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Cytomegalovirus Vaccine Development

Mark R. Schleiss
Division of Pediatric Infectious Diseases, Department of Pediatrics, Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota Medical School, 2001 6th Street SE, Minneapolis, MN 55455, Telephone: 612-626-9913, Facsimile: 612-624-8927

Mark R. Schleiss: schleiss@umn.edu

Abstract

Although infection with human cytomegalovirus (HCMV) is ubiquitous and usually asymptomatic, there are individuals at high risk for serious HCMV disease. These include solid organ and hematopoietic stem cell (HSC) transplant patients, individuals with HIV infection, and the fetus. Since immunity to HCMV ameliorates the severity of disease, there have been efforts made for over thirty years to develop vaccines for use in these high-risk settings. However, in spite of these efforts, no HCMV vaccine appears to be approaching imminent licensure. The reasons for the failure to achieve the goal of a licensed HCMV vaccine are complex, but several key problems stand out. First, host immunology correlation to protective immunity consist is not yet clear. Secondly, the viral proteins that should be included in a HCMV vaccine are uncertain. Third, clinical trials have largely focused on immune compromised patients, a population that may not be relevant to the problem of protection of the fetus against congenital infection. Fourth, the ultimate target population for HCMV vaccination remains unclear. Finally, and most importantly, there has been insufficient education about the problem of HCMV infection, particularly among women of child-bearing age and in the lay public. This review considers the strategies that have been explored to date in development of HCMV vaccines, and summarizes both active clinical trials as well as novel technologies that merit future consideration toward the goal of prevention of this significant public health problem.

1 Spectrum of HCMV Disease, Rationale for Vaccine, and Target Population

1.1 Congenital HCMV Infection: A Major Public Health Problem

The problem of congenital HCMV infection is unquestionably the major driving force behind efforts to develop a HCMV vaccine. In the developed world, HCMV is the most common congenital viral infection (Whitley 1994). Estimates of the prevalence of congenital HCMV infection suggest that between 0.5–2% of all newborns in the developed world are infected *in utero* (Demmler 1996). In the United States alone, this corresponds to approximately 40,000 infected newborn infants born annually with HCMV infection. The concern is particularly acute for HCMV-seronegative women of child-bearing age. Based on recent HCMV incidence estimates, approximately 27,000 new infections are believed to occur among seronegative pregnant women in the United States each year (Colugnati et al. 2007). Approximately 10% of congenitally infected infants have clinically evident disease in the newborn period, including visceral organomegaly, microcephaly with intracranial calcifications, chorioretinitis, and skin lesions including petechiae and purpura. Although the majority of congenitally-infected infants appear normal at birth, these children are nonetheless at risk for neurodevelopmental sequelae, in particular sensorineural hearing loss (SNHL). Antiviral therapy in infected newborns with neurologic involvement is of value in ameliorating the severity and progression of SNHL

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(Kimberlin et al. 2003), but the toxicities of available antiviral agents are of concern, and the benefits of therapy are limited. Therefore, there are few medical interventions currently available to prevent or limit HCMV-induced neurological morbidity in infants, underscoring the urgent need for vaccine development.

1.2 Health Care Costs Associated with Congenital HCMV Infection: A Compelling Argument for Vaccine Development

The economic burden on the healthcare system in caring for neurodevelopmental disability in early childhood caused by congenital HCMV infection is substantial. Congenital HCMV infection is the most common infectious cause of brain damage in children, and HCMV causes more hearing loss in children than did *H. influenzae* meningitis in the pre-Hib vaccine era (Pass 1996). The economic costs to society associated with congenital HCMV infection present a compelling argument for vaccine development. In the early 1990s, the expense to the US health care system associated with congenital HCMV infection was estimated at approximately $1.9 billion annually, with a average cost per child of over $300,000 (Arvin et al. 2004). Children with congenital HCMV infection often require long-term custodial care and extensive medical and surgical interventions. A recent economic analysis by the Institute of Medicine (IOM) examined the theoretical cost-effectiveness of a hypothetical HCMV vaccine based on “quality adjusted life years” (QALYs). QALYs quantify the acute and chronic problems caused by an illness. Employing this model, the more severe or permanent the sequelae, the larger will be the potential benefit conferred by an effective intervention. Not surprisingly, a hypothetical HCMV vaccine administered to 12-year-olds was in the “Level 1” group (the group for which a vaccine development strategy would save society money), and in fact was the single most cost-effective vaccine identified (Stratton et al. 1999). Thus, the economic benefit of HCMV vaccination holds the highest priority for any hypothetical new vaccine.

1.3 HCMV Vaccine: What is the Ideal Target Population?

1.3.1 Perinatal and Early Childhood HCMV Infection—One strategy for vaccine-mediated prevention of HCMV would be to target acquistion of primary infection in infancy and early childhood. Perinatal acquisition of HCMV may occur by one of three different routes: exposure to HCMV in the birth canal during labor and delivery, transmission of HCMV by blood transfusion, or transmission by breast-feeding. In a prospective study in premature infants receiving breast milk containing HCMV, transmission was observed in 33 of 87 exposed infants, and approximately half of these babies developed disease, including hepatitis, neutropenia, thrombocytopenia, and sepsis-like state (Maschmann et al. 2001). It is uncertain if HCMV infection of low-birth weight premature infants by this route carries any risk of long-term sequelae, and highly speculative as to whether maternal immunization programs would play a role in elimination of transmission by breast milk in this vulnerable population.

