Med Microbiol Immunol (2016) 205:537–547
DOI 10.1007/s00430-016-0472-z

REVIEW

Prospects of a vaccine for the prevention of congenital cytomegalovirus disease

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Received: 29 July 2016 / Accepted: 3 August 2016 / Published online: 12 August 2016

Abstract Congenital human cytomegalovirus (HCMV) infection is one leading cause of childhood disabilities. Prevention of congenital HCMV disease by vaccination has consequently been identified as a priority public health-care goal. Several vaccine candidates have been introduced in the past that aimed at the prevention of primary HCMV infection in pregnancy. None of these has provided complete protection, and no licensed vaccine is thus far available. An additional level of complexity has been reached by recent studies indicating that the burden of HCMV transmission and disease following non-primary infections in pregnancy may be higher than previously anticipated. Substantial progress in our understanding of the immunobiology of HCMV infection in pregnancy has fostered studies to test revised or novel vaccine strategies. Preventing HCMV transmission has been identified a surrogate endpoint, rendering the conduction of vaccine studies feasible with reasonable effort. Identification of the glycoprotein complex gH/gL/UL128-131 as a mediator of HCMV host cell tropism and evaluation of that complex as a major target of the neutralizing antibody response made manufacturers consider vaccine candidates that include these proteins. Detailed structural analyses of the neutralizing determinants on HCMV glycoprotein B (gB) have revived interest in using this protein in its pre-fusion conformation for vaccine purposes. Studies in pregnant women and in animal models have provided evidence that addressing the T lymphocyte response by vaccination may be crucial to prevent HCMV transmission to the offspring. CD4 T lymphocytes may be of particular importance in this respect. A simultaneous targeting of both the humoral and cellular immune response against HCMV by vaccination thus appears warranted in order to prevent congenital HCMV infection. There is, however, still need for further research to be able to define an immunological correlate of protection against HCMV transmission during pregnancy. This brief review will highlight recent developments in our understanding of the natural history and immunobiology of HCMV infection in pregnancy and their possible impact on the strategies for the development of an HCMV vaccine.

Keywords Cytomegalovirus · Vaccine · Congenital cytomegalovirus infection

Introduction

Human cytomegalovirus (HCMV) infection during pregnancy and subsequent transmission to the developing fetus remains one of the most prompting problems in prenatal care. Congenital infection rates range, according to data from more recent newborn screening programs, between 0.05 and 1 % [1, 2]. These rates differ depending on variables such as geographic location, socioeconomic status, race, and other characteristics of the maternal population [3]. Most of the congenitally infected infants will not suffer from lasting sequelae. Still roughly 25 % of infected infants from mothers with primary HCMV infection in pregnancy will present with sequelae, mostly affecting hearing, eyesight, and central nervous system functions ([4–6], reviewed in [7–9]). The considerable medical burden of congenital HCMV disease becomes apparent when comparing its incidence with those of trisomy 21, cystic fibrosis, or fetal alcohol syndrome, which are all in the

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same range [8, 10]. The development of a vaccine against congenital HCMV infection and disease has thus been identified as a top-level priority goal by healthcare agencies [11, 12].

First attempts to develop a vaccine for the prevention of congenital HCMV disease date back to the early seventies of the past century [13–16]. Numerous HCMV vaccine candidates have been developed and tested in preclinical studies since then, and some of them have entered clinical trials (reviewed in [15, 17–20]). However, none of these has been licensed for the prevention of congenital HCMV infection or disease yet. One reason for this may have been the impracticality of vaccine studies using the prevention of congenital HCMV disease as an endpoint. This is related to the complexity of the disease, the large number of participants that would have to be enrolled to reach statistical significance, and the time frame that would be needed for follow-up [12]. During a recent multidisciplinary meeting held to discuss priorities in the development of an HCMV vaccine, participants agreed on using congenital infection rather than disease as an endpoint for clinical studies [12]. Detection of HCMV in urine or saliva samples by standardized PCR protocols was suggested as a tool to measure infection immediately after birth [1]. As study protocols based on congenital infection appear acceptable, there is increasing interest in testing established or novel vaccine candidates in phase III clinical trials. This brief review will not focus on the different candidates that are currently under consideration, as these have been addressed by several recent reviews [15–20]. Rather a few aspects from studies on the biology and epidemiology of HCMV infections shall be highlighted, as they may have impact on the prospect of HCMV vaccine development.

