EDITORIAL

IS HCMV VACCINE AN UNMET NEED? THE STATE OF ART OF VACCINE DEVELOPMENT

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Received March 8, 2012 – Accepted November 22, 2012

Congenital HCMV infection is the most frequent congenital infection, with an incidence of 0.2- 2.5% among all live births. About 11% of infected newborns show symptoms at birth, including hepato-splenomegaly, thrombocytopenia, neurologic involvement, hearing impairment and visual deficit. Moreover, 5-25% of the asymptomatic congenital HCMV-infected neonates will develop sequelae over months or even years. The relevant social burden, the economic costs of pre-natal screening, post-natal diagnosis, follow-up and possible therapy, although still limited, are the major factors to be considered. Several types of vaccines have been explored in order to develop an effective and safe HCMV vaccine: live attenuated, subunit, vectored, peptide, DNA, and subviral ones, but none are available for use. This review illustrates the different vaccine types studied to date, focusing on the possible vaccination strategy to be implemented once the HCMV vaccine is available, in terms of target population.

Human cytomegalovirus (HCMV) is an opportunistic, ubiquitous, human-specific pathogen of the Herpesviridae family responsible for severe congenital disease when it affects, through the placenta, the fetus. The reported incidence of congenital HCMV (cHCMV) infection varies between 0.3% and 2.3% of all live births, being the most frequent congenital infection (1). About 11% of congenitally infected newborns show symptoms at birth and more than 70% of these will develop permanent sequelae, in particular neurological, deafness or hearing impairment and visual deficit or blindness. The mortality rate in the perinatal period varies from 2% to 30% in the most severe cases. Most infants born with cHCMV infection do not exhibit clinical abnormalities at birth, but 5-15% of them will develop symptoms over months or even years (1, 2). The risk of severe consequences is greater when HCMV infection is acquired when the pregnant woman experiences a primary infection. Reactivation or reinfection, related with elevated seroprevalence of HCMV infection in reproductive age (Fig. 1), is responsible for 60-75% of congenital HCMV infections and can provoke severe disease and intrauterine fetal death (2). Many efforts have been focused on prevention of maternal transmission of HCMV to the fetus and its treatment by *in utero* therapy but currently no therapeutic options during pregnancy are available (1). Controversial opinions on postnatal therapy still persist and a clear consensus on treatment has not yet been reached (3).

Key words: HCMV, vaccine, target population

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0394-6320 (2013)

