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Synthetic DNA Approach to Cytomegalovirus Vaccine/Immune Therapy

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Abstract

There is no licensed vaccine or cure for human cytomegalovirus (CMV), a ubiquitous β-herpes virus that infects 60–95 % of adults worldwide. Infection is a major cause of congenital abnormalities in newborns, contributes to development of childhood cerebral palsy and medulloblastoma, can result in severe disease in immunocompromised patients, and is a major impediment during successful organ transplantation. While CMV has been increasingly associated with numerous inflammatory diseases and cancers, only recently has it been correlated with increased risk of heart disease in adults, the number-one killer in the USA. These data, among others, suggest that subclinical CMV infection, or microinfection, in healthy individuals may play more of a causative role than an epiphenomenon in development of CMV-associated pathologies. Due to the myriad of diseases and complications associated with CMV, an efficacious vaccine would be highly valuable in reducing human morbidity and mortality as well as saving billions of dollars in annual health-care costs and disability adjusted life years (DALY) in the developing world. Therefore, the development of a safe efficacious CMV vaccine or immune therapy is paramount to the public health. This review aims to provide a brief overview on aspects of CMV infection and disease and focuses on current vaccine strategies. The use of new synthetic DNA vaccines might offer one such approach to this difficult problem.

Introduction

Human cytomegalovirus (CMV) is a ubiquitous β-human herpes virus, also known as human herpes virus type 5, with broad clinical implications in both the developing and developed world. It is the largest member of the human herpes viruses with a linear, double-stranded DNA genome of ~230 kbp coding for 200–250 open reading frames (ORF)s [1]. It is highly seroprevalent in the human population and establishes lifelong latency within the host with periodic reactivation. Reports of seropositivity in the USA range from 36.3 % in 6–11 year old children to 90.8 % in those aged ≥80 years [2]. Worldwide annual seroconversion rates among pregnant women and health-care workers were found to be around 2.3 % and 8.5 %, respectively [3]. CMV is transmitted primarily via saliva, placental transfer, breast-feeding, blood transfusion, sexual contact, solid-organ transplantation (SOT), or hematopoietic stem cell transplantation (HSCT) [4]. While acquired CMV infection is asymptomatic in the vast majority of immunocompetent hosts, the consequences of infection in fetuses and immunocompromised patients make CMV an important public health concern [5].

Furthermore, infection is a major impediment to successful organ transplantation [6–8]. Despite over 50 years of clinical research, there is no vaccine or cure available.

Overt Danger: CMV Infection and Its Burden to Public Health

CMV is estimated to infect 60–95 % of adults worldwide. The most common overt CMV-related disease is congenital CMV, which is a major cause of neurological and sensory impairments in children [9]. Newborns may develop cytomegalic inclusion body disease, a severe disease characterized by jaundice, petechiae, hepatosplenomegaly, microcephaly, motor disability, chorioretinitis, cerebral calcification, and multiple organ involvement [10]. Permanent physical sequelae include microcephaly, hearing loss, vision loss, and mental retardation. Furthermore, there is evidence that intrauterine CMV infection is significantly associated with cerebral palsy [11]. Between 20,000 and 40,000 children are born with congenital CMV infections in the USA each year, resulting in 100–200 deaths and 4,000–8,000 individuals developing permanent neurological sequelae [12, 13]. Sensorineural hearing loss is the most common symptom of CMV infection, occurring in 10–15 % of symptomatic children.

Immunocompromised adults including AIDS and transplant patients are also at major risk for CMV disease. In AIDS patients, viral disease is most commonly manifested as retinitis during which CMV causes a complete-thickness infection of retinal cells. If left without treatment, this infection results in subacute progressive retinal destruction and permanent blindness [14]. CMV disease can also less commonly involve other organ systems, including the central nervous system (resulting in polyradiculopathy and ventriculoencephalitis) and the respiratory system (causing pneumonitis) [13].

