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CRITICAL SURVEY

Controversies in the natural history of congenital human cytomegalovirus infection: the paradox of infection and disease in offspring of women with immunity prior to pregnancy

William Britt

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Abstract Human cytomegalovirus (HCMV) is the most common virus infection in the developing fetus. A fraction of infants infected in utero develop significant life-threatening and organ-threatening disease with over 90 % of infected infants exhibiting no clinical evidence of infection in the newborn period. However, about 10 % of all infected infants will develop long-term sequelae. Early studies stressed the importance of primary maternal HCMV infection during pregnancy as a critical determinant of intrauterine transmission and outcome. This concept serves as the foundation for the development of prophylactic vaccines and biologics such as HCMV immune globulins. More recently, studies in maternal populations with high HCMV seroprevalence have challenged the concept of protective maternal immunity. Findings from multiple studies suggest that preexisting maternal HCMV immunity provides at best, partial protection from disease in the infected offspring and similarly may have limited impact on intrauterine transmission. This brief review will provide some considerations about the apparent paradox of maternal HCMV immunity and congenital infection.

Keywords Congenital CMV · Maternal immunity · CMV

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W. Britt (✉)
Charles Alford Professor of Pediatrics, Departments of Pediatrics, Microbiology, and Neurobiology, Childrens Hospital of Alabama, University of Alabama School of Medicine, Room 160CHB, Birmingham, AL 35233, USA
e-mail: wbritt@peds.uab.edu

Introduction

Human cytomegalovirus (HCMV) remains the most common virus infection in the developing fetus. Intrauterine transmission of HCMV to the fetus results in congenital (present at birth) infection of the newborn infant. Studies from several sites in the world have documented the commonplace occurrence of this perinatal infection with rates ranging from 0.2 % to as high as 3–6 % in live births [1–3]. More valid results from studies using newborn screening have reported congenital HCMV infection rates between 0.05 and 1 % depending on characteristics of the maternal populations [4, 5]. To place the prevalence of newborns with congenital HCMV infections in context with other well-recognized diseases of childhood, cystic fibrosis occurs in about 1/3000 live and trisomy 21 (Down's syndrome) in about 1/700 live births. Thus, congenital HCMV infection occurs in a large number of infants and children with an estimated 40,000 new infections each year in the USA, 250,000 in India and 35,000 in Brazil based on birth rates in these countries. Although the vast majority of infants with congenital infection exhibit no clinical symptoms or well-recognized long-term sequelae, up to 10 % of infants will have manifestations of this infection during early development, particularly neurological sequelae [2, 5, 6]. As a result, prevention and treatment of this intrauterine infection have been identified by private and government agencies as a priority for programs aimed at improving child health [7, 8].

Technological advances provided by modern molecular biology coupled with increasing interest in vaccine development led to early optimism for the rapid development of a prophylactic vaccine for congenital HCMV infection. In addition, early studies using an attenuated HCMV vaccine in transplant patients suggested that HCMV-induced

disease could be modulated in immune suppressed hosts by vaccination and raised the possibility that similar approaches could also limit disease in infants infected in utero with HCMV [9]. More recently, hopes for a successful vaccine to limit disease associated with congenital HCMV infection were bolstered by the results from a clinical trial using an adjuvanted recombinant protein as a candidate vaccine [10]. Although initially this study was heralded as supportive of the potential of such a vaccine platform, more careful analysis of the findings in this study indicated that protection from maternal acquisition of HCMV was transient and that the differences in outcome between vaccine and placebo groups was less than robust raising the possibilities that unrecognized confounders could have impacted the results of this trial. Furthermore, the study was not powered to allow the investigators to determine whether this vaccine platform would prevent intrauterine transmission or impact disease associated with fetal infection. As a result, further testing of this vaccine has been limited and newer vaccine platforms are being pursued by investigators in academia and industry.