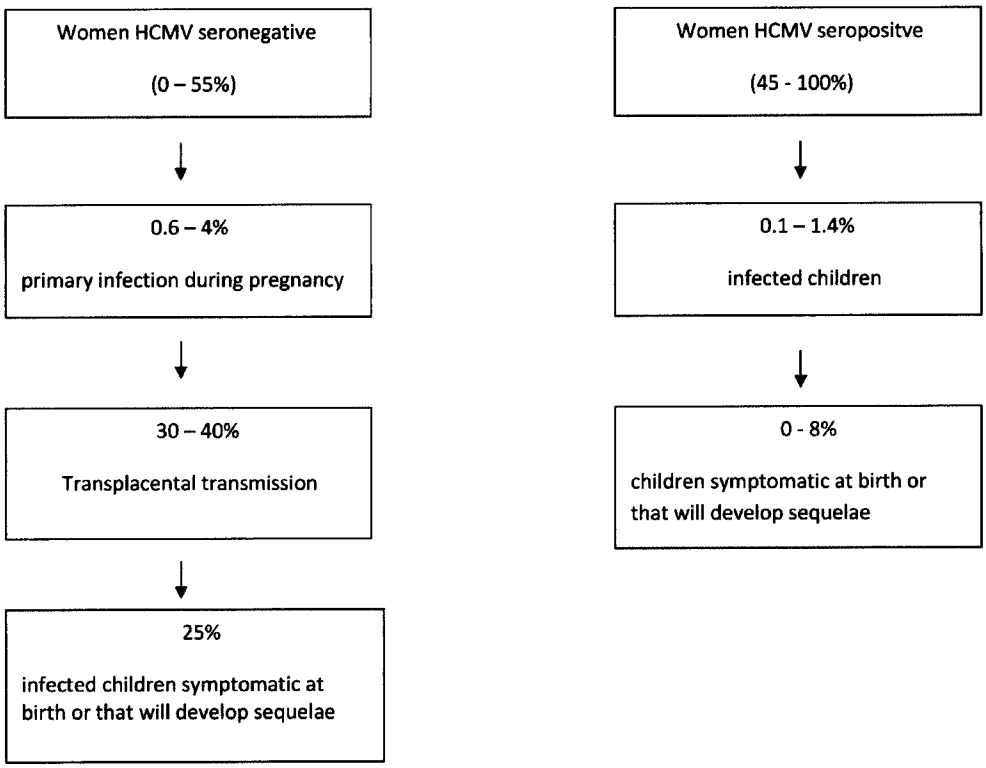

Fig. 1. *Epidemiology of congenital HCMV infection and disease.*

Social and economic burden of HCMV congenital infection

It has been estimated that yearly in USA 40,000 children are born with congenital HCMV infection. According to a study published in 2004, in the early 1990s, the expenses of the United States Health Care System associated with congenital human cytomegalovirus infection was estimated at approximately $1.9 billion annually, with an average cost per child of over $300,000. A vaccine for prevention of cHCMV infection should be among the priorities for both health care cost and improvement in quality adjusted life years (4). Several authors have evaluated the possible scenarios of a HCMV vaccine in terms of biologic feasibility and decrease or eradication of virus from the population. Griffiths et al., using a mathematical modeling approach, showed that HCMV has a low force of infection, and calculated that the critical vaccination proportion required for the eradication of HCMV is between 59 and 62% (5). Even according to data reported by V.C. Emery, the critical vaccination proportion needed to eradicate HCMV, is between 41 and 62% (6).

TYPES OF VACCINE

Several vaccines were evaluated and are summarized in Table I.

Live attenuated virus vaccines

The first human studies on HCMV vaccine were developed by Elek and Stern in 1970s testing AD169, a laboratory-adapted strain of HCMV. In two different trials AD169 was shown to be well tolerated and safe, being undetectable in vaccinated seronegative persons and not transmitted to seronegative contacts of the vaccine recipients. Moreover, it was able to elicit good antibody HCMV production in seronegative individuals, whereas there was no humoral response in seropositive subjects. Nevertheless, evaluation of some of vaccinated subjects 8 years later revealed that only half of them had detectable HCMV antibodies. Almost simultaneously, another live attenuated

virus vaccine was developed with the Towne strain. Several trials showed it was safe, able to elicit both binding and neutralizing antibodies, which showed similar specificities to the antibodies arising from natural HCMV infection but were lower and/or weaned over the course of a year. Interestingly, Towne vaccine elicited not only humoral but also cell-mediated responses in healthy seronegative adults. Furthermore, other studies showed that also seropositive vaccinees developed IgM antibodies, raising the possibility that the Towne virus strain may reinfect persons who had previously been infected with another strain of HCMV. Like AD169, Towne vaccine was not excreted and was unable to persist in vaccinated persons. With regard to vaccine efficacy, series of studies in renal transplant recipients showed that although vaccination did provide a protective impact on HCMV disease, reducing its severity, Towne vaccine failed to prevent HCMV infection after transplantation. Moreover, a placebo controlled study was performed in seronegative women with children in daycare showing that Towne vaccination failed to protect women from HCMV infection, while natural infection was highly protective against re-infection with HCMV (7). To enhance the immunogenicity of Towne vaccine, several efforts have been performed such as the adjuvated with recombinant interleukin-12 Towne HCMV vaccine obtaining interesting results (8). Further attempts to produce live HCMV vaccine candidates with increased immunogenic potential were performed by recombining the genomes of the Towne virus strain and the unattenuated HCMV Toledo strains to yield 4 different chimeric vaccines supposing that the high level of attenuation exhibited by the Towne vaccine was presumably due to genetic mutations introduced during its manifold passages in cultured cells. Data showed that none of the vaccine candidates was isolated from the blood, urine or saliva cultures of any vaccinee or any of their close contacts, suggesting that systemic infection did not occur in this population and that all four vaccines were safe and well-tolerated (9).

