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Maternal immune correlates of protection against placental transmission of cytomegalovirus

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Abstract

Human cytomegalovirus (HCMV) is the most common congenitally transmitted pathogen worldwide, impacting an estimated 1 million newborns annually. In a subset of infected infants, congenital HCMV causes severe, long-lasting sequelae, including deafness, microcephaly, neurodevelopmental delay, and even death. Accordingly, a maternal vaccine to prevent congenital HCMV infection continues to be a top public health priority. Nevertheless, all vaccines tested to date have failed to meet clinical trial endpoints. Maternal immunity provides partial protection against congenital HCMV transmission, as vertical transmission from seropositive mothers is relatively rare. Therefore, an understanding of the maternal immune correlates of protection against HCMV congenital infection will be critical to inform design of an efficacious maternal vaccine. This review summarizes our understanding of the innate and adaptive immune correlates of protection against congenital transmission of HCMV, and discusses the advantages and applications of a novel nonhuman primate model of congenital CMV transmission to aid in rational vaccine design and evaluation.

Keywords

Congenital cytomegalovirus; Correlates of protection; Maternal immunity; Human CMV; Rhesus model

1. Introduction

Human cytomegalovirus (HCMV) congenital infection impacts 1 in every 150 (0.7%) live-born infants [1–3]. Nearly 40,000 cases of congenital HCMV (cCMV) occur in the United States annually, resulting in up to 7,000 infants with permanent sequelae such as sensorineural hearing loss (SNHL), growth restriction, and intellectual disability (Fig. 1) [1–5]. cCMV is the leading cause of hearing loss and cognitive deficits in infants, accounting for up to 25% of all pediatric hearing loss [6]. Although not all infected infants will develop

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Conflict of interest statement

S.R.P. provides consulting services to Pfizer Inc. for their preclinical human cytomegalovirus vaccine program and associated animal models. The other authors declare no conflict of interest.

permanent sequelae, an estimated 13% will exhibit neurological disabilities at birth and another 10–15% will develop SNHL or other neurologic sequelae within the first two years of life. Despite this burden of disease, there are no effective interventions to reduce the incidence of intrauterine transmission. A maternal vaccine to prevent cCMV has been a “tier 1 priority” of the National Academy of Medicine for over 15 years, but all vaccines clinically tested thus far have failed to achieve desired clinical endpoints [5].

Vertical transmission of HCMV *in utero* is thought to begin with infection of the placenta from the maternal circulation, though infection ascending from the genital tract may also be possible [7,8]. The hematogenous route of congenital infection, which is more widely described than the ascending route, posits that virus from infected maternal uterine cells replicates in decidual cells and spreads to the placenta, leading to focal infection of cytotrophoblasts [9–12]. It has been hypothesized that, during mid to late gestation, weakly neutralizing IgG antibodies form IgG-virion immune complexes that undergo transcytosis via the Fc receptor neonatal (FcRn) that is expressed on placental syncytiotrophoblasts on the villous core surface [11,13]. In the villous core, infection could spread to stromal fibroblasts and fetal blood vessels, leading to fetal infection. Therefore, a maternal HCMV vaccine that blocks this initial chain of infection events could prevent cCMV transmission.

Interestingly, in industrialized countries, cCMV transmission only occurs in 1–2% of pregnant women with non-primary infection, which include women who acquired their first HCMV infection prior to pregnancy (Fig. 1) [1]. Congenital transmission in mothers with non-primary infection occurs during reinfection by another strain of HCMV or viral reactivation [4]. In contrast, up to 30–40% of seronegative women who acquire primary HCMV infection during pregnancy transmit the virus to their fetus, suggesting a partially protective role of maternal immune factors against cCMV [1]. Therefore, an understanding of the maternal immune responses to both primary and non-primary infection will be critical for determining the maternal immune correlates of protection against cCMV and informing future maternal vaccine development (fetal immune responses reviewed in Ref. [14]). This review summarizes what is known about the role of maternal innate and adaptive immunity in the prevention of cCMV transmission, gaps in the current knowledge of maternal immune correlates of protection, and the recent advancement of a relevant cCMV nonhuman primate (NHP) animal model.

2. Innate immunity

2.1. NK cells

cCMV occurs in the setting of an altered immune system, as typical immune responses are suppressed during pregnancy to establish immune tolerance to prevent fetal rejection [16]. The gravid uterus contains decidual natural killer cells (dNK) that are less cytotoxic than peripheral NK cells and are responsible for maintenance of physiologic placental conditions via cytokine, chemokine, and growth factor secretions [17]. dNK cells account for ~70% of the immune cells in the decidua during the first trimester of pregnancy, with the remaining cells being macrophages (20%), T cells (10%), and dendritic cells (2%) [18]. Despite the high prevalence of non-effector phenotype NK cells during early gestation, cCMV infection risk is decreased during the first trimester (30% transmission risk) compared to the third

trimester (72% transmission risk) [19]. A recent study investigated the function of dNK cells in inhibiting viral spread and found that exposure to HCMV-infected decidual fibroblasts induced a dNK cell receptor repertoire shift and, importantly, a cytotoxic effector phenotype in dNK cells that was specific against HCMV-infected cells, but not fetal fibroblasts [20]. Though dNK cells typically have high expression of inhibitory receptors, such as CD94/NKG2A, LIR-1, and KIRs specific to HLA-G, exposure to HCMV-infected fibroblasts induced expression of the activating receptors NKG2D and CD94/NKG2C or α 2E and this shift was essential for dNK cells exhibiting cytotoxic function [16,20,21]. Moreover, CD56^{pos} dNK cells effectively infiltrated HCMV-infected tissue *in vivo*, suggesting that the phenotypic switch in dNK cells could help prevent congenital transmission.