Along with the potential for significant morbidity and mortality, CMV disease, in addition to medical consequences, also places an extraordinarily high economic burden on the US health-care system. The economic burden of congenital disease alone exceeds $2 billion annually in the USA [12]. In liver transplant recipients, CMV disease is associated with a roughly 49 % increase in medical charges [15]. Congenital CMV is a significant contributor to the lifetime costs associated with mental retardation, hearing loss and vision impairment, estimated to be $51.2 billion, $2.1 billion, and $2.5 billion respectively [16]. A severely affected, CMV-infected child has been estimated to have additional lifetime health-care costs of ~1 million dollars [17]. All told, overt CMV disease is estimated to cost the US health system at least $4 billion annually [18]. Therefore, CMV morbidity and mortality among immune-compromised patients (such as those infected with HIV), solid-organ and HSCT patients, as well as fetuses and newborns, calls for the development of an efficacious vaccine to combat this infectious disease.

CMV Microinfection: The Silent Threat

While it was widely held that latent or asymptomatic CMV infection was virtually benign in healthy individuals, it has now become increasingly clear that subclinical infection with CMV may play a greater role in a variety of diseases. This low-grade “microinfection” has been only recently detectable through the use of advanced techniques [19, 20] and has been implicated as a causative factor rather than an epiphenomenon in certain cancers,

inflammatory, and hypertensive and pulmonary diseases [20–24]. This may be due to CMV's polytrophic nature, large proteome and immunomodulatory activity, allowing CMV to exert significant effects in a variety of organ systems. Recently, CMV microinfection has been correlated to increased risk of essential hypertension. Through quantitative reverse-transcription polymerase chain reaction, Li et al. have identified the presence of CMV microRNA in individuals with hypertension, finding a significant correlation between the presence of CMV DNA and diagnosed hypertension [20]. Essential hypertension is a prevalent risk factor for a variety of cardiovascular diseases including stroke, coronary heart disease and renal and heart failure, affecting >1 billion adults worldwide.

Low-grade CMV infection has also recently been associated with various forms of cancer, including medulloblastoma [24], colon cancer, malignant glioblastoma, EBV-negative Hodgkin's lymphoma, prostatic carcinoma, and breast cancer [25]. In medulloblastomas, which are the most frequent malignant brain tumors in children, inhibition of CMV replication activity with the antiviral drug valganciclovir was reported to reduce tumor growth both in vitro and in vivo [24]. The molecular basis for such oncogenesis and "oncomodulation" has been described in broad terms. Several CMV-encoded gene products have been shown to control cellular pathways that may be involved in oncogenesis, including cellular differentiation, cell cycle regulation, DNA damage and repair, epigenetic functions, apoptosis, cellular migration, angiogenesis, and immune evasion [26].

CMV microinfections have also been implicated in a number of inflammatory diseases. Studies have found that approximately 90 % of patients with inflammatory bowel diseases have an active CMV infection in their bowel [27]. While infected cells were rare, they were present in the deep mucosa of the bowel and only in inflamed areas. CMV reactivation has also been seen in the inflamed, but not non-inflamed, tissues of patients with rheumatoid arthritis (RA), Sjögren's syndrome, dermatomyositis and polymyositis, psoriasis, Wegener's granulomatosis, ulcerative colitis, and Crohn's disease [25]. These viral microinfections were thus invariably associated with disease pathology and inflammation. This association may in part be the result of elicitation of $CD4^+CD28^-$ T cell populations, which have only been described in CMV-infected individuals. In patients with RA, only CMV-seropositive patients, which constitute the majority of all RA patients, carried $CD4^+CD28^-$ T cells. These $CD28^-$ T cells were found to be enriched in RA patients, as well as in patients with dermatomyositis and polymyositis, but found in a lower frequency in healthy CMV-seropositive controls [28–30]. Indeed, in patients with myositis, 60–90 % of all infiltrating T cells in inflamed muscle were $CD28^-$. These T cells responded in vitro to CMV antigen (Ag) stimulation, suggesting that CMV may drive the accumulation of such $CD28^-$ T cells in inflamed tissues during the course of an inflammatory disease [25]. Thus, a link between low-grade CMV infection and numerous inflammatory diseases has emerged in recent studies.

