD4^+$ and $CD8^+$ effector T cells producing multiple cytokines in response to HCMV peptide stimulation were induced in all subjects immunized with AVX601. The vaccine elicited only mild to moderate local reactogenicity, although erythema and induration began or persisted more than 7 days in half of the subjects after s.c. injection. These results demonstrate favorable safety and immunogenicity profiles, supporting the further evaluation of AVX601 in phase III trials.

Live, attenuated HCMV vaccines: Towne and Towne-Toledo chimeras

In contrast to subunit vaccines, live, attenuated HCMV vaccines have, in principle, the potential to elicit a broad range of immune responses to multiple viral proteins. The potential importance of this is underscored by recent studies demonstrating the unexpectedly large number of HCMV gene products to which the host mounts T-cell responses following infection (35, 36). Although a live, attenuated HCMV vaccine has the potential to elicit a broad range of immune responses to viral antigens, this theoretical advantage must be weighed against the safety concerns intrinsic to a live herpesvirus vaccine, including the potential for replication in the inoculated host, the possibility of transmission from a vaccinated to an unvaccinated individual and the potential for establishment of latency. The most extensively tested live, attenuated HCMV vaccine is based on a strain referred to as the "Towne" strain of HCMV (37-39), which was generated following serial passage of a clinical isolate in cell culture. Immunization with the Towne vaccine prevented HCMV disease in seronegative renal transplant recipients, although it did not prevent infection in these patients or in parents of HCMV-infected children (37, 39).

Recent evidence suggests that the relative defect in the Towne vaccine may be related to inadequate antigen-specific IFN-γ responses generated by $CD4^+$ and $CD8^+$ T cells following vaccination (40). Thus, approaches to improve the immunogenicity of the Towne vaccine are under evaluation. One approach is to coadminister Towne with recombinant human IL-12 (rhIL-12). The immunogenicity and safety of the Towne vaccine coadministered with rhIL-12 were evaluated in a dose-escalation, randomized phase I clinical trial in HCMV-seronegative healthy volunteers (41). The adjuvant effect of rhIL-12 was associated with dose-related increases in peak anti-gB titers and $CD4^+$ T-cell proliferation responses. Adjuvant rhIL-12 at doses up to 2 mg was well tolerated and rhIL-12 coadministration with Towne did not lead to viral shedding or latent infection. Immunogenicity of the Towne vaccine can also be augmented by a "prime–boost" approach, in which VCL-CT02, a plasmid DNA vaccine containing the *UL83, IE1* and *gB* genes, is administered along with the Towne vaccine challenge (42). In a safety and immunogenicity analysis in HCMV-seronegative subjects, although the DNA vaccine alone had minimal immunogenicity, vaccination with VCL-CT02 was found to be able to safely prime for an anamnestic response to administration of Towne. Local reactions were all mild and brief in duration, and no evidence of viral shedding could be demonstrated. Further studies attempting to increase and optimize immunogenicity of this approach are warranted.

Lastly, another approach to improve the immunogenicity of the Towne vaccine is the generation of the Towne/Toledo so-called "chimera" vaccines (43). The Toledo strain of HCMV represents a minimally attenuated isolate capable of inducing disease in human challenge studies (44-46). Using overlapping closmid libraries of cloned genomic segments from each virus, a series of recombinant vaccines was generated containing regions from the genome of the unattenuated Toledo strain of HCMV, substituted for the corresponding regions of the Towne genome (47). The chimeras retain some, but not all, of the mutations that apparently contribute to Towne vaccine attenuation, and were hypothesized to be less attenuated, and hence presumably more immunogenic, than the Towne vaccine. Four independent chimeric vaccines were produced and tested in a double-blind, placebo-controlled study (43). All of the vaccines were well tolerated, and none were shed by vaccinees, as demonstrated by viral culture and PCR analyses of blood and body fluids. Thus, these vaccines are sufficiently attenuated to warrant studies in HCMV-seronegative individuals.

VACCINES OF THE FUTURE: PRECLINICAL HCMV VACCINE DEVELOPMENT

A multitude of HCMV gene products utilizing a variety of expression approaches have been proposed for vaccine development in recent years, and some have been validated, to varying degrees, in animal models. The following section summarizes some of the preclinical approaches in active development for the generation of an HCMV vaccine.