Beyond the immediate neonatal period, an extremely important population for primary HCMV infection – and a potential target for implementation of a vaccine program – is the early childhood population, in particular infants and toddlers attending group day-care. Although primary HCMV infection may occasionally cause mild disease in the toddler, a far greater concern is that the child may serve as a vehicle for subsequent infection of a parent. Should a pregnant mother become infected, the resulting newborn would then be at significant risk of HCMV disease and its attendant sequelae. Such child-to-parent transmission of HCMV has been well documented: day-care workers are in particular at increased risk for primary HCMV infection (Pass et al. 1990; Murph et al. 1991). Thus, interruption of HCMV transmission in the day-care environment could serve as an important efficacy endpoint for vaccine programs. Behavioral interventions and improved education about the risks of transmission can also likely play a role in decreasing the likelihood of this mode of transmission (Cannon and Davis 2005), but behavioral interventions alone are unlikely to completely eliminate the risk of

transmission in this setting. Development and implementation of HCMV serologic screening programs for women of child-bearing age may be of benefit in identifying those women and families who might benefit most from behavioral and vaccination strategies aimed at interrupting this type of transmission.

Clearly, immunization of infants and toddlers prior to acquisition of primary HCMV infection is a strategy that should be considered for HCMV disease control. Immunization of the infant or toddler could result in secondary benefits for adult subjects, particularly mothers, who would be at decreased risk for acquiring infection from their child. Such an immunization approach has been utilized for rubella vaccine, which is routinely administered to young children; in this setting, the primary benefit of vaccination is not the prevention of rubella per se in the child, but the prevention of rubella transmission to young women, with the secondary benefit of prevention of congenital rubella syndrome in subsequent pregnancies. This occurs, in part, through 'herd' immunity, which ultimately benefits all women of child-bearing age. Use of mathematical modeling suggests that such an approach for a HCMV vaccine would produce benefits similar to those realized by rubella vaccination (Griffiths et al. 2001). Thus, the strategy of universal immunization of young children against HCMV deserves further consideration.

Moreover, universal immunization against HCMV in early life may confer health benefits that extend ultimately to men as well as women of child-bearing age. Increasingly, HCMV infection has been tied to an increased lifetime risk of illnesses such as atherosclerosis, malignancies, inflammatory and autoimmune diseases, and the phenomenon of immune senescence in later life (Soderberg-Naucler 2006). Prevention of HCMV infection, and conceivably elimination of infection through herd immunity, could provide widespread benefits for human health.

1.3.2 Adolescent HCMV Infections—Adolescents acquire primary HCMV infections at a high frequency. In a prospective study of HCMV-seronegative adolescents, an annual HCMV infection rate of 13.1% was observed (Zhanghellini et al. 1999). Onset of sexual activity and exposure to young children, particularly in child care settings, have been proposed as potential sources of primary HCMV infection in this population. Therefore, adolescence may also be an important target population for eventual implementation of HCMV vaccination programs. In recognition of the importance of the adolescent period in acquisition of primary HCMV infection, the Institute of Medicine (IOM) modeled its analysis of the potential benefits of a HCMV vaccine program upon hypothetical administration of vaccine to the adolescent patient (Stratton et al. 1999).

1.3.3 HCMV Infection and Disease in the Immunocompromised Patient—The potential value of HCMV vaccines is not limited to prevention of congenital infection. Bone marrow/stem cell transplant and solid organ transplant patients are at high risk for HCMV disease, pneumonitis, enteritis, retinitis, and viremia. Although the availability of effective prophylactic and preemptive antiviral therapy has made HCMV a rare cause of mortality in the HSC transplantation setting, HCMV-seropositive transplant recipients and seronegative recipients of a positive graft have a mortality disadvantage when compared with seronegative recipients with a seronegative donor (Boeckh et al. 2003). HCMV seropositivity is an important risk factor for impaired graft survival, increased risk of graft-versus-host disease, and other opportunistic infections such as invasive fungal infections. Therefore, prevention strategies that employ vaccines capable of stimulating both humoral and cell-mediated immune responses to HCMV may be of value in further decreasing the incidence and severity of HCMV disease, as well as these other complications of transplantation. Such vaccines could be administered to either the transplant recipient, or to the HSC or living-related solid organ donor prior to transplantation. Whether the same vaccines that might prove successful in this patient population would protect against HCMV transmission in women of child-bearing age is uncertain.