While the costs of overt CMV disease are substantial, the recent interest in micro-infections in a variety of other disease outcomes has broadly expanded the potential economic implications of CMV infections. In the USA, cardiovascular diseases are estimated to have cost $444 billion in 2010, with treatments accounting for 1/6 of all health-care expenditures (http://www.cdc.gov/chronicdisease/resources/publications/AAG/dhdsp.htm). Total direct

medical costs of cardiovascular disease are projected to triple from $273 billion in 2010 to $818 billion in 2030. Real indirect costs due to lost productivity for all cardiovascular disease are estimated to increase from $172 billion in 2010 to $276 billion in 2030, a 61 % increase [31]. A recent model estimated that life-time costs of patients diagnosed with prostate cancer are $110,520 per patient, with prostate-cancer related costs estimated to be $34,432 or roughly 31 % of total costs [32]. With over 660,000 new cases diagnosed each year, including 186,300 in the USA alone, prostate carcinoma represents a significant economic burden on the health-care system [33]. Furthermore, the potential role of latent CMV infection in inflammatory bowel disease represents significant costs to the health-care system. A study by Feagan et al. found that the median annual costs for Crohn's disease patients was $3,668 per patient with the subset of hospitalized patients having a median annual cost of $21,671 per patient [34]. As the CDC estimates that as many as 1.4 million persons in the USA suffer from Crohn's disease or ulcerative colitis, both thus cause significant costs to the health-care system and patients [35]. Thus, when considering the impact of CMV microinfection to CMV-associated diseases, development of an efficacious vaccine is of the utmost importance and has the potential to dramatically reduce associated health-care costs.

Quest for a CMV Vaccine/Immune Therapy

Due to significant human and economic costs, the need for an effective vaccine against CMV has been ranked as of the highest priority by the US Institute of Medicine. Further emphasizing the need for an effective vaccine is the emerging evidence implicating CMV microinfection in a number of other diseases, including cancers and inflammatory conditions. While numerous attempts have been made for over 5 decades in this regard, there is no currently licensed CMV vaccine or cure. However, the ability of the immune response to suppress virus for long intervals of time during CMV infection provides evidence of protective immune correlates and suggests that the development of a CMV vaccine may be feasible. Therefore, the understanding of immunological markers that can predict protection from CMV along with the identification of immunogenic CMV antigen targets may be essential for improving future vaccine immunogenicity and duration of protection.

CMV Immunology

A better understanding of protective immune responses against CMV is pivotal in the quest for a CMV vaccine. Suppression of CMV within otherwise healthy individuals is an active process mediated by antiviral CMV-specific immune responses. Both promising clinical [36] and preclinical [37] data suggest that both neutralizing antibodies (NAbs) and cell-mediated immunity contribute to protection against CMV disease [38]. Therefore, a vaccine should aim to elicit both CMV-specific NAbs and cell-mediated immunity.

CMV induces a strong humoral response, which serves to restrict viral dissemination and limit disease severity. Glycoprotein B (gB), which is involved in cell attachment and penetration, has been found to be a major target for NAbs and is responsible for at least 50 % of the NAbs in CMV-infected individuals [39, 40]. Glycoprotein H (gH), which is

involved in the fusion of the viral envelope with the host cell membrane, also has been found to induce potent NAbs [41]. This antibody (Ab) response is likely to be important in controlling infection, as transfer of Abs from CMV-seropositive mothers to newborn infants was shown to be protective against CMV infection from seropositive blood transfusion [42].

