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Preventing Congenital Cytomegalovirus Infection: Protection to a “T”

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Abstract

A lack of knowledge of the correlates of maternal immunity required for protection of the placenta and fetus against congenital cytomegalovirus (CMV) transmission has complicated vaccine development. New work from Bialas and colleagues demonstrates a critical role for maternal CD4(+) T cells in controlling viremia and preventing CMV-associated fetal disease.

Keywords

congenital cytomegalovirus infection; cytomegalovirus vaccine; animal models; cytomegalovirus immunity; placenta; pentameric complex

The most common infectious cause of disability in the developed world, and probably globally, is congenital infection with cytomegalovirus (CMV). Congenital CMV occurs in approximately 0.6% of all births in the United States. The percentage of infants with symptoms at birth with permanent neurological and neurodevelopmental sequelae is as high as 60%, and even infants without symptoms at birth will have sequelae in up to 13.5% of cases, most commonly manifest as sensorineural hearing loss [1]. Thus, the development of strategies to prevent congenital CMV infection is a major public health priority.

A leading strategy for dealing with the problem of congenital CMV infection is the concept of developing a pre-conception vaccine against the virus. A vaccine capable of preventing congenital infection would clearly have a profound impact on maternal and child health. Vaccine development against CMV is complicated, however, by the increasing recognition that re-infections frequently occur in women in spite of preconception immunity to the virus. These infections can result in fetal transmission and attendant neurological sequelae [2]. Re-infection appears to be related to the plethora of immune evasion genes encoded by CMV, which interfere with class I and class II major histocompatibility complex (MHC) pathways, natural killer (NK) cell function, antibody-mediated clearance of infection, and host cytokine and chemokine functions [3]. Although immunity to CMV provides only partial protection against later re-infection, clearly preconception immunity does reduce the rate of CMV transmission to the fetus in women with non-primary infections during pregnancy: the

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risk of transmission for a primary infection during pregnancy is 30–40%, compared to ~1% for re-infection. Preconception immunity probably also decreases the risk of neurodevelopmental sequelae when fetal transmission occurs. In an analysis of whether prior infection with CMV significantly reduced the risk of congenital CMV infection in future pregnancies, it was observed that naturally acquired immunity resulted in a 69% reduction in transmission risk [4], providing a useful metric for future comparisons of the relative impact of ‘natural’ immunity and vaccine-derived immunity on the risk of vertical transmission in pregnancy.

A major knowledge deficit in the quest for a CMV vaccine is the uncertainty about the key correlate(s) of protective immunity that limit virus transmission to the placenta and developing fetus. Both humoral and cellular immune responses are involved in recovery from CMV infection and establishment of immunity, but the relative importance of these two pathways in preventing congenital transmission is incompletely defined [3]. A major effort in the pharmaceutical industry has been focused on developing passive antibody-based immunotherapies for administration to women at high risk for congenital CMV transmission, based on the premise that a neutralizing antibody to CMV can block placental and fetal transmission. Although ex vivo models suggest a role for neutralizing antibody in prevention of CMV transmission in syncytiotrophoblasts [3], the results of a randomized, blinded, placebo-controlled trial recently demonstrated that the efficacy of high-titer anti-cytomegalovirus immune globulin was no different than placebo with respect to the percentages of women who transmitted CMV during pregnancy, the levels of virus-specific antibodies, the T cell-mediated immune response, or the levels of viral DNA in maternal blood [5]. The clinical outcome of congenital infection at birth was also similar in both groups, and the number of obstetrical adverse events was actually higher in the hyperimmune globulin group than the placebo group. These observations call into question the future therapeutic role of passive administration of virus-neutralizing antibodies for prevention of fetal CMV transmission during pregnancy.

Against this backdrop, new work recently reported by Bialas et al. has shed some important insights on immune effectors of protection against maternal, placental, and fetal CMV infection that are highly relevant to vaccine design [6]. This work demonstrates a key role for the CD4⁺ T cell in protection in a relevant primate model. Using a model of CMV transmission in rhesus macaques, Bialas, along with multiple collaborators and under the overall leadership of Sallie Permar at the Duke Human Vaccine Institute, examined determinants of placental CMV transmission by performing selective depletion of CD4⁺ T cells in animals which then underwent experimentally induced primary CMV infection in the early second trimester of pregnancy. Infection was also modeled in seronegative dams that did not undergo CD4⁺ depletion. In a parallel experiment, CD4⁺ depletion was also performed in rhesus CMV seropositive animals, followed by viral challenge. In this model, intrauterine infections were observed in all experimental groups. Disease was, perhaps predictably, more severe in seronegative CD4⁺-depleted dams, with placental infection, fetal death, and severe maternal and fetal CMV end-organ injury (including myocarditis and hepatic calcifications) noted. Animals that received the CD4⁺ T cell-depleting antibody also exhibited higher plasma and amniotic fluid viral loads, demonstrated reduced virus-specific CD8⁺ T cell responses, and were noted to have delayed production of neutralizing

