Nonprimate Models of Congenital Cytomegalovirus (CMV) Infection: Gaining Insight into Pathogenesis and Prevention of Disease in Newborns

Mark R. Schleiss

Abstract

Congenital and perinatal infections with cytomegalovirus (CMV) are responsible for considerable short- and long-term morbidity in infants. CMV is the most common congenital viral infection in the developed world, and is a common cause of neurodevelopmental injury, including mental retardation and sensorineural hearing loss (SNHL). Antiviral therapy has been shown to be valuable in ameliorating the severity of SNHL, but CMV disease control in newborns ultimately depends on successful development of a vaccine. Because CMVs are extremely species specific, preclinical evaluation of vaccines must be performed in animal models using the appropriate CMV of the animal being studied. Several small animal models available for CMV vaccine and pathogenesis research are described. The discussion focuses on the guinea pig model because guinea pig cytomegalovirus (GPCMV), which crosses the placenta and causes infection in utero, is uniquely useful. Examination of vaccines in the GPCMV and other nonprimate models should provide insights into the determinants of the host response that protect the fetus, and may help to prioritize potential vaccine strategies for use in human clinical trials related to this important public health problem.

Key Words: animal models of CMV vaccine; cytomegalovirus (CMV) vaccines; glycoprotein B; guinea pig; guinea pig cytomegalovirus; murine cytomegalovirus; placenta; TORCH infection

Rationale for the Use of Animal Models to Study Congenital Cytomegalovirus Infection

Developing strategies to control and prevent congenital infection with cytomegalovirus (CMV\(^1\)) represents a major priority in perinatal medicine. CMV infection is the most common congenital infection in the developed world. The condition occurs in 0.5 to 2% of all births and is responsible for a wide range of neurodevelopmental disabilities in newborns (Demmler 1994). Of these disabilities, the most common is sensorineural hearing loss (SNHL\(^1\)), which occurs in up to 15% of all congenitally infected children (Dahle et al. 2000). The outcome of SNHL is improved when antiviral therapy (ganciclovir) is administered to infants with neurological involvement who are congenitally infected with CMV (Kimberlin et al. 2003). However, other forms of neurological injury associated with symptomatic congenital CMV may be irreversible.

Because preconceptual maternal immunity to CMV reduces the severity of injury caused by congenital CMV, the development of vaccines is considered to be of high priority (Arvin et al. 2004; Stratton et al. 1999). A number of CMV vaccines are currently being evaluated in clinical trials, including live attenuated vaccines and subunit recombinant vaccines (Schleiss and Heineman 2005). Better elucidation of the determinants of the maternal immune response that result in protection of the fetus will help in prioritizing future vaccine studies for prevention of congenital infection.

Ideally, immunizations for CMV would first be evaluated in animal models prior to human clinical trials. Unfortunately, the strict species specificity of CMVs precludes any meaningful evaluation of candidate human CMV vaccines for protection in animal models. Although laboratory animals will engender immune responses to the candidate vaccines being evaluated in clinical trials, which allows study of safety and immunogenicity, laboratory animals cannot be infected with human CMV. As a result, the ability to analyze the protective effect of vaccines against experimental disease is restricted, and investigators have turned to species-specific animal CMVs to generate models of pathogenesis and immunity (Staczek 1990).

In Table 1 the various animal CMV models available for experimental study are summarized, and their strengths and relevance to human health are compared. For a review of nonhuman primate models of CMV infection and their unique suitability to the study of CMV pathogenesis and vaccines, the reader is referred to the article of Dr. Peter Barry and colleagues in this issue of ILAR Journal (Barry et al. 2006).

In the text below, several nonprimate models of CMV are described briefly, including but not limited to the murine and guinea pig models. The respective roles and advantages of these and other nonprimate models in the study of CMV pathogenesis and immunobiology of fetal and newborn infection are summarized briefly.

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1Abbreviations used in this article: BAC, bacterial artificial chromosome; CCMV, chimpanzee cytomegalovirus; CMV, cytomegalovirus; CNS, central nervous system; GPCMV, guinea pig cytomegalovirus; IE, immediately early; MCMV, murine cytomegalovirus; NK, natural killer; PCMV, porcine cytomegalovirus; RMCMV, rat cytomegalovirus; SNHL, sensorineural hearing loss.
Murine Cytomegalovirus

Murine cytomegalovirus (MCMV\(^1\)) is the best characterized of the animal CMVs, both at the level of the molecular biology of the virus and at the level of host susceptibility to infection and host immune response. The pathogenesis and immunobiology of MCMV infection are beyond the scope of this review but have been the subject of several recent reviews (Krmpotic et al. 2003; Reddehase et al. 2002, 2004).

Remarkably, there are dramatic differences among different strains of mice in their susceptibility to MCMV infection. These differences in host susceptibility to MCMV-induced disease are dependent, in part, on interactions between the virus and host natural killer (NK) cells. A specific genetic locus, \(Cmv\)-1, has been associated with NK cell-mediated protection against murine CMV infection (Forbes et al. 1997; Scalzