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REVIEW

The immunological underpinnings of vaccinations to prevent cytomegalovirus disease

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A universal cytomegalovirus (CMV) vaccination promises to reduce the burden of the developmental damage that afflicts up to 0.5% of live births worldwide. An effective vaccination that prevents transplacental transmission would reduce CMV congenital disease and CMV-associated still births and leave populations less susceptible to opportunistic CMV disease. Thus, a vaccination against this virus has long been recognized for the potential of enormous health-care savings because congenital damage is life-long and existing anti-viral options are limited. Vaccine researchers, industry leaders, and regulatory representatives have discussed the challenges posed by clinical efficacy trials that would lead to a universal CMV vaccine, reviewing the links between infection and disease, and identifying settings where disrupting viral transmission might provide a surrogate endpoint for disease prevention. Reducing the complexity of such trials would facilitate vaccine development. Children and adolescents are the targets for universal vaccination, with the expectation of protecting the offspring of immunized women. Given that a majority of females worldwide experience CMV infection during childhood, a universal vaccine must boost natural immunity and reduce transmission due to reactivation and re-infection as well as primary infection during pregnancy. Although current vaccine strategies recognize the value of humoral and cellular immunity, the precise mechanisms that act at the placental interface remain elusive. Immunity resulting from natural infection appears to limit rather than prevent reactivation of latent viruses and susceptibility to re-infection, leaving a challenge for universal vaccination to improve upon natural immunity levels. Despite these hurdles, early phase clinical trials have achieved primary end points in CMV seronegative subjects. Efficacy studies must be expanded to mixed populations of CMV-naive and naturally infected subjects to understand the overall efficacy and potential. Together with CMV vaccine candidates currently in clinical development, additional promising preclinical strategies continue to come forward; however, these face limitations due to the insufficient understanding of host defense mechanisms that prevent transmission, as well as the age-old challenges of reaching the appropriate threshold of immunogenicity, efficacy, durability and potency. This review focuses on the current understanding of natural and CMV vaccine-induced protective immunity.
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EPIDEMIOLOGY AND CONGENITAL DISEASE PATHOGENESIS

Several recent perspectives[1–7] and modeling efforts[8] provide thoughtful background on cytomegalovirus (CMV) transmission *via* mucosal contact with infected body fluids as well as the desirability of a CMV vaccine. The CMV transmission parameters and congenital disease risks are well established,[9–12] even though details of transmission parameters and the worldwide distribution of this disease have only recently come to light.[13,14] Approximately half of the US and EU populations escape CMV infection during childhood,[13,15] leaving approximately half of the population susceptible to primary CMV infection during their childbearing years. Epidemiological evaluations of representative US populations have not identified a single major contributor to efficient CMV transmission,[16] although large family size, day care and frequent exposure to young children (who may be asymptomatic virus shedders for months or years),[17,18] as well as adult sexual

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contact,[19] continue to be the recognized risks. Due to the nature of CMV congenital disease pathogenesis, females are the principle target population for vaccination. Once risk behavior is described, protective measures, such as hand washing, would dramatically reduce child-to-mother transmission.[20] Like other infectious diseases acquired from young children, primary CMV infections are effectively reduced by hand washing (http://www.cdc.gov/CMV/index.html).[21]

Transplacental transmission results in an estimated 40,000 CMV-infected newborns each year in the United States.[13,15] Projections suggest at least a million annual CMV congenital infections worldwide. Hearing, eyesight and IQ compromises have consistently been the most common manifestations of congenital disease. Approximately 25% of infected newborns exhibit sensorineural deficits, with half being evident at birth and half developing these deficits over the first year or so of life. Only a small proportion of CMV-infected newborns (roughly 1/10,000 live births) show classical cytomegalic inclusion disease features, which are characterized by hepatosplenomegaly, thrombocytopenic purpura, microcephaly and sensorineural deficit.[22] Even though CMV is the most common infectious cause of congenital hearing loss in the United States,[15] awareness of this disease remains very low in the general population and among practicing physicians.[23]

Primary CMV infection during pregnancy is associated with an increased risk of transmission to the fetus, while prior natural infection with CMV provides protection from transplacental transmission.[24–26] Primary maternal infection is also more frequently associated with severe congenital disease than disease following reactivation or re-infection.[9–12] The transplacental transmission rates reported for CMV seropositive women (ranging from 0.5% to 2%) are very low compared with the rates for women who first encounter the virus during pregnancy (ranging from 30 to 40%), implicating adaptive immunity in reducing the risk of transplacental CMV transmission. While protective, this natural immunity is incomplete.[27–29] Recent studies in Brazil, where almost all congenital infections occur in infants born to CMV-experienced women,[30] are consistent with a significant worldwide burden of CMV congenital disease due to recurrent infections.[13,14] Recurrent infections are the result of reactivation of resident viruses as well as the acquisition of new virus strains (re-infection or super-infection). Either mechanism clearly reveals the shortcomings in the natural protective immune response to this virus. Given that naturally infected individuals are susceptible to re-infection with additional CMV strains,[24,27–29] and re-infections occur despite the robust cellular immunity[31] known to control this virus,[32] vaccine strategies must reach a level of immunity that blocks transmission in CMV-seropositive populations as well as CMV-naive populations.

When a newborn, child or adult becomes exposed to CMV, typically *via* a mucosal route, initial replication starts at this local site. Systemic infection then follows within susceptible blood monocytes that provide a vehicle for dissemination to the kidney and salivary gland ductal epithelia. Viral pathogenesis steps are incompletely understood, but replication in ductal epithelia contributes to shedding in saliva, urine, breast milk and other body fluids.[22,33] CMV is shed sporadically for life,[22] an outcome that may be related to stress levels based on limited data from astronauts.[34] Active viral replication is strongly suppressed by adaptive immunity, which is primarily metered by CD4 and CD8 T cells.[35,36] Following adaptive immune control, CMV maintains a latent reservoir within bone marrow-derived progenitors of blood monocytes.[22,37] CMV rarely causes disease in the immunocompetent host even in situations such as trauma, which leads to systemic reactivation.[38] Defects in T-cell host defense mechanisms are associated with life-threatening CMV disease, making CMV one of the most significant opportunistic diseases in immunocompromised individuals. Immunosuppression promotes the reactivation of latent viruses, persistent shedding and end-organ disease.[35,36] Individuals with defects in humoral immunity do not succumb to CMV disease, suggesting that antibodies play a supportive role in the normal host.

Transmission to the fetus across the placenta follows blood flow.[39] The humoral and cellular immune mechanisms that engage CMV at the maternal–fetal interface remain to be fully elaborated, although both are likely to contribute. These immune correlates remain difficult to evaluate because the proportion of CMV seropositive pregnant women who develop an active infection sufficient to transmit the virus is unknown. It seems that the process of pregnancy may contribute to an altered immune status that is associated with recurrent infection[40] and likely facilitates transmission. In settings where transmission follows transplantation, CMV is carried by the organ or tissue and viremia levels in the recipient predict the likelihood of disease. Recurrent infections in CMV seropositive women are not associated with detectable viremia despite occasional transmission to the fetus. CMV DNA has been readily detected in placentas from CMV seropositive women,[39,41,42] suggesting that more comprehensive studies may uncover the mechanisms of innate as well as adaptive host defense at the maternal–fetal interface. Once virus transmission occurs, disease pathogenesis follows from the infection of sensory neurons in the brain and cochlea,[43,44] with consequent damage most commonly manifesting as compromised sensorineural hearing and eyesight.[22]

The lack of a universally accepted clinical definition of CMV congenital disease adds to the difficulty in summarizing disease pathogenesis. CMV infection diagnoses *in utero* have been undertaken; however, they remain risky and have not gained widespread acceptance. Thus, clinical CMV disease diagnoses *in utero* by imaging methods commonly applied during pregnancy remain controversial. Viral load quantification at the time of birth provides unambiguous evidence that transplacental transmission has occurred, which is the defining predisposing attribute of CMV congenital disease. CMV acquisition *in utero* is readily diagnosed within the first two weeks of life by measuring virus or viral DNA levels in the bloodstream, saliva or urine. High viral indicator levels (e.g., virus, DNA or antigens) in the blood are a guide to disease severity in

immunocompromised patients and provide invaluable guides to therapeutic intervention.[22] In the case of congenital disease, however, viral DNA levels in the fetal or newborn blood have been controversial as disease risk predictors, although this is a promising area for further study in combination with fetal immune response parameters.[45,46] Primary or recurrent maternal infections contribute to the sensorineural hearing damage incidence.[47,48] Phase III clinical vaccine evaluations are expected to shed light on specific mechanisms that contribute to protection from transmission and disease. Regardless of the range of clinical disease manifestations, a successful universal CMV vaccine will need to address the risks of CMV transplacental transmission in both CMV seropositive and CMV-naive populations.

CMV INFECTION AND DISEASE PREVENTION

Hand washing is important in preventing infection[21] and may also reduce re-infection.[30,49,50] Clearly, hand washing would not be expected to prevent congenital diseases that follow a pattern of reactivation or long-term infections with multiple virus strains that reactivate independently during pregnancy. Antiviral chemotherapy with ganciclovir in newborns provides a benefit in severe cytomegalic inclusion disease; however, it does not reverse damage or impact progressive diseases, such as hearing loss.[51,52] Poor drug penetration into the CNS may underlie this failure, but is also consistent with immunopathogenesis independent of persistent virus. Regardless, ganciclovir is considered too toxic to risk long-term treatment of infants with modest disease levels.

Immune clearance at the maternal–fetal interface is apparently bolstered by pooled human gammaglobulin, selected to have high levels of CMV-specific binding antibodies (CMV-IVIG) and administered to pregnant women diagnosed with CMV-damaged fetuses. Early observations indicated that CMV-IVIG both prevents and ameliorates cytomegalic inclusion disease,[53] outcomes that have been associated with improvements in newborns and placental health.[54] Without an untreated control group, a comparison of low and high titer CMV-IVIG, or further mechanistic insight into the role of antibodies, the success remains enigmatic even if attractive.[55] This has been further complicated by a recent randomized trial that trended in the same direction but failed to reach statistical significance.[56] Much remains to be learned about the control of CMV congenital disease by immune mechanisms that act at the maternal–fetal interface or in the fetus.[57] Optimism for CMV-IVIG studies[55] has rekindled efforts to intervene in congenital disease with defined CMV-specific neutralizing antibodies.[58,59] Several candidates are in preclinical development. It remains as challenging to predict mechanisms of antibody immunoprophylaxis as it is for selecting CMV vaccine candidates to take into clinical development.[60]

