

## Prospects for Development and Potential Impact of a Vaccine Against Congenital Cytomegalovirus (CMV) Infection

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A recent review made note of the 50th anniversary of the identification of human cytomegalovirus (CMV) in cell culture, an important milestone that paved the way to today's understanding of the molecular biology, immunology, and clinical importance of this ubiquitous viral infection.<sup>1</sup> In the past 50 years, much progress has been made in the characterization of disease caused by CMV. Solid organ and hematopoietic stem cell (HSC) transplant patients have significant problems with CMV disease, including pneumonia, retinitis, and graft rejection, and there are extensive efforts to identify immune correlates of protection in these populations.<sup>2-4</sup> However, the major concern that pediatricians face with respect to CMV disease is the problem of *congenital CMV infection*. CMV is the most common congenital viral infection in the developed world, and disease in newborn infants is associated with mental retardation, neurodevelopmental disability, and sensorineural hearing loss (SNHL). Preconceptual maternal immunity to CMV, though imperfect, provides protection against infection and disability in newborn infants. This knowledge has driven great interest in the development of candidate CMV vaccines that could, in principle, decrease the underrecognized and underappreciated disease burden associated with this disabling infection.

### SCOPE OF THE POTENTIAL IMPACT OF A VACCINE AGAINST CONGENITAL CMV INFECTION

Current estimates suggest that congenital CMV infection occurs in 0.5% to 2% of all deliveries in the United States and Europe.<sup>5,6</sup> Congenital CMV infections can occur in association with either primary maternal infection during pregnancy, or in the setting of reinfection during pregnancy. Primary maternal infection is associated with the highest risk of congenital transmission. Primary CMV infection occurs in up to 2% of CMV-seronegative women during pregnancy, and the virus may be transmitted to the fetus in up to 40% of cases.<sup>7</sup> The risk of primary CMV infection is increased in mothers who have young children attending group day care, with annual acquisition rates of a primary CMV infection ranging from 8% to 20%.<sup>8-10</sup> Preconceptual maternal immunity confers a reduced risk of congenital infection and its attendant sequelae. In a cohort study of approximately 3500 multiparous women from a population with a high risk of congenital CMV infection, naturally acquired immunity resulted in a 69% reduction of congenital CMV transmission,<sup>11</sup> an observation supporting the theoretical benefits of a proposed maternal vaccination strategy. In addition to preconceptual immunity, other factors that impact both the likelihood of infection and severity of sequelae include the timing of maternal infection relative to pregnancy,<sup>12</sup> the avidity index of maternal IgG antibodies to CMV,<sup>13,14</sup> and molecular genotype of the infecting CMV strain.<sup>15</sup>

The disease burden conferred by congenital CMV infection is substantial. A recent report of the National Vaccine Advisory Committee summarized information about the health impact of congenital CMV infections, and emphasized the impact that an effective vaccine could have on CMV-associated disabilities.<sup>16</sup> Among infants born to women with primary infection during pregnancy, 25% will have one or more serious sequelae,<sup>10</sup> including mental retardation, neurodevelopmental disability, and SNHL. CMV is the most common infectious cause of SNHL, and is responsible for more hearing impairment than was *Haemophilus influenzae* type B (Hib) meningitis in the pre-Hib vaccine era.<sup>17</sup> Congenital CMV is responsible for up to one-third of all cases of non-syndromic SNHL in children, and hearing loss may evolve in congenitally infected infants who have asymptomatic infection and normal hearing at birth.<sup>18-21</sup> Overall, it is estimated that up to 15% of congenitally infected infants will have some degree of SNHL demonstrable either at birth or at some point in early childhood, making prevention of this disability one

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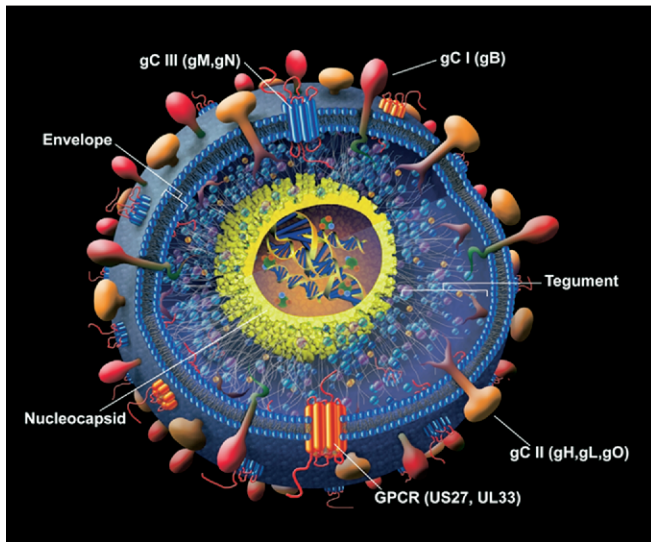
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CMV	Cytomegalovirus	SNHL	Sensorineural hearing loss
HSC	Hematopoietic stem cell		