Perhaps the most perplexing aspect of the natural history of congenital HCMV infection and one that clearly distinguishes it from other perinatal infection such as congenital rubella or congenital toxoplasmosis is the incomplete protection from infection, transmission or disease afforded by maternal HCMV immunity acquired prior to conception [4–6, 11–15]. Very early observations identified this unique characteristic of the natural history of congenital HCMV infection and when these older findings were coupled with more recent observations, clearly documented that maternal immunity is not completely protective in terms of the prevention of virus transmission to the fetus nor it is protective in terms of limiting disease following fetal infection [11, 12, 16, 17]. Although the mechanism(s) that accounts for the lack of protective immunity in women with naturally acquired immunity to HCMV is unknown, several observations derived from studies in pregnant women with primary HCMV infection have suggested testable hypotheses that could explain the failure of preconceptional maternal immunity to HCMV to limit intrauterine transmission and disease.

Natural history of congenital HCMV infections: the importance of preconceptional maternal HCMV immunity and intrauterine transmission

The natural history of congenital HCMV infections has been described in publications spanning 3–4 decades [1, 5, 18, 19]. More recently, the application of rigorous epidemiological tools and study design has greatly improved our understanding of this complex maternal–fetal infection. Unfortunately, many of the concepts that continue to drive programs to prevent and/or modify congenital HCMV infections are based on results from early, less rigorously performed studies that were understandably limited by methodologies that were available when these studies were completed. Several characteristics of congenital HCMV infection have helped establish a framework for the interpretation of data derived from studies of the natural history of this infection. The first is the classification of the maternal infection that is associated with the congenital HCMV infection. Maternal infections have been classified as primary infections (HCMV acquired in pregnancy in the absence of any evidence of serological immunity to HCMV with the de novo development of HCMV-specific IgG antibodies) and non-primary infections in which a congenitally infected infant is born to a woman who had documented serological immunity to HCMV prior to pregnancy. Older terminology for this latter category was reactivated/recurrent maternal infection even though no definitive evidence was available to support such designations. In fact, more recent findings have argued that the women with non-primary infections are frequently reinfected (superinfected) with serologically distinct strains of HCMV [20–22]. The second epidemiological classification is based on the expression by a congenitally infected newborn infant of clinical findings that are consistent with congenital HCMV infections, a so-called symptomatic congenital infection as compared to infants that have no symptoms of congenital infection (asymptomatic infection). These two classifications simplified study design and assessment of the outcome of maternal and fetal infections. Unfortunately, the attempt to simplify the epidemiology of this complex maternal–fetal infection also resulted in the arbitrary application of these epidemiological classifications to diverse maternal populations and their offspring and led to delays in fully investigating the unique biology of this perinatal infection.

A summary of the natural history of congenital HCMV infection is depicted in Fig. 1. Approximately 30 % of women experiencing a primary infection with HCMV during pregnancy will transmit virus to their developing fetuses, and current estimates argue that transmission is more common in the later part of pregnancy [1, 5, 23, 24]. Of the infants infected in utero, about 5–10 % will exhibit some symptomatology at birth that is consistent with congenital HCMV infection (symptomatic infection), including hepatosplenomegaly, hepatitis, petechial rashes, chorioretinitis, microcephaly, and neurological findings, including seizures [25, 26]. Laboratory findings include elevated liver transaminases, direct hyperbilirubinemia, thrombocytopenia, abnormal neuroradiological findings and hearing loss [25–28]. More recently, some authors have included intrauterine growth restriction as a clinical finding of symptomatic congenital HCMV infection. Long-term