Primary versus non-primary HCMV infection

Primary HCMV infection in pregnancy has long been identified as a major risk factor for the development of permanent sequelae in children [4, 5]. Transplacental transmission as a consequence of primary maternal infection occurs in roughly 30 % of cases [21–23], with transmission rates increasing during later stages of pregnancy [22]. It remains unclear what prevents transmission of HCMV in the remaining 70 % of women with primary infection. Congenital HCMV disease may be apparent at birth or may develop later in life. A spectrum of clinical manifestations in newborns and infants has been reported as a consequence of primary maternal HCMV infection [6]. For those children who were symptomatic at birth, rates of up to 50 % were reported for the development of neurologic sequelae [4, 5]. These numbers may, however, represent some overestimation, related to enrollment bias [6, 8]. Even in that case, congenital HCMV infection after primary infection is a significant healthcare problem. For that reason, prevention of primary infection has been a long-standing goal in the development of an HCMV vaccine.

The lack of HCMV-specific immune responses at the time of virus encounter, represented by the lack of virus-specific antibodies, is considered a major risk factor for subsequent transmission of the virus. In contrast, HCMV-specific immunity, acquired prior to pregnancy, was taken as marker for protection. Consequently, secondary or non-primary infection was generally considered to be less critical with respect to the development of a severe congenital HCMV disease. There are indeed some indications that the most severe manifestations of congenital HCMV disease may not be seen as a consequence of transmission following non-primary infection, although the data basis for this argument may be weak [8]. A number of reports have, however, documented that the consequences of transmission after non-primary infection are similar to those after primary infection [2, 24–27]. It thus has become increasingly clear that a preexisting HCMV-specific immune response in the case of non-primary infection in pregnancy is incompletely protective against the development of HCMV disease in the offspring. This has been stressed already in early reports [25, 28, 29]. Recent studies on populations with high HCMV antibody seroprevalence rates confirmed these findings [2, 27, 30, 31].

There is, however, discussion about the level of protection against transmission that is imparted by preexisting immunity of the mother [8, 18, 20]. It has been established that higher numbers of congenitally infected infants are seen in populations with higher HCMV antibody seropositivity rates [9, 32]. This supports the idea of an incomplete immunity against transmission induced by prior infection. However, the level of protection against transmission remains an issue of discussion. It is believed that non-primary infection results in HCMV transmission at rates between 1 and 2 %, opposed to 30 % in primary infections. This would argue in favor of considerable protection of natural immunity against transplacental HCMV transmission. Still there is a lack of data about the frequency of non-primary maternal infections in HCMV antibody-seropositive populations, which makes it complicated to measure the risk of transmission. It is further unclear whether non-primary infection is the result of reactivation of latent virus or superinfection with a novel strain [18, 29]. If superinfection was the sole basis for non-primary infection, then one could possibly assume an infection rate comparable to the 2–3 % seen in primary infection [33]. A recent study performed in a highly HCMV-seropositive population in Brazil reported an estimated superinfection rate of 4.2 % [27]. In that case, transmission rates as measured by the incidence of congenital infection could be as high as the rates seen during primary infection [8]. If in

contrast non-primary infections were caused by reactivation of an endogenous HCMV strain, transmission rates would depend on the frequency of viral reactivation rates in pregnancy, which are poorly defined. Consequently, the individual risk of transmission following reactivation cannot accurately be defined at this time. The situation may get even more complicated if one assumes that both reinfection and reactivation contribute to non-primary infection and, eventually, to transmission. In any way, these recent findings suggest that trying to stimulate immune responses similar to those seen in natural infection may not suffice to protect all women against HCMV transmission to their offspring. More research has to be performed especially on the biology and immunology of non-primary infections to define immunological parameters that are associated with prevention of transmission in that group. This will likely provide valuable information on how to improve current vaccine strategies in order to prevent transplacental transmission in both primary and non-primary infections.