Subunit vaccines

Glycoprotein B (gB) is present on all human cytomegaloviruses; it is the most abundant membrane protein in the HCMV envelope and highly conserved. This protein is necessary for viral infectivity, mediating attachment and entry to infected cells, cell-to-cell transmission of virus and syncytium formation (10). gB seems to be the most immunogenic HCMV protein; in fact, all sera from HCMV-seropositive subjects contain antibodies to gB, and up to 70% of the neutralizing antibody response in convalescent sera is gB-specific (11). Given its features, for several years gB has been a relevant vaccine target. Positive results on animals have encouraged human trials assessing gB-based vaccines. During the 1990s a series of phase 1 and phase 2 studies were performed evaluating CMVgB vaccine safety, immunogenicity, antigen dose and immunization schedule. In over 700 subjects (mostly healthy seronegative adults plus a limited number of seronegative children and seropositive adults) who received HCMV gB vaccine in different trials, safety, immunogenicity and reactogenicity were evaluated, showing reassuring data. Some of these studies found that the highest antibody titer was induced when the vaccine was administered at 0, 1 and 6 months with 5 mcg dose of gB/MF59. Based on the results of these early studies, a phase 2, placebo-controlled, randomized, double-blind efficacy trial was performed giving 3 doses of the gB MF59 vaccine or placebo at 0, 1, and 6 months to HCMV-seronegative women within 1 year after they had given birth, considered the most at-risk population. The gB MF59 vaccine showed an overall vaccine efficacy of 50%, thus resulting that vaccine recipients were more likely to remain uninfected than placebo recipients (P=0.02). Furthermore, congenital HCMV infections were investigated in children born to women who had become pregnant during the trial, resulting in an incidence of 1% (1) and 3% (3) among infants born to mothers in the vaccine and placebo group, respectively (P=0.41). These data are of interest, however, the overall evidence is too limited to allow any conclusion regarding the vaccine's ability to prevent congenital infection beyond its efficacy for prevention of maternal infection (12). Noteworthy, it has to be considered that the study population was composed of only seronegative women and was not representative of the whole target population; in fact, as described above, there is a quite relevant number of congenital HCMV-infected children born to mothers who experienced a secondary infection

Table I. *Types of vaccine.*

LIVE ATTENUATED VIRUS VACCINES	PRO	CONS
AD169	• good antibody HCMV production in seronegative individuals • safe (undetactable in body fluids of vaccinated seronegative persons and not transmitted to seronegative contacts)	• no humoral response in seropovitives • limited persistence of immunity
Towne	• elicits humoral and cell mediated response that can be enhanced with adjuvant recombinant IL-12 • safe (undetactable in body fluids of vaccinated seronegative persons and not transmitted to seronegative contacts) • reduces severity of HCMV disease in renal transplant recipients	• Abs production lower than natural infection and suboptimal cell response • limited persistence of immunity • fails to prevent HCMV infection in women and in transplanted recipients
Towne/Toledo chimeric vaccines	• safe (undetactable in body fluids of vaccinated seronegative persons and not transmitted to seronegative contacts) • attenuated compared to Toledo but less attenuated than Towne	• immunogenicity data are needed
SUBUNIT VACCINES		
gB/MF59 vaccine	• interesting data in preventing congenital infection • induces high humoral and cell mediated responses that can be boostered in S+ after vaccination • safe	• too small sample evalueted
gH/gL/pUL128-pUL130-pUL131 complex	• might be effective in preventing viral acquisition through mucosal epithelia	Further data are needed
VECTORED VACCINES		
ALVAC/gB vaccine	• controversial data on its ability of "prime-boost" the immune response when administered with an attenuated vaccine • good safety profile	• very low humoral response in humans
ALVAC/pp65 vaccine	• induces strong CD8+ CTL response • good safety profile	Further data are needed
alphavirus/gB-pp65-IE1	• good safety profile • induces neutralizing antibody and multifunctional T cell responses against the 3 antigens	Further data are needed
modified vaccine virus Ankara (expressing gB; expressing gB-pp65-UL123/e4; expressing pp65-e4 of IE1)	• excellent immune response in mice and in human peripheral blood • immunogenic and efficaious in reducing plasma viral loads in rhesus macaques	Further data are needed (no human data are available)
Ad-gBCMVpoly	• wider repertoire of HCMV specific immune responses	Further data are needed