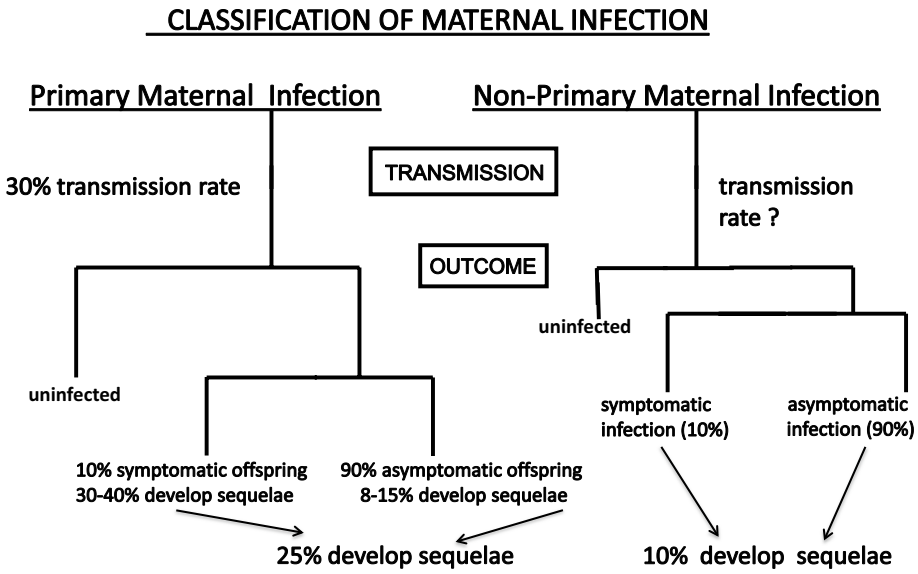

Fig. 1 Natural history of congenital HCMV infections. Figure depicts the importance of the type of maternal infection during pregnancy to intrauterine transmission and the resulting outcome of the fetal infection

neurological sequelae develop in about 35–45 % of infected infants with clinical symptoms at birth, most often abnormal hearing [25, 26, 29]. However, it is important to note that initial estimates on number of infants with severe long-term sequelae in symptomatically infected infants were based on observations from large populations of congenitally infected infants that contained both patients enrolled through screening studies and patient referred specifically secondary to symptoms consistent with congenital CMV infection. As a result, the true prevalence of disease and long-term sequelae in infants with symptomatic congenital HCMV infection was overestimated secondary to the contamination of the study population by the referral population (enrollment bias) [30]. Thus, some of the reported prevalence of specific clinical abnormalities in infants with symptomatic infections likely will require revision and additional natural history studies based on this more recent data [30].

Similarly, much of what is described about the outcome of infections in pregnant women undergoing a non-primary maternal infection is based on inferences from studies comparing outcomes to those of women following primary infection. As an example, it has been often stated that the rate of HCMV transmission from a women with preconceptional serological immunity to her offspring is about 1–2 % because this number reflects the overall rate of infants with congenital HCMV infection in a number of different maternal study populations with high HCMV seroprevalence. However, this concept is based on the assumption that every woman with preconceptional serological immunity in a population has the same risk of acquisition of HCMV during their pregnancy (reinfection) or perhaps, a similar chance virus reactivation during pregnancy. There is no published data to support either of these assumptions. In fact, data from studies of serologically non-immune women from the several maternal populations with differing HCMV seroprevalence have demonstrated an annual seroconversion (infection) rate in non-serologically immune women of only about 2–3 %, suggesting that only a small minority of women will be exposed to infectious HCMV during their pregnancy [31]. Thus, without a more definitive understanding of the natural history of HCMV infections in women with HCMV serological immunity prior to pregnancy, it remains uncertain whether preconceptional serological immunity to HCMV can efficiently limit intrauterine transmission of HCMV following a reinfection with a new strain/family of virus or reactivation of a virus population in HCMV immune pregnant women.

To illustrate the potential variance of ranges of intrauterine transmission in women with non-primary HCMV infections during pregnancy, we analyzed data derived from a population of women in Brazil who have near universal (>96 %) seroimmunity to HCMV prior to pregnancy [4, 22]. Utilizing several serologic assays, we determined that overall, about 4.2 % of this population of over 7800 women had evidence of a reinfection with a new strain/family of HCMV during pregnancy [22]. The congenital infection rate of offspring in this population was 1.1 % (87 infected infants), and in women who delivered an infected infant, the rate of reinfection with a new strain/family of HCMV was about fourfold higher (17 %) [4, 22]. As illustrated in Fig. 2, the rate of intrauterine transmission in this maternal population could be as high as 27 % if the 4.2 % risk of reinfection with a new strain of HCMV extends equally over this population, a rate very similar to that seen following primary maternal infection (Fig. 2). Conversely, if the risk of reinfection is higher and reflected by the 17 % rate of reinfection in women who delivered an infected infant,