Adaptive, memory-like traits have recently been described in NK cells (reviewed in Ref. [22]). Though innate immune cells cannot somatically rearrange receptors to achieve antigen specificity, multiple mouse studies have found that NK cells with a murine CMV (MCMV)-specific receptor are preferentially expanded during acute MCMV infection [22]. Moreover, these virus-specific NK cells can undergo contraction to maintain a reservoir of “adaptive” NK cells for potential future secondary expansion, similar to T cells. Recently, Foley and colleagues characterized a memory-like potential for NKG2C⁺ NK cells in response to HCMV [23]. In adult allogeneic hematopoietic cell transplants and of patients that experienced HCMV reactivation, recipients of a seropositive donor graft had a higher proportion of NKG2C⁺ NK cells and higher capacity for target cell-induced IFN- γ production. NKG2C⁺ NK cell expansion was also found in the absence of detectable HCMV viremia in blood when donor and recipient were seropositive, suggesting an educated, memory-like expansion for NKG2C⁺ NK cells. However, another similar study investigated NK cell maturation in seropositive patients who experienced reactivation after umbilical cord blood transplants from donors with a homozygous deletion of *NKG2C* [24]. Reactivation induced NKG2A⁺ KIR⁺ NK cell expansion, NK cell cytotoxicity, and IFN- γ production, even in the absence of NKG2C expression. Therefore, activating KIRs, possibly in addition to NKG2C, may be involved in maturation of memory-like NK cells in response to HCMV infection. The role of memory-like NK cells on HCMV viremia and replication remains to be fully understood, and future studies should work to determine the presence and function of these cells in the maternal-fetal interface during cCMV infection.

2.2. Cytokines and chemokines

In addition to NK cells, cytokines and chemokines have been evaluated in the setting of cCMV infection (toll-like receptors reviewed in Refs. [8,25]). In a study that compared cytokine expression profiles in amniotic fluid between HCMV seropositive pregnant women with and without cCMV transmission, several cytokines were upregulated in the amniotic fluid of infected women, including TNF- α , IL-1 β , IL-1RA, IL-10, IL-12, IL-15, IL-17, CCL2, CCL4, CXCL10, GM-CSF, and PDGF-bb [26]. Additionally, IL-4 was downregulated. Though there was a general trend towards upregulation of pro-inflammatory markers, a few anti-inflammatory markers such as IL-1RA and IL-10 were also upregulated. The authors attributed these conflicting results to the complex nature of HCMV immune escape mechanisms, such as production of an IL-10 homolog [27]. Nevertheless, this robust

and diverse cytokine response suggests that an altered amniotic fluid cytokine milieu may be suggestive of HCMV congenital infection.

Another study investigating cytokine expression in placental tissue demonstrated that HCMV-infected placentas had significantly elevated CCL2 and TNF- α , as compared to uninfected placentas and placentas infected with other microorganisms, such as enterovirus, herpes simplex virus, human herpes viruses, and various bacteria [28]. Elevated CCL2 and TNF- α expression was also observed *ex vivo* in placental explant histocultures infected with HCMV. Together, these studies highlight that HCMV infection alters cytokine and chemokine profiles at the maternal-fetal interface. However, it remains unclear if the upregulation of these cytokines and chemokines protects against HCMV infection, or whether this modulation could also be responsible for placental and/or fetal disease during HCMV infection [29].

In addition to analyzing cytokine expression levels, researchers have also assessed the prevalence of genotype variants in proinflammatory cytokine genes in fetuses and neonates with cCMV [30]. In this study, a “CT haplotype” (*IL1A* -889 C > T; *IL1B* +3954 C > T) was associated with cCMV infection and development of disease-associated sequelae, which suggests that polymorphisms in certain cytokine genes could result in differently-functional cytokines and thereby promote cCMV transmission. However, it is unclear if the fetal and neonatal clinical samples used for genotyping contained maternal contamination, which could impact the reported correlation. To understand the protective role of other chemokines and cytokines in the intrauterine compartment, future work should analyze expression profiles and genotype variant frequencies in amniotic fluid and placentas with relation to fetal transmission and disease outcome (See Table 1 for summary of innate immunity responses).

3. Adaptive immunity

3.1. T cells

Adaptive immune responses in the setting of cCMV infection have been more thoroughly characterized than innate responses. For example, a study found that pregnant women with non-primary HCMV infection had a significantly higher frequency of peripheral HCMV-specific CD4⁺ T cells than seropositive normal pregnant women [31]. cCMV transmission did not occur in any of these cases, leading the authors to speculate that increased CD4⁺ T cell frequency in seropositive women experiencing reinfection or reactivation may be protective against transmission. To further elucidate the relationship between T cells and cCMV transmission, Lilleri and colleagues compared the lymphoproliferative response (LPR) to HCMV within one year of primary infection in cCMV transmitting pregnant women, non-transmitting pregnant women, and non-pregnant women [32]. Importantly, HCMV-specific CD4⁺ and CD8⁺ T cell LPR was correlated with peripheral viral clearance, and delayed CD4⁺ LPR, but not CD8⁺ LPR, was associated with congenital transmission. In a similar study comparing transmitting to non-transmitting mothers during the first 3 months after primary HCMV infection, an increased and early general LPR to HCMV in non-transmitting mothers was the only significant immunological difference between the groups [33]. These studies implicate a protective role for HCMV-specific CD4⁺ T cell frequency

and/or proliferation to prevent congenital transmission of HCMV. In contrast to these findings, two recent studies have reported that lower IFN- γ responses to HCMV and lower HCMV ELISpot levels were independently associated with absence of congenital transmission, which suggests that HCMV-specific T cell responses are not protective [34,35]. Nevertheless, differences in assay techniques and clinical sample types and time points across these studies limit the comparison of these results. Future work should standardize the measurement of HCMV-specific Tcell frequency and proliferation to enable the comparison of various cohorts and studies.