PEPTIDE VECCINES	PRO	CONS
Epitope-based vaccine	• vigorous CMV-specific CTL response in mice	Further data are needed
DNA VACCINES		
pp65+gB	• VCL-CB01 in humans was demonstrated be safe and immunogenic in S- ; in S+ able to booster existing pp65 T-cell response	• no able to boost gB antibody responses in S+
MCMV expressing NK-cell receptor ligand	• induces strong and long-lasting efficacious immunity and protects the offspring of immunized mice from MCMV disease	Further data are needed (no human data are available)
Bacterial Artificial Chromosome (BAC) vaccines	• efficacious and safe in mice and guinea pigs	Further data are needed (no human data are available)
complex II (gM + gN)	• induces virus neutralizing antibodies in experimental animals	Further data are needed (no human data are available)
SUBVIRAL PARTICLES/DENSE BODIES VACCINES		
Subviral particles/dense bodies	• able to induce an excellent immune response in mice • safe • recombinant db to enhance immune response	Further data are needed (no human data are available)

during pregnancy. In this regard, some efforts have recently been made to include seropositive women in the vaccine target population. Sabbaj et al. demonstrated by a placebo-controlled study that both HCMV-specific antibody and CD4+ T-cell responses can be boosted after vaccination with an HCMV gB/MF59 vaccine in women who had chronic HCMV infection (13). To confirm and expand these data, in 2011 a phase 2 randomized placebo control trial on adults awaiting kidney or liver transplantation was carried out, assessing safety and immunogenicity of HCMV gB/MF59 vaccine showing that seronegative recipients with seropositive donors had a reduced duration of viremia when given the vaccine and, as a result, a shorter duration of antiviral therapy (14).

Considering the relevant results of the gB vaccine trials, studies on other glycoproteins have been performed. In particular, the gH/gL/pUL128-pUL130-pUL131 pentameric complex required for CMV entry into epithelial/endothelial cells, has been investigated. The results of these studies suggest that a potential vaccine which incorporates gH/gL/UL128-131 epitopes might be effective in preventing viral acquisition through mucosal epithelia (15). Moreover, other authors have evaluated the role of the glycoproteins M and N in the induction of immune response with interesting results.

Vectored vaccines

Canarypox. Among the several viruses that have been used as vectors to express potential vaccine antigens, the attenuated ALVAC strain of canarypox has been most extensively employed due to its specific features. In fact, it is known that the ALVAC genome permits the insertion of large exogenous DNA fragments and it can replicate productively in avian species but only abortively in mammalian cells with a low risk of vaccine-associated complications. Furthermore, foreign antigens expressed by ALVAC are transported and processed within cells allowing their presentation in the context of MHC class I molecules that, in turn, may facilitate the stimulation of CTL responses that mimic those of natural infection. Given these features, in 1995 Gonczol et al. tested the immunogenicity of a recombinant ALVAC (canarypox)-HCMV-gB (ALVAC-gB) in

mice and guinea pigs showing that it was capable of inducing both humoral and cell-mediated immune responses to HCMV in both immune and non-immune individuals (16) but the positive results obtained in animal studies were not confirmed in human trials. Other studies have evaluated the phosphoprotein 65 (pp65) as an additional target for subunit vaccine development using the canarypox vector. In 2001 Berencsi et al. published a phase I placebo-control clinical trial on 21 HCMV-seronegative adult volunteers, evaluating a canarypox–CMV pp65 recombinant's ability to induce HCMV pp65-specific CTL, helper T lymphocytes, and antibodies and showing that HCMV pp65, when expressed by an ALVAC recombinant, induces strong CD8+ CTL responses in humans (17).