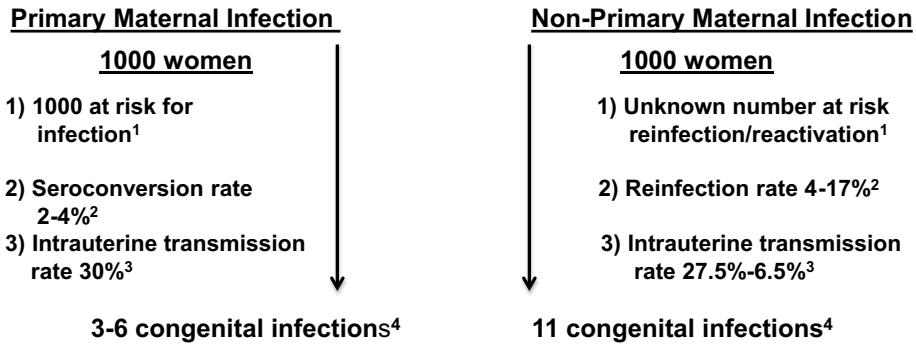

Fig. 2 Estimated transmission rates following different types of maternal HCMV infection during pregnancy. (1) Assumption that 100 % of non-immune women (primary) susceptible to HCMV infection. Unknown number of immune women (non-primary) susceptible to infection and/or reactivation; assume 100 % non-immune women susceptible to infection. (2) Seroconversion rates for women with primary infection based on reported rates of maternal seropositivity 60–80 % [31]. Reinfection rates of 4 % for entire population of women with non-primary infection and 17 % of immune women who delivered congenitally infected infants [22]. (3) Transmission rates following primary infection documented in several publications. Transmission rates following non-primary infection are not known; rates calculated based on reinfection rates during pregnancy (4 and 17 %) [22]. (4) Congenital infections following non-primary maternal infections from screened newborn populations in Brazil [4]. Congenital infections following primary maternal infection approximated based reported rates of congenital infections in newborn infants from maternal populations with annualized seroconversion rates of 2–4 %. Note that the number of infected infants following non-primary maternal infection is about threefold to fourfold greater than number following primary maternal infection [14]

the intrauterine transmission rate would drop to about 7 % (Fig. 2). Thus, preexisting maternal immunity to HCMV could potentially offer little to minimal protective activity from HCMV transmission to the developing fetus. If this is the case, then establishing levels of serological immunity that mimic those seen in mothers with naturally acquired serological immunity by prophylactic vaccines or passive transfer of immune serum could be expected to provide only limited protection from HCMV infection in the developing fetus. Consistent with this possibility has been the description of the failure of treatment with HCMV hyperimmune immunoglobulin to prevent intrauterine transmission of HCMV in pregnant women undergoing a primary infection [32].

Natural history of congenital HCMV infections: the importance of preconceptional maternal HCMV immunity to the outcome of intrauterine HCMV infection

Although the lack of complete protection from intrauterine transmission provided by HCMV seroimmunity has been recognized for over three decades, many investigators have continued to argue that maternal immunity to HCMV prior to pregnancy will prevent damaging congenital HCMV infections. This concept has been supported by studies comparing outcomes of infants infected following primary maternal infections to congenitally infected offspring of women with non-primary infections during pregnancy [11, 33]. However, these early studies were often composed of populations of infants from referral populations as well as infants from screened populations and included women who could not be rigorously classified as having a primary or non-primary infection. Subsequent studies of the outcome of infants infected following a non-primary maternal infection from screened populations in which maternal HCMV serological status prior to pregnancy could be firmly established have demonstrated that the outcome of infants infected following primary and non-primary infections is remarkably similar [4, 15–17, 34, 35]. Comparisons of outcomes from three larger studies are provided in Table 1 and illustrate that maternal immunity prior to pregnancy cannot be viewed as protective in terms of altering long-term outcome. It is important to note that these studies shown in Table 1 were conducted at three different institutions during three different time periods yet have provided similar conclusions. In contrast, others have argued that maternal immunity prior to pregnancy can prevent the most severe congenital infections associated with in the most striking long-term sequelae. Although this claim may indeed be true, no well-conducted studies have been performed to support such a conclusion. Furthermore, infants with the most severe congenital HCMV infections with multiorgan system disease and severe CNS damage represent perhaps <5 % of the total number of infants with congenital HCMV infections. Thus, any protection that is offered by preexisting maternal immunity must be viewed as being of benefit in only a limited number of infected infants and results in only a minimal impact on the overall disease burden from congenital HCMV infections. Together, available data fail to provide a convincing