2 Evidence that Immunity Protects Against HCMV Infection and Disease

2.1 Role of Preconception Maternal Immunity in Protection Against Congenital HCMV Transmission and HCMV Disease in the Newborn

Preconceptual maternal immunity to HCMV clearly provides some degree of protection against the most devastating forms of congenital infection. In a comparison of outcomes of HCMV-infected infants born to mothers who acquired primary infection during pregnancy with those of infected infants born to mothers with preconception immunity, only infants born in the primary-infection group had symptomatic disease at birth. These infants were at the highest risk for long-term sequelae (Fowler et al. 1992). A recent study suggests that preconceptual immunity does not completely eliminate the risk of symptomatic congenital transmission. In this study, some women who were seropositive for HCMV were nonetheless susceptible to reinfection with a new HCMV strain during pregnancy, and such reinfections did lead in some cases to symptomatic disease in the neonate (Boppana et al. 2001). In light of these data, a HCMV vaccine may not completely eliminate the potential for congenital HCMV transmission. Substantial evidence nonetheless strongly suggests that a HCMV vaccine program would protect many newborns. Other lines of evidence indicate that preconceptual immunity reduces both the incidence of congenital transmission, and the severity of disease if transmission occurs. In a recent study that followed over 3,000 women from one pregnancy to the subsequent pregnancy and delivery, the rate of congenital HCMV infection was 3 times higher in offspring of women who initially were HCMV seronegative (Fowler et al. 2003). Preconception immunity was clearly protective in this study, and resulted in a 69% reduction of congenital HCMV infection. Protection against congenital HCMV infection is enhanced by longer time intervals between pregnancies (Fowler et al. 2004). This effect is likely due to maturation of antibody avidity against HCMV (Revello and Gerna 2002). Therefore, emphasis should be placed on developing HCMV vaccines that are capable of mimicking the protective components of natural immunity, particularly antibody avidity, and such vaccines would likely have a significant impact on preventing symptomatic congenital HCMV infections.

2.2 Lessons from Adoptive Transfer Studies

Additional evidence supporting the protective role of immunity in preventing symptomatic congenital HCMV transmission comes from recently described passive immunization studies, using high-titer anti-HCMV immunoglobulin. In this study (Nigro et al. 2005), pregnant women with a primary HCMV infection were offered intravenous HCMV hyperimmune globulin, in two different dose regimens (“therapy” and “prevention” groups). In the therapy group, only 1/31 women gave birth to an infant with HCMV disease (defined as an infant who was symptomatic at birth and handicapped at two or more years of age), compared with 7 of 14 women in an untreated control group. In the prevention group, 6/37 women who received hyperimmune globulin during pregnancy had infants with congenital HCMV infection, compared with 19/47 women who did not receive the high-titer HCMV globulin. Although uncontrolled, these data support the protective effect of humoral immunity in prevention of fetal HCMV-associated disease. Additional randomized controlled trials of immune globulin are warranted in high-risk pregnancies, to further validate the protective effect of passive immunization.

3 HCMV Vaccines in Clinical Trials

A number of HCMV vaccines have been evaluated in clinical trials. These vaccine candidates are summarized in Table 1. A variety of strategies have been employed, but generally HCMV vaccines can be conceptually subdivided into the categories of live, attenuated vaccines, and subunit vaccines that target individual proteins. Progress in study of these vaccines is considered below.

3.1 Live, Attenuated HCMV Vaccines

HCMV has been the target of live, attenuated vaccine development efforts since the 1970s (reviewed in Schleiss and Heineman 2005). The first live, attenuated HCMV vaccine candidate tested in humans was based on the laboratory-adapted AD169 strain. Subsequent trials with another laboratory-adapted clinical isolate, the Towne strain, confirmed that live attenuated vaccines could elicit neutralizing antibodies, as well as CD4+ and CD8+ T lymphocyte responses. The efficacy of Towne vaccine was tested in a series of studies in renal transplant recipients. Although Towne failed to prevent HCMV infection after transplantation, vaccination did provide a protective impact on HCMV disease (Plotkin et al. 1994). Towne vaccine was also evaluated in a placebo-controlled study in seronegative mothers who had children attending group daycare. This study indicated that immunization with Towne failed to protect these women from acquiring HCMV infection from their children. The apparent failure of Towne vaccine was in contrast to the protection against re-infection observed in women with pre-existing immunity, who were protected against acquiring a new strain of HCMV from their children (Adler et al., 1995). One interpretation of this study is that a HCMV vaccine that induced immune responses comparable to natural infection could provide protection of a high-risk patient population, but that the Towne vaccine may be overattenuated for this purpose. The molecular basis for the apparent over-attenuation of the Towne vaccine remains unknown. Recent evidence suggests that the relative defect in Towne vaccine may be related to inadequate antigen-specific interferon gamma responses by CD4+ and CD8+ cells following vaccination (Jacobsen et al. 2006a). An approach to improve the immunogenicity of the Towne vaccine is currently being explored, in which recombinant interleukin-12 (rhIL-12) is co-administered with Towne vaccine. The adjuvant effect of rhIL-12 was associated with increases in antibody titer to glycoprotein B and improved CD4+ T cell proliferation responses in this recently reported phase I study(Jacobsen et al. 2006b).

Another approach to improve the immunogenicity of the Towne vaccine has recently been reported, in which a series of genetic recombinant vaccines were generated containing regions from the genome of the unattenuated Toledo strain of HCMV, substituted for the corresponding regions of the Towne genome. These Towne/Toledo ‘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-blinded, placebo controlled study (Heineman et al. 2006). All of the vaccines were well-tolerated, and none were shed by vaccinees, as assessed by viral culture and PCR analyses of blood and body fluids. Thus, these vaccines are sufficiently attenuated to warrant future studies in seronegative individuals. Concerns about the potential risk of establishing a latent HCMV infection have hindered the progress of live, attenuated vaccine studies, although to date there has been no evidence that any of these approaches have resulted in latent or persistent infections in any subject.

