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SUPPLEMENT ARTICLE

Human Cytomegalovirus Infection in Women With Preexisting Immunity: Sources of Infection and Mechanisms of Infection in the Presence of Antiviral Immunity

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Human cytomegalovirus (HCMV) infection remains an important cause of neurodevelopmental sequelae in infants infected in utero. Unique to the natural history of perinatal HCMV infections is the occurrence of congenital HCMV infections (cCMV) in women with existing immunity to HCMV, infections that have been designated as nonprimary maternal infection. In maternal populations with a high HCMV seroprevalence, cCMV that follows nonprimary maternal infections accounts for 75%–90% of all cases of cCMV infections as well as a large proportion of infected infants with neurodevelopmental sequelae. Although considerable effort has been directed toward understanding immune correlates that can modify maternal infections and intrauterine transmission, the source of virus leading to nonprimary maternal infections and intrauterine transmission is not well defined. Previous paradigms that included reactivation of latent virus as the source of infection in immune women have been challenged by studies demonstrating acquisition and transmission of antigenically distinct viruses, a finding suggesting that reinfection through exposure to an exogenous virus is responsible for some cases of nonprimary maternal infection. Additional understanding of the source(s) of virus that leads to nonprimary maternal infection will be of considerable value in the development and testing of interventions such as vaccines designed to limit the incidence of cCMV in populations with high HCMV seroprevalence.

Keywords. congenital CMV infection; maternal non-primary infection; recurrent infection; CMV infection in pregnancy.

Human cytomegalovirus (HCMV) infections are ubiquitous in all populations and in most regions of the world are acquired during infancy and childhood. Although HCMV infection results in lifelong persistence, there are limited definitive data linking HCMV infection to definable clinical syndromes in the vast majority of individuals with intact adaptive and innate immune systems. However, there is an extensive literature describing associations between HCMV infections and human cancers, cardiovascular disease, and immunosenescence [1–6]. Although far from definitive, several lines of evidence are often presented that convincingly argue for a role of HCMV in either the development and/or phenotypic expression of these diseases. Ongoing studies will hopefully clarify the role of HCMV in these diseases. In contrast, HCMV infections in individuals with compromised immune systems, secondary to untreated HIV infection, treatment with immunosuppressive agents to limit allograft rejection, or following treatments for autoinflammatory or autoimmune diseases, often result in disease in multiple organ systems that can be directly attributed to HCMV replication. In addition, severe multisystem disease can be observed in newborn infants who are infected in utero and in infants with severe immunodeficiencies, particularly those with deficits in T-lymphocyte function [7, 8].

The source of HCMV infections in most normal hosts in community settings can be traced to close contact with individuals shedding virus. Because young infants infected with HCMV commonly shed large amounts of virus for prolonged periods of time, observational studies have frequently identified exposure to young children as a major risk factor for HCMV infection [9–11]. Other studies have demonstrated the transmission of HCMV through sexual contact with increased HCMV seroprevalence being observed in couples discordant for HCMV infection, women attending sexually transmitted infection (STI) clinics, and in early studies of homosexual men [12–14]. Perhaps the most common route for HCMV transmission in many populations in the world is through ingestion of breast milk from a previously infected mother, with reported rates of transmission being as high as 50%–70% [15–18].

In contrast to community sources of HCMV, infections identified in hospital settings such as those in transplant recipients can frequently be traced to specific sources such as the transplanted organ, blood products from donors with HCMV