Table 1 Outcome of congenital HCMV infection as function of maternal infection

Study	Maternal infection	Symptomatic infection	Sequelae/Poor outcome
Townsend, et al. [15]	Primary	8/82 (9.8 %)	5/82 (6.1 %)
	Non-Primary	6/45 (13.3 %)	9/45 (20 %)
Ahlfors, et al. [16]	Primary	9/30 (30 %)	5/23 (22 %)
	Non-Primary	9/232 (28 %)	8/23 (35 %)
Ross, et al. [34]	Primary	19/176 (11 %)	19/176 (11 %)[a]
	Non-Primary	14/124 (11 %)	14/124 (11 %)[a]

[a] Hearing loss. Preliminary analysis did not show significant differences between overall long-term outcome in offspring of women with primary or non-primary infections during pregnancy that were included in this study

argument for a protective role of maternal immunity in the outcome of congenital HCMV infections.

The natural history of congenital HCMV infection: Insights into the lack of protection provided by maternal immunity

Early in the study of the epidemiology of congenital HCMV infections investigator determined that the number of infants with congenital HCMV infection following non-primary maternal HCMV infection greatly outnumbered infants infected following a primary maternal infection [11]. Various estimates have been made, but early data by Stagno and colleagues suggested that there were threefold to fourfold more congenitally infected infants delivered to women with non-primary HCMV infection than from women with primary infection in the same maternal population, a value that has been confirmed in more recent reports [11, 14]. Subsequent studies have confirmed the larger contribution of infected offspring of women with non-primary infections to the total number of infants with congenital HCMV infections, and meta-analysis of primary data has shown that the major contribution to the disease burden associated with congenital HCMV infection is from infected infants born to women with non-primary infections during pregnancy [1, 14]. Such findings are of particular importance in the developing world because limited studies in these population have reported that the HCMV seroprevalence in maternal populations in countries such as the Ivory Coast, Gambia, Brazil and India is >90 %, findings that indicate that almost all congenitally infected infants in these populations will result from non-primary maternal infections [3, 4, 36–40]. It is of interest that the incidence of congenital HCMV infection is highest in populations with the highest maternal HCMV seroprevalence [41, 42]. This characteristic of the natural history of HCMV is striking and best appreciated graphically (Fig. 3a). These data clearly demonstrated that as the HCMV seroprevalence of the maternal population increases, the rate of congenital HCMV infection increases and does not reach a threshold at which the incidence of congenital HCMV infections begins to fall. Thus, in the case of highly HCMV seroimmune maternal populations, it unlikely that a phenomena exists similar to community's immunity to rubella that has been described as contributing to the decline of congenital rubella syndrome following epidemic outbreaks prior to the introduction of the rubella vaccine (see below).

The lack of protective immunity provided by preexisting maternal immunity is unique to HCMV when compared to other microbes associated with perinatal infections such as toxoplasma gondii, rubella virus and herpes simplex virus (HSV). Exposure of pregnant non-immunocompromised women with preconceptional immunity to toxoplasma gondii and rubella rarely results in damaging infection of the fetus. Scattered case reports have described rubella reinfection of pregnant women with existing rubella immunity, thus suggesting an infrequent occurrence of reinfections and transmissions. Similarly, transmission of toxoplasma has been reported in pregnant women with underlying deficits in immunity such as those with HIV [43, 44]. Herpes simplex virus infection do occur in newborn infants born to women with seroimmunity to HSV, but the rate of transmission and the clinical course of the ensuing infection are markedly modified by maternal immunity that is transplacentally transferred to the newborn infant [45, 46]. Thus, HCMV is unique within this group of agents associated with clinically significant perinatal infections in that maternal immunity provides only limited protection from infection and from disease in the offspring. An informative contrast to the natural history of congenital HCMV infection is provided by the natural history of congenital rubella syndrome and its control by a successful vaccine [47]. Rubella virus infection in women of child-bearing age occurred in periodic epidemics, and studies have suggested that these increases in rates of infection could be traced to falling levels of immunity in the population [47–49]. Similarly, the incidence of congenital rubella syndrome followed increasing rates of rubella infection in population until maternal seroimmunity exceeded about 85 % [49]. At this level of