3.2 Subunit Vaccines

Subunit vaccine approaches emphasize specific immunogenic viral proteins, expressed by a variety of techniques, and administered either singly or in combination. The candidate subunit vaccines that are in clinical or preclinical development are described below, along with an overview of the expression techniques being employed.

3.2.1 Glycoprotein B (gpUL55) Vaccine—The humoral immune response to HCMV is dominated by responses to viral glycoproteins, present in the outer envelope of the virus particle. Of these, the most fully characterized is the glycoprotein complex I (gcI) consitisting of gB (gB; UL55). All sera from HCMV-seropositive individuals contain antibodies to gB, and up to 70% of the neutralizing antibody response is gB-specific (Britt et al. 1990).

Recombinant vaccines based on gB demonstrate efficacy against disease in murine and guinea pig models of cytomegalovirus infection (Rapp et al. 1993; Schleiss et al. 2004), providing further support for human efficacy testing. Accordingly, the gB protein is the leading candidate for subunit vaccine development and testing, and the vaccine currently most actively studied in clinical trials.

One formulation of HCMV gB currently being explored in clinical trials is a recombinant protein expressed in Chinese Hamster Ovary (CHO) cells. In contrast to native gB, this formulation of gB is a truncated, secreted form of the protein, modified in two ways to facilitate its expression and purification. First, the proteolytic cleavage site, R-T-K-R, at which gB is normally cleaved into its amino and carboxyl moieties, was modified to prevent cleavage of the protein; secondly, a stop mutation was introduced prior to its hydrophobic transmembrane domain, resulting in a truncated, soluble form of gB (Spaete 1991). The resulting secreted protein is purified from CHO cell culture supernatants and used, with adjuvant, as a vaccine. Purified recombinant gB vaccine has undergoing safety, immunogenicity, and efficacy testing in several clinical trials. The first study of this vaccine was a phase I randomized, double-blind, placebo-controlled trial, in adults, in which recombinant gB was combined with one of two aduvants, MF59 or alum (Pass et al. 1999). Levels of gB-specific antibodies and total virus-neutralizing activity after the third dose of vaccine exceeded those observed in HCMV-seropositive controls. Antigen dose was evaluated in a phase I study of 95 HCMV-seronegative adult volunteers (Frey et al. 1999), and the immunogenicity and safety of the vaccine has been studied in a limited number of toddlers (Mitchell et al. 2002). In all studies reported to date, the safety profile of the vaccine has been favorable, although injection-site discomfort has been observed. There is currently a double-blinded, placebo controlled phase II study of gB/MF59 vaccine ongoing in young, HCMV-seronegative, women who are at high risk for acquisition of primary infection (Zhang et al. 2006). This study should provide insights into the potential protective efficacy of this vaccine in young women. A recombinant gB study is also currently in progress in renal transplant patients. In this study, subjects awaiting transplantation receive gB vaccine, to test whether the antibody responses engendered will contribute to reduction of HCMV viral load following transplantation (PD Griffiths, personal communication).

Another formulation of recombinant gB has also been evaluated in clinical trials using a “vectored” vaccine expression system based on a canarypox vector, ALVAC, an attenuated poxvirus that replicates abortively in mammalian cells. Clinical trials have focused on using ALVAC-gB in a ‘prime-boost’ approach, in which ALVAC vaccine is administered to “prime” immune responses for subsequent “boost” with live, attenuated vaccine, or recombinant protein. In the first such “prime-boost” study, ALVAC-gB was evaluated alone or in combination with live, attenuated Towne vaccine. ALVAC-gB vaccine induced low neutralizing and ELISA antibodies in seronegative adults, but subjects primed with ALVAC-gB and then boosted with a single dose of Towne developed binding and neutralizing antibody titers comparable to naturally seropositive individuals (Adler et al. 1999). A subsequent study compared three immunization regimens: subunit gB vaccine; ALVAC-gB followed by gB/MF59; or both vaccines administered concomitantly (Bernstein et al. 2002). All 3 vaccine approaches induced high-titer antibody and lymphoproliferative responses, but no benefit for priming was detected. Thus, ALVAC-gB priming appears to result in augmented gB-specific responses following a boost with Towne vaccine, but not subunit gB/MF59.

Another approach used to express gB as a vaccine is the use of an alphavirus replicon system. This approach results in generation of virus-like replicon particles (VRPs) based on an attenuated Venezuelan Equine Encephalitis (VEE) expression system. Advantages of the VRP approach include the expression of high levels of heterologous proteins, the targeting of expression to dendritic cells, and the induction of both humoral and cellular immune responses to the vectored gene products of interest. HCMV gB has been expressed in the VRP system,

and these VRPs have undergone protein expression analyses in cell culture as well as immunogenicity studies in mice. These studies demonstrated that protein expression levels are highest in VRPs expressing the extracellular domain of gB. BALB/c mice immunized with VRP expressing gB developed high titers of neutralizing antibody to HCMV (Reap et al. 2007). Based on these encouraging results, a phase I study of a trivalent vaccine including gB has recently been commenced in humans.