3.2. Antibodies

Maternal HCMV-specific IgG has also been characterized as critical for passive immunity and protection against congenital transmission [11,36–39]. During congenital HCMV transmission, IgG-CMV virion complexes can be transported across the syncytiotrophoblast by FcRn and EGFR [13]. Notably, cCMV transmission risk is only 30% during the first trimester when FcRn and EGFR are not fully expressed, compared to a 72% transmission risk during third trimester when these receptors are expressed [13,19,40]. To assess the role of maternal antibody neutralization titer on infection spread, Maidji and colleagues tested IgG-virion complexes from placentas infected *in utero* in a model for virion complex transcytosis [11]. Weakly neutralizing maternal IgG resulted in HCMV infected human fibroblasts, but no infection was detected with potently neutralizing IgG. The correlation between high neutralization titer and protection against infection has also been examined in transmitting and non-transmitting seropositive mothers [36]. In this study, HCMV-specific IgG neutralization and avidity, but not total IgG concentration, were positively correlated with protection against congenital transmission. Furthermore, we recently observed a significant correlation between HCMV neutralizing titers against epithelial-tropic viruses and reduced cCMV transmission in the setting of non-primary cCMV transmission in HIV/CMV co-infected pregnant women [41] (HIV/CMV coinfections in Africa reviewed in Ref. [42]). Although another study has reported that high neutralization titers were associated with sequelae development among infected infants from mothers with first trimester primary infection, the high neutralization titers in these individuals may be a result of increased maternal systemic virus replication with higher HCMV viral load, which is independently associated with symptomatic infection [43,44]. Altogether, these findings suggest that high antibody neutralization titer contributes to protection against congenital transmission, though in cases of transmission, neutralization titer may be predictive of poor outcome.

Multiple HCMV surface glycoproteins (gB, gM, gN, gH, gL, gO, UL128-131A) are involved in viral entry to host cells and contain neutralizing epitopes [45]. Within glycoprotein B, distinct epitopes and protein domains have been characterized as targets for neutralizing antibodies, including antigenic domain 1 (AD-1), AD-2, domain I (AD-5), and domain II (AD-4) [46–49]. Additionally, the pentameric complex (gH/gL/UL128-131A) has been implicated as the target of the most potently neutralizing antibodies [50]. In sera from transmitter and non-transmitter mothers with primary infection, absorption with the pentameric complex, but not gH/gL or gB, resulted in a dramatic reduction in neutralizing activity in epithelial cells at all post-infection time points. Moreover, anti-pentamer and anti-

gH/gL IgG titers, but not anti-gB IgG titers, were elevated in non-transmitting mothers less than 30 days post-infection [50].

However, several recent studies have suggested that gB-directed antibodies remain important to prevent viral spread. Treatment with monoclonal antibodies against gB, but not the pentameric complex, can block placental trophoblast infection, and an HCMV mutant strain lacking the pentameric complex was able to infect human trophoblast progenitor cells, suggesting the pentameric complex is not essential for placental cell entry [51]. Furthermore, in the setting of non-primary cCMV transmission in HIV-infected women, we observed a weak association between protection and maternal IgG binding to gB AD-2, but not the pentameric complex, gH/gL, or gH/gL/gO [41]. Thus, there are multiple neutralization epitopes on HCMV glycoproteins, yet their association with placental transmission has varied across studies. Eliciting antibody responses against multiple epitopes may be essential for cCMV prevention. See Table 1 for summary of adaptive immunity responses.

4. cCMV vaccine development and current challenges

For the past several decades, preclinical development of a cCMV vaccine has relied upon a guinea pig animal model system to test candidate vaccine strategies (cCMV vaccine field reviewed in Refs. [52,53]). Although the requirement of CMV species-specificity is a challenge for cCMV animal models, guinea pig CMV (GPCMV) can be vertically transmitted to the developing pup following maternal infection and can cause intrauterine growth restriction, labyrinthitis, and SNHL, all of which reflect human cCMV pathogenesis [54–58]. Additionally, GPCMV encodes an HCMV UL128-131 homolog, which is critical for efficient infection for both HCMV and GPCMV [59,60]. Therefore, several studies have tested vaccination strategies in guinea pigs and have demonstrated the importance of preexisting antibody in the prevention of congenital GPCMV transmission. For instance, infusion of potently GPCMV-neutralizing monoclonal antibodies or antisera prior to GPCMV infection can protect against placental GPCMV transmission and fetal death [39,61]. Additionally, both live-attenuated virus and adjuvanted subunit vaccines have been efficacious in protecting against congenital transmission in the guinea pig model [62,63]. Yet, two phase II clinical trials of a gB subunit vaccine have demonstrated only 40–50% protection against HCMV acquisition [64,65]. Despite the observed partial protection against virus acquisition, this vaccination strategy ultimately failed to reach target clinical endpoints and was never assessed for efficacy against congenital transmission.

The therapeutic efficacy of passively infused antibodies has also been assessed in the guinea pig model. Administration of neutralizing antisera to GPCMV and infusion of antibody against gB post-infection were effective in decreasing placental disease and fetal loss in guinea pigs [38,61]. Likewise in humans, small-scale studies and phase I clinical trials found that post-infection hyperimmune gamma globulin (HIG) infusions were associated with decreased cCMV transmission, placental disease, and neonatal disease severity and prevalence [66–69]. Nevertheless, in a large-scale, placebo-controlled phase II trial, HIG treatment did not yield significant protection against congenital transmission or reduce the

severity of disease [70]. A similar trial of HIG as therapy for cCMV is currently ongoing in the United States.

