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Perspective

Congenital cytomegalovirus infection and advances in murine models of neuropathogenesis

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Congenital human cytomegalovirus (cHCMV) has been a well-known infectious cause of morbidity and mortality in newborns and a contributing factor for neurological disorders in infants (Crough and Khanna, 2009). In a recent study, 65% of patients that contracted cHCMV developed neurological sequelae that included psychomotor impairment, microcephaly, hearing loss, motor disorders, epilepsy, and chorioretinitis, with symptomatic and neurological imaging providing significant evidence that neurological disease had occurred in these patients (de Juan Gallach et al., 2020). Because of these sequelae, it is vital to screen for possible infection, especially in high risk newborns. To better understand this phenomenon, animal models have been developed to examine the neuropathogenesis associated with cHCMV. Because HCMV is known as the "silent pandemic," it is vital to use as many available resources to determine the mechanistic causes for the clinical manifestations that have been observed. Murine models are a great tool for adding to this field of study (Tsutsui et al., 2008; Moulden et al., 2021; Slavuljica et al., 2015). Due to the species specificity of HCMV (i.e., HCMV can only productively infect humans), animal models for direct HCMV infection are lacking to study HCMV pathogenesis. Studies of murine cytomegalovirus (MCMV) infections of mice have served a major role as a model of CMV biology and pathogenesis. MCMV infection in mice remains the best small animal model because: 1) there are significant similarities between MCMV infection in mice and the HCMV infection in humans; 2) MCMV contains homologues (and/or at least functional homologues) of many HCMV genes and gene products; 3) the MCMV genome and proteome have been well-defined, the MCMV genome can be easily manipulated to either delete or insert genes, and it is easy to use mice as experimental animals; and 4) the mouse has a well-characterized immune system, short gestational period, large litter size, and there are numerous immunological reagents available including transgenic and knockout mice. Vertical transmission of HCMV is a result either of transplacental virus infection of the fetus, intrapartum infection from delivering a child, or infection via breastfeeding a newborn. The MCMV infection of newborn mice has been successfully used as a model of perinatal CMV infection. However, long-term sequelae are mostly linked to transplacental transmission of the virus. One significant disadvantage of mouse model is the inability of MCMV to cross the placental barrier and infect mouse embryos.

In the recent issue of *JCI Insight* (Zhou et al., 2022), Zhou et al. established a novel mouse system to model cHCMV infection by intracranially (i.c.) infecting fetal mice at embryonic day 13.5 (E13.5) with the virulent MCMV strain K181. A detailed study was conducted to optimize the infectious dose and the infection time in order to get the maximal number of surviving mice for a long term investigation of congenital MCMV infection (cMCMV) induced sequelae. Especially, the model system contains a consistently high numbers of pups showing clear neurological deficits. Thereby, Zhou et al. developed an approach to utilize mechanistic and neuroimaging analyses to understand cMCMV-induced diseases in a mouse model. Using the established infection model, the authors showed that the cMCMV positive pups presented significant size and weight differences compared to mock infected controls with a long-term observation until adulthood. The cMCMV-infected mice also demonstrated behavioral difference than the mock-infected mice, and presented neurological symptoms including hearing loss and learning deficits similar to those observed in cHCMV-caused phenomena in humans. A comprehensive study using multiple biological and pathological methods was employed to investigate the connections between the symptoms and histopathology. Histopathological studies revealed differences in the brain structure in the affected animals. Furthermore, the presence of high viral genome copy numbers in the brain of newborn mice correlate with high levels of neuroinflammation, causing the secretion of cytokines, and accumulation of leukocytes in the brain tissue. The inflammation in turn causes apoptosis and pyroptosis of MCMV infected cells that are probably related to the calcification in brain that was detected by CT and MRI and confirmed by histopathology, resembling pathological finding observed in human patients. Some highlights from the study include the high survival rate (~82%) and increased length of survival (~65% by P14, and ~45% through P98) following intracranial infection of E13.5 embryos, physiological findings consistent with the clinical observations of