A final expression approach that has been applied to HCMV gB is DNA vaccination. In preclinical studies of HCMV gB DNA vaccines in mice, both the full-length gB, as well as a truncated, secreted form expressing amino acids 1–680 (of a total of 906 gB residues), were evaluated. Immunization with both constructs induced neutralizing antibodies, but titers were higher in mice immunized with the DNA encoding the truncated form of gB, which predominately elicited IgG1 antibody. In contrast, the full-length gB construct primarily elicited IgG2a antibodies (Endresz et al. 1999). The gB plasmid vaccine that has moved forward in human clinical trials is accordingly based on a construct encoding a truncated, secreted form of the protein. For clinical trials, HCMV DNA vaccines are currently formulated using the poloxamer adjuvant, CRL1005 and benzalkonium chloride. In a phase I trial using a bivalent vaccine consisting of gB and pp65 (see below), 1 mg and 5 mg doses were studied in HCMV seropositive and seronegative subjects, and appeared to be safe and well-tolerated (Evans et al. 2004; R. Moss, personal communication). There is currently an ongoing multicenter study in HSC patients of this adjuvanted bivalent HCMV DNA vaccine, toward the goal of reducing HCMV viremia and disease in this high-risk patient population.

3.2.2 pp65 (ppUL83) Vaccines—The cellular immune response to HCMV infection includes MHC class II restricted CD4+ and MHC class I restricted, cytotoxic CD8+ T lymphocyte responses to a number of viral antigens, many of which are found in the viral tegument, the region of the viral particle that lies between the envelope and nucleocapsid. For vaccination strategies aimed at eliciting T-cell responses, most attention has focused on the pp65 protein (ppUL83). This is in part based on the apparent dominance of pp65 in the cellular immune response to HCMV: this protein elicits the majority of CD8+ T lymphocyte responses following HCMV infection (McLaughlin-Taylor et al. 1994; Wills et al. 1996). The observation that adoptive transfer of pp65-specific CTL ameliorates HCMV disease in high-risk transplant patients provides further support for the study of pp65-based vaccines (Walter et al. 1995).

Many of the same expression strategies described for development of candidate gB vaccines in clinical trials have been employed for generation of pp65-based vaccines. An ALVAC vaccine expressing pp65 was administered to HCMV seronegative adult volunteers in a placebo-controlled trial (Berencsi et al. 2001). The ALVAC/pp65 recipients developed HCMV-specific CD8+ CTL responses at frequencies comparable to those seen in naturally seropositive individuals. A pp65-based alphavirus/VRP vaccine has also been developed, using the approach described above for VRP-gB (Reap et al. 2007). Support for a VRP-pp65 vaccine approach was garnered in a recent guinea pig study, in which the guinea pig CMV (GPCMV) homolog of pp65, the GP83 gene product, was studied as a vaccine against congenital GPCMV infection. In this study, the VRP-GP83 vaccine improved pregnancy outcomes and reduced maternal viral load following early third-trimester viral challenge (Schleiss et al. 2007). The VRP-pp65 vaccine has entered phase 1 trials in humans, administered in a trivalent formulation with gB and IE1 (UL123) VRPs. As noted above, pp65 has also been expressed in a DNA vaccine, and is currently being evaluated in a phase II study in BMT recipients, co-administered in a bivalent formulation with gB DNA vaccine.

3.2.3 IE1 Vaccines—Based on the observation that the HCMV IE1 gene is an important target of the CD8+ T-cell response to HCMV infection, with IE1-specific responses being identified in up to 40% of HCMV-seropositive subjects (Slezak et al. 2007), this gene product

is also being evaluated in a number of clinical trials. These studies to date have not involved administration of IE1 vaccine alone, but have consisted of trivalent vaccines that also contain pp65 and gB. As noted, one expression approach that has undergone phase 1 evaluation is that of DNA vaccination. A trivalent DNA vaccine targeting gB, pp65, and IE1 (Vilalta et al. 2005) was evaluated in a Phase 1 trial involving a total of forty healthy adult subjects (24 HCMV-seronegative, 16 HCMV-seropositive). Subjects received a 1 mg or 5 mg dose of trivalent vaccine in several multidose regimens; safety and immunogenicity studies are ongoing (R. Moss, personal communication). IE1 has also been expressed using the alphavirus/VRP approach. A trivalent VRP vaccine, consisting of the IE1 gene product along with gB and pp65, is currently being evaluated in a phase I study (Reap et al. 2007).

4 HCMV Vaccine Approaches in Preclinical Development

4.1 Alternative Expression Strategies for HCMV gB, pp65, and IE1

In addition to the expression strategies outlined above that have made their way into human clinical trials, there are other modes of expression of HCMV subunit vaccine candidates that appear useful in preclinical study. These approaches are summarized in Table 2. One particularly promising approach is based on a recombinant attenuated poxvirus, modified vaccinia virus ‘Ankara’. A recombinant Ankara vaccine has been constructed that expresses a soluble, secreted form of HCMV gB, based on the AD169 strain sequence (Wang et al. 2004). In preclinical studies, high levels of gB-specific neutralizing antibodies, equivalent to those induced by natural HCMV infection, were induced in immunized mice. Recombinant MVA have similarly been generated expressing pp65 and IE1, and are capable of inducing robust cell-mediated immune responses in preclinical studies in mice. A trivalent MVA expressing gB, pp65, and IE1 has been developed and proposed for clinical studies (Wang et al. 2006).