Though there are numerous benefits of a small-animal model of transplacental CMV transmission, including low cost, large experimental size, and short gestation period, there may be a disconnect in the clinical translation between interventions for GPCMV and HCMV congenital disease, as evidenced by the inconsistency of clinical trials in recapitulating the efficacy of interventions in the guinea pig model. GPCMV has limited sequence homology to HCMV, which may contribute to these observed differences between guinea pigs and humans [71]. Therefore, there is a need for a highly relevant, translatable animal model to study maternal immunity against cCMV transmission and to test future immunotherapeutic strategies.

5. Nonhuman primate model of cCMV

5.1. Rhesus monkeys for an NHP model of cCMV infection

The rhesus monkey (*Macaca mulatta*) model has previously been used to study acute CMV infection and persistence during primary and non-primary CMV infection [72,73]. Rhesus CMV (RhCMV) has higher sequence homology to HCMV than small-animal CMV variants, and causes persistent and asymptomatic infection, similar to that of HCMV [71,74,75]. Yet, likely due to the high seroprevalence of RhCMV among breeding colonies, no reports previously documented congenital RhCMV transmission [76,77]. Nevertheless, rhesus monkey fetuses directly inoculated with RhCMV in the first and second trimesters developed similar brain lesions and neurologic sequelae as seen in human cCMV infants, supporting the applicability of a potential congenital transmission model for RhCMV [78,79].

5.2. Proof-of-concept for congenital RhCMV transmission

We recently reported the first NHP model of cCMV transmission, demonstrating that systemic RhCMV inoculation of either immunocompetent or CD4⁺ T cell-depleted seronegative, pregnant rhesus monkeys could result in congenital RhCMV infection [80]. Dams were challenged with a high-titer swarm of RhCMV variants, including fibroblast-passaged 180.92 and epithelial cell-tropic UCD52 and UCD59, to simulate HCMV infection in humans [81,82]. In our model, 4 out of 4 (100%) CD4⁺ T cell-depleted animals and 2 out of 3 (66%) immunocompetent seronegative animals were positive for RhCMV DNA in amniotic fluid. Positive staining for RhCMV-IE1 protein was also found in placental tissue (Fig. 2). Importantly, neither of the infected fetuses from immunocompetent seronegative dams displayed disease-associated sequelae, whereas 3 of the 4 CD4⁺ T cell depleted seronegative animals had spontaneous abortions and the surviving fourth infant exhibited CMV-associated sequelae, including liver lesions and neutropenia. Also, we observed that in CD4⁺ T cell-depleted dams, neutralizing antibody production was significantly delayed and antibody responses targeting RhCMV gB and the pentameric complex were reduced compared to those in immunocompetent dams. This study not only demonstrates a proof-of-concept of systemic maternal RhCMV infection leading to placental infection and intrauterine transmission, but also supports findings from *in vitro* and HCMV models by

implicating important, protective roles for CD4⁺ T cells and potentially maternal antibody in controlling congenital transmission and disease.

5.3. Applications of the rhesus monkey cCMV model

The rhesus monkey cCMV transmission model is ideal to test cCMV-candidate vaccines and immune modulation strategies, as it bears biologic relevance to HCMV infection and disease. Ongoing investigations include defining the role of HIG on the prevention of RhCMV transmission in CD4⁺ T cell-depleted dams. In addition, the role of HIG in the treatment of maternal and fetal CMV infection is being explored in the rhesus monkey model to define the dose and timing at which the intervention is still effective. Finally, the rhesus monkey cCMV model could be used in the future to assess the efficacy of vaccine candidates for prevention of placental and fetal CMV transmission, to ultimately yield more rigorous cCMV candidate vaccine testing for future clinical development.

6. Conclusions and future studies

A comprehensive understanding of both innate and adaptive responses to cCMV infection and protection is essential for vaccine development. Elucidation of protective innate and adaptive immune correlates of protection through human cohort studies and animal models could guide rational design of a vaccine that elicits immune responses that are protective of cCMV transmission. With the recent development of an NHP cCMV model, in addition to the existing guinea pig cCMV model, CMV vaccine candidates and immune approaches can be more rigorously assessed for their ability to block cCMV infection prior to clinical trials, thereby de-risking the development of an effective cCMV vaccine.

References

1. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev Med Virol.* 2007; 17(4):253–276. [PubMed: 17579921]
2. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The “silent” global burden of congenital cytomegalovirus. *Clin Microbiol Rev.* 2013; 26(1):86–102. [PubMed: 23297260]
3. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev Med Virol.* 2007; 17(5):355–363. [PubMed: 17542052]
4. Wang C, Zhang X, Bialek S, Cannon MJ. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. *Clin Infect Dis.* 2011; 52(2):e11–13. [PubMed: 21288834]
5. Stratton, KR. Durch, JS., Lawrence, RS., editors. *Vaccines for the 21st Century: a Tool for Decisionmaking.* Washington DC: 2000.
6. Morton CC, Nance WE. Newborn hearing screening—a silent revolution. *N Engl J Med.* 2006; 354(20):2151–2164. [PubMed: 16707752]
7. Coonrod D, Collier AC, Ashley R, DeRouen T, Corey L. Association between cytomegalovirus seroconversion and upper genital tract infection among women attending a sexually transmitted disease clinic: a prospective study. *J Infect Dis.* 1998; 177(5):1188–1193. [PubMed: 9593002]
8. Muller WJ, Jones CA, Koelle DM. Immunobiology of herpes simplex virus and cytomegalovirus infections of the fetus and newborn. *Curr Immunol Rev.* 2010; 6(1):38–55. [PubMed: 20467462]
9. Pereira L, Maidji E, McDonagh S, Genbacev O, Fisher S. Human cytomegalovirus transmission from the uterus to the placenta correlates with the presence of pathogenic bacteria and maternal immunity. *J Virol.* 2003; 77(24):13301–13314. [PubMed: 14645586]