While humoral responses are an important part of the adaptive immune response against CMV, T-cell-mediated immune responses are considered the predominant mechanism by which CMV replication is controlled. $CD8^+$ and/or $CD4^+$ T cells are directed toward more than 70 % of the CMV proteins, indicating the importance of T cell responses in controlling CMV infection [43, 44]. Mature and functional fetal $CD8^+$ cytotoxic T lymphocytes (CTL) in humans expand in utero in response to primary CMV infection [45]. In patients with AIDS, IFN-γ CMV-specific $CD8^+$ T cells are protective against CMV-associated retinitis [46]. Similarly, in bone marrow transplant patients, the development of CMV-specific $CD8^+$ T cell responses was correlated with protection and recovery from CMV disease [47, 48]. Furthermore, infusion of donor-derived CMV-specific $CD8^+$ T cells effectively restored Ag-specific cellular immunity in allogeneic bone marrow transplant recipients and protected from CMV-associated complications [49]. This correlation between CMV-specific CTL responses and protection against CMV disease has also been demonstrated in solid organ transplant (SOT) patients. CMV-specific $CD8^+$ T cells make up a huge proportion of all $CD8^+$ T cells in adult infected humans populations, with a median of 10 % of $CD8^+$ T cells in the peripheral blood of healthy virus carriers and up to 40 % in elderly individuals devoted to the anti-CMV response [4, 44]. Furthermore, the relative contributions of reactivation and reinfection to CMV disease are not yet clear, and the role of antibody or cellular immunity in preventing them still needs to be elucidated. A more comprehensive literature review on cell-mediated immunity on CMV is addressed in the following reviews [50–52].

The importance of $CD4^+$ T cells in controlling CMV has become increasingly evident. Low levels of CMV-specific $CD4^+$ T cells have been found to be significantly correlated with susceptibility to infectious complications with CMV in lung and renal transplant recipients, as well as prolonged viral urinary and salivary shedding in otherwise healthy children [53, 54]. In bone marrow transplant recipients, a detectable $CD4^+$ T-helper response has been shown to correlate with protection from CMV disease [55]. Additionally, the adoptive transfer of $CD4^+$ CMV-specific T cell lines dramatically reduced CMV viral load in allogeneic HSCT recipients [56]. As with $CD8^+$ T cell populations, anti-CMV immunity occupies a significant proportion of the total $CD4^+$ T cell population in healthy seropositive individuals, with individuals devoting a median of 9.1 % of their circulating $CD4^+$ memory T-cell population to control CMV [44]. Most frequently detected in healthy individuals is a large proportion of the $CD4^+$ CTL response specific for highly conserved regions of the gB and gH proteins [57]. Overall, currently it is assumed that CD4 T cells, CTL, and Nabs are essential for the control of CMV disease. Nevertheless, a better understanding of how the immune system keeps CMV under control will eventually lead to identification of established immune correlates for protection. The correlates perhaps will only be identified from the evaluation of potential vaccine candidates in future clinical trials.

Vaccine Target Selection

One major limitation to development of a successful CMV vaccine has been the lack of relevant animal models, which are typically proven crucial in the development of new vaccines. Unlike many other viruses, the cytomegaloviruses are highly species-specific, and CMV's specificity to humans and low infectivity in other species present a significant challenge to vaccine development. Although in vitro models may be useful, animal studies ultimately are required to determine vaccine efficacy. Currently, mouse, guinea pigs, and rhesus macaques and their corresponding, species-specific viruses serve as the model systems in which CMV vaccine immunogenicity is studied [58]. Of these, guinea pigs and guinea pig CMV (gpCMV) are believed to be the most clinically relevant models as gpCMV, similar to CMV, crosses the placenta in utero and causes infection through vertical transmission [37]. Species-specific model viruses provide some utility as challenge models, but fundamental differences in the structure and biology between CMV and these viruses limit their predictive power when assessing potential efficacy of a human vaccine. Thus, regarding the development of CMV vaccines, the lack of reliable CMV infection mouse models has limited progress in the field of CMV vaccines. However, this issue will benefit strongly from studies aimed at developing better small animal models of human CMV infection. In addition, a better understanding of CMV structure, replication cycle, and specific mechanisms of immune suppression may be critical to identifying viable targets for vaccine development.