Alphavirus. In parallel with canarypox vectors, alphaviruses were investigated as potential vectors in both animal and human studies aimed to develop an HCMV vaccine. Animal positive data have supported the development of a Phase 1, randomized, placebo-control study in which the safety and the immune response to the vaccine expressing three HCMV proteins (gB, pp65 and IE1) in seronegative healthy volunteers were tested. Forty subjects received a lower dose or higher dose of vaccine or placebo by intramuscular or subcutaneous injection at Weeks 0, 8 and 24. In all recipients the administration of this vaccine proved to be safe and able to induce neutralizing antibody and multifunctional T-cell responses against the three evaluated CMV antigens (18).

Poxvirus. A different approach was developed in 2004 by Z. Wang et al. who constructed an attenuated poxvirus, modified vaccine virus Ankara (MVA) initially expressing gB (gB680-MVA), with positive results. Consequently, the same authors generated a recombinant MVA (rMVA) simultaneously expressing gB (UL55), pp65 (UL83) and UL123/e4 (nuclear protein) and, four years later, a new rMVA expressing three immunodominant antigens (pp65-IE1-IE2) at high levels. The final data of this study showed that pp65- IE1-IE2 MVA was safe, able to induce robust primary cell-mediated immunity to all three antigens in both CD4 and CD8 subsets and stimulate vigorous expansion of memory T-lymphocyte responses to all the antigens in mice and PBMC of HCMV-positive donors (19).

Adenovirus. More recently, an HCMV vaccine using the modified adenoviral vector Ad5F35, in which amplified antigenic gB domain-1 (AD-1) was cloned, was developed, showing to be a promising candidate for clinical trials (20). Furthermore, other adenoviral vectored vaccines targeting gB or gH were experimented via mucosal immunization. Although immunological results were interesting with both good systemic and mucosal humoral responses, mucosal immunization failed to elicit cellular immune responses and prevent acquisition of a new infection in immunized mice (21). Recently, a novel chimeric vaccine composed of the extracellular domain of HCMV-encoded glycoprotein-B covalently linked to multiple HLA class I and class II-restricted T-cell epitopes from multiple HCMV antigens as a contiguous polypeptide in a replication-deficient adenoviral vector Ad5/F35 (referred to as Ad-gBCMVpoly), able to induce both cellular and humoral immune response has been developed. Compared with the other vaccine formulations, Ad-gBCMVpoly, being replication-deficient, does not have safety concerns related to reactivation and it is more efficient in inducing virus-specific T-cell responses. Moreover, including gB protein and CD4 T-cell epitopes from eight different antigens, this vaccine can induce a much wider repertoire of HCMV specific immune responses (22).

Peptide vaccines

Some peptide fragments of immunogenic proteins can stimulate the CTL response and have thus been used as target to develop vaccines. In 2000 BenMohammed et al. provided a rational model for the design and assessment of new epitope-based vaccines. They used transgenic mice expressing both HLA class I (A*0201 or A2.1) and class II (DRB1*0101 or DR1) molecules for immunization with an HLA A*0201-restricted and CMV-specific CTL epitope and different TH epitopes, obtaining a vigorous CTL response (23). In 2005 Gopal et al. evaluated the immune response in mice to an immunodominant MCMV epitope presented as a nasal peptide vaccine in combination with cholera toxin adjuvant. The results of the study showed that this type of nasal peptide vaccine was immunogenic (in particular, a CD8 cell response) in MCMV-infected and naïve mice, and capable of reducing

viral titer in naive mice after virulent MCMV challenge (24).

Recently, given the interesting results obtained in animal studies, La Rosa et al. evaluated two candidate CMV peptide vaccines composed of the HLA A*0201 pp65(495-503) cytotoxic CD8(+) T-cell epitope fused to 2 different universal T-helper epitopes [either the synthetic Pan DR epitope (PADRE) or a natural Tetanus sequence] in HLA A*0201 healthy volunteers. This trial demonstrated an acceptable safety profile and vaccine-driven expansion of pp65(495-503) T cells supporting further evaluation of CMV peptide vaccines combined with PF03512676 in the hemopoietic cell transplantation (HCT) setting (25).