10. McDonagh S, Maidji E, Ma W, Chang HT, Fisher S, Pereira L. Viral and bacterial pathogens at the maternal-fetal interface. *J Infect Dis.* 2004; 190(4):826–834. [PubMed: 15272412]
11. Maidji E, McDonagh S, Genbacev O, Tabata T, Pereira L. Maternal antibodies enhance or prevent cytomegalovirus infection in the placenta by neonatal Fc receptor-mediated transcytosis. *Am J Pathol.* 2006; 168(4):1210–1226. [PubMed: 16565496]
12. Pereira L, Maidji E, McDonagh S, Tabata T. Insights into viral transmission at the uterine-placental interface. *Trends Microbiol.* 2005; 13(4):164–174. [PubMed: 15817386]
13. Pereira L, Maidji E. Cytomegalovirus infection in the human placenta: maternal immunity and developmentally regulated receptors on trophoblasts converge. *Curr Top Microbiol Immunol.* 2008; 325:383–395. [PubMed: 18637517]
14. Schleiss MR. Cytomegalovirus in the neonate: immune correlates of infection and protection. *Clin Dev Immunol.* 2013; 2013:501801. [PubMed: 24023565]
15. Martin JA, Hamilton BE, Osterman MJ. Births in the United States, 2015. *NCHS Data Brief.* 2016; 258:1–8.
16. Trowsdale J, Betz AG. Mother's little helpers: mechanisms of maternal-fetal tolerance. *Nat Immunol.* 2006; 7(3):241–246. [PubMed: 16482172]
17. Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S, Mandelboim O. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med.* 2006; 12(9):1065–1074. [PubMed: 16892062]
18. Whitelaw PF, Croy BA. Granulated lymphocytes of pregnancy. *Placenta.* 1996; 17(8):533–543. [PubMed: 8916201]
19. Enders G, Daiminger A, Bader U, Exler S, Enders M. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. *J Clin Virol.* 2011; 52(3):244–246. [PubMed: 21820954]
20. Siewiera J, El Costa H, Tabiasco J, Berrebi A, Cartron G, Le Bouteiller P, Jabrane-Ferrat N. Human cytomegalovirus infection elicits new decidual natural killer cell effector functions. *PLoS Pathog.* 2013; 9(4):e1003257. [PubMed: 23592985]
21. Vacca P, Mingari MC, Moretta L. Natural killer cells in human pregnancy. *J Reprod Immunol.* 2013; 97(1):14–19. [PubMed: 23432867]
22. Sullivan TEO, Sun JC, Lanier LL. Natural killer cell memory. *Immunity.* 2015; 43(4):634–645. [PubMed: 26488815]
23. Foley B, Cooley S, Verneris MR, Curtsinger J, Luo X, Waller EK, Anasetti C, Weisdorf D, Miller JS. Human cytomegalovirus (CMV)-induced memory-like NKG2C(+) NK cells are transplantable and expand in vivo in response to recipient CMV antigen. *J Immunol.* 2012; 189(10):5082–5088. [PubMed: 23077239]
24. Della Chiesa M, Falco M, Bertaina A, Muccio L, Alicata C, Frassoni F, Locatelli F, Moretta L, Moretta A. Human cytomegalovirus infection promotes rapid maturation of NK cells expressing activating killer Ig-like receptor in patients transplanted with NKG2C–/– umbilical cord blood. *J Immunol.* 2014; 192(4):1471–1479. [PubMed: 24442432]
25. Wujcicka W, Wilczynski J, Nowakowska D. Alterations in TLRs as new molecular markers of congenital infections with Human cytomegalovirus? *Pathog Dis.* 2014; 70(1):3–16. [PubMed: 23929630]
26. Scott GM, Chow SS, Craig ME, Pang CN, Hall B, Wilkins MR, Jones CA, Lloyd AR, Rawlinson WD. Cytomegalovirus infection during pregnancy with maternofetal transmission induces a proinflammatory cytokine bias in placenta and amniotic fluid. *J Infect Dis.* 2012; 205(8):1305–1310. [PubMed: 22383678]
27. Spencer JV, Lockridge KM, Barry PA, Lin G, Tsang M, Penfold ME, Schall TJ. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. *J Virol.* 2002; 76(3):1285–1292. [PubMed: 11773404]
28. Hamilton ST, Scott G, Naing Z, Iwasenko J, Hall B, Graf N, Arbuckle S, Craig ME, Rawlinson WD. Human cytomegalovirus-induces cytokine changes in the placenta with implications for adverse pregnancy outcomes. *PLoS One.* 2012; 7(12):e52899. [PubMed: 23300810]