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infections, or from reactivation of an existing latent infection in the immunocompromised host [19–24]. Notably, infections acquired from the infected allograft and/or blood products can occur in individuals with preexisting immunity to HCMV, although these new infections are established in the presence of deficits in adaptive and innate immune responses. Similarly, observations made in decade-old studies of HCMV infection in homosexual men reported that these individuals were not infrequently infected with genotypically diverse strains of HCMV, a finding that suggested reinfection in this population [14, 25]. However, the underlying immunostatus of these populations of homosexual men was not well defined, so it could be argued that reinfections in this population also resulted from deficits in adaptive immunity. Yet it is important to note that infection (reinfection) of the previously immunocompetent host, including pregnant women, also appears to take place [26, 27]. Studies in solid organ allograft recipients have provided definitive evidence that reinfections with HCMV occur and contribute to HCMV-associated disease in these individuals, yet mechanisms leading to reinfection in the presence of existing adaptive immunity to HCMV remained incompletely defined in allograft recipients and even less well understood in previously infected, immunocompetent women. In contrast to allograft recipients, reinfections that presumably follow community acquisition of HCMV are similar to those occurring in nonimmune individuals in that they are rarely, if ever associated with clinical symptomatology or documented laboratory abnormalities. Yet, reinfections in pregnant seroimmune women are believed to represent a major source of infection leading to intrauterine transmission and congenital HCMV (cCMV) infection [28–30]. Thus, understanding the source(s) of reinfection in pregnant seroimmune women and mechanisms responsible for acquisition of a new virus are of considerable importance in the design and potential testing of prophylactic vaccines as well as other interventions that could limit the incidence of cCMV, particularly in populations with high HCMV seroprevalence [31].

REINFECTION IN SEROIMMUNE WOMEN: INFECTIONS FOLLOWING EXPOSURE TO NEW VIRAL VARIANTS

Early studies in women attending STI clinics provided evidence that women could be infected with multiple genotypes of HCMV as defined by comparison of viral DNA fragment size following restriction endonuclease digestion [32]. Because individual women shed different viral genotypes at different times, the authors concluded that reinfection from an exogenous source of HCMV was relatively frequent [32]. Similarly, studies in children in group care facilities demonstrated shedding of different HCMV genotypes over time in individual children [33]. Interestingly, in this study about 16% (6/37) of the children who shed virus in longitudinal samples were found to have different viral genotypes in sequential specimens collected during the study [33]. These findings were interpreted as evidence that some children were reinfected multiple times within the time frame of this study [33]. Last, a number of investigators documented infections with multiple strains of HCMV in homosexual men utilizing similar methodologies, thus providing additional evidence of reinfection with genotypic variants of HCMV [14, 25].

Reinfection of seroimmune pregnant women with new genotypic variants of HCMV as a source of nonprimary maternal infections and resultant cCMV infection was described nearly 20 years ago [26]. Results from these studies were based on the presence of polymorphisms in a major antigenic site of the virion envelop glycoprotein H (gH) that allowed serological detection of new infections in previously HCMV seroimmune women [34]. Using antigens derived from this region of gH, acquisition of new viruses during pregnancy were described as a seroconversion, that is, the detection of new antibody reactivities to a specific antigenic site on gH [26]. In this study, the authors documented that women transmitted newly acquired viruses to their fetuses using sequence analysis of the UL75 viral gene (gH) from viruses recovered from infants with cCMV [26]. Subsequently, combinations of polymorphisms in antigenic sites on both envelope proteins gB and gH of HCMV have been used to further increase the sensitivity of serological detection of reinfection in pregnant seroimmune women [35, 36]. Using this assay system, the incidence of serologically defined reinfections in normal women during an intrapartum period was estimated to be about 30% over a period of 36 months [27]. Thus, reinfection of immunocompetent women as defined by the development of antibody reactivity to new antigenic determinants appears to be relatively frequent in some populations.

REINFECTION IN SEROIMMUNE WOMEN: PERSISTENT INFECTION VERSUS INFECTION WITH EXOGENOUS VIRUS

Two sources of virus that can result in infection of HCMV immune women have been proposed: (1) reactivation of a latent virus infection leading to recurrent infection and (2) infection following exposure to an exogenous source of virus. Although either mechanism could lead to intrauterine transmission and cCMV, distinguishing between these two sources of virus is of considerable importance in the design of strategies to limit the incidence of cCMV following nonprimary maternal infections, regardless if such strategies will rely on vaccine-induced immunity or behavioral interventions to limit exposures.