A reasonable expectation of vaccination is to provide immunity sufficient to reduce transplacental transmission rather than to induce immunity in the developing fetus or to prevent primary infections in women. With the worldwide transmission of CMV most often occurring during childhood, a CMV vaccine administered well before the childbearing years would achieve the most benefit. Because neutralizing antibody and cell-mediated immunity have been associated with modest protection during pregnancy,[61–63] vaccines that induce both arms of the adaptive immune response are of interest. Vaccine strategies most proficient at this dual challenge would be expected to have the greatest promise and commercial interest; however, this has really not yet been put to the test. In studies of primary maternal infection, anti-gH pentamer antibody responses as well as antiviral CD4 and CD8 T-cell responses develop more slowly in a subset of women who transmit virus to their offspring. Although limited, these data suggest that the presence of anti-CMV immunity matters more than any particular mechanism. Late-gestation fetal immunity may and newborn immunity does contribute to the host defenses that prevent disease pathogenesis. This area is sketchy even though response parameters have been documented.[64–70] Fetal immunity late in gestation or in the newborn occurs at times when susceptibility to congenital disease declines, although more needs to be learned from studies that consider the contribution of overall development relative to immunological development for this to decline. Transmission takes place throughout pregnancy, with the highest frequency occurring during the third trimester,[22] which is coincident with the development of fetal immunocompetence. Because the highest risk of disease occurs with transmission in the first trimester when the fetus has not yet developed the capacity to mount either innate or adaptive immunity, maternal immune mechanisms must predominate in the control of transmission early in pregnancy. This adds to the evidence that women are the appropriate target for vaccine strategies and the proposal that preventing transplacental transmission will prevent congenital disease. Thus, vaccination with a goal of mimicking the benefit of natural CMV infection-induced cellular and humoral immunity must drive comparable humoral (i.e., neutralizing antibody) and cellular (i.e., cytotoxic T cell (CTL)) immune responses. Given that natural immunity to CMV is complicated and the virus manipulates the immune system at many levels, the precise adaptive immune mechanism(s) that protect from transplacental transmission may only become clear with the successful completion of phase III vaccine trials when thorough analyses of specific contributions of neutralizing antibodies, CTLs, and other mechanisms (e.g., antibody dependent cellular cytotoxicity) can be addressed.

Due to the low force of infection combined with benefits of herd immunity, a universal vaccine with 50% efficacy might be sufficient to interrupt patterns of person-to-person CMV transmission.[2] This, along with well-established societal health and economic benefits,[71] make a CMV vaccine highly attractive. Such a vaccine would be expected to enter the vaccine schedule during the childhood years or in pre-teens where the highly effective human papillomavirus vaccine,[72,73] a virus-like particle (VLP) vaccine, has had remarkable success. A vaccine strategy combining a neutralizing antibody target, soluble CMV envelope glycoprotein B (gB), in a stable oil-in-water

emulsion (MF59) adjuvant completed a phase II trial, which showed protection in half of the subjects, manifesting as reduced acquisition of CMV during pregnancy.[74] This trial, more than any other, supports the continued evaluation of a viral subunit antigen vaccine, despite the long list of challenges that have confronted CMV vaccine development.

CMV-SPECIFIC PROTECTIVE IMMUNE RESPONSE PARAMETERS

The vaccine challenge is to identify the CMV-specific immune response parameters that prevent transplacental transmission in pregnant women undergoing primary infection, reactivation or re-infection. Observational studies of natural immune responses as well as natural protective mechanisms against CMV in the immune-suppressed host will inform this presentation. CMV remains an important opportunistic pathogen in patients receiving a hematopoietic cell transplant (HCT) or a solid organ transplant (SOT) where protective immunity correlates have received more extensive evaluation. CMV seropositive solid organ donors are likely to transmit viruses to any recipient independent of their CMV serostatus; however, the resultant viral levels and disease are highly dependent on the status of the adaptive cellular CD4 and CD8 T-cell responses to the virus.[22] Importantly, CMV infection and clinical disease are distinct, and protection from disease does not require protection from infection. Reduced viral levels tend to correlate with clinical benefit. On the one hand, adoptive transfer of CMV antigen-specific T cells has been a long-recognized, though costly, approach in HCT recipients.[75] Control of CMV in SOT also depends on effective cellular immunity. On the other hand, CMV-specific antibodies delivered by hyper-immune globulin have little or no independent impact in this particular transplant setting.[76] Translating these observations to congenital infection is difficult because of the unique features of the maternal–fetal interface, where antibodies may be sufficient to impact transplacental infections as well as their symptoms.[58,63] Naturally, the contribution of maternal cellular immunity cannot be discounted.[62,64]