DNA vaccines

DNA vaccines are based on the *in vivo* expression of heterologous genes carried by plasmid vectors; they are extremely stable and can be produced en masse at low cost. The first report in literature detailing the use of DNA vaccine for CMV involved the immunization of mice with plasmid DNA encoding the tegument protein pp65 of CMV demonstrating its ability to elicit an antigen-specific immune response (26). Subsequently a second generation of CMV DNA vaccines was designed to stimulate at the same time both humoral and cell-mediated immunity consisting of a cocktail of plasmids encoding gB and pp65. In order to enhance immune responses, several methods have been applied to CMV DNA vaccines such as co-administration of various immunomodulators (cytokines, chemokines, costimulatory molecules), delivery of plasmids in liposomes, and the use of experimental adjuvants and type I interferon genes leading to contrasting data (26-28).

Not only adaptive response but also the role of NK was evaluated. In particular, in a recent study, Slavuljica et al. engineered a recombinant mouse cytomegalovirus (MCMV) expressing the high-affinity NKG2D ligand RAE-1g demonstrating it was able to induce strong and long-lasting efficacious immunity and to protect the offspring of immunized mice from MCMV disease. These interesting data support the possible future use of a recombinant virus encoding the NK cell receptor ligand, although safety concerns (reactivation) have to be taken into account (29). Given the interesting results in animal studies, in 2008, Wloch et al. assessed the ability of a candidate HCMV DNA vaccine (VCL-CB01), containing plasmids encoding HCMV phosphoprotein 65 (pp65) and glycoprotein B (gB) in a phase 1 clinical trial. The results of this study suggest that the vaccine was well tolerated and immunogenic, eliciting both T-cell responses and gB antibodies and the priming of memory T cells in a majority of HCMV-seronegative subjects. Moreover, in seropositive subjects, VCL-CB01 was demonstrated to be able to booster existing pp65 T-cell responses, even if not gB antibody responses (30). Recently, Kharfan-Dabaja et al., in a double-blind, placebo-control, parallel-group, phase 2 trial, tested a vaccine containing plasmids encoding CMV gB and pp-65 in 94 HCT recipients and 14 paired donors. Compared to placebo, the DNA vaccine was shown to be well-tolerated and able to reduce the occurrence, recurrence and duration of episodes of cytomegalovirus, viremia and to improve time-to-event at 1-year follow-up, although the rates of clinically significant viremia requiring CMV-specific antiviral treatment after vaccine did not differ from those noted for placebo (31).

Another assessed DNA vaccine target was glycoprotein complex II consisting of two glycoproteins, gM and gN. In particular, in 2007 Shen et al. demonstrated that a DNA vaccine expressing the CMV glycoproteins M and N can induce virus neutralizing antibodies in experimental animals against heterologous strains of CMV. Furthermore, this DNA vaccine could be used as a primer of the host immune responses subsequently amplified by a boost consisting in other forms of vaccine (32).

Subviral particles/dense bodies

Studies performed in the seventies showed that following HCMV infection, not only infectious virions but also non-infectious particles are released. These latter can be non-infectious enveloped particles or dense bodies, enveloped spherical structures composed of glycoproteins and viral tegument (the most important of which are gB and pp65 respectively), but lacking viral capsid and DNA. Having been demonstrated that HCMV-seropositive individuals' sera reacted with these particles, attention was focused on dense bodies (DBs) as possible targets of HCMV vaccines, given

that they could be potentially immunogenic and safe, not being infectious and able to establish a latent infection. Pepperl et al. in 2000 and then in 2002, using a mouse model, demonstrated that DB was able to induce a neutralizing antibody response and a significant anti-CMV CTL and T-helper lymphocyte response making it appear as an ideal carrier system for the development of multivalent vaccines against HCMV infection (33). Subsequent studies have proved that genetic modifications of dense body (recombinant subviral dense bodies, recDB) vaccine, including additional antigens not normally present in these particles and which can be introduced successfully into the MHC class I presentation pathway, can enhance the immune response.