Reactivation of latent HCMV has long been argued to be the source of recurrent or nonprimary maternal infections leading to cCMV [37]. This paradigm is based on early studies that relied entirely on restriction enzyme digestions and comparison of restriction fragment length polymorphisms (RFLPs) as a methodology to genotype viral isolates, including studies designed to establish genetic relatedness of viruses recovered

following transmission from an index case [38]. This approach represented a major advance in epidemiological studies of HCMV infection because early attempts to develop serological grouping of HCMV to aid epidemiological investigations of HCMV infections were unsuccessful, even though serological differences could be demonstrated [39, 40]. However, results from many of the published studies that utilized RFLPs to genotype viral isolates can no longer be considered as definitive evidence of genetic relatedness of HCMV isolates as the use of limited combinations of restriction enzymes that were often included in these studies sampled very limited amounts of the genetic diversity of HCMV. Furthermore, in these early studies virus isolates were often extensively passaged in vitro. More recent studies have shown that even brief passaging in vitro can introduce a variety of mutations into viral isolates, including deletions of regions of the genome. Such mutations could further confound a definitive assignment of genetic relatedness between viral isolates. Thus, even though the reactivation of latent virus leading to nonprimary infections in pregnant women remains consistent with an established paradigm in the biology of HCMV in the immunocompromised transplant recipient, this paradigm has not been rigorously validated in pregnant women in studies using contemporary technologies. Although definitive data are lacking, it is important to note that the frequency of reactivation of latent HCMV infections during pregnancy has not been defined but could be significant if intermittent reactivations of latent infections account for virus shedding in seroimmune women during pregnancy. However, available data that would argue that virus shedding during pregnancy reflects frequent reactivations of latent infections are confounded because most studies of virus shedding in pregnant seroimmune women have included women with increased risk for exposure to HCMV from known sources such as young children.

More recent findings from population studies have provided epidemiological data that are inconsistent with reactivation of latent virus as a sole source of nonprimary infections in pregnant seroimmune women. An epidemiological feature of cCMV infection that was noted decades ago is that as the seroprevalence increases in a maternal population, the rate of cCMV infections also increases, such that populations with the highest seroprevalence also have the highest prevalence of cCMV infections [31, 41, 42]. This observation is consistent with reactivation of latent maternal infections and resulting intrauterine transmission being a stochastic event such that as more women in the population are persistently infected, the prevalence of cCMV could be expected to increase. However, studies in some maternal populations argue against such a direct relationship between seroprevalence, reactivation, and cCMV infection. This is illustrated by the results from a recent study in Finland in which the prevalence of cCMV was calculated as 2 per 1000 based on results from screening of 20 000 newborn infants for HCMV shedding [45]. This result was somewhat surprising as

Table 1. Human Cytomegalovirus (HCMV) Seroreactivity and Congenital HCMV Prevalence in Maternal Populations

Location	Maternal Seroreactivity	cCMV Prevalence
Brazil (Mussi-Pinhata, 2018) [29]	98%	6.1
China (Wang, 2011) [28]	96%	7
Japan (Tanimura, 2017) [43]	71%	6.4
France (Leruez-Ville, 2017) [44]	61%	3.7
Finland (Puhakka, 2018) [43][a]	71%	2.0

Abbreviation: cCMV, congenital human cytomegalovirus.

[a]Maternal seroprevalence in Finland estimated based on data reported by Puhakka et al, 2016.

the overall maternal seroprevalence in Finland has been reported to be about 70%, a rate that has been associated with a much higher prevalence of cCMV in many countries, including urban populations in the United States [46] (Table 1). Second, comparison of the seroprevalence in women of childbearing age and the prevalence of cCMV in 2 different ethnic groups in the United States again revealed a population-specific discrepancy between maternal seroprevalence and the prevalence of cCMV, such that the prevalence of cCMV is about 3-fold higher in black maternal populations as compared to Hispanic women [47, 48] (Table 2). Thus, a simple explanation based on a stochastic reactivation of latent HCMV in pregnant women as a source of infection is not entirely consistent with existing epidemiological data and suggests that other risk factors specific to maternal populations are associated with the delivery of an infant with cCMV in women with HCMV seroimmunity prior to conception.