The CMV virion consists of an icosahedral capsid, tegument, and cellular lipid layer [59]. The major capsid protein, pUL86, forms the penton and hexons of the icosahedral capsid and is the most abundant protein component of the capsid [60, 61]. In the tegument, ppUL53 and ppUL83 (pp65) are expressed in the nucleus of host cells early after infection but become localized primarily in the cytoplasm later in the replicative cycle of CMV [62]. While the structural functions of these tegument proteins are poorly defined, pp65 is believed to inhibit the expression of genes associated with induction of interferon responses [1, 63]. It has also been shown to elicit strong T cell responses and is a major component of many current CMV vaccine strategies [37]. The lipid membrane is comprised by a number of envelope glycoproteins including gB, gH, gL, gM, gN, and gO, among others. These more abundant CMV glycoproteins have been shown to exist as disulfide-linked complexes within the virion as gCI (gB homodimer), gCII (gM/gN), and gCIII (gH/gL/gO) [1]. In terms of composition, gM/gN have been shown using mass spectroscopy to be the most abundant, followed by gB and gH/gL/gO [1]. Since the envelope glycoproteins are anchored to the surface of the virion and exposed to binding by Abs they are attractive vaccine targets for induction of NAbs, which are considered more likely to prevent or attenuate primary infection. Moreover, since these antigens could also elicit cell-mediated responses (essential to mediate lifelong control of virus replication after infection has established) they are considered key targets for future CMV vaccines.

Glycoproteins M and N

As one of the most abundant glycoproteins in CMV, gM, the product of *UL100*, appears to exhibit very little amino acid variation among different strains of CMV and may therefore

be a good candidate for vaccine target selection. While its structure has not yet been defined, this conservation of amino acid sequence suggests that either there is little selective pressure on this viral envelope protein or that it is structurally constrained such that it cannot tolerate significant amino acid variation with major loss of function [1]. In contrast, the *UL73* product gN displays a high degree of amino acid sequence variability, although the total number of O-linked carbohydrate modification sites appears to be relatively conserved [1]. The variation in gN's primary structure may indicate positive selective pressure during the evasion of the Ab response by CMV. The extensive glycosylation of gN, then, may serve to shield this protein from Ab recognition in a similar fashion to that shown for the envelope protein of HIV-1 [64]. gM forms a heterodimeric infectivity complex with gN in the endoplasmic reticulum through a network of covalent disulfide bonds and non-covalent interactions [65]. Complex formation is required for the native folding and intracellular transport of both gM and gN and studies show that infectious virus cannot be recovered from viral genomes with deletions in either *UL100* or *UL73* [65, 66]. Encouragingly, this gM-gN infectivity complex has been shown to elicit binding Abs during natural human infection [67]. These anti-gM/gN Abs appear to react specifically with the gM/gN complex and were found to efficiently neutralize infectious CMV in vitro [67].

Glycoprotein B

gB is an integral membrane protein that homodimerizes to form a type 1 membrane protein. This homodimer is expressed on the surface of both infected cells and virions [68]. Posttranslational modifications of gB have been shown to enable this glycoprotein to interact with components of the endosomal recycling system, particularly phosphofurin acidic cluster sorting protein-I (PACS-I). These interactions between PACS-I and gB may result in the retention of gB in the trans-Golgi network, a possible site of virion envelopment [69]. gB has been observed to play a crucial role in the initial virion-tethering, attachment and fusion, necessary for cell entry [70]. Importantly, gB is a major target for NAbs and has been the subject of intense investigation as a core component of CMV prophylactic vaccine strategies [37, 71, 72].

Glycoproteins H, L, O

The gCIII complex is formed by gH, gL, and gO. Similarly to gM/gN, gH requires coexpression of gL for intracellular transport and terminal carbohydrate modification [73]. In the absence of gH, gL remains localized in the endoplasmic reticulum. These virion surface proteins are crucial for viral entry into host cells. Recent reports demonstrate that a complex formed by gene products UL128, UL130, and UL131A, along with gH and gL is required for viral entry into endothelial and epithelial cells [74]. By contrast, a gH/gL/gO complex has been implicated in viral entry into fibroblasts [74]. Importantly, gH appears to function in a post-attachment event during infection such as membrane fusion or virus penetration [75, 76]. gH is a significant target of NAbs, which seem to block this function. Interestingly, the primary structure of gH is more than 95 % conserved between CMV strains and anti-gH monoclonal Abs are broadly reactive. To evade these NAbs, CMV can modulate gH expression and, under Ab selection, infectious virion containing limiting amounts of gH could be positively selected [77, 78]. Deletion of the gO gene does not prevent assembly and release of infectious virus, but does appear to impair growth [66].