Recombinant modified vaccinia virus Ankara (MVA)

The attenuated poxvirus MVA was developed as a safer alternative to licensed vaccinia virus derivatives as a potential smallpox vaccine, and has been well established as a safe and potent antigen delivery

system (48). An attractive feature of MVA for the delivery of heterologous (foreign) antigens as potential vaccines is its potential for accommodating a large amount of additional genetic material, probably because the MVA genome has undergone major deletions during tissue culture passage. A recombinant MVA vaccine has been constructed that expresses a soluble, secreted form of gB (49). In preclinical studies, high levels of gB-specific neutralizing antibodies were induced in mice. A trivalent MVA expressing gB, pp65 and IE1 has also been developed and proposed for use in clinical studies (50). Support for human studies comes from experiments in cell culture demonstrating the responsiveness of peripheral blood mononuclear cells stimulated with the trivalent MVA vaccine (50, 51).

A proof-of-concept analysis was evaluated using recombinant MVA vaccine in a rhesus macaque model of CMV infection. In this study, an MVA expressing the rhesus CMV gB, pp65-2 and IE1 homologs was generated and administered to rhesus macaques with or without prior priming with DNA plasmid vaccines for the same antigens (52). Macaques primed with only a single dose of DNA plasmids elicited earlier and stronger antibody and cellular immune responses than those without priming. Following challenge of vaccinated and control animals, plasma peak viral loads were reduced in both vaccine groups compared to untreated controls. These promising data obtained from nonhuman primates highlight the potential of MVA-based HCMV vaccines and justifies further investigation using this expression system

Replication-deficient adenovirus-vectored polyepitope vaccine

Another vectored vaccine approach that has recently been pursued in preclinical studies is that of utilization of a replication-deficient adenoviral vector (53-57). The potential of this expression approach was demonstrated by studies using murine cytomegalovirus (MCMV). Systemic and mucosal immunity to MCMV could be induced by intranasal immunization using a replication-deficient adenoviral vector expressing MCMV gB or glycoprotein H in a murine model (54, 55). Adenoviral vaccines have been engineered for HCMV genes as well. Recently, a modified adenoviral vector expressing the immunodominant antigenic domain-1 epitope of HCMV gB has been developed (56). A novel replication-deficient adenoviral vectored vaccine, Ad-gBCMVpoly, was designed to induce a broad repertoire of HCMV-specific immune responses. Ad-gBCMVpoly encodes 46 HCMV T-cell epitopes from multiple antigens that may play a role in the immune response to infection. This HLA class I- and class II-restricted T-cell polyepitope was covalently linked to the extracellular domain of gB, allowing the expression of the polypeptide and gB proteins as a single fusion protein (57). Immunization with this chimeric vaccine elicited neutralizing antibody responses and virus-specific $CD4^+$ and $CD8^+$ T-cell responses in mice (53, 57). Since the Ad-gBCMVpoly vaccine has the potential to induce both humoral and cellular immunity to a wide range of antigens, it represents an attractive option for future clinical development.

Bacterial artificial chromosome (BAC) mutagenesis of cloned CMV genomes as a novel vaccine strategy

The successful cloning of CMV genomes as infectious bacterial artificial chromosomes (BACs) in *Escherichia coli* has allowed the development of reliable targeted deletion approaches to remove CMV genes potentially involved in pathogenesis or evasion of the host immune system (58, 59). Such vaccines have the potential to elicit immune responses to a wide range of viral proteins, while obviating safety concerns intrinsic to immunization with viruses with a more "wild-type" genome content. An example of the powerful potential of this approach has been reported in the MCMV model. A recombinant MCMV lacking a total of 32 genes as 31.2 kb at the left and right genome termini of the wild-type genome, *Dm01-17+m144-158*-MCMV, was generated by BAC mutagenesis. This mutant has deletions of most of the known immune modulators regulating functions in MHC-1 presentation (*m04*, *m06* and *m152*) and natural killer (NK) cell response (*m144*, *m145*, *m152*, *m155* and *m157*). The deletion virus replicated to wild-type level in cell culture, but was severely attenuated in animals, including the SCID/bg mouse, which lacks NK, B and T cells and is exquisitely susceptible to MCMV. The recombinant deletion mutant induced MCMV-specific antibodies and a specific cellular immune response detectable by tetramer staining of peripheral cytotoxic $CD8^+$ T lymphocytes and protected mice from a challenge with infection by wild-type MCMV (58). Targeted deletion of HCMV genes by BAC mutagenesis may offer an approach to the rational design of immunogenic and safe live, attenuated vaccines (60).