In contrast to limited definitive data supporting reactivation of latent HCMV infection as a source of infection in immune pregnant women, reinfection of immunocompetent pregnant women with a new variant of HCMV defined by detection of new antibody reactivities has been described in several maternal populations and represents a plausible mechanism of nonprimary maternal infections leading to intrauterine transmission and cCMV [26, 35, 36]. However, it is important to stress that these studies remain incomplete, as the source of virus leading to reinfections in the pregnant women has not been definitively identified in available studies. Notably, studies that have described reinfection of pregnant women have been carried out in populations with high HCMV seroprevalence and in one study, women experiencing nonprimary infections

Table 2. Maternal Seroreactivity and Congenital Human Cytomegalovirus Prevalence in 2 Maternal Populations

Maternal Population	Maternal Seroimmunity[a]	cCMV Prevalence[b]
Black	75%–80%	9.5/1000
Hispanic	75%–80%	3.0/1000

Abbreviation: cCMV, congenital human cytomegalovirus.

[a]colugnati, 2007 [48].

[b]Fowler, 2018 [47].

leading to intrauterine transmission were more likely to be exposed to children <3 years of age, a well-described risk factor for virus exposure and infection (Figure 1) [35]. Interestingly, this risk factor for reinfection for pregnant women aligned with results from studies of virus infection in seronegative maternal populations in which exposure to young children has been the most consistently reported risk factor for HCMV infection and delivery of an infant with cCMV [49–54]. Thus, it appears that an established risk factor for HCMV infections during pregnancy—that is, exposure to young children—is common to both nonimmune and immune women undergoing HCMV infection during pregnancy. These observations would argue that reinfections (nonprimary) infections in seroimmune women during pregnancy can be explained by exposure to new viruses, presumably from household contacts and not solely by reactivations/recurrences of existing infections. Last, an alternative explanation for reinfections detected by development of new antibody reactivity has also been proposed. In this mechanism, the production of new serotype specific antibody reactivity that have been used to define reinfections in immune women is proposed to be generated following de novo expansion of minor populations of resident viruses in persistently infected women. This mechanism requires the establishment of persistent infection by multiple genotypic viral variants following initial infection and then, at a later time, expansion of a minor population to a sufficient level that can induce a measurable antibody response. Although current data cannot exclude this potential explanation, recent studies from next-generation sequencing of longitudinal specimens from immunocompromised children and congenitally infected infants have shown remarkable stability of virus populations within a host (intrahost) without periodic expansion of previously undetectable populations [55, 56]. Interestingly, the genetic complexity of virus populations in these individuals were markedly altered when new viral populations were introduced into the host, presumably as the result of reinfection with a new virus population [55, 56].

	Transmitters	Non-Transmitters
Number[1]	43 (23)	109 (23)
Number seropositive	40 (93%)	109 (100%)
Number sexual partners	2	2
Exposure to children <3	7/40 (17.5%)	5/109 (4.6%) $P = .01$
Seroconversion to 2nd strain during pregnancy[2]	7/40 (22.5%)	5/109 (5%) $P = .02$

Figure 1. Increased rate of intrauterine transmission of human cytomegalovirus (HCMV) in seroimmune women with seroconversion to new antigenic variants. Sequential serum samples from women who transmitted virus to their fetuses (transmitters) and matched controls who did not transmit virus (nontransmitters) from a highly HCMV-seroimmune population were assayed for polymorphic antigenic determinants on glycoprotein B and glycoprotein H to determine reinfection with new HCMV variants during pregnancy. [a]Approximately 4000 women were enrolled in this prospective study of HCMV infection during pregnancy. Demographic features of the population including number (median age at enrollment), HCMV seroreactivity, number of sexual partners, and exposure to children <3 years of age are shown. [b]The rate of seroconversion to a new antigenic variant of HCMV is shown (22.5% in transmitters and 5% in nontransmitters). Note that exposure to children <3 years of age was associated with seroconversion to a new variant of HCMV. Adapted from Yamamoto et al [35].