Broad CMV-specific antibody responses develop during primary infection and accompany recurrence.[27,30,77,78] The development of CMV-neutralizing antibodies and viral epitope-specific antibodies has been used to distinguish settings that support or prevent CMV infection of the fetus.[79,80] Antibodies that target envelope gB, gH/gL, gH/gL/gO, gH/gL/UL128/UL130/UL131A (gH pentamer) and gM/gN complexes all show neutralization potential against the virus and block infection of cultured cells.[79,81–83] The distinct role of the gH pentamer complex in promoting attachment or entry into epithelial and endothelial cells known to be involved in CMV pathogenesis has raised interest and focus on this complex. One report,[84] in particular, implicated gH pentamer-specific antibodies over other CMV glycoprotein-specific antibodies in the neutralizing potential of CMV-IVIG. Technical limitations of the study complicate interpretation of the simple comparisons described. Any clinical benefit of CMV-IVIG[53] may come from an antibody effector function, such as neutralization, another gH pentamer-specific antibody activity or another benefit provided by gammaglobulin. Given that CMV-IVIG preparations are qualified based on antibodies that bind to CMV antigen rather than in a biological assay, there has been no way to evaluate or control batch-to-batch variation or standardize for qualities known to protect the fetus. The fact that a randomized controlled clinical evaluation that assessed the benefit of CMV-IVIG in preventing transmission did not find any clear benefit, makes it difficult to rely on an independent role of antibodies.[56] CMV-IVIG failed to reduce viral loads, alter clinical course or reduce obstetrical complications. Although CMV-IVIG represents large plasma pheresis pools used in the collection of gammaglobulin, methods to evaluate lot-to-lot variability must be deployed more effectively. Additional immunological studies of CMV-IVIG may provide greater insight into the path forward and the comparisons to undertake in the clinic.

Preclinical cell culture models continue to provide evidence that antibodies prevent infection,[85,86] which is an important backdrop to vaccine strategies aiming to mimic or improve on natural immunity. Specifically, the predominance of gH pentamer-specific antibodies in immune sera combined with a broader understanding of cell types that support initial infection, dissemination and transplacental transmission have spawned efforts to improve the response directed at the gH pentamer complex as well as efforts to improve gB-specific antibody titers to more efficiently neutralize viruses in epithelial and endothelial cells. Given that CMV-IVIG approaches to prevent congenital disease [53–55,87,88] have been driven by clinical impact rather than mechanism and that, when administered, CMV-IVIG reaches sites of infection in the woman as well as the fetus, it remains impossible to ascribe any impact to a particular step in transmission or disease pathogenesis. This area will be facilitated as therapeutic monoclonal antibody approaches reach the clinic.

The natural cellular immune response to CMV is broad, intense and protective. Profiling of cellular responses provides an important backdrop, especially for evaluating immunogenicity of vaccine candidates. A large proportion of CMV-encoded proteins are immunogenic and drive readily assessed virus-specific $CD4^+$ and $CD8^+$ T-cell responses[31] that are sustained for life in naturally infected individuals. Indeed, T-cell immunity intensifies with time over the course of life.[89] A hierarchy of viral proteins encoded by UL55 (gB), UL83 (pp65), UL122 (IE2), UL48, UL32 (pp150), UL123 (IE1), UL99 (pp28) and UL82 (pp71) drive the high-frequency antigen-specific CD8 T-cell responses that maintain control of natural infection. The reason for such a broad response and the relative protective benefit of this response are not known, although the pattern suggests frequent boosting through natural reactivation or re-infection. The breadth of responses required for protection by CD8-derived CTLs has not been sufficiently investigated in clinical settings. Notably, however, early reports showing clinical benefits of CMV epitope-specific CD8 T cells in immunocompromised HCT recipients[32,90] changed thinking regarding

transplants. In this setting, CD8 CMV-specific T cells, where immunodominance is determined by pp150 or pp65 epitopes, together with CD4 T cells, provide immune-mediated protection from CMV disease.