Induction of humoral responses

Detectability of an HCMV-specific antibody response before pregnancy was taken, for a long time, as a correlate of protection against severe congenital disease. The protective role of HCMV antibody seropositivity has been challenged by reports describing severe HCMV disease in newborn babies from mothers undergoing non-primary infection [2, 27, 30, 31]. Still the clinical relevance of infection in antibody-seropositive mothers versus infection in antibody-naïve mothers with regard to HCMV transmission and fetal disease remains controversial [8, 20]. In any way, it appears generally accepted that preexisting immune responses to HCMV are only partially protective against HCMV transplacental transmission. Consequently, HCMV-specific antibodies present in mothers with non-primary infection are insufficient to reliably prevent transmission. This leaves us with the question whether there is, at all, a role of antibody effector functions in the prevention of HCMV transmission and disease. There are, of course, arguments in favor of a positive effect of HCMV-specific antibodies in this respect. In murine models, the application of CMV-specific antibodies has been shown to have therapeutic potential [34, 35]. Maidji and colleagues showed that the application of immunoglobulins enriched for HCMV-specific antibodies (hyperimmune globulin; CMV-HIG) suppresses HCMV replication in placental tissue and prevents placental dysfunction [36]. Both CMV-HIG and a monoclonal antibody directed against the HCMV glycoprotein B (gB) neutralized the virus in placental cell cultures [37]. Furthermore, application of CMV-HIG to pregnant women undergoing primary HCMV infection has been suggested to be effective in preventing congenital infection [38]. Yet no such benefit of CMV-HIG could be confirmed in a subsequent placebo-controlled study [39]. CMV-HIG batches are prepared based on HCMV-specific antibody titers rather than on antiviral biological activity. It is thus difficult to appreciate at this stage, if there is a lack of antibody function in preventing transmission following CMV-HIG application or, alternatively, there is simply too little biological activity contained in these preparations. In addition, CMV-HIG application was performed only after virus infection ensued in a therapeutic approach [39]. Thus, antibody application may have simply been too late to be effective. Recent interest is focusing on the use of monoclonal antibodies instead of CMV-HIG [40–42].

HCMV envelope glycoproteins and the antibody response against these proteins have been investigated in numerous studies (reviewed in [43]). Functionality and epitope specificity of the antibodies targeting these proteins are, however, only partially characterized.

The gB is a component of the core fusion machinery of HCMV. It has been identified as a dominant target of the humoral immune response [44, 45]. Early work showed a delay in the induction of gB-specific antibodies following primary HCMV infection [46, 47]. Recent studies indicate that this was related to a limited induction of effector memory B cells, a process that may add to the plethora of HCMV-induced immune evasion mechanisms ([48], reviewed in [49]).

Vaccination based on recombinant gB resulted in partial protection, both in seronegative girls and women and in solid organ transplant recipients [50–52]. Pötzsch and colleagues have shown that potent neutralizing antibodies in human sera against gB are targeted against two previously unknown determinants [53]. The crystal structure of one of these determinants with a human neutralizing monoclonal antibody attached to it was recently published [54]. Data from that work suggest that virus neutralization by this antibody was mediated either by blocking the pre- to post-fusion transition of gB or by precluding the interaction with additional regulators.

The issue has been raised that the gB-specific neutralizing antibody response after vaccination with recombinant gB may differ from the response seen after natural infection [43, 54]. Following gB vaccination, the neutralizing potential of the sera was considerably lower on epithelial cell cultures [55]. This could possibly be explained by preferential induction of gB-specific post-fusion antibodies, in contrast to pre-fusion antibodies induced after natural infection. Such differences may have significant impact on vaccine design, as vaccine candidates containing gB in its natural pre-fusion conformation may be more potent in inducing a broad neutralizing antibody response. More research on that issue is certainly warranted.