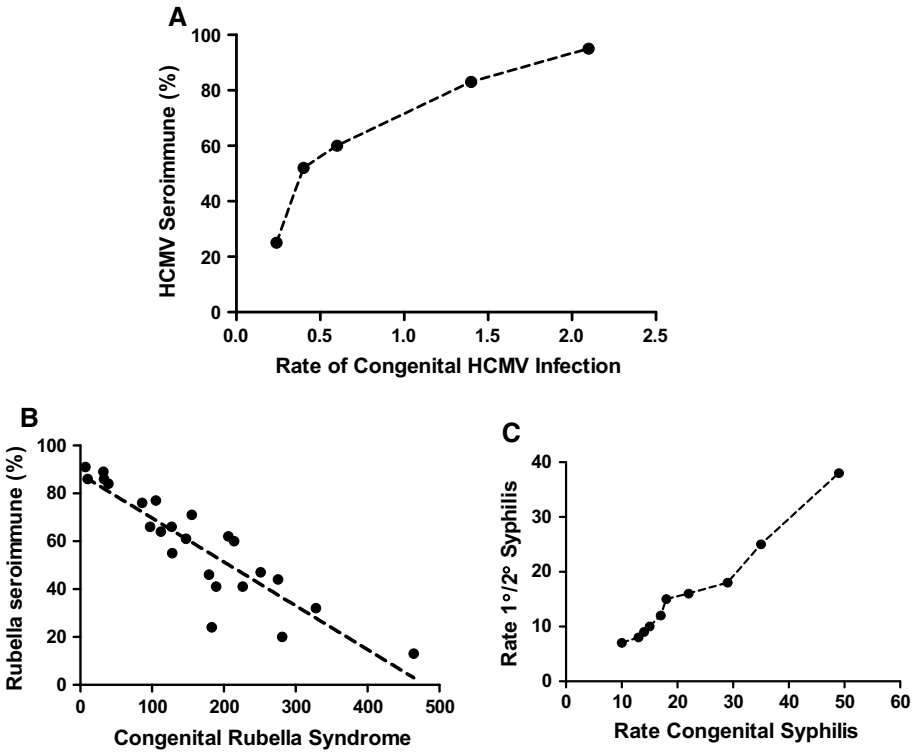

Fig. 3 HCMV seroprevalence and congenital HCMV infection. **a** Rates of HCMV maternal seropositivity obtained from Stagno et al. [41]. **b** Values for rates of rubella seroreactivity (%) and congenital rubella syndrome (cases/10^5) from Cutts et al. [49]. **c** Estimates for case of congenital syphilis and community syphilis activity (cases/10^5) from MMWR [50]

rubella seroprevalence, the incidence of congenital rubella fell to nearly zero suggesting that vaccine coverage >85 % would be sufficient to dramatically reduce the incidence of congenital rubella syndrome. Such a threshold of maternal HCMV seroprevalence that would lead to the elimination of congenital HCMV infection does not appear to exist (Fig. 3b). In fact, modeling of the impact of primary and non-primary maternal HCMV infection and congenital HCMV infections suggests that the burden of congenitally infected infants born following primary maternal infection will not significantly impact the much larger burden of infants infected following non-primary infection [42]. Alternatively, the natural history of congenital syphilis is at least superficially, remarkably similar to that of congenital HCMV infections. The prevalence of congenital syphilis infections is closely related to the incidence of syphilis in the community as measured by serology that reflects the presence of active infection in infected individuals such that an increasing prevalence of active syphilis in women of child-bearing age results in increasing incidence of cases of congenital syphilis (Fig. 3c) [50]. Similar to congenital HCMV infections, protective immunity to *T. Pallidum*, the spirochete that causes syphilis, is not well understood but apparently non-existent in community-acquired infections as evidenced by a well-recognized occurrence of reinfections with *T. Pallidum* even in women with recently treated infections that have documented *T. Pallidum* specific IgG antibodies [51]. Importantly, reinfections in these previously infected women can lead to transplacental transmission and congenital syphilis. The lack of the development of protective immunity following infection has not been fully defined but sequence variability within the TprK surface protein of *T. Pallidum* among isolates recovered from clinical samples suggests the possibility that protective immunity is limited to homologous challenge [51–53].