Novel strategies

A totally new approach for HCMV vaccine was recently developed by N.J. Logsdon and coll. based on human cytomegalovirus expressing a viral ortholog (CMVIL-10) of human cellular interleukin-10 (cIL-10). Despite being mostly different in their structure, both CMVIL-10 and cIL-10 exhibit comparable immunosuppressive activity on multiple lymphoid cell types, especially dendritic cells (DC), which link innate and adaptive immunity, thus, they attenuate HCMV antiviral immune responses and contribute to lifelong persistence within infected hosts. Moreover, both CMVIL-10 and cIL-10 engage the IL-10R1 and IL-10R2 cell surface receptor chains to induce their biological activities. A recent study on the role of viral IL-10 *in vivo* demonstrated that primary infection of rhesus (Rh) macaques with a variant of RhCMV lacking the RhCMVIL-10 gene led to an increased innate responses at the site of inoculation, and increased long-term B and T cell responses to RhCMV antigens, compared to infection with the parental variant expressing RhCMVIL-10. Taking into account all these data, a non-functional immunogenic RhCMVIL-10 was designed for vaccination in a non-human primate model that closely mimics HCMV infection in humans. It was established that immunizing animals with signaling-incompetent RhCMVIL-10 mutants is able to generate, or boost, RhCMVIL-10-NAb titers in seropositive rhesus macaques. Moreover, RhCMVIL-10-NAbs do not react with cellular RhIL-10, thus not appearing to harm the animals. These results provide a rationale for a new vaccine strategy that needs confirmation in efficacy trials (34).

Furthermore, the role of recently described CMV microRNAs, small non-coding RNA that regulate both viral and cellular gene expression, should be investigated as a potential target for prevention strategies (35).

TARGET POPULATION

In the perspective of developing a vaccine for HCMV congenital infection, two major issues have to be considered: the absence of any licensed vaccine along with the identification of the ideal target population for a vaccine campaign. The ideal target population is still controversial at present (Table II).

Children

Frequently, HCMV is transmitted from child to child, especially among children in large group day care, often being a source of HCMV infection for their parents and for their professional caretakers. Thus, it is reasonable that a vaccination in the early age, providing a very high population coverage, could lead to a significant reduction of HCMV transmission, with consequent benefit for pregnant women, and consequently for their offspring. On one hand, a vaccination strategy including males and females in the young age, could secondarily reduce congenital infection, avoiding any potential viral transmission to pregnant women among the closest contacts. On the other hand, should the persistence of vaccination-induced protection be demonstrated, male coverage would allow an indirect protection of pregnant women. In this perspective, Numazaki et al. showed a significant relationship between seroconversion in women during pregnancy and prevalence of IgG antibody against HCMV in their husbands, thus inferring that transmission of HCMV by sexual contact may play an important role in the pathogenesis of congenital infection. Addressing "all children" as the target population, the problem of vaccine efficacy in seropositive subjects could be mostly cleared. In order to strengthen the hypothesis of vaccinating all children, positive data on safety and immunogenicity were reported in a study by Mitchell et al. in which 18 HCMV-seronegative children between 12 and 35 months of age were included. In this trial, an open label phase in which HCMV gB/MF59 was given

to 6 children to evaluate safety was followed by an observer-blinded randomized portion characterized by a randomization of other 12 children to receive HCMV or hepatitis vaccine. The results showed that, although infants required the same three doses of vaccine to obtain maximal antibody responses compared to adults, they had 6-fold higher mean titers, thus demonstrating a high immunogenicity of the vaccine (36). Together with these reasons, other issues have to be considered in the overall risk/benefit profile of a vaccine devoted to the pediatric population. Firstly, vaccine efficacy should persist until and throughout child-bearing age, in order to reach the final goal of the reduction of congenital HCMV infection and disease. Secondly, it has to be considered that all children who will not become parents, could be exposed to the potential side effects of the vaccine with negligible, if any, direct benefits from the vaccination.