Another vectored vaccine approach that has been pursued in preclinical studies is that of utilization of a recombinant adenovirus vector for expression of HCMV subunit vaccine candidates. The observation that a recombinant adenovirus vaccine expressing the related murine CMV (MCMV) gB demonstrated protection in a mouse model of MCMV disease provides support for further studies of this strategy in human clinical trials (Shanley and Wu 2003).

In addition, HCMV gB has been successfully expressed in transgenic plants (Tackaberry et al. 1999), offering the possibility of a novel vaccination approach through oral/mucosal immunization.

4.2 Potential Role of Other Viral Proteins in HCMV Vaccine Design

As noted, most efforts in clinical trials of candidate subunit HCMV vaccine development and testing have focused on the envelope glycoprotein gB and the T-cell targets, pp65 and IE1. However, a plethora of other HCMV-encoded proteins play key roles in the host immune response and these warrant consideration in future clinical trials. To date, only animal model data are available to validate the potential role of proteins as vaccines. This information is summarized in Table 3.

4.2.1 HCMV Glycoproteins—In addition to gB, other envelope glycoproteins have been considered for vaccine development, although to date no candidates have been tested in human trials. Among the other HCMV glycoproteins, the gcII complex, consisting of gN (UL73) and gM (UL100), is of particular interest. Proteomic analyses of the HCMV virion have demonstrated that gcII is the most abundantly expressed glycoprotein in virus particles, emphasizing its potential importance in protective immunity (Varnum et al. 2004). HCMV

infection elicits a gcII-specific antibody response in a majority of seropositive individuals (Shimamura et al. 2006), and DNA vaccines consisting of gcII antigens gM and gN are able to elicit neutralizing antibody responses in rabbits and mice (Shen et al. 2007).

Constituents of the gcIII complex, consisting of glycoproteins gH (UL75), gO (UL74), and gL (UL115), are also targets of neutralizing antibody responses, and these proteins may also merit consideration in future vaccine studies. Since acquisition of new antibody specificities to gH in the setting of re-infection with a new HCMV is associated with symptomatic congenital transmission in pregnant patients (Boppana et al. 2001) and with adverse outcomes following renal transplantation (Ishibashi et al. 2007), these observations might be important for potential gcIII-based vaccine design. Proof-of-concept has been studied with vaccines based on the MCMV gH homolog using recombinant vaccinia (Rapp et al. 1993) and adenovirus (Shanley and Wu 2005) vectors; of these two approaches, the adenovirus-expressed gH vaccine was more effective as a vaccine against MCMV.

4.2.2 Regulatory/Structural HCMV Proteins Involved in T-cell Response—Most attention on HCMV vaccine candidates that elicit potentially protective T-cell responses has been focused on the pp65 and IE1 gene products. However, recent evaluation of the T-cell responses in HCMV-seropositive individuals identified a plethora of additional, previously unrecognized CD4+ and CD8+ T lymphocyte targets encoded by the HCMV genome. Other potential CD8+ T cell targets, in addition to pp65 and IE1, are just beginning to be investigated. A recently described bioinformatics and *ex vivo* functional T-cell assay approach revealed that CD8+ T cell responses to HCMV often contained multiple antigen-specific reactivities, which were not just constrained to pp65 or IE1 antigens. These studies identified structural, early/late antigens, and HCMV-encoded immunomodulators (pp28, pp50, gH, gB, US2, US3, US6, and UL18) as potential targets for HCMV-specific CD8+ T cell immunity (Elkington et al. 2003). An elegant and comprehensive analysis of T-cell responses to HCMV infection was recently conducted using cytokine flow cytometry in conjunction with overlapping peptides comprising 213 HCMV open reading frames: this study demonstrated that 151 HCMV ORFs were immunogenic for CD4+ and/or CD8+ T cells (Sylwester et al. 2005). Recently, an approach to vaccination has been examined in the MCMV model in which essential, nonstructural proteins that are highly conserved among the CMVs were explored as a novel class of T-cell targets. These studies found that DNA immunization of mice with the murine CMV (MCMV) homologs of HCMV DNA polymerase (M54) or helicase (M105) was protective against virus replication following systemic challenge, and that gamma interferon staining of CD8+ T cells from mice immunized with either the M54 or M105 DNAs showed strong primary responses that recalled rapidly after viral challenge (Morello et al. 2007). These conserved, essential proteins thus may represent a novel class of CD8+ T-cell targets that may contribute to a successful HCMV vaccine.