29. Chaiworapongsa T, Romero R, Tolosa JE, Yoshimatsu J, Espinoza J, Kim YM, Kim JC, Bujold E, Kalache K, Edwin S. Elevated monocyte chemotactic protein-1 in amniotic fluid is a risk factor for pregnancy loss. *J Matern Fetal Neonatal Med.* 2002; 12(3):159–164. [PubMed: 12530612]
30. Wujcicka W, Wilczynski J, Paradowska E, Studzinska M, Nowakowska D. The role of single nucleotide polymorphisms, contained in proinflammatory cytokine genes, in the development of congenital infection with human cytomegalovirus in fetuses and neonates. *Microb Pathog.* 2017; 105:106–116. [PubMed: 28219832]
31. Fujikawa T, Numazaki K, Asanuma H, Kudo R, Tsutsumi H. Frequency of human cytomegalovirus-specific T cells during pregnancy determined by intracellular cytokine staining. *J Med Virol.* 2003; 71(4):527–531. [PubMed: 14556265]
32. Lilleri D, Fornara C, Furione M, Zavattoni M, Revello MG, Gerna G. Development of human cytomegalovirus-specific T cell immunity during primary infection of pregnant women and its correlation with virus transmission to the fetus. *J Infect Dis.* 2007; 195(7):1062–1070. [PubMed: 17330798]
33. Revello MG, Lilleri D, Zavattoni M, Furione M, Genini E, Comolli G, Gerna G. Lymphoproliferative response in primary human cytomegalovirus (HCMV) infection is delayed in HCMV transmitter mothers. *J Infect Dis.* 2006; 193(2):269–276. [PubMed: 16362891]
34. Eldar-Yedidia Y, Bar-Meir M, Hillel M, Abitbol G, Broide E, Falk R, Assous M, Schlesinger Y. Low interferon relative-response to cytomegalo-virus is associated with low likelihood of intrauterine transmission of the virus. *PLoS One.* 2016; 11(2):e0147883. [PubMed: 26881863]
35. Saldan A, Forner G, Mengoli C, Gussetti N, Palu G, Abate D. Strong cell-mediated immune response to human cytomegalovirus is associated with increased risk of fetal infection in primarily infected pregnant women. *Clin Infect Dis.* 2015; 61(8):1228–1234. [PubMed: 26175520]
36. Boppana SB, Britt WJ. Antiviral antibody responses and intrauterine transmission after primary maternal cytomegalovirus infection. *J Infect Dis.* 1995; 171(5):1115–1121. [PubMed: 7751685]
37. Fowler KB, Stagno S, Pass RF. Maternal immunity and prevention of congenital cytomegalovirus infection. *JAMA.* 2003; 289(8):1008–1011. [PubMed: 12597753]
38. Chatterjee A, Harrison CJ, Britt WJ, Bewtra C. Modification of maternal and congenital cytomegalovirus infection by anti-glycoprotein b antibody transfer in Guinea pigs. *J Infect Dis.* 2001; 183(11):1547–1553. [PubMed: 11343203]
39. Auerbach MR, Yan D, Vij R, Hongo JA, Nakamura G, Vernes JM, Meng YG, Lein S, Chan P, Ross J, Carano R, Deng R, Lewin-Koh N, Xu M, Feierbach B. A neutralizing anti-gH/gL monoclonal antibody is protective in the Guinea pig model of congenital CMV infection. *PLoS Pathog.* 2014; 10(4):e1004060. [PubMed: 24722349]
40. Simister NE, Story CM, Chen HL, Hunt JS. An IgG-transporting Fc receptor expressed in the syncytiotrophoblast of human placenta. *Eur J Immunol.* 1996; 26(7):1527–1531. [PubMed: 8766556]
41. Bialas KM, Westreich D, Cisneros De La Rosa E, Nelson C, Kauvar LM, Fu TM, Permar SR. Maternal antibody responses and nonprimary congenital cytomegalovirus infection of HIV-1 exposed infants. *J Infect Dis.* 2016; 214(12):1916–1923. [PubMed: 27923951]
42. Gronborg HL, Jespersen S, Hønge BL, Jensen-Fangel S, Wejse C. Review of cytomegalovirus coinfection in HIV-infected individuals in Africa. *Rev Med Virol.* 2017; 27(1)
43. Boppana SB, Miller J, Britt WJ. Transplacentally acquired antiviral antibodies and outcome in congenital human cytomegalovirus infection. *Viral Immunol.* 1996; 9(4):211–218. [PubMed: 8978017]
44. Revello MG, Gerna G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus, and newborn infant. *Clin Microbiol Rev.* 2002; 15(4):680–715. [PubMed: 12364375]
45. Compton, T., Feire, A. Early events in human cytomegalovirus infection. In: Arvin, A.Campadelli-Fiume, G.Mocarski, E.Moore, PS.Roizman, B.Whitley, R., Yamanishi, K., editors. *Human Herpesviruses, Biology, Therapy, and Immunoprophylaxis.* Cambridge: 2007.
46. Wieggers AK, Sticht H, Winkler TH, Britt WJ, Mach M. Identification of a neutralizing epitope within antigenic domain 5 of glycoprotein B of human cytomegalovirus. *J Virol.* 2015; 89(1):361–372. [PubMed: 25320309]