In conclusion, numerous CMV gene products including several glycoproteins and non-structural proteins have been identified as B- and T-cell targets, although protective Ab levels have not been established [18]. While gB is a major target of NAbs, gH and glycoprotein M-glycoprotein N (gM-gN) have also been identified as important Ab targets along with pp65, IE1, pp150, pp28, pp71, and pp52, which are targets of cell-mediated immunity. The most immunodominant Ags to which CMV-specific $CD8^+$ T cells are directed have been identified as IE-1, IE-2, and pp65, although it is unclear whether magnitude of responses directly correlate with efficacy in restricting CMV replication [4]. In particular, pp65, IE-1, IE-2, gH, gL, gM, gN, gO, and gB were found to be recognized at high frequency by both $CD4^+$ and $CD8^+$ T cells, making these particularly tempting vaccine targets [44, 79].

The Road So Far: Vaccine Platforms Under Development

Viral Vaccines

Several attenuated CMV vaccines have been studied. The Towne strain of CMV, a strain passaged 125 times in WI-38 human diploid fibroblasts, has been the most extensively studied of these replicating, attenuated vaccines. Intramuscular injection of Towne has been shown to result in seroconversion of seronegative adults and the elicitation of NAbs. These Ab levels, however, waned over the course of a year [80]. Towne vaccination has also been shown to elicit CMV-specific $CD4^+$ and $CD8^+$ T cell responses in immunocompetent individuals [18]. Challenge studies (using a less passaged CMV strain, Toledo) showed that Towne afforded some protection against infection, but this protection was inferior to natural infection. Additionally, Towne failed to protect seronegative women with children in daycare (a population at high risk of CMV exposure) against CMV infection while natural infection was highly protective against reinfection with CMV [81]. The lack of protective efficacy afforded by Towne has led to the development of genetic recombinants attempting to achieve a level of attenuation between the Towne strain and wild-type virus. Various Towne/Toledo chimeras have been produced and tested in double-blind, placebo controlled clinical trials and found to be safe, well-tolerated, and appear attenuated [18]. This phase 1 trail is currently in progress.

More recently, a potential CMV vaccine option is based on noninfectious subviral particles of HCMV termed dense bodies (DB). DB are derived by the infection of cultured fibroblasts which then leads to the production of not only infectious virions, but also defective noninfectious particles [82]. These noninfectious DB particles contain enveloped structures consisting of viral tegument proteins and glycoproteins but lacking a capsid, and noninfectious enveloped particles, which resemble normal virions, but lack infectious DNA. This strategy in HLA-A2 transgenic mice was found to yield high virus neutralization titers and developed Abs against a variety of CMV Ags, including gB, gH, pp65, and pp150 when immunized with these dense bodies [83–85]. Interestingly, dense bodies have also been shown to *elicit* high levels of CMV-specific CTLs in mice. Further evaluation, development, and optimization of this potential CMV vaccine approach are currently ongoing [85].

Nonviral Vaccines

Subunit vaccines in which select proteins are used in combination with an immune adjuvant to augment immunity, has also been explored extensively for CMV. The most potential promising subunit CMV vaccine targets the CMV gB, a highly conserved CMV antigen that induces potent neutralizing antibodies. In healthy sero-negative adults, CMV gB with MF59 (an oil and water adjuvant) was found to elicit levels of binding and NAbs comparable to those induced by natural CMV infection with anti-gB IgG and IgA evident in saliva or nasal washes of subjects [86]. NAb titers fell rapidly following vaccination, possibly due to an insufficient CD4+ T cell response, but rebounded significantly following a boosting dose of vaccine [87, 88]. Furthermore, vaccination with gB/MF59 induced strong anti-gB and anti-CMV lymphocyte proliferative responses which persisted for the year following vaccination [88]. A gB vaccine with MF59 adjuvant recently completed a Phase 2 study and has been found to be safe in seronegative women within 1 year after giving birth. The vaccine was found to be 50 % efficacious in this population. Immunized patients did not experience significant differences in adverse event frequency or severity [89].