4.3 Dense Body Vaccines

A novel candidate for vaccination against HCMV currently in preclinical development is the “dense body” vaccine. Dense bodies (DBs) are enveloped, replication-defective particles formed during replication of CMVs in cell culture. These structures are a promising vaccine because they contain both envelope glycoproteins and large quantities of pp65 protein, two key targets of the protective immune response to infection. DBs are non-infectious and therefore, in principle, would be immunogenic, but incapable of establishing latent HCMV infection in the vaccine recipient, providing a useful safety feature. DBs have been shown to be capable of inducing virus neutralizing antibodies and T-cell responses after immunization of mice, including human HLA-A2.K(b) transgenic mice, in the absence of viral gene expression. Based on these studies, DBs may represent a promising, novel approach to the development of a subunit vaccine against HCMV infection (Pepperl et al. 2000).

4.4 Peptide-Based Vaccines

Another potential approach to HCMV vaccination is the use of peptide vaccination employing synthetic peptides comprising immunodominant cytotoxic-T cell epitopes. This approach may ultimately prove to be most useful in the vaccine-mediated prevention of HCMV disease in the transplant setting, where specific peptide vaccine regimens could be tailored for donor-recipient pairs based on HLA genetics. Nasal peptide vaccination with the immunodominant MCMV epitope, YPHFMPTNL, in combination with cholera toxin adjuvant, protected mice against virulent MCMV challenge (Gopal et al. 2005). In a preclinical study relevant to HCMV vaccines, a pp65 human leukocyte antigen (HLA)-A2.1–restricted CTL epitope corresponding to an immunodominant region spanning amino acid residues 495–503, fused to the carboxyl terminus of a pan-DR T-help epitope, was capable of eliciting CTL responses from mice transgenic for the same human HLA molecule (BenMohamed et al. 2000). Since this epitope is highly conserved in clinical isolates, this vaccine could conceivably be broadly protective against multiple HCMV strains. Other peptide-based vaccine studies are envisioned in future clinical trials. A subset of epitopes from a group of important CTL targets has been nominated for inclusion in a polyepitope HCMV vaccine on the basis of human immune responsiveness and population coverage (Khanna and Diamond 2006).

4.5 Novel Vaccine Approaches

Several other vaccination approaches have been proposed for HCMV, and have been validated in varying degrees in animal models. One approach is based on exploitation of viral genomes cloned in *E. coli* as bacterial artificial chromosomes (BACs). Vaccination of mice with bacteria containing the MCMV genome cloned as a BAC conferred protective immunity against subsequent challenge (Cicin-Sain et al. 2003). In guinea pigs, a non-infectious BAC generated by transposon mutagenesis induced immune responses that protected against congenital GPCMV infection and disease (Schleiss et al. 2006). Given the ease of manipulation of BACs using mutagenesis techniques available for *E. coli*, future BAC studies provide the opportunity to generate recombinant, “designer” vaccines with specific genomic deletions or insertions that could modify the immune response or improve the safety profile of the candidate vaccine. Such modified BACs are being employed as immunocontraceptive vaccines for population control in mice (Hardy 2007), although the likelihood of these moving forward in clinical trials is at present unclear.

Another novel strategy that has been validated in the MCMV model and proposed for clinical trial evaluation is a “prime-boost” approach, in which priming with DNA vaccination is followed by boosting with formalin-inactivated viral particles. Mice were immunized with a cocktail of 13 MCMV-containing plasmids followed by boosting with formalin-inactivated, alum-adjuvanted MCMV. This approach elicited high levels of neutralizing antibodies as well as CD8+ T cells specific for the virion-associated antigen (Morello et al. 2002). Subsequent studies examined whether similar protection levels could be achieved by priming with a pool of plasmids encoding MCMV proteins IE1, M84, and gB. This approach was found to elicit CD8+ T lymphocyte responses and, following boost with inactivated MCMV, high levels of virus-neutralizing antibody. Following MCMV challenge, titers of virus were either at or below the detection limits for the salivary glands, liver, and spleen of most immunized animals (Morello et al. 2005). These results support further study of “prime-boost” approaches for vaccination in other animal models, and, potentially, for future clinical trials of HCMV vaccines.

5.0 Perspectives

Although it has been suggested that it is time to consider alternative options for HCMV vaccination, the principle barrier to licensure of a vaccine is not a paucity of candidate vaccines

from which to currently choose. Rather, the major barrier to progress is the lack of knowledge about the public health significance of congenital HCMV infection and the disabilities it produces in children. Increased public awareness about the risks of HCMV is urgently needed: this in turn will drive the social, political and economic forces necessary to increase the pace of progress of clinical trials. Vaccine manufacturers need to increase emphasis on research and development of novel strategies at the same time that clinical trials of existing candidates move forward.

Although it has been asserted that a better understanding of the correlates of protective immunity must be achieved before the goal of a HCMV vaccine can be realized, in fact licensure and implementation of any of the vaccines tested to date would likely impact significantly on disease. The force of HCMV infection is low (Colugnanti et al. 2007) and sterilizing immunity of the maternal-placental-fetal unit is not likely to be necessary for prevention of disease and disability from congenital HCMV infection. Indeed, insights from the GPCMV model of congenital infection suggest that reduction of viral load below a critical threshold, and not sterilizing immunity, is sufficient to ensure improved pregnancy outcomes following vaccination in the setting of maternal viremia. Therefore, the pace of research should be substantially accelerated with existing vaccine candidates, even as research continues to more fully explore the correlates of protective immunity. Although expensive to perform and logistically challenging to conduct, it is imperative that trials be powered to examine symptomatic congenital HCMV infection as an efficacy endpoint. Industry-sponsored clinical trials are currently largely focusing on evaluation of HCMV vaccines in HSC and solid organ transplant patients at high risk for HCMV viremia and disease. While such studies advance the field, negative data from these studies should be interpreted cautiously, and such data cannot automatically be extrapolated toward the problem of prevention of congenital HCMV infection and disease. Industry sponsors, funding agencies, and regulatory bodies must work together to dramatically accelerate the pace of clinical trials for this urgent public health priority.