The role of gB being the primary target of the neutralizing humoral immune response against HCMV has been recently challenged. Laboratory strains of the virus have been used for a long time for the evaluation of neutralizing antibodies, using fibroblasts as target cells. Results showing that an envelope protein complex consisting of gH, gL, and UL128-131 (referred herein as PC) was absent from laboratory strains [56] raised the issue about the relevance of antibodies against these proteins for HCMV-specific immunity. This was related to the finding that the PC was required for the infection cells like endothelial cells or epithelial cells ([57, 58], reviewed in [59]). Evidence for the importance of antibodies against the PC was provided by comparing human convalescent sera with sera from individuals that had been vaccinated either with the life Towne vaccine [60] or with recombinant gB [61]. Both vaccines induced levels of neutralizing antibodies against HCMV infection of epithelial cells that were well below the titers seen in natural infection [55]. Antibodies against the PC comprise the major faction of virus-neutralizing activity in CMV-HIG [62]. Human monoclonal antibodies directed against the PC showed neutralizing activity against HCMV infection of epithelial and endothelial cells at strikingly low concentrations [63]. Recent data provided evidence that a delayed induction of PC-specific antibodies during primary infection in pregnancy correlates with transmission [64]. In addition, PC-specific antibodies induced by immunization of animals with a Modified Vaccinia Ankara recombinant expressing the PC showed high levels of neutralization on human placental trophoblast cells [65]. These data may suggest that PC-specific antibodies provide protection of the fetus following primary maternal infection. Still this has to be further evaluated. The benefit of such PC-specific antibodies with regard to reactivation or transmission in non-primary infections during pregnancy is unclear, also requiring further investigation. The data generated remarkable interest in the PC to be included in HCMV vaccine candidates, and several strategies have been developed to provide such vaccines [66–71]. It will be interesting to learn about their immunological potential in clinical studies.

Besides gB and the PC, other viral glycoproteins may also be considered as vaccine components [43, 72, 73]. Targeting the glycoprotein O (gO) for instance may assist in preventing infection, but may fail to prevent spread, as shown in a murine model [74, 75]. Furthermore, there may be other antibody effector functions that have not been thoroughly investigated with respect to their antiviral role in pregnancy [76], reviewed in [43]. Considering the knowledge that HCMV has evolved multiple strategies to evade the control of the immune system, including the antibody responses (reviewed in [43]), alternative antibody targets may become important. One such target may be the viral IL-10 molecule. Antibodies against this protein were suitable to modify CMV infection in a rhesus macaque model to the benefit of the host ([77], reviewed in [78]). In summary, there is evidence that antibodies contribute to protection against HCMV infection. However, more research will have to be performed on the relevance of different humoral effector functions and the molecular mechanisms and epitopes that mediate HCMV neutralization by antibodies.