DNA Vaccines

DNA vaccines, which involves the direct injection of purified DNA encoding specific Ags has been shown to induce levels of protective immunity especially in small animals. Although poor immunogenicity of “first-generation” DNA vaccines in animal models tended to compromise the potential uses for DNA as a vaccine platform, the development of new optimization and delivery strategies, however, have revived DNA vaccines as a viable vaccine vector [90]. These improvements have significantly boosted DNA vaccine immunogenicity and efficacy far beyond “first-generation DNA vaccines.” As such, these improved platforms are collectively termed “second-generation DNA vaccines.” Gene-level optimization such as codon-optimization to improve RNA stability, and transcriptional and translational efficiency have significantly boosted DNA vaccine immunogenicity against a variety of Ags through increased in vivo expression. Furthermore, Ag design has improved the breadth of protection to target highly variable pathogens such as CMV. These optimized immunogenic sequences can be created based on a collection of target Ag protein sequences. In response to polymorphism, likely due to spontaneous mutations or immune selective pressure [79], immunity can be altered to target multiple circulating strains by “consensus-engineering” of the amino acid sequence of the DNA vaccine immunogens [91]. Finally, a cocktail of DNA constructs could be used to drive the immune response against a plethora of variable antigens.

Furthermore, the development of new delivery methods to increase transfection efficiency has dramatically improved DNA vaccine immunogenicity. The delivery of Ag-encoding plasmids adsorbed to gold beads using gene guns has been shown to be efficacious in inducing NAbs against the gM and gN proteins of CMV [92]. Delivery of DNA plasmid with adjuvants such as aluminum salts has been shown to increase Ab responses in mice. In particular, a DNA vaccine containing the CMV gB gene and administered with aluminum phosphate gel and CpG oligodeoxynucleotides was found to elicit a significantly higher Ab response and greater NAb titers compared to DNA alone [93]. The use of molecular adjuvants has also been shown to boost DNA vaccine efficacy. Mice co-immunized with the

MCMV gB and type I interferon genes exhibited enhanced protection against MCMV challenge compared to mice immunized with the MCMV gB gene alone [94]. Finally, the use of in vivo electroporation with DNA vaccination has been shown to significantly increase antigen-specific immune responses in a variety of animal models against a wide array of pathogens [95–98]. The electroporation process makes use of probes that deliver square-wave pulses after inoculation with DNA plasmid. This electroporation and inoculation procedure can be administered intramuscularly, subcutaneously, or intradermally. This delivery method has been shown to dramatically improve both humoral and cellular immunogenicity of DNA vaccines. As a result of the “second-generation” DNA platform optimizations, DNA vaccines have been shown to been potently immunogenic against a variety of CMV proteins [79].

In addition, the advantages of DNA vaccines extend far beyond their immunogenic potential. Since DNA vaccines are DNA plasmids whose function is not dependent on thermodynamically stabilized secondary, tertiary, and quaternary structures, they are more temperature-stable and do not require the same cold-chain transportation that is essential for protein-based vaccines (viral-vectored vaccines, recombinant protein vaccines). This consideration reduces transportation costs and is particularly important for vaccine delivery to developing countries, where electricity and proper refrigeration may not be readily available. As these nations are often the most affected by epidemics, ease of distribution is a crucial factor in the success of any vaccine.