**Figure.** Schematic representation of the CMV virion with a depiction of potential vaccine targets. CMV is a herpesvirus with an outer envelope containing virally encoded glycoproteins (gC I-III) that are the major targets of host neutralizing antibody responses. The virus also contains an inner nucleocapsid consisting of the viral genome and associated proteins, and a tegument layer consisting of multiple virally encoded proteins, including the phosphoprotein pp65 (the product of the UL83 gene). The pp65 protein is a major target of both CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocyte responses in the setting of infection, and is a leading subunit vaccine candidate. Original image by Andrew Townsend, Extreme Images, and reproduced with permission (Caister Academic Press). Available in color at [www.jpeds.com](http://www.jpeds.com).

of the most important endpoints for vaccine efficacy analyses.<sup>20</sup> In addition to reducing SNHL, an effective preconceptual vaccine also could result in better outcomes for other disabilities caused by congenital CMV.

## THE CMV VIRION: ELUCIDATING THE TARGETS OF PROTECTIVE IMMUNITY

CMV is the largest human herpesvirus, containing a double-stranded DNA genome of ~225 to 230 kbp that encodes approximately 165 genes.<sup>22</sup> Recently a proteomic analysis has been performed of the CMV virion.<sup>23</sup> This information has not only provided important information about the protein content of the viral particle, but also the stoichiometry of viral-encoded proteins within the virion, information that should prove to be of value in future vaccine design. The structure of the CMV virion is represented in the [Figure](#), and key proteins of potential importance in vaccine design are indicated.

Immunity to CMV is complex, and involves humoral and cellular responses. The *humoral immune response to CMV* is dominated by responses to viral *glycoproteins*, present in the outer envelope of the virus particle ([Figure](#)). Of these, the most fully characterized is the gB complex (gB; UL55). All sera from CMV-seropositive individuals contain antibodies to gB, and up to 70% of the neutralizing antibody response in convalescent sera is gB-specific.<sup>24-27</sup> Hence, this protein is a highly attractive candidate for subunit vaccine development.

The gCII complex, consisting of gN (UL73) and gM (UL100), and the gCIII complex, consisting of glycoproteins gH (UL75), gO (UL74), and gL (UL115), are also targets of neutralizing antibody responses,<sup>28-33</sup> and these proteins may merit consideration in future vaccine studies. The *cellular immune response to CMV* includes MHC class II restricted CD4<sup>+</sup> and MHC class I restricted, cytotoxic CD8<sup>+</sup> 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 ([Figure](#)). For eliciting T-lymphocyte responses through vaccination, most attention in vaccine design has been focused on the pp65 protein (UL83), which elicits the majority of CD8<sup>+</sup> T-lymphocyte responses following CMV infection.<sup>34-37</sup> Other proteins that elicit T-lymphocyte responses include the immediate early-1 (IE1) protein (UL123) and pp150 (UL32).<sup>38-41</sup> Recent evaluation of the T-lymphocyte responses in CMV-seropositive individuals identified additional, previously unrecognized CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocyte targets encoded by the CMV genome<sup>42,43</sup> and these may form the basis for future vaccine evaluation.

## CMV VACCINES IN CLINICAL TRIALS

A number of candidate CMV vaccines have been evaluated in clinical trials. These vaccine candidates are summarized in the [Table](#). A wide variety of expression strategies have been used, but generally CMV vaccines can be conceptually subdivided into the categories of *live*, *attenuated vaccines*, and *subunit vaccines* that target individual proteins.

### Live, Attenuated CMV Vaccines

CMV has been the target of live, attenuated vaccine development efforts since the 1970s. The first live CMV vaccine candidate tested in humans was a laboratory-adapted strain referred to as “AD169.”<sup>44</sup> Subsequent trials with another laboratory-adapted clinical isolate, referred to as the “Towne” strain, confirmed that that this vaccine approach could elicit neutralizing antibodies, as well as CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocyte responses.<sup>45-52</sup> The efficacy of this vaccine was tested in a series of studies in kidney transplant recipients, and although Towne failed to prevent CMV infection after transplantation, vaccination did provide a protective impact on CMV disease.<sup>53-56</sup> A placebo-controlled study of Towne vaccine in seronegative mothers who had children attending out-of-home group childcare indicated that immunization failed to protect these women from CMV infection. In this study, pre-existing immunity conferred by natural infection was highly protective against re-infection with new strains of CMV introduced into the family household by toddlers attending these group daycare centers.<sup>46</sup> This study therefore validated the concept that a CMV vaccine that induced immune responses comparable to natural infection could provide protection of a high-risk patient population. One interpretation of these studies was that Towne vaccine may have been overattenuated, compared to clinical strains of CMV causing natural infection.