Role of the T lymphocyte response in immunity against congenital HCMV infection

Early adoptive transfer experiments, both in animal models and in clinical studies, have convincingly documented the importance of the CMV-specific T lymphocyte response for the prevention of viral reactivation and disease following hematopoietic cell transplantation ([79–84], reviewed in [85], see also the accompanying article by N.A.W. Lemmermann and M.J. Reddehase in this issue of MMI). This has been confirmed in many studies on patients receiving either hematopoietic cell or solid organ transplants [9, 86–89]. In contrast, surprisingly little is known about the role of T lymphocytes in the prevention of congenital HCMV infection. A weak or delayed, HCMV-specific lymphoproliferative response was found to be associated with viral transmission in cases of primary infection during pregnancy [90–92]. In a more recent study, a significant delay in CD4 T lymphocyte proliferation and a trend toward a delayed CD8 T lymphocyte response in women who transmitted HCMV in the first or second trimester of gestation, as compared to non-transmitting women, were reported [93]. Furthermore, the level of CD4 T lymphoproliferation was significantly lower in those women who transmitted the virus to their offspring. The polyfunctional profile of the HCMV-specific T memory response was reached only months or years after primary infection [94]. This was confirmed and extended in a study on women with primary and non-primary infection during pregnancy, where transmission rates appeared to correlate with low IL-2 production by CD4 T lymphocytes and with a lower frequency of both CD4 T lymphocytes and CD8-CD45RA+ T lymphocytes [95]. A functional exhaustion of CD4 T lymphocytes due to the extended duration of HCMV replication after primary infection in pregnancy was reported by Marchant and coworkers [96]. Although adaptive cellular immunity to HCMV appears to be important, the molecular mechanisms how T lymphocyte responses interfere with HCMV transmission to the fetus are still unclear. It will be necessary to investigate whether such cells have an impact on placental infection and subsequent impairment of placental function [97].

The role of CD4 T lymphocytes will likely become of particular interest in future efforts for vaccine development,

as these cells have also been suggested to be important for controlling maternal viremia and preventing severe disease in the offspring in a recently introduced non-human primate model of rhesus cytomegalovirus infection [98]. In this study, CD4 T lymphocytes were depleted by antibody application. RhCMV antibody-seropositive and RhCMV antibody-seronegative pregnant animals were then challenged by i.v. application of pools of different RhCMV strains. CD4 T lymphocyte depleted, seropositive animals showed some reduction in the levels of viral DNA in the blood following infection, compared to immune competent controls. Viral transmission to the offspring was found in 4/4 seronegative animals, compared to 2/3 seropositive animals. Three out of four seronegative animals experienced fetal loss, whereas all the seropositive animals carried their fetuses to term. There appeared to be more severe RhCMV-induced pathology in the infants of seronegative dams. In addition, CD4 T lymphocyte depletion led to an impairment and delay of CMV-specific antibody and CD8 T lymphocyte responses. As likely expected, all of these results point toward a central role of CD4 T lymphocytes in the overall antiviral response and in the control of RhCMV infection in pregnant animals. There are, however, some limitations in this study, including a confounding effect of CD4 T lymphocyte depletion on maternal health and the numbers of included animals [98, 99]. Thus, the results do not allow a final conclusion about a possible impact of CD4 T lymphocyte function on CMV transmission and disease. Still this and other animal models, like the guinea pig CMV model, may become important to address the question about a possible correlate of protection against HCMV congenital infection in future studies [100, 101].

Prospects of an HCMV vaccine

Congenital HCMV infection is a considerable healthcare burden. As with vaccines against agents like HIV, herpes simplex virus, or Mycobacterium tuberculosis, prophylaxis against prenatal HCMV infection and disease by vaccination represents an urgent, but still unmet clinical need [12, 102, 103]. Consequently, there is substantial interest in both academic institutions and the pharmaceutical industry to develop an effective vaccine against HCMV. A number of candidates have been introduced, some of which have been tested in clinical studies (reviewed in [16–18, 104, 105]. Although there are promising results with some of these vaccines, there is none so far that would have the prospect of providing full protection. Consequently, there is a need for optimization. To provide a scientific basis for this, still open questions have to be addressed regarding the natural history of HCMV infection and the immune responses that have to be targeted by a vaccine.

One issue that was recently raised by the results of studies on populations with high levels of HCMV antibody seropositivity was the relevance of non-primary infections during pregnancy with respect to transmission and disease. Estimates of fetal infection rates of 1–2 % after non-primary infection were recently challenged. Based on biases related to the composition of the study populations, a much higher rate of transmission following non-primary infection was discussed as being conceivable [8]. Even though the most severe consequences may only be seen following maternal primary infection, the occurrence of congenital infection and disease after non-primary infection widely outnumbers that seen after primary infection in low-seroprevalence populations [32]. In high-seroprevalence populations, most congenital HCMV infections are related to non-primary infections [2, 30, 31]. This leads to the conclusion that most cases of congenital HCMV infection worldwide are the consequence of non-primary infection. However, there is still controversial discussion about the relevance of non-primary infection with relation to the severity and frequency of symptoms. In any way, a successful vaccine will have to target both primary and non-primary infections during pregnancy [8, 18]. Vaccine strategies that formerly focused on the prevention of HCMV infection may have to be modified in order to stimulate immune responses that reduce levels of viral replication or other still poorly defined parameters that affect viral transmission at the maternal–fetal interface.