In pregnant women, natural primary infection is characterized by the emergence of antigen-specific $CD4^+$, $CD8^+$ and $CD45RA^+$ effector memory T cells,[62,91] which is a type of immune response that is associated with viral replication control. If these studies are predictive, viral transmission to the fetus may be more likely to occur when CMV-specific $CD4^+$ and possibly $CD8^+$ T-cell responses are delayed during primary infections or sub-optimal in women who are already infected. Nevertheless, the emergence of $CD45RA^+$ effector memory may be associated with control of viremia and prevention of transplacental transmission. These observations suggest that cellular immunity in women provides a level of protection for the fetus by reducing viral load and transplacental transmission risk, which follow the principles that emerged long ago in HCT transplantation.[32,90] Only further observational studies on the immune parameters that dictate steps in transplacental transmission will flesh this area out.

CMV VACCINE STRATEGIES

CMV congenital disease vaccine advances and strategies have been recently reviewed from a wide variety of perspectives, including academic[1,5,7,35,92–94] and commercial[4,95] research groups from major institutions around the world. One human herpesvirus, varicella zoster virus, is controlled by universal vaccination. Two different formulations, one (Varivax) to prevent chickenpox in children and another (Zostavax) to prevent shingles in the elderly, are available. Both include viral antigens that accumulate in infected cells as well as attenuated virus, a design reminiscent of effective veterinary vaccines used to prevent herpesvirus diseases in domestic animals. The Towne and AD169 CMV strains, attenuated by serial propagation, showed excellent safety and some efficacy in clinical trials,[28,29,96–101] as described in detail by Schleiss and Heineman.[102] Experience with AD169 is limited; however, experience with the Towne live-attenuated CMV vaccine (LACV) provided important information and perspective on efficacy. Recent revelations regarding high levels of CMV genome variation within infected individuals,[103,104] frequent recurrence and transmission in largely CMV seropositive populations [14,30,49,50]and co-infection frequency,[105] all present direct confounders to vaccination with any live attenuated CMV. After all, vaccination seeks to mimic (or exceed) the protection afforded by natural infection and depends on a set of conserved antigens that are represented in circulating strains.

LIVE ATTENUATED VIRUS VACCINE

Thirty years ago, the Towne LACV set a hallmark for efficacy, whereby the vaccination prevented severe CMV disease in kidney transplant recipients.[98] Since then, the availability of antiviral therapy, however imprecise and damaging itself, eclipsed the potential benefit of LACV vaccination in SOT recipients. The current perception is that a live vaccine strain, however attenuated, would pose some risk to immunocompromised patients. At about the same time, the Towne LACV was shown to be deficient for reactivation in immunosuppressed SOT recipients, a pattern that differs from frequent reactivation of natural CMV infections in such settings.[96] Inadequate efficacy of the Towne LACV in healthy women of childbearing age,[29] however, together with the demonstration that the Towne LACV had been over-attenuated by acquisition of large deletions[106] prompted interest in alternate LACVs. These have included attempts by Aviron (now MedImmune/AstraZeneca) to 'dial up' the Towne strain by swapping genome segments from a less-attenuated strain (Toledo, used as challenge virus in Towne-LACV studies[28]), in an effort to add back genes that the LACV stocks had lost on passage. This 'dial-up' effort has progressed slowly over the past twenty years due to safety concerns raised by experts in the field during an FDA review in 1999. This, combined with the inability of Towne-Toledo chimeras to boost CMV immunity in seropositive vaccines,[107] resulted in termination of clinical development by MedImmune. The evaluation of the chimeras is now in an investigator-initiated phase I study to evaluate safety and immunogenicity properties in CMV-naive subjects. A different 'dial-up' strategy seeks to improve the AD169 LACV by repairing mutations that prevent expression of the gH pentamer complex,[108,109] with the intention of broadening cell tropism and the quality of neutralizing antibody responses. One challenge is the loss of gH pentamer expression during propagation in fibroblasts,[110,111] so the LACV candidate V160 must be propagated on the ARPE19[109,112] epithelial cell line. The V160 LACV shows promise and induces pentamer-specific responses in rabbits and rhesus macaques[113,114] in preclinical studies that support an ongoing phase I evaluation.