Adolescents and women in child-bearing age

The possibility of transmitting human cytomegalovirus by saliva and genital fluids makes adolescents another potential target of anti-HCMV vaccination, especially considering that seroconversion rate in this subset is high as to 13.1% per year (37). Petty et al. assessed the parental acceptance, through a self-administered, internet-based survey, of a potential HCMV vaccine given to their adolescent daughters. Data from 516 parents were analyzed showing a generally high acceptance (38). Moreover, Dempsey et al., who made a cost-effectiveness analysis of vaccination in this subset which showed a positive balance, assumed that vaccine achieves 61% reduction in the incidence of HCMV disease in neonates (39). The considerations made for children as the target population of HCMV vaccine could be applied also to this subset. In this scenario, a vaccination campaign targeting adolescents and women in child-bearing age could reduce the HCMV infection in a population with high risk of transmission and could be performed in a subset of mostly seronegative subjects limiting the potential issue of vaccine efficacy in seropositives.

However, as discussed above, adolescents who will not become parents may not have direct benefit from the vaccination while being exposed to possible adverse events of the vaccine. Vaccine efficacy should persist during all child-bearing age. If not, vaccination in children, adolescents and women who are not willing to become pregnant could paradoxically lead to the risk of an increased maternal and thus congenital HCMV infection. It has to be taken in account that some women who had received the vaccination in the past might underestimate the risk of CMV infection for the rest of their lives, including gestational periods.

Women willing to be pregnant

Bearing in mind the ultimate appointees of vaccination, women willing to be pregnant could be a reasonable target population. Among the advantages of choosing this subset as the appropriate target population, there is the benefit in terms of increase of compliance and reduction of costs. In fact, no data are

Table II. *Target population.*

	PRO	CONS
CHILDREN	• High population coverage • Suppression of a relevant epidemic source • Seronegative population	• Costs • Ethical issue • Uncertainty of long term vaccine immunogenicity
ADOLESCENTS + WOMEN IN CHILD-BEARING AGE	• Suppression of a relevant epidemic source • Mostly seronegative population	• Costs • Ethical issue • Uncertainty of long term vaccine immunogenicity
WOMEN WILLING TO BE PREGNANT	• Ethical issue • Certainty of vaccine immunogenicity • Costs	• Low prevalence of family planning • Seropositive population

currently available on the long persistence of vaccine efficacy, therefore, several boosters could be necessary to guarantee protection until the pregnancy period in women who received the vaccine during childhood or adolescence. On the other hand, it has to be considered that not all women plan their pregnancy, in particular in some countries, therefore, this subset would not benefit from the vaccine. Furthermore, it is likely that the schedule of such a vaccine is extended for a period of 6 months meaning that, in order to achieve the best immune protection, women seeking medical advice before pregnancy could be counseled to wait for the last dose of vaccine before becoming pregnant. These problems could be partially solved by widespread informative campaigns on congenital HCMV infection and disease addressed to physicians and women. In fact, there is some evidence that the level of information on HMCV congenital infection and correlated risks among physicians and women in child-bearing age is still limited. Additional concerns are raised regarding the high level of HCMV seropositivity in this population subset. It is, in fact, estimated that HCMV infection is common among women in reproductive age, with seroprevalence ranging from 45 to 100%. Moreover, 75% of HCMV-positive children have mothers who are HCMV-seropositive before pregnancy, thus reflecting the need to develop methods of preventing recurrent HCMV infections (40). However, recent studies by Sabbaj et al. and Griffith et al. provide encouraging data on the efficacy of vaccination even in seropositive individuals. In particular, although additional evaluations are needed, the data showed that both HCMV-specific antibody and CD4 T-cell responses can be boosted after vaccination with an HCMV gB/MF59 vaccine in women who had persistent HCMV infection, suggesting that this kind of vaccine may prevent mother-to-child transmission of HCMV (13, 14). Finally, an ethical concern would have a relevant role in the choice of women willing to become pregnant as the target population for the HCMV vaccine. In fact, the potential positive effect of the vaccine in preventing congenital HCMV infection in the offspring would constitute an indirect positive effect for the vaccine recipients, balancing the possible adverse events of the vaccine in this subset population.

CONCLUSIONS

In conclusion, considering the high economical and social burden of congenital HCMV infection, the need of a vaccine appears to be unquestionably urgent. As discussed in this review, different strategies have been investigated and subunit vaccines seem to be the nearest to becoming licensed. In our view, women willing to be pregnant would be the best vaccine target population at least in the first period post-licensure and until further results on long term persistence of vaccine immunity are available.

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