The definition of such parameters will be essential for the optimization of current vaccine strategies. Studies on the immunobiology of non-primary infection may be instructive. The frequencies of transmission following non-primary infection have been an issue of debate. Whatsoever the truth will be, there is a substantial number of women with non-primary infection who do not transmit the virus. A more detailed analysis of the differences in the immune responses in transmitters versus non-transmitters, using available sophisticated immunological technologies, should provide valuable information to define correlates of protection against viral transmission. In this context, phase III efficacy studies of currently pursued vaccine strategies may provide additional information. Based on such results, vaccine strategies may be adapted in a way to control HCMV after both primary and non-primary infection.

A major fraction of women worldwide live in areas where coinfections with other agents during pregnancy are common. One study from The Gambia showed that the prevalence of congenital CMV, mainly due to non-primary infection, was higher than in industrialized countries and that this was associated with active placental malaria [31]. Coinfections with HIV would be another example. In women not displaying advanced stages of HIV disease, the HCMV transmission rates appear to be comparable to

non-infected women [106, 107]. This may, however, be different in HIV-infected women with advanced immunosuppression, where HCMV transmission rates may be high [108]. There are a number of highly prevalent coinfections that will have to be considered when designing HCMV vaccines for widespread application.

A vaccine that induces an immune response matching that induced after natural infection will likely fail to prevent congenital infection in many vaccinees. Even though the consequences of HCMV infection in pregnancy may be mitigated, there still will be public discussion about the healthcare value of such a vaccine. This will have impact on the general acceptance, e.g., in parents who consider having their child immunized. As a consequence, immunity afforded by a vaccine should possibly not only match natural immunity, but should provide a higher level of immunity. This certainly is a formidable task and easily proposed in a paper. The question remains how to reach that goal and to devise strategies to optimize current vaccine candidates. As alluded to above, more research about the immunobiology of HCMV infection in pregnancy will be essential.

HCMV strain variations may become increasingly important for vaccine. A major breakthrough was achieved by demonstrating that laboratory HCMV strains are crippled as they lack large portions of the wt-genome [109]. This challenged text book knowledge in a number of areas about HCMV, as most of the previous studies had been carried out with laboratory strains. One such field was the neutralizing antibody response against HCMV, as outlined above. In addition, there is increasing evidence of wt-strain heterogeneity. The possible impact of different wt HCMV variants on viral pathogenesis and neutralizing antibody responses was already discussed in earlier studies, based on methods that were available at the time ([110–113], reviewed in [114]). More recent data, using advanced polymerase chain reaction and next-generation sequencing technologies, revealed that HCMV exhibits a high genomic diversity, even in the same host ([115–118], reviewed in [119]). Based on these findings, vaccine development has to appreciate HCMV genetic diversity in order to avoid immune escape. Consequently, antigen selection for vaccine purposes may have to focus on those proteins that are conserved in all HCMV variants.

Although not the primary target of a vaccine, effectors of the innate response should be more thoroughly investigated. There is limited information on the role of natural killer (NK) cells or other innate lymphoid cells in the control of HCMV transmission during pregnancy. However, such cells may be important as support to the adaptive response or may even be operative in limiting viral transmission. Decidual natural killer cells, for instance, are known to be essential for placental health and do also provide effector functions to limit HCMV transmission through the placenta [120]. Induction of cytokines like interferon-γ has been reported to be induced in decidual tissues following HCMV infection [121]. Mast cells have been shown to recruit CD8 T lymphocytes to sites of cytomegalovirus infection and may thus support adaptive antiviral functions [122, 123]. The understanding how innate responses intercept with the adaptive immune system at the maternal–fetal interface will provide important information for vaccine design. In addition, more detailed knowledge about the multiple immune evasion functions that HCMV uses on different levels for replication and spread will be necessary to antagonize those restrictions by a vaccine [49, 124–126].

