Animal models of human cytomegalovirus congenital infection

Helen Farrell
School of Chemistry and Molecular Biology
University of Queensland
St Lucia, Qld 4067, Australia
Centre for Children’s Health Research
University of Queensland
South Brisbane, Qld 4101, Australia
Email: h.farrell1@uq.edu.au

Human cytomegalovirus (HCMV) infection is highly species-specific, which means that it is unable to productively infect laboratory animals. Despite this caveat, studies of animal CMV counterparts in their natural hosts have revealed significant correlations with observed neuropathological effects of congenital HCMV infection and have improved our understanding of host responses to vaccination. The biological relatedness between human and animal CMVs has been confirmed by phylogenetic analyses; the conservation of ‘core’ genes that are essential for virus replication as well as genes that contribute similar mechanisms for virus persistence in their respective host species. The common animal models of HCMV congenital infection include Rhesus CMV (RhCMV), guinea-pig CMV (GPCMV) and mouse CMV (MCMV). Whilst animal models of CMV do not fully recapitulate HCMV infection, they each offer specific advantages in understanding HCMV congenital/perinatal infection (summarised in Table 1).

Transplacental transmission and neonatal infections

The placentae of the guinea-pig and the rhesus macaque are structurally similar to the human placenta. Experimental infections with RhCMV and GPCMV result in foetal infection, with clinical manifestations that include CNS involvement and (for GPCMV) sensorineural hearing loss (SNHL). Systemic maternal infection causes syndromes deleterious for the developing foetus, with the incidence of foetal morbidity and mortality being highest when transmission occurs during early gestation. These key pathological features are similar to congenital HCMV infection.

Despite poor transplacental transmission of MCMV in the laboratory setting, direct injection into the foetus or the newborn pup has been shown to mimic HCMV-induced congenital disease. Similar to RhCMV, the susceptibility of neuronal stem cells to MCMV infection is maturation stage-dependent, with a rapid resistance to infection of the brain developing after birth. MCMV also infects the auditory nerve spiral ganglion and cochlea of newborn pups with measurable cytopathic effects and neuronal loss, and thus offers an amenable model for studying viral and host factors that contribute to SNHL.

Evaluation of antiviral therapies to ameliorate effects of congenital infection

Current antiviral therapies for HCMV target the viral replicative machinery (e.g. ganciclovir, valganciclovir, foscarnet and cidifllov). However, due to their toxicity, none are licenced for use during pregnancy and only ganciclovir/valganciclovir (that target the
HCMV-encoded UL97 kinase) are administered to symptomatic HCMV-infected newborns at high risk of SNHL. Despite similarities in the pharmacokinetics between humans and rhesus macaques, and similar sensitivities to HCMV antivirals, assessments of drug efficacies have not been performed due to the expense of this model. By comparison, GPCMV model has proved a highly valuable model to evaluate the action of hexadecyloxypropyl-cidofovir (brincidofovir or CMX001) in the reduction of foetal morbidity, virus load and the manifestation of SNHL. Although GPCMV is resistant to ganciclovir, the generation of GPCMV/HCMV UL97 chimaeric viruses will enable future antiviral testing of ganciclovir in vivo.

Vaccination studies
Because maternal immunity reduces the severity of congenital CMV disease, the development of a vaccine is a high priority. The GPCMV model has been used extensively for evaluating vaccine efficacy, by virtue of the ability to quantify the maternal immune responses, virus loads, as well as developmental sequelae. Several GPCMV vaccines (live attenuated, subunit and DNA) administered before conception, have been evaluated. Whilst sterilising immunity to any vaccination program has not been demonstrated,
the model has informed HCMV vaccination strategies with respect to choice of the immunogen and adjuvant as well as identifying diagnostic correlates of foetal protection, such as the magnitude of the maternal neutralising antibody response to vaccination and reduction in maternal viraemia\cite{5,6,11,23}. The use of vectored approaches for the delivery of subunit GPCMV vaccines that provide both cellular and humoral immunity also significantly reduce the incidence of congenital GPCMV infection\cite{5,24}. Notably, transplacental transmission of GPCMV has been observed in dams that had been previously infected, a feature in common with the evidence of symptomatic HCMV congenital infections resulting from maternal re-infections during pregnancy\cite{25}.

To date, there have been few RhCMV vaccination studies, confined to immunogenicity, rather than efficacy studies. This is due in part by the paucity of seronegative colonies. Nevertheless, the RhCMV model has been useful to identify optimal vaccination regimes and immunogens that elicit strong cellular and humoral immune responses, using heterologous DNA prime-protein boost approaches\cite{5}. Notably, recent studies of RhCMV have uncovered novel, diverse and highly promiscuous CD8+ T-cell repertoires from macaques immunised with a live RhCMV vaccine deleted of HCMV counterparts responsible for cell tropism. The results have implications for the use of HCMV deletion mutants in directing the CD8+ T-cell repertoire and for their use as a vector for delivery of other immunogens\cite{26}.

The MCMV model has been instrumental in our understanding of mechanisms of innate and adaptive mechanisms of host resistance to infection\cite{14}. The availability of immunological reagents has facilitated the characterisation of both humoral and cell-mediated responses to MCMV using live attenuated, subunit, DNA and vectored vaccines\cite{27,28}. Although poor transplacental transmission precludes laboratory studies of vaccine efficacy, there is potential for the MCMV model to measure the potency of maternal immunity from vaccinated dams in restricting perinatal infection of newborn pups.

**Other animal models**

The potentials of rat CMV (RCMV) or porcine CMV (PCMV) as models of HCMV congenital infection have not been explored. PCMV is of interest because natural maternal infection results in predilection of maternal viremia\cite{5,6,11,23}. The use of vectored approaches for the delivery of subunit GPCMV vaccines that provide both cellular and humoral immunity also significantly reduce the incidence of congenital GPCMV infection\cite{5,24}. Notably, transplacental transmission of GPCMV has been observed in dams that had been previously infected, a feature in common with the evidence of symptomatic HCMV congenital infections resulting from maternal re-infections during pregnancy\cite{25}.

**Future Perspectives**

Several advances are facilitating refinement of the above animal models for congenital HCMV:

- The implementation of chimaeric CMVs that express authentic HCMV immune/drug targets and ‘humanised’ animal models that dissect protective immune responses;\cite{29,31}.
- The exploitation of viral ‘immune evasion’ proteins either as targets for the immune response or their deletion in live attenuated viral vaccines\cite{22,23}.
- The implementation of live imaging technologies to track virus dissemination *in vivo*;\cite{27}.
- The identification of viral and cellular determinants that dictate HCMV species specificity may allow future cross-species studies of HCMV infection.\cite{7}

**References**


**Biography**

**Helen Farrell** completed her PhD at the University of Western Australia under the mentorship of Geoff Shellam in 1989, exploring virus-host relationships using the mouse CMV model. She has continued to identify and characterise herpesvirus determinants of pathogenesis and persistence during her postdoctoral career in the UK and in Australia. She is currently a Senior Research Officer at the School of Chemistry and Molecular Biology and the Centre for Children’s Health Research at the University of Queensland.