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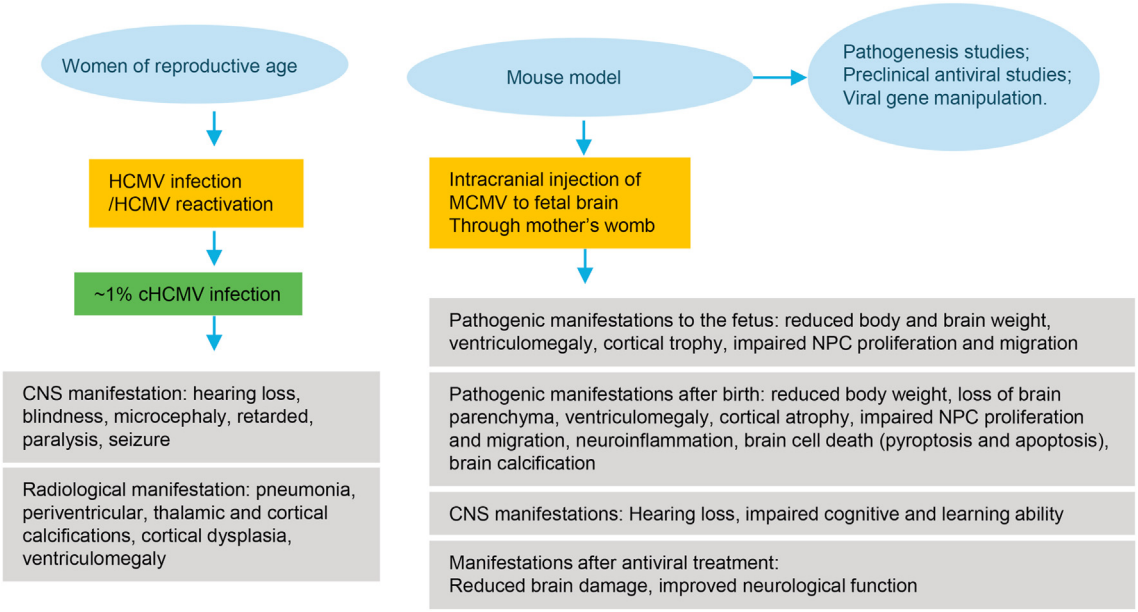

Fig. 1. Comparison of cHCMV infection in human (left) and cMCMV infection in mouse (Right).

congenital HCMV sequelae including growth retardation, abnormal cognitive (i.e., learning and memory) abilities, sensorineural hearing loss, as well as structural changes in the brain including brain hemorrhage, loss of parenchyma, ventriculomegaly, cortical atrophy, impaired proliferation and migration of neural progenitor cells (NPCs), and calcification.

The most significant usefulness of the cMCMV system, in our opinion, is probably that it provides a tool for seeing how efficacious future therapeutics and vaccines may be at mitigating or preventing neurological complications in newborns (Zhou et al., 2022). Ganciclovir is a nucleoside analogue and has been regularly administered to newborn babies diagnosed with cHCMV infection right after birth. Zhou et al. treated the cMCMV newborn mice with ganciclovir and found a significant reversal of all observed pathological symptoms and pathological presentations, which not only indicates that the cMCMV infection and the resulting neuroinflamation is causative for the observed neuropathology, but also demonstrate this cMCMV system is a gold tool for the future anti-CMV drug screening and preclinical trial. After comparison of the cHCMV-caused human pathogenesis and symptoms to those in the cMCMV model as shown in the Fig. 1, the mouse model has advantages in studies of congenital cytomegalovirus (cCMV) in the aspects of pathogenesis, preclinical antiviral test, and viral gene manipulation.

Although this study does not mimic natural infection due to, with the exception of the severe combined immune-deficiency mouse model (Woolf et al., 2007), the inability of MCMV to cross the placenta, it still can be used as a tool to understand the neuropathology associated with cHCMV. Hearing loss as a result of cHCMV is highly complex, and not just solely involving the central nervous system (CNS) anomalies. Furthermore, clinical manifestation of hearing loss can be variable and can include presentations such as delayed onset hearing loss, unilateral hearing loss, and progressive hearing loss post infection (Moulden et al., 2021). Week 7–8 mice demonstrated significant hearing loss following infection with MCMV at E13.5 (Zhou et al., 2022). This could have been in part due to the previously mentioned CMV-induced structural damage of the CNS. It would be interesting to see if structural damage to the middle and inner ear could also be observed with this model to further explain CMV associated hearing loss. A Balb/c mouse model for CMV sensorineural hearing loss (SNHL) was develop after intracerebral infection within 24 hours after delivery. In this study, mice demonstrated both unilateral (24%) and bilateral (29%) hearing loss that progressed with time with 79% of the mice demonstrating that they had developed bilateral hearing loss by the age of 6 weeks. Antigens and DNA for MCMV appeared in the spiral ganglion and cells surrounding the meninges and scala tympani at the age of week one but disappeared at the age of two weeks. Additionally, myosin VI expression in the outer hair cells was absent at three weeks of age. There ultimately was a lag time between the detection of MCMV antigen and DNA positive cells and the development of SNHL and myosin VI-negative hair cells (Ikuta et al., 2015). In addition to these findings, varying degrees of degeneration have been observed in the cochlear vasculature following CMV infection (Carraro et al., 2017). Treatment with ganciclovir was able to ameliorate the hearing loss (Zhou et al., 2022; Haller et al., 2020), and protection was provided to prevent outer hair cell loss. These types of studies are vital as CMV-induced hearing loss is highly prevalent in children with symptomatic infection, and they are more likely to develop bilateral hearing loss (Riga et al., 2018).

Footnotes

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