As detailed above, the antiviral antibody response has been extensively studied in congenital HCMV infection and has been the primary target of vaccine research. This is based on the finding that HCMV antibody seropositivity is associated with at least partial protection against congenital infection. The recent identification of the gH/gL/UL128-131 pentameric complex as prime target of the antiviral neutralizing antibody response has fostered attempts to include these proteins in a vaccine. Although this appears obvious, some caution is warranted, given recent findings from the rhesus model of CMV infection that the absence of the PC in a live vaccine may alter the cytotoxic T cell response to a more broadly targeted CD8 T lymphocyte response [127]. It remains to be determined whether such a broad CD8 response would possibly be beneficial to a novel HCMV vaccine.

In addition, other targets of the neutralizing antibody response should possibly be further investigated. The most important candidate is certainly gB. The gB is one of the major targets of the humoral response of infected individuals, still the bulk of these antibodies is non-neutralizing (reviewed in [43]). Yet potent neutralizing determinants were recently identified [53, 54]. Although there was only limited protection conferred by application of recombinant gB as a vaccine in clinical studies, further evaluation of that protein and the antibodies targeting different domains is warranted. This is related to the fact that some of the dominant neutralizing determinants on gB may be conformation dependent and may only be displayed on the protein in the pre-fusion, not in the post-fusion conformation [54]. These determinants may have been inappropriately displayed in recombinant gB preparations.

Besides the PC and gB, other envelope glycoproteins, such as the gH/gL/gO or gM/gN complexes, may also be further evaluated for their use in a vaccine preparation. In addition, besides neutralization of the virus, other antibody effector functions such as Fcγ-receptor activation or alternative antibody targets such as cmv-IL10 may also become relevant for vaccine development [76, 77].

One field of research that should certainly be extended is related to the role of T lymphocytes for the prevention

of transmission. The CD4 T lymphocyte response should be more thoroughly investigated, as time of induction and functionality of these cells may correlate with HCMV transmission and with the induction of the antibody and the CD8 T lymphocyte responses. [93, 95, 96]. CD8 T lymphocytes should also be addressed, especially for their function at the maternal–fetal interface. The non-human primate model that was recently introduced may aid in these analyses [98]. Interestingly, the rhesus model was also used to show that the well-established evasion from CD8 T lymphocyte responses by the virus was essential for horizontal rhCMV reinfection, rendering the rhesus model even more attractive [128]. The detailed knowledge available from studying T lymphocyte responses against HCMV in hematopoietic cell or solid organ transplant recipients may also be helpful to elucidate which effector functions should be targeted and which antigens should be included in a vaccine. In addition, both humoral and cellular responses should be more thoroughly studied for their impact on placental health and on their functionality to prevent transmission at the maternal–fetal interface [129, 130].

Concluding remarks

The development of a prophylactic vaccine to prevent the consequences of congenital HCMV infection is a high-priority goal. Recent results both from clinical studies and from animal models suggest that protection against viral transmission to the offspring is incomplete in the presence of naturally acquired immunity. Defining the immunological and virological parameters that correlate with protection against transmission at the maternal–fetal interface is essential for the optimization of available or future vaccine candidates. Comparing immune responses of HCMV-seropositive mothers who transmit the virus against the responses of mothers who do not transmit will help to define such parameters and provide information for the ultimate definition of a correlate of protection against congenital HCMV infection.

Acknowledgments The work in the author's laboratory is supported by a grant on vaccine development funded by the Else Kröner-Fresenius Foundation.

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