Other evolving HCMV vaccine strategies

A number of recent advances in the understanding of the biology of HCMV have resulted in the development of other innovative vaccine strategies. Recently, it has been demonstrated that entry of HCMV into epithelial and endothelial cells requires an endocytic pathway of cell entry involving a complex of HCMV proteins gH, gL, and UL128, UL130 and UL131. Evidence suggests that an HCMV vaccine targeting this endocytic pathway of entry would provide advantages over existing HCMV vaccine strategies (61, 62), because the majority of the neutralizing activity of convalescent human sera from HCMV-seropositive individuals targets the endocytic pathway of entry. Accordingly, a strategy of recombinant expression of the gH/gL/UL128/UL130/UL131 proteins as potential vaccine candidates merits consideration for future preclinical development. Vaccine strategies to block viral entry could also include approaches aimed at blocking cellular receptors, such as the platelet-derived growth factor receptor α (PDGF-R-α). Receptor blockade with a PDGF-R-α-blocking antibody inhibits HCMV infection in epithelial, endothelial and fibroblast cells, and anti-gB-neutralizing antibodies inhibit PDGF-R-α phosphorylation, inhibiting HCMV replication (63). Exploiting this knowledge derived from the elucidation of these mechanisms of binding and entry to design innovative active and/or passive immunization strategies is a high priority for future research.

Strategies designed to improve current HCMV vaccine approaches with respect to the ability to elicit more robust cytotoxic T-cell responses also deserve further study. This will be of particular importance if neutralizing antibody approaches currently in clinical trials prove to be inadequate in disease control and prevention. A recombinant, secreted form of pp65 was recently demonstrated to be able to elicit strong HCMV-specific CD4 and CD8 responses following culture of PBMCs of diverse HLA background in the presence of the protein (64). Examination of this secreted form of pp65 as a therapeutic vaccine for augmenting immunity in HCMV-seropositive

individuals may represent a particularly compelling approach to the problem of reinfection.

Another vaccine strategy under active investigation exploits the elucidation of the function of viral genes that inhibit the protein kinase R (PKR) host defense pathway. These gene products play a critical role in HCMV immune evasion. A key element of this host defense cascade is the production of double-stranded RNA (dsRNA) that is produced during infection by many viruses, including CMVs (65). The presence of dsRNA functions as an "alarm signal" to the host, resulting in activation of PKR to inhibit viral replication. Evolution of dsRNA-activated host antiviral pathways has been matched by the evolution of viral mechanisms that thwart these pathways (66, 67). One such strategy is the sequestration of dsRNA by dsRNA-binding proteins (68). In the case of the HCMV, two genes, *TRS1* and *IRS1*, each bind to dsRNA, resulting in suppression of PKR activation, and deletion of these genes results in highly attenuated HCMV, the replication of which is severely impaired by activation of the PKR pathway (69, 70). The notion that elimination of a viral PKR antagonist can result in a highly attenuated but effective vaccine has been demonstrated in vaccinia (71). Extension of these observations toward the long-term aim of designing a fully protective but highly attenuated HCMV vaccine warrants further investigation.

CONCLUSIONS

There has been substantial recent progress in the development of an effective vaccine against HCMV infection. For the first time, a clinical trial has demonstrated that an HCMV vaccine (based on the gB glycoprotein) can protect against acquisition of infection. The positive results observed in this trial have accelerated interest in future studies toward the goal of preventing congenital HCMV transmission. A vaccine capable of protecting the fetus would represent a major public health breakthrough, given the severe disabling injury that can occur in infected infants. A variety of expression strategies, including DNA vaccines, adenovirus-vectored vaccines and alphavirus replicon vaccines, are being considered for future clinical trials. Newly recognized pathways of HCMV entry into cells have been elucidated, opening the door for novel subunit vaccines targeting heretofore uncharacterized proteins. Live, attenuated vaccines also continue to be explored in preclinical models and clinical trials. It is not yet clear what the HCMV vaccine(s) of the future will look like, but recent progress in the field has taken us one step closer to developing a vaccine strategy to deal with this major public health problem.

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DISCLOSURES

The author states no conflicts of interest.

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