antibodies compared with immunocompetent macaques. These data indicate a critical role for maternal CD4⁺ T cell immune responses during primary CMV infection, and suggest that these responses may be a key correlate of protective immunity required in controlling maternal viremia and preventing severe CMV-associated fetal disease. Notably, CMV transmission also was observed in seropositive, CD4⁺-depleted animals, an important observation in light of the known transmission of human CMV that can occur in the context of re-infection of seropositive women during pregnancy [2]. Thus, the observations noted in the Bialas study will be broadly applicable to future experimental modeling of vaccines against transplacental CMV infection in both seropositive and seronegative females – and is highly relevant to the challenges of CMV vaccine design in humans.

Although these data represent a significant advance in the field, a number of notes of caution are appropriate. First, Bialas and colleagues challenged these macaques with mixed workpools of rhesus CMV virus with variable genome configurations. Some of these challenge viruses in the rhesus model did not contain fully wild-type sequences in the so-called ULb' region of the viral genome. This region consists of sequences spanning CMV open reading frames UL128-151, sequences that are present in all low-passage primary clinical isolates but undergo extensive deletion, rearrangement and mutation after serial passage of virus in cell culture, particularly in fibroblast cells [7]. Some of these gene products in this region function as important targets of the host antibody responses, particularly the UL128, 130 and 131 proteins (components of the CMV pentameric complex that also includes glycoproteins H and L). The pentameric complex is essential for entry of virus into endothelial and epithelial cell types, is a major target of neutralizing antibody response, and accordingly is a leading subunit vaccine candidate [8]. Other genes in the ULb' region encode mediators of latency and pathogenicity [9]. Interestingly, when pregnant macaques were challenged with mixed strain workpools of rhesus macaque CMV in the Bialas study [6], only a single, more 'wild-type' virus strain was identified in amniotic fluid. This observation underscores the importance and relevance of using full-length, wild-type strains in future challenge studies that are aimed at defining immune correlates that block fetal transmission. A second limitation of these studies is intrinsic to the challenges in breeding CMV-seronegative colonies of rhesus macaques. As a result, these primate studies are extraordinarily expensive, limiting the number of dams that can be evaluated (and hence the overall statistical power). A total of seven CMV-seronegative pregnant animals were challenged: four had undergone CD4⁺ depletion, and three were not depleted. Larger studies with more animals will be required for statistically meaningful comparisons of vaccine strategies in this model in the future. Finally, the challenge viruses used in this study were administered to pregnant macaques via an intravenous route. This route of infection is non-physiologic, insofar as most human CMV infections occur at mucosal surfaces, and not through an intravascular portal of entry. Future studies employing a more physiologic route of viral challenge would better mimic the pathogenesis of most maternal infections.

These minor limitations aside, the results reported in this study are nonetheless highly significant to the CMV vaccine field. This is the first study to report intrauterine CMV transmission in a nonhuman primate animal model. In addition, this study provides a strong rationale for examining vaccine strategies that elicit robust CD4⁺ responses as a key correlate of placental and fetal protection. The examination of placental infection is a

particularly important aspect of this model. In the only other animal model of congenital CMV infection, the guinea pig model, placental infection is also used as an endpoint for the evaluation of CMV vaccines and immunotherapies (Figure 1), but the guinea pig model is limited by the lack of immunological reagents and the lack of genetic relatedness of the guinea pig CMV to human CMV [10]. The rhesus CMV is genetically very similar to human CMV, and therefore provides a model that more faithfully mimics the pathogenesis of congenital CMV infection in infants. This work opens the door to the exciting possibility that vaccines against congenital CMV infection and disease can be evaluated in the rhesus model in a manner that can help inform and direct future clinical trials for this highly significant and unsolved challenge in reproductive health.

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Fig. 1.

Placental infection with CMV in a Guinea Pig congenital CMV transmission model. Placenta was harvested from a GPCMV-infected dam near term after early second trimester challenge with guinea pig cytomegalovirus. Panel A, Hematoxylin and eosin stain demonstrates syncytiotrophoblast in term CMV-infected placenta from congenitally infected pup. Panels B and C, Immunofluorescence using anti-guinea pig CMV antibody to glycoprotein B using FITC-conjugate (panel B) and Texas Red conjugate (panel C) secondary antibody [13] at 40× magnification with DAPI counterstain. Arrows indicate regions of cytoplasmic staining with gB antibody.