The rhesus macaque CMV strain 68.1 has proven safe and effective as a vaccine vector, stimulating interest and providing information on primate CMV determinants that control the super-infection potential and induction of novel MHC class II restricted CTL responses.[115] In this model, expression of the gH pentamer-like complex undermines the vaccine immunogenicity while broadening cell tropism.[116] The ability to super-infect is dependent on viral down modulation of MHC class I expression.[117] Natural immunity to CMV in rhesus macaques does not afford protection to this vector, whereas natural immunity to CMV in humans provides dose-dependent resistance to super-infection with the Toledo strain,[28] a virus that lacks the gH pentamer complex but sustains MHC class I down-regulation. Indeed, the ability of a low-dose challenge with Toledo to super-infect Towne LACV-vaccinated individuals, but not naturally immune individuals, is generally considered a shortcoming in the Towne LACV vaccine. Despite being constructed to carry representative segments of the Toledo genome, Towne–Toledo chimeras do not exhibit a Toledo-like super-infection quality in CMV seropositive individuals even at a relatively high dose.[107] Given the apparent inability of natural immunity to protect from or be boosted by exposure to the vector,[118] together with differences in gene function and

pathogenesis,[119] there really is a need for additional evaluation of LACV candidates in humans.

CMV VACCINE EXPERIENCES—SUBUNIT, DNA AND NON-LIVE CMV VACCINE STRATEGIES

Non-live approaches to CMV vaccination have focused on the benefit of neutralizing antibodies directed at the major envelope glycoprotein B (gB) or the more recently characterized gH pentamer complex. Humoral immune targets have been pursued alone as well as in combination with cellular immune targets, predominantly immediate early (IE)1 protein and virion tegument protein, pp65.[31] A combination of gB, IE1 and pp65 provides broad major histocompatibility-mediated antigen presentation covering the diverse human population. Substitution of gH for gB would also provide coverage, though not as much as gB.[31] Whether delivered as subunit, DNA, VLP or other non-live CMV vaccine formats, this subset of viral antigens has been viewed as promising, and several human phase I and II trials have succeeded in eliciting immune responses to some combination of gB, IE1 and/or pp65.

The subunit/adjuvant vaccine strategy developed at Chiron (and licensed to Sanofi Pasteur by Novartis) progressed through phase II trials with clinical efficacy in CMV-naive women[120] as well as in both CMV-naive and seropositive SOT recipients.[121] This subunit vaccine is composed of gB, which was produced as a secreted, soluble derivative in Chinese hamster ovary cells, combined with an oil-in-water emulsion, called MF59,and is currently under development at Sanofi Pasteur. In Europe, the MF59 adjuvant has been included in a Novartis influenza vaccine where its immune properties and safety profile have been characterized.[122] A three-dose regimen of a CMV gB/MF59 vaccine prevented CMV acquisition in 50% of CMV-naive women,[120] an impressive level of protection from infection that validates gB as an important immune target. Infection prevention is short-lived, whereby the durability of only slightly more than one year following the last dose of vaccine suggests that a more potent adjuvant or formulation may be required. This gB/MF59 vaccine also successfully reduced viral load in SOT recipients[121] where reduced viremia and increased gB antibody titers were shown as control correlates. The vaccine was effective in recipients of CMV-positive organs whether they were CMV seropositive or seronegative, consistent with a potential to prime as well as to boost responses. Another soluble gB vaccine, formulated by GlaxoSmithKline to include a TLR4 agonist adjuvant, has undergone phase I evaluation (NCT00435396 and NCT01357915) with encouraging results (A. Marchant, personal communication).