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Table 1

HCMV vaccines that have undergone evaluation in clinical trials.

CMV Vaccines Evaluated in Clinical Trials	
Live, Attenuated Vaccines	
AD169 Vaccine	• Elicited HCMV-specific antibody responses in seronegative vaccine recipients • Significant injection-site and systemic reactogenicity • No ongoing studies active
Towne (+/− rhIL12)	• Elicits broad-based humoral and cellular immune responses • Favorable safety profile; no evidence for latency or viral shedding in recipients • Lack of efficacy for HCMV infection; reduced HCMV disease in renal transplant recipients • Augmentation of immunogenicity by inclusion of recombinant IL-12 in phase 1 studies
Towne/Toledo Chimera Vaccines	• Favorable safety profile; no evidence for latency or viral shedding in recipients • Attenuated compared to Toledo strain of HCMV • No efficacy data available
Subunit Vaccines	
Glycoprotein B/MF59 Adjuvant (CHO Cell Expression)	• Favorable safety profile • High-titer neutralizing antibody and strong cell- mediated immune responses • Efficacy studies ongoing in young women, adolescents, renal transplant patients
Glycoprotein B/Canarypox Vector	• Favorable safety profile • Suboptimal immunogenicity • “Prime-Boost” effect when administered in combination with Towne vaccine
pp65 (U83)/Canarypox Vector	• Favorable safety profile • Strong antibody and cell-mediated immune responses • No efficacy data available
gB/pp65/IE1 Trivalent DNA Vaccine gB/pp65 Bivalent DNA Vaccine	• DNA vaccine adjuvanted with poloxamer adjuvant and benzalkonium chloride • Phase I studies completed • Phase 2 study ongoing with bivalent gB/pp65 vaccine in HSC transplant recipients
gB/pp65/IE1 Alphavirus Replicon Trivalent Vaccine	• Engineered using replication-deficient alphavirus technology • Generation of virus-like replicon particles (VRPs) • Phase 1 clinical trial recently initiated

Table 2

Alternative subunit vaccine expression strategies proposed for HCMV gB, pp65, and IE1.

Alternative Expression Strategies Proposed for HCMV gB, pp65, and IE1 Vaccines	
Modified Vaccinia Virus Ankara (MVA)	• High-level protein expression • Excellent immunogenicity (humoral and cellular responses) in mice • Ability to express multiple immunogens (bivalent or trivalent vaccines) in single construct • Pre-existing immunity to poxvirus does not limit immune response (utility for vaccinees who have received smallpox vaccine)
Recombinant Adenovirus	• Potential for induction of mucosal immune responses • Replication-deficient adenoviruses available to ensure vector safety • Efficacy in murine model using MCMV gB homolog
Transgenic Plants	• Recombinant HCMV gB successfully expressed in transgenic rice • Offers potential for oral vaccination • Potential for induction of mucosal immune responses • No animal immunogenicity data yet reported

Table 3

Potential novel HCMV vaccine strategies that have been explored in preclinical/animal model studies.

Novel HCMV Vaccine Approaches Currently in Preclinical Models	
gM/gN (gcII Complex)	• Major glycoprotein constituent of virion • Majority of human sera contain anti-gcII antibodies • gM/gN DNA vaccine immunogenic in mice
gH/gL/gO (gcIII Complex)	• Target of neutralizing antibody response in setting of HCMV infection • gH vaccine based on MCMV homolog protective in murine model when expressed as recombinant adenovirus
Essential/Nonstructural Gene Products as Novel CTL Targets	• DNA polymerase (UL54) and helicase (UL105) as novel T-cell targets • Protective in MCMV model when expressed as DNA vaccines
Prime/Boost Strategy	• Prime with cocktail of plasmid DNA vaccines • Boost with formalin-inactivated viral particles • Induces “sterilizing immunity” in MCMV model
Bacterial Artificial Chromosome (BAC) Vaccines	• Protective in MCMV model following delivery in bacteria with reconstitution of virus in vivo • Protective in GPCMV model when administered as replication-disabled DNA vaccine • Offers potential for specifically engineered vaccines
Peptide Vaccines	• Effective in MCMV model following mucosal immunization with cholera toxin • Allows simultaneous immunization against broad range of CTL epitopes: “polyepitope” vaccine • Requires knowledge of HLA status; best suited to HCMV vaccination in transplantation setting?
“Dense Body” Vaccines	• Noninfectious particles enriched for envelope glycoproteins and pp65, major subunit vaccine candidates • Highly immunogenic in animal models • Humoral and cell-mediated immune responses • Can be engineered to express heterologous genes