Variability in the sequence of envelope proteins of the HCMV virion has been described as strain-specific antibody responses that suggest incomplete protection from heterologous viruses [20, 54–56]. In addition, non-specific mechanisms that limit virus neutralizing antibody recognition of infectious virions including the existence of a glycan shield have been reported [57]. Similarly, a multitude of virus-encoded immune evasion functions has been shown to limit CD8+ and CD4+ T lymphocyte as well as NK cell recognition of HCMV-infected cells [58, 59]. In experimental animal models, these immune evasion functions have been shown to facilitate virus dissemination and persistence [60–62]. Recently, the critical role played by the immune evasion functions encoded by rhesus macaque CMV (RhCMV) that target class I MHC presentation of viral antigens in the reinfection of previously immune animals has been described [63]. Thus, the capacity of HCMV to reinfect previously immune hosts and to be transmitted to the developing fetus can be readily reconciled by the capacity of the virus to evade immune control. The ease with which these primate CMVs can reinfect an immune

host is somewhat unanticipated but has been elegantly shown in studies utilizing RhCMV as a replicating vaccine vector platform to deliver simian deficiency virus (SIV) antigens to RhCMV immune rhesus macaques [64]. These studies have provided very convincing evidence of the capacity of vectored SIV antigens to generate a protective effector/memory CD8+ T lymphocyte response and clearly demonstrated that animals could be repeatedly reinfected with the homologous RhCMV vector backbone containing different components of SIV [65]. These data have convincingly demonstrated that immunity induced following infection with RhCMV cannot prevent subsequent infection with the homologous strain of RhCMV, a finding consistent with the relatively frequent reinfection of immune women with new strains of HCMV.

Incomplete protective maternal immunity and congenital CMV infection: implications for development of prophylactic vaccines for congenital HCMV infections

The bulk of available evidence argues that simply inducing natural immunity to HCMV will not alter the current natural history of congenital HCMV infection in any population [42]. The mechanisms that account for the incomplete protection by maternal immunity to HCMV are unknown even though numerous potential explanations that account for the failure of immunity have been described. Yet the question remains can protective immunity be induced by increasing the magnitude or quality of natural immunity? Published studies have reported differences in both the magnitude and quality of antibody responses in women who transmitted viruses as compared to those who did not transmit virus to their fetuses following a primary infection [66–68]. However, these responses followed a primary infection, and results from these studies cannot be directly compared to responses in women with existing serological immunity to HCMV who may have been immunologically boosted by reinfection multiple times before pregnancy. An alternative possibility is that HCMV has evolved mechanisms to evade conventional immune responses, including responses against structural envelop proteins that appear to be the primary targets of virus neutralizing antibodies. If so, then immunization against novel targets that modify the early events of HCMV infection could potentially induce protective immunity, albeit non-conventional in the sense of conventional vaccines. One possibility that has been recently described is immunity to the HCMV-encoded IL-10. In model systems to investigate such an approach, investigators-induced immunity to the viral IL-10 encoded by the closely related RhCMV and then analyzed RhCMV replication in rhesus macaques [69]. The production of antibodies reactive with the viral IL-10 modified the course of virus replication and excretion suggesting that inhibiting the function of virus-encoded molecule thought to be important for the early phases of virus infection could shift control of virus infection to be in favor of the host [69]. Other approaches currently under consideration could potentially lead to prophylactic vaccines that could prevent HCMV infection and limit maternal as well as fetal infection.

Implications of incomplete protection from transmission and disease afforded by preconceptional maternal HCMV immunity

In this brief review of existing data, it could be argued that maternal immunity to HCMV that develops following natural infection provides only limited protection from intrauterine transmission and in contrast to a widely held concept, from symptomatic congenital infection. Although it could be debated whether the most severely affected congenitally infected infants are only born to women with primary infection during pregnancy, such infants with severe congenital infections contribute only a minor component to the overall burden of disease associated with congenital HCMV infection. Careful reviews of older reports and more recent studies clearly demonstrate the need for more comprehensive studies of the natural history of congenital HCMV infections that follow non-primary maternal infections. The results of such studies could be of great value to vaccine development programs and to other interventions such as universal counseling of pregnant women to limit exposure to HCMV. Finally, deployment of candidate vaccines that induce adaptive immune responses that parallel those seen in women with naturally acquired HCMV infection could have limited benefit in many maternal populations as the vast majority of women in these populations have established immunity to HCMV acquired during childhood and in early adolescence.

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