MECHANISMS OF REINFECTIONS: VIRUS ESCAPE FROM HCMV-SPECIFIC ADAPTIVE IMMUNITY

Regardless if a serologically defined reinfection in a previously immune woman can be attributed to exposure to a new virus from a close contact or alternatively from expansion of a minor variant from a site of persistence, it can be inferred that new viral variants escape control by existing adaptive immunity, presumably virus-specific antibodies. Proposed mechanisms that facilitate evasion of HCMV from existing antiviral antibodies range from variations in primary sequence of virion proteins that are targets of protective antibodies to virus-encoded immune evasion functions, findings that suggest that HCMV acquires polymorphisms in virus-encoded targets of protective immune responses to allow escape and persistence in individuals and in populations. Sequence variations in several major HCMV envelope glycoproteins including gB, gH, gO, gN, and UL128 could limit recognition by potentially protective antiviral antibodies as each of these proteins has been shown to either be a target of functional antibodies or a component of protein complex recognized by functional antibodies, findings consistent with the critical role of these envelope proteins in the replication and infectivity of HCMV [57]. Examples of these include gH and gN in which infection with a new virus that results in seroconversion to a new gH or gN genotype results in the development of functional antibody responses reactive with the new antigenic variants (Figure 2) [26, 58, 59]. Other mechanisms that could limit the activity of existing antiviral antibodies include the presence of extensive carbohydrate modifications in several abundant envelope glycoproteins, including gB, gO, and gN in which carbohydrate modifications comprise approximately 40%, 50%, and 75%, respectively, of the mass of the virion protein [57]. Evidence of the importance of the carbohydrate modifications in the recognition of HCMV by antiviral antibodies against several glycoproteins including, gB, gH, and gN has been demonstrated by generation of recombinant viruses lacking a portion of the carbohydrate modifications of gN [57, 60]. Although variations in linear antibody binding sites could limit recognition of variant viruses, it is perhaps more likely that variations in the potential multitude of conformation-dependent antibody binding sites present on gB, gH, gN, and components of the pentamer (gH, gL, UL128-131) and trimer (gH, gL, gO)

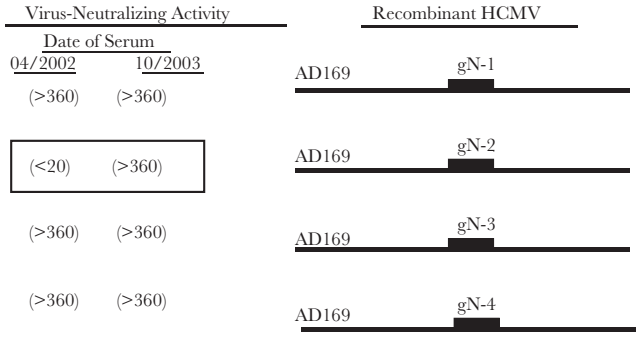

Figure 2. Seroconversion to new glycoprotein N (gN) genotype. Congenic human cytomegalovirus (HCMV) was derived from the HB-5 HCMV BAC clone with replacement of UL73 with UL73 encoding gN genotypes 1–4 (Burkhardt, 2009) [56]. These viruses differed only in the amino acid sequence encoded by UL73 (gN). These viruses were used in a microneutralization assay to define neutralizing activity of sequential sera from an immunocompetent woman during the intrapartum period. This is unit of virus neutralizing activity the reciprocal of the serum dilution resulting in 50% reduction in infectivity is shown. Note the increase in neutralizing capacity of sera from the later date for AD169 gN2 indicating the development of new antibody reactivity for this gN genotype.

complexes could provide additional modes of escape from antiviral antibodies in the immune host. Findings from an early study demonstrating that anti-gB virus-neutralizing antibodies were directed at strain-specific sites in clinical HCMV isolates are consistent with variations in conformation-dependent antibody binding sites on gB [61]. Similarly, potent anti-gH virus neutralizing monoclonal antibodies have been shown to exhibit considerable differences in neutralization activity when assayed on different strains of HCMV, suggesting that viral strain–specific conformation-dependent epitopes could impact functional antibody activity in the host with existing immunity to HCMV [62, 63]. Last, investigators analyzing responses to a subunit gB vaccine have suggested that initial response of the immune system to nonprotective epitope of gB limited the subsequent development of functional antibody and potentially protective responses to virus infection, that is, a concept referred to as original antigenic sin in older literature [64].