47. Spindler N, Rucker P, Potzsch S, Diestel U, Sticht H, Martin-Parras L, Winkler TH, Mach M. Characterization of a discontinuous neutralizing epitope on glycoprotein B of human cytomegalovirus. *J Virol*. 2013; 87(16):8927–8939. [PubMed: 23740990]
48. Potzsch S, Spindler N, Wiegers AK, Fisch T, Rucker P, Sticht H, Grieb N, Baroti T, Weisel F, Stamminger T, Martin-Parras L, Mach M, Winkler TH. B cell repertoire analysis identifies new antigenic domains on glycoprotein B of human cytomegalovirus which are target of neutralizing antibodies. *PLoS Pathog*. 2011; 7(8):e1002172. [PubMed: 21852946]
49. Burke HG, Heldwein EE. Crystal structure of the human cytomegalovirus glycoprotein B. *PLoS Pathog*. 2015; 11(10):e1005227. [PubMed: 26484870]
50. Lilleri D, Kabanova A, Revello MG, Percivalle E, Sarasini A, Genini E, Sallusto F, Lanzavecchia A, Corti D, Gerna G. Fetal human cytomegalovirus transmission correlates with delayed maternal antibodies to gH/gL/pUL128-130-131 complex during primary infection. *PLoS One*. 2013; 8(3):e59863. [PubMed: 23555812]
51. Zydek M, Pettit M, Fang-Hoover J, Adler B, Kauvar LM, Pereira L, Tabata T. HCMV infection of human trophoblast progenitor cells of the placenta is neutralized by a human monoclonal antibody to glycoprotein B and not by antibodies to the pentamer complex. *Viruses*. 2014; 6(3):1346–1364. [PubMed: 24651029]
52. Bialas KM, Permar SR. The march towards a vaccine for congenital CMV: rationale and models. *PLoS Pathog*. 2016; 12(2):e1005355. [PubMed: 26866914]
53. Rieder F, Steininger C. Cytomegalovirus vaccine: phase II clinical trial results. *Clin Microbiol Infect*. 2014; 20(Suppl 5):95–102. [PubMed: 24283990]
54. Choi YC, Hsiung GD. Cytomegalovirus infection in Guinea pigs. II. Trans-placental and horizontal transmission. *J Infect Dis*. 1978; 138(2):197–202. [PubMed: 210239]
55. Staczek J. Animal cytomegaloviruses. *Microbiol Rev*. 1990; 54(3):247–265. [PubMed: 2170830]
56. Harrison CJ, Myers MG. Relation of maternal CMV viremia and antibody response to the rate of congenital infection and intrauterine growth retardation. *J Med Virol*. 1990; 31(3):222–228. [PubMed: 2167945]
57. Katano H, Sato Y, Tsutsui Y, Sata T, Maeda A, Nozawa N, Inoue N, Nomura Y, Kurata T. Pathogenesis of cytomegalovirus-associated labyrinthitis in a Guinea pig model. *Microbes Infect*. 2007; 9(2):183–191. [PubMed: 17208485]
58. Park AH, Gifford T, Schleiss MR, Dahlstrom L, Chase S, McGill L, Li W, Alder SC. Development of cytomegalovirus-mediated sensorineural hearing loss in a Guinea pig model. *Arch Otolaryngol Head Neck Surg*. 2010; 136(1):48–53. [PubMed: 20083778]
59. Hahn G, Revello MG, Patrone M, Percivalle E, Campanini G, Sarasini A, Wagner M, Gallina A, Milanese G, Koszinowski U, Baldanti F, Gerna G. Human cytomegalovirus UL131-128 genes are indispensable for virus growth in endothelial cells and virus transfer to leukocytes. *J Virol*. 2004; 78(18):10023–10033. [PubMed: 15331735]
60. Nozawa N, Yamamoto Y, Fukui Y, Katano H, Tsutsui Y, Sato Y, Yamada S, Inami Y, Nakamura K, Yokoi M, Kurane I, Inoue N. Identification of a 1.6 kb genome locus of Guinea pig cytomegalovirus required for efficient viral growth in animals but not in cell culture. *Virology*. 2008; 379(1):45–54. [PubMed: 18656220]
61. Bratcher DF, Bourne N, Bravo FJ, Schleiss MR, Slaoui M, Myers MG, Bernstein DI. Effect of passive antibody on congenital cytomegalovirus infection in Guinea pigs. *J Infect Dis*. 1995; 172(4):944–950. [PubMed: 7561214]
62. Bia FJ, Miller SA, Lucia HL, Griffith BP, Tarsio M, Hsiung GD. Vaccination against transplacental cytomegalovirus transmission: vaccine reactivation and efficacy in Guinea pigs. *J Infect Dis*. 1984; 149(3):355–362. [PubMed: 6325549]
63. Schleiss MR, Choi KY, Anderson J, Mash JG, Wettendorff M, Mossman S, Van Damme M. Glycoprotein B (gB) vaccines adjuvanted with AS01 or AS02 protect female Guinea pigs against cytomegalovirus (CMV) viremia and offspring mortality in a CMV-challenge model. *Vaccine*. 2014; 32(23):2756–2762. [PubMed: 23867012]
64. Pass RF, Zhang C, Evans A, Simpson T, Andrews W, Huang ML, Corey L, Hill J, Davis E, Flanigan C, Cloud G. Vaccine prevention of maternal cytomegalovirus infection. *N Engl J Med*. 2009; 360(12):1191–1199. [PubMed: 19297572]