DNA plasmid vaccines have held promise to induce CD8 T-cell immunity in addition to humoral immunity. Clinical trials have evaluated a combination of gB and pp65 or gB, pp65 and IE1 in an immunopotentiating formulation. A phase II trial of the bivalent vaccine by Vical/Astellas reduced CMT reactivation in HCT recipients at risk of serious CMV disease.[123] This strategy is continuing in a phase III study that seeks to protect HCT recipients from markers of CMV disease progression. Clinical evaluations may be extended to other settings, such as SOT, where CMV levels are regularly monitored and the virus is an opportunist. Although DNA vaccines have shown limited promise in clinical studies targeting other pathogens, the studies focused on CMV promise to validate particular antigens that may also be relevant to vaccine control of congenital disease.

Several additional non-live CMV vaccine candidates have been in clinical trials, including different replication-defective poxvirus, adenovirus, and alphavirus vectors,[2] all with limited success. The best developed of these is a dual alphavirus replicon that expresses the key viral antigen gB plus a IE1–pp65 fusion protein that has been evaluated in phase I safety and immunogenicity studies by AlphaVax[124–126] and was acquired by Novartis. This vaccine platform has been harnessed to express the gH pentamer complex, which has shown promising preclinical immunogenicity characteristics.[127,128] This platform may be able to bring the gH pentamer complex together with antigens that stimulate cellular immunity together in a phase I trial.

There is clearly a high level of interest in strategies to prevent infection of epithelial and other cell types where the gH pentamer affords entry.[114,129] Antigens gH and the other components of the pentameric complex are conserved across CMV strains. gB is also highly conserved in CMV strains and is a crucial viral fusion protein necessary for entry into all cell types.[22] Although the gH pentamer has attracted recent interest, gB has long been known to drive a broad neutralizing antibody response in humans recognizing well-defined linear as well as conformational epitopes,[81,130] spawning strategies to better harness the response to gB.[131–133] Despite positive results in clinical trials,[120] there remains the question of whether gB-induced antibodies are able to efficiently neutralize the virus in all biologically relevant cell types. Recently, two preclinical vaccine strategies, each devoid of the gH pentamer, reported the induction of antibodies in mice that can prevent epithelial cell infection. VLP-like capsidless particles, called dense bodies, derived from the Towne LACV, were shown to induce gB-dependent neutralizing antibodies sufficient to block infection of epithelial and fibroblast cells[134] and a specifically engineered gB-containing VLP was shown to induce neutralizing antibodies with equivalent activity on epithelial or fibroblast cells.[135] These results provide a reminder that the fashion in which viral glycoproteins are presented to the immune system may have a strong impact on the results.

SUMMARY

Given the widespread interest in immunoprophylaxis to prevent CMV disease, there has been an insufficient level of objective research into the immune mechanisms governing transplacental transmission of CMV during pregnancy in humans. Vaccination efforts have been slow to acknowledge the importance of recurrent infections due to reactivation or re-infection in the pathogenesis of CMV congenital disease. The goal of controlling primary infection in women must be balanced with a goal of boosting immunity to prevent recurrence. In order for

vaccination to become a reality, greater emphasis must be invested in the generation and interpretation of clinical data. Natural immunity is effective at reducing super-infection, but a significant proportion of CMV seropositive women nevertheless transmit CMV to their offspring. To eliminate the scourge of CMV congenital disease, research and vaccine strategies will need to focus on identifying effective prime and boost approaches. Trials to date suggest that the simplest of CMV vaccine designs may prevent infection, reduce viral amplification and subdue dissemination in a pattern that would tilt the balance and protect the developing fetus. The challenge is to establish effective and durable strategies to prime immunity in CMV seronegative individuals while also achieving a clinical benefit by boosting immunity in CMV seropositive women. A deeper understanding of immune mechanisms that function specifically at the placental interface is warranted. Given the worldwide impact of congenital CMV disease in children of mothers who were already infected with CMV prior to pregnancy, adaptive immune memory acting at the placental interface remains a significant research question whose answer will provide a cornerstone for a universal vaccine.

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