**Table. Summary of pros and cons of various CMV vaccine strategies that have been tested in human clinical trials. The table indicates the type of vaccine, the expression technology (for subunit vaccines) employed, theoretical benefits and shortcomings, and the current status of the vaccine in ongoing trials**

CMV vaccine	Pros	Cons	Current status
Live, attenuated vaccines	<ul style="list-style-type: none"> <li>Elicits broad-based immunity to multiple CMV proteins</li> </ul>	<ul style="list-style-type: none"> <li>Safety concerns about live-virus vaccines</li> <li>Incomplete understanding of mechanisms of attenuation of virus</li> </ul>	<ul style="list-style-type: none"> <li>AD169: No studies currently active</li> <li>Towne vaccine: Safety and immunogenicity studies ongoing</li> <li>Towne/Toledo chimera vaccines: Phase I study recently completed</li> </ul>
Purified recombinant glycoprotein B (gB)	<ul style="list-style-type: none"> <li>Elicits neutralizing antibody responses</li> <li>Excellent safety profile</li> </ul>	<ul style="list-style-type: none"> <li>Excludes other glycoproteins targets of neutralizing antibody response</li> <li>Excludes other important T-lymphocyte targets</li> <li>Requires MF59 adjuvant to optimize immunogenicity (not licensed in United States)</li> <li>Not highly immunogenic</li> </ul>	<ul style="list-style-type: none"> <li>Efficacy study ongoing in postpartum women of childbearing age</li> <li>Studies active in renal transplant patients</li> <li>Efficacy studies anticipated in adolescents (high risk for primary CMV infection)</li> <li>No clinical studies of canarypox vaccines currently active</li> </ul>
Canarypox and other vectored expression systems	<ul style="list-style-type: none"> <li>Has been used for both CMV gB and pp65 (UL83)</li> <li>Vector does not replicate in mammalian host cells. Excellent safety profile</li> </ul>	<ul style="list-style-type: none"> <li>For gB/canarypox chimera, “prime-boost” approach with Towne vaccine required for optimal immunogenicity</li> <li>No “prime-boost” effect noted when administered with recombinant gB</li> </ul>	<ul style="list-style-type: none"> <li>Related ‘vectored’ approaches undergoing evaluation in preclinical models</li> </ul>
DNA vaccines	<ul style="list-style-type: none"> <li>Ease of expression and purification</li> <li>Elicits strong humoral and cellular responses</li> </ul>	<ul style="list-style-type: none"> <li>DNA vaccines not highly immunogenic in humans</li> <li>Theoretical safety concerns regarding use of DNA vaccines</li> </ul>	<ul style="list-style-type: none"> <li>Efficacy study ongoing in hematopoietic stem cell transplant patients: bivalent DNA vaccine with pp65 (UL83) and gB (glycoprotein B)</li> </ul>

\*Information about the current status of the vaccine is provided, including the industry sponsor/manufacturer.

To explore the possibility of optimizing immunogenicity of a live, attenuated CMV vaccine, MedImmune Vaccines recently constructed a series of genetic recombinants in which regions from the genome of the unattenuated “Toledo” strain of CMV were substituted for the corresponding regions of the Towne genome, toward a goal of constructing one or more Towne/Toledo “chimeras” that contain some, but not all, of the mutations that contribute to Towne vaccine attenuation.<sup>57</sup> Four independent chimeric vaccines were produced and tested in a double-blinded, placebo-controlled trial.<sup>58,59</sup> All of the vaccines were found to be well-tolerated, and none were shed by vaccinees, as assessed by viral culture and PCR analyses of blood and body fluids. Thus, these vaccines appear to be sufficiently attenuated to warrant studies in seronegative individuals. Whether regulatory bodies such as the Food and Drug Administration will be supportive of future clinical trials of these vaccines is a matter open to speculation.

### Subunit CMV Vaccines

The leading subunit CMV vaccine candidate is based on the envelope glycoprotein, gB, due to this protein’s ability

to elicit high-titer, virus-neutralizing antibody responses during natural infection. Other viral proteins being evaluated as subunit vaccine candidates include pp65 and IE1, both of which elicit T-cell responses. The current status of individual subunit vaccine candidates is summarized in the Table and below.