A large number of virus-encoded immune evasion functions have been shown to target both adaptive and innate immune responses to HCMV. In general, these immune evasion functions have been shown to target cellular effector functions of the immune response and less so humoral responses to HCMV. However, it is of interest that studies in a rhesus macaque model of CMV reinfection demonstrated that immune evasion functions encoded by rhesus CMV (RhCMV) that target $CD8^+$ T-cell responses played an important role in reinfection of immune animals [65]. Deletion of these viral genes limited the capacity of a mutant RhCMV to reinfect immune animals, a finding that argues for a contribution of T-cell immunity in prevention of infection [65]. Similarly, the expression of a virus-encoded interleukin 10 functional homolog in RhCMV has also been shown to play a role in the early events of infection [66]. The importance of this immune modulating function of RhCMV in reinfection of animals with existing immunity remains to be determined.

Much of the preceding discussion is based on in vitro assay functional antibody activities that are projected to be protective in vivo. Yet there are few, if any, well-studied in vitro measures of protective antibody activity that can be assigned to a specific function(s) of antibody. Perhaps the most obvious example of the redundancy of in vitro antiviral antibody activities is virus neutralization. HCMV neutralizing antibodies have been shown to target gB, gH, gN, the trimer complex, and the pentamer complex [58, 67–70]. In addition, virus-neutralizing antibodies reactive with these virion envelope proteins or protein complexes can bind virus and in many cases, also bind to cells infected with HCMV and thus potentially lead to antibody dependent cellular cytotoxicity (ADCC)–mediated destruction of infected cells and potentially antibody-dependent phagocytosis (ADP) of virus. Of note, antiviral antibodies that participate in ADCC or ADP can be directed at epitopes present on viral envelope proteins that do not lead to in vitro virus neutralization and potentially, nonenvelope viral proteins (ADCC). In agreement with these possibilities, informative animal models and studies in recipients of a gB subunit vaccine have clearly demonstrated that in vitro virus-neutralizing activities do not correlate with in vivo protection and that in some cases antibodies that are nonneutralizing in vitro can have substantial protective activity in vivo [71, 72]. Finally, in assays quantifying the capacity of anti-envelope antibodies to limit cell-to-cell virus spread, a significant discrepancy was reported between the relative potency of antibodies that limit cell-to-cell spread as compared to their capacity to neutralize cell-free virus [73]. Thus, it appears that there are a number of mechanisms through which HCMV can potentially escape preexisting adaptive humoral immunity when antibody activity is measured by conventional assays, and it is an almost certainty that other mechanisms of escape from control by existing antiviral antibodies will be defined as additional mechanisms of functional antiviral antibody activity are defined.

CONCLUSIONS

The frequent occurrence of nonprimary maternal infections leading to cCMV infections and the similar long-term outcomes in infants with cCMV born to women undergoing primary and nonprimary infections presents one of the most vexing questions in the design, testing, and deployment of prophylactic HCMV vaccines [31, 74]. Current strategies in HCMV vaccine development have been forced to rely on the benchmark of adaptive immune responses that follow community-acquired primary infections of immunocompetent women. Even if these responses could be protective in the face of limited exposures to HCMV, it is possible that these responses will provide

incomplete protection in maternal populations in which exposures to HCMV are more frequent and potentially of greater magnitude. In these populations, protective responses could require the induction of a greater magnitude and breadth of antiviral antibody reactivity to limit reinfection by new viruses of differing antigenic content. Thus, the performance of prophylactic vaccines that induce adaptive immune responses similar to those following naturally acquired infections may be quite different in highly seroimmune populations such as are present in South America, Asia, Africa, and in some urban populations in the United States when compared to maternal populations with low to intermediate seroprevalence and presumably less frequent exposures to HCMV. Additional understanding of the source(s) of nonprimary HCMV infections in seroimmune women should be considered a critical parameter in the design and eventual testing of candidate vaccines in seroimmune populations.

Notes

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