65. Bernstein DI, Munoz FM, Callahan ST, Rupp R, Wootton SH, Edwards KM, Turley CB, Stanberry LR, Patel SM, McNeal MM, Pichon S, Amegashie C, Bellamy AR. Safety and efficacy of a cytomegalovirus glycoprotein B (gB) vaccine in adolescent girls: a randomized clinical trial. *Vaccine*. 2016; 34(3):313–319. [PubMed: 26657184]
66. La Torre R, Nigro G, Mazzocco M, Best AM, Adler SP. Placental enlargement in women with primary maternal cytomegalovirus infection is associated with fetal and neonatal disease. *Clin Infect Dis*. 2006; 43(8):994–1000. [PubMed: 16983610]
67. Nigro G, Adler SP, La Torre R, Best AM. Passive immunization during pregnancy for congenital cytomegalovirus infection. *N Engl J Med*. 2005; 353(13):1350–1362. [PubMed: 16192480]
68. Nigro G, Adler SP, Parruti G, Anceschi MM, Coclite E, Pezone I, Di Renzo GC. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy—a case-control study of the outcome in children. *J Infect Dis*. 2012; 205(2):215–227. [PubMed: 22140265]
69. Visentin S, Manara R, Milanese L, Da Roit A, Forner G, Salviato E, Citton V, Magno FM, Orzan E, Morando C, Cusinato R, Mengoli C, Palu G, Ermani M, Rinaldi R, Cosmi E, Gussetti N. Early primary cytomegalovirus infection in pregnancy: maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. *Clin Infect Dis*. 2012; 55(4):497–503. [PubMed: 22539662]
70. Revello MG, Lazzarotto T, Guerra B, Spinillo A, Ferrazzi E, Kustermann A, Guaschino S, Vergani P, Todros T, Frusca T, Arossa A, Furione M, Rognoni V, Rizzo N, Gabrielli L, Klersy C, Gerna G. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. *N Engl J Med*. 2014; 370(14):1316–1326. [PubMed: 24693891]
71. Kanai K, Yamada S, Yamamoto Y, Fukui Y, Kurane I, Inoue N. Re-evaluation of the genome sequence of Guinea pig cytomegalovirus. *J Gen Virol*. 2011; 92(Pt 5):1005–1020. [PubMed: 21270288]
72. Yue Y, Zhou SS, Barry PA. Antibody responses to rhesus cytomegalovirus glycoprotein B in naturally infected rhesus macaques. *J Gen Virol*. 2003; 84(Pt 12):3371–3379. [PubMed: 14645918]
73. Antoine P, Olislagers V, Huygens A, Lecomte S, Liesnard C, Donner C, Marchant A. Functional exhaustion of CD4+ T lymphocytes during primary cytomegalovirus infection. *J Immunol*. 2012; 189(5):2665–2672. [PubMed: 22865914]
74. Hansen SG, Strelow LI, Franchi DC, Anders DG, Wong SW. Complete sequence and genomic analysis of rhesus cytomegalovirus. *J Virol*. 2003; 77(12):6620–6636. [PubMed: 12767982]
75. Yue Y, Barry PA. Rhesus cytomegalovirus a nonhuman primate model for the study of human cytomegalovirus. *Adv Virus Res*. 2008; 72:207–226. [PubMed: 19081492]
76. Asher DM, Gibbs CJ Jr, Lang DJ, Gajdusek DC, Chanock RM. Persistent shedding of cytomegalovirus in the urine of healthy Rhesus monkeys. *Proc Soc Exp Biol Med*. 1974; 145(3):794–801. [PubMed: 4131945]
77. Vogel P, Weigler BJ, Kerr H, Hendrickx AG, Barry PA. Seroepidemiologic studies of cytomegalovirus infection in a breeding population of rhesus macaques. *Lab Anim Sci*. 1994; 44(1):25–30. [PubMed: 8007656]
78. Tarantal AF, Salamat MS, Britt WJ, Luciw PA, Hendrickx AG, Barry PA. Neuropathogenesis induced by rhesus cytomegalovirus in fetal rhesus monkeys (*Macaca mulatta*). *J Infect Dis*. 1998; 177(2):446–450. [PubMed: 9466534]
79. Barry PA, Lockridge KM, Salamat S, Tinling SP, Yue Y, Zhou SS, Gospe SM Jr, Britt WJ, Tarantal AF. Nonhuman primate models of intrauterine cytomegalovirus infection. *ILAR J*. 2006; 47(1):49–64. [PubMed: 16391431]
80. Bialas KM, Tanaka T, Tran D, Varner V, Cisneros De La Rosa E, Chiuppesi F, Wussow F, Kattenhorn L, Macri S, Kunz EL, Estroff JA, Kirchherr J, Yue Y, Fan Q, Lauck M, O'Connor DH, Hall AH, Xavier A, Diamond DJ, Barry PA, Kaur A, Permar SR. Maternal CD4+ T cells protect against severe congenital cytomegalovirus disease in a novel nonhuman primate model of placental cytomegalovirus transmission. *Proc Natl Acad Sci U S A*. 2015; 112(44):13645–13650. [PubMed: 26483473]

81. Kaur A, Daniel MD, Hempel D, Lee-Parritz D, Hirsch MS, Johnson RP. Cytotoxic T-lymphocyte responses to cytomegalovirus in normal and simian immunodeficiency virus-infected rhesus macaques. *J Virol.* 1996; 70(11):7725–7733. [PubMed: 8892893]
82. Oxford KL, Strelow L, Yue Y, Chang WL, Schmidt KA, Diamond DJ, Barry PA. Open reading frames carried on UL/b' are implicated in shedding and horizontal transmission of rhesus cytomegalovirus in rhesus monkeys. *J Virol.* 2011; 85(10):5105–5114. [PubMed: 21389128]

Fig. 1. Estimates of the annual incidence of congenital HCMV infection and associated sequelae in the United States. The reported incidence and rates are based on estimates from multiple meta-analyses and systematic reviews [1–5,15].

Fig. 2.
Detection of RhCMV infection in placental tissue. Placenta tissue from two of the CD4⁺ T cell depleted seronegative dams positively stained for RhCMV-IE1 protein. Adapted from Bialas et al., 2015 [80].

Table 1

Summary of immune responses to cCMV and their implicated role in protection against congenital transmission and fetal disease.

Innate Immunity			
Response type	Implicated role	Reference	
NK Cell	• Cytotoxic switch in dNK cells could help prevent congenital transmission	•	Siewiera et al., 2013 [20]
	• Memory-like NK cells respond specifically to HCMV reactivation. Are there memory-like responses in the intrauterine compartment?	•	O'Sullivan et al., 2015 [22]
Cytokines and Chemokines	• A diverse range of cytokines and chemokines are upregulated in amniotic fluid during cCMV infection	•	Foley et al., 2012 [23]
	• CCL2 and TNF- α are upregulated in infected placentas—result or correlate of infection?	•	Scott et al., 2012 [26]
	• Genotype variants in proinflammatory cytokines are associated with cCMV infection	•	Hamilton et al., 2012 [28]
Adaptive Immunity			
Response type	Implicated role	Reference	
T cells	• CD4+ T cell frequency may be protective against congenital transmission and fetal disease	•	Wujcicka et al., 2017 [30]
	• Robust and early CD4+ T cell LPR is correlated with viral clearance from blood and reduced transmission, though there is some discrepancy when different techniques are used	•	Scott et al., 2012 [26]
	• CD4+ T cell frequency may promote neutralizing antibody response	•	Hamilton et al., 2012 [28]
		•	Wujcicka et al., 2017 [30]
Antibody	• High-titer and avidity antibodies are correlated with reduced transmission	•	Fujikawa et al., 2003 [31]
	• In cases of congenital transmission, high-titer antibodies may predict sequelae development	•	Lilleri et al., 2007 [32]
	• gB and the pentameric complex are major targets of high-titer antibodies	•	Revello et al., 2006 [33]
		•	Eldar-Yedidia et al., 2016 [34]
		•	Saldan et al., 2015 [35]