**ADJUVANTED PROTEIN VACCINES.** The formulation of CMV gB currently being used in clinical vaccine trials is a recombinant protein expressed in Chinese hamster ovary (CHO) cells.<sup>60,61</sup> Purified recombinant gB vaccine is manufactured by Sanofi-Pasteur Vaccines, and is undergoing safety, immunogenicity, and efficacy testing in several active clinical trials. The first study of gB vaccine was a phase I, randomized, double-blinded, placebo-controlled trial in adults, in which gB was combined either with a novel adjuvant, MF59, or alum.<sup>62</sup> Levels of gB-specific antibodies and total virus-neutralizing activity after the third dose of vaccine exceeded those observed in CMV-seropositive control subjects. Antigen dose and immunization regimen were further evaluated in a phase I study of 95 CMV-seronegative adult volunteers,<sup>63</sup> and the immunogenicity and safety of recombinant gB has also been studied in toddlers.<sup>64</sup>

The toddler study is significant because young children may represent an ideal population for vaccination, given the ubiquitous nature of CMV transmission within group daycare centers. In all studies to date, the safety profile of the vaccine is favorable, with injection site discomfort being the only significant adverse event observed. There is currently a phase II study of gB/MF59 vaccine ongoing at the University of Alabama, Birmingham.<sup>65</sup> This study is being conducted in young, CMV-seronegative women who are vaccinated post-partum. A valuable aspect of this study is that it will provide safety data regarding the adjuvant, MF59, which is not yet licensed for use in the United States.

**DNA VACCINES.** DNA vaccines elicit robust cellular and humoral immune responses and are well suited to specificity and precision in vaccine design. DNA vaccines have been developed for CMV<sup>66</sup> and have focused on the gB, IE1, and pp65 proteins as the candidate target immunogens. There are currently phase 1 clinical trials underway of both a bivalent CMV DNA vaccine candidate, using plasmid DNA encoding pp65 and gB, and a trivalent vaccine candidate that also includes a third plasmid encoding the IE1 gene product, developed and produced by Vical Vaccines.<sup>67,68</sup> A study is currently ongoing with the bivalent DNA vaccine in an HSC transplant population. Both donors and recipients are being vaccinated, with the goal of reducing CMV disease, viral load, and use of antiviral therapy in the post-transplant period.

**VECTORED VACCINES.** In a 'vectored' vaccine approach, the gene product of interest is expressed in the context of a non-replicating (usually viral) carrier. One example of such a vaccine vector is a canarypox vector known as ALVAC, developed by Virogenetics and Sanofi-Pasteur Vaccines. This vector is an attenuated poxvirus that replicates abortively in mammalian cells. ALVAC expressing CMV gB has been studied in a "prime-boost" approach, in which ALVAC vaccine was administered to "prime" immune responses for subsequent "boost" with either live, attenuated vaccine (Towne strain), or recombinant gB protein vaccine.<sup>69,70</sup> An ALVAC vaccine expressing pp65, the major CD8<sup>+</sup> T-lymphocyte target in naturally CMV seropositive persons, has also been evaluated in human trials. This vaccine was administered to CMV seronegative adult volunteers in a placebo-controlled trial.<sup>71</sup> The ALVAC/pp65 recipients had CMV-specific CD8<sup>+</sup> T-lymphocyte responses at frequencies comparable to those seen in naturally seropositive individuals. Other vectored CMV vaccine expression strategies include an approach based on the modified vaccinia virus Ankara,<sup>72</sup> manufactured at the City of Hope Cancer Center, and a Venezuelan equine encephalitis (VEE) virus vectored vaccine, manufactured by AlphaVax Vaccines. Both approaches show promise in pre-clinical models, and the VEE-vectored vaccine approach provided protection against congenital CMV-associated disease in an animal model of vertical transmission.<sup>73</sup>