Finally, DNA vaccines have been shown to have favorable safety profiles in the preclinical and clinical settings. As of 2011, 43 clinical trials were underway to evaluate the effectiveness of DNA vaccines against various viral and nonviral diseases [91]. These vaccine targets include HIV, various cancers, influenza, hepatitis B and C, HPV, and malaria [91]. In addition, an important anti-CMV DNA vaccine currently undergoing clinical trials is the TransVax vaccine by Vical, a vaccine consisting of plasmids encoding CMV gB and pp65 formulated with poloxamer CRL1005 and benzalkonium chloride [36]. TransVax is being tested as a CMV therapeutic DNA vaccine. In a recently completed Phase 2 double-blind, placebo-controlled, parallel group trial, the TransVax or placebo were given to CMV seropositive recipients undergoing allogeneic HSCT, a population at high-risk for CMV reactivation or reinfection. Safety of the vaccine compared to placebo as well as rates of CMV viremia resulting in initiation of cytomegalovirus-specific antiviral therapy were assessed as primary endpoints. The immunogenicity of vaccine compared with placebo was measured using interferon-γ enzyme-linked immunosorbent spot (ELISPOT) responses to pp65 and gB and gB-specific Ab concentrations measured in an indirect binding IgG ELISA against full-length gB protein [36]. The TransVax vaccine was well-tolerated by patients, with only mild adverse reactions and one allergic reaction reported, indicating favorable safety for the DNA vaccine [36]. Although the randomized Phase 2 study was not designed to demonstrate potential effects on CMV diseases, the TransVax vaccine elicited gB and pp65 cell-mediated immunity responses and reduced the rate of viremia in CMV-seropositive HSCT recipients [36]. Furthermore, the number of pp65 interferon-γ-producing T cells was increased in the TransVax group compared to placebo group at all time points following HSCT. Additionally, the longitudinal anti-pp65 T-cell responses were higher in the TransVax group. However, anti-gB T-cell responses were the same at all time points

between the TransVax and placebo groups while no significant increase in anti-gB IgG concentrations were observed in TransVax group compared to the placebo group [36].

Overall, the TransVax DNA favorable safety profile is indicative of the safety of an anti-CMV DNA vaccine. Nevertheless, through genetic optimization, improved delivery methods such as electroporation, and the use of different molecular adjuvants, the efficacy of DNA vaccines can likely be significantly improved while maintaining a similar safety profile to TransVax. While TransVax was not highly immunogenic, its ability to elicit anti-pp65T-cell responses indicates that DNA vaccines can induce cellular responses against a plasmid-encoded Ag. This is likely to be an important factor in the success of any CMV vaccine, especially in a therapeutic vaccine, given the importance of cellular immunity in natural control of CMV infection and reactivation in healthy seropositive individuals. However, promising clinical and preclinical data support that an effective vaccine will need to induce both humoral and cellular immune responses. Thus, DNA vaccines are an extremely promising platform for the future development of both therapeutic and prophylactic vaccines against CMV. Given DNA vaccines' safety profile in clinical settings and their ability to drive both humoral and CMI, which are considered essential for CMV immunity, makes DNA a suitable platform for use in immunocompromised populations. This platform is germane for CMV, since immunocompromised patients comprise the vast majority of the at-risk population for CMV disease and would be the target population for a CMV vaccine.

Conclusion

The development of a CMV vaccine would be highly effective to reduce congenital diseases, to improve longevity of transplant patients, and to address the significant unmet public health issues caused by CMV infections. However, CMV's sophisticated mechanisms of immune evasion, the relative complexity of its genome, its numerous glycoproteins associated with cell tropism, and due to the lack of identified CMV immunogens has stunted CMV vaccine development. However, the identification of new target CMV immunogens and further studies of our understanding of immune responses to CMV should inevitably lead to the establishment of immunological correlates that could aid future rational vaccine design. The results of the most currently advanced ongoing clinical trials (Table 1) should identify correlates of protection for revolutionizing the next generation of CMV vaccines.

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Table 1

Most advanced CMV vaccines currently in clinical trials

Vaccine	Vaccine platform	Viral antigens	Stage
Towne	Attenuated	Whole virus	Phase 2
gB/MF59	Subunit protein	gB	Phase 2
TransVax	DNA vaccine	gB, pp65	Phase 2