## PASSIVE IMMUNIZATION AGAINST CONGENITAL CMV INFECTION

Clinical trials of a *passive immunization* approach designed to target pregnancies at high risk for CMV transmission are also being conducted. In one such study, Nigro et al<sup>74</sup> studied pregnant women with a primary CMV infection. These women were offered intravenous CMV hyperimmune globulin, in two different dose regimens, a "therapy" regimen or a "prevention" regimen. In the therapy group, only 1 of 31 women gave birth to an infant with CMV disease (defined as an infant who was symptomatic at birth and handicapped at 2 or more years of age), compared with 7 of 14 women in an untreated control group. In the prevention group, 6 of 37 women who received hyperimmune globulin during pregnancy had infants with congenital CMV infection, compared with 19 of 47 women who did not receive the high-titer CMV globulin. Overall, the CMV hyperimmune globulin (CMV-IGIV) therapy was associated with a significantly lower risk of congenital CMV infection ( $P = .04$ ). In a follow-up study,<sup>75</sup> ultrasonographic findings that were present in a group of 92 pregnant women with primary CMV infection were compared with those observed in 73 control patients with evidence of preconceptual immunity to CMV. The administration of CMV-IGIV to women in the primary infection group was associated with significant reductions in placental thickness, suggesting that a major component of the CMV-IGIV effect was mediated by protection at the placental level.<sup>76</sup> These studies are encouraging, although uncontrolled. Additional randomized controlled trials of CMV-IGIV are warranted in high-risk pregnancies, to further validate the protective effect of passive immunization.

## FUTURE DIRECTIONS AND MAJOR PRIORITIES FOR CMV VACCINE RESEARCH

One of the barriers hindering progress in the area of vaccination against CMV is the lack of consensus on what should be the *target population* for immunization. If the goal of a vaccination program is to prevent congenital CMV infection, immunization of young women just before entering their child-bearing years would be a rational approach, and would support the concept of evaluating adolescent immunization strategies. Several studies have identified adolescence as a high-risk period for CMV transmission, with reported annual attack rates of over 13%.<sup>77,78</sup> Of particular concern is the potential for acquisition of a primary infection in a pregnant adolescent. In a prospective study of 3253 adolescent women, 1% acquired CMV infection during pregnancy, with a transmission rate to the fetus of 50%.<sup>79</sup> These studies suggest that the adolescent may be an ideal target for implementation of a CMV vaccine program. Consistent with this hypothesis, the Institute of Medicine analyzed the potential economic impact of a hypothetical CMV vaccine that would target the adolescent patient,<sup>80</sup> and concluded that such an approach would represent the single most cost-effective vaccine of any of the candidate infectious disease vaccines cur-

rently in pre-clinical development (excluding HIV vaccines). Whether CMV vaccination should be offered exclusively to young women or instead should be universally incorporated into the routine childhood vaccination schedule remains unclear. The problem of congenital rubella syndrome has been largely solved in the developed world by a universal early childhood vaccination strategy capable of inducing herd immunity to this virus, suggesting that this might similarly be the preferred approach for CMV vaccination.<sup>81</sup> Mathematical modeling has suggested that such an early childhood immunization approach would be a useful strategy for implementation of a CMV vaccine.<sup>82</sup> In this model, universal immunization of infants, particularly emphasizing those attending group day care, would confer broad protection to the population, and decrease the impact of congenital CMV infection on society. As more is learned about the long-term health consequences of CMV infection, which may include atherosclerosis, cancer, and immunosenescence, a case can be made that the benefits of a CMV vaccine could extend to all individuals, not just those who will become pregnant.<sup>83</sup>

The other barrier to progress in testing CMV vaccines is the major need for increased knowledge about the public health significance of congenital CMV infection and the disabilities it produces in children. The major contribution of the Centers for Disease Control<sup>6,84</sup> in increasing public awareness about the risks of CMV should help focus more attention on the problem of congenital CMV infection. An increased awareness of the value of maternal and newborn screening programs is greatly needed,<sup>85-87</sup> and the American College of Obstetrics and Gynecology (ACOG) must be involved in promoting education of physicians who care for women of childbearing age about the importance of CMV in reproductive health. Existing ACOG policies regarding education of pregnant women on good hygienic practices should be more aggressively employed.<sup>88</sup> Industry also needs to increase its emphasis on research and development of preclinical and clinical vaccine studies. Several industry-sponsored studies are currently focusing on clinical evaluation of CMV vaccines in high-risk HSC and solid organ transplant patients at high risk for CMV disease.<sup>2-4</sup> Although such studies advance the field, it is not clear that CMV efficacy studies in this population are applicable to the problem of prevention of congenital infection. Thus, negative data from cancer/oncology vaccine studies should be interpreted cautiously, and such studies cannot substitute for efficacy evaluation of CMV vaccines in women of child-bearing age. Public education is of paramount importance. With better public awareness of the scope of the problem of congenital CMV infection, the necessary economic and social forces will be in place to drive an increased sense of urgency on the immediate need for more aggressive clinical trial testing of CMV vaccines. Through promoting increased awareness of the disabilities caused by congenital CMV infection, pediatricians can take a leading role in serving as advocates for CMV vaccine research. Industry sponsors and regulatory bodies must work together to accelerate the pace of clinical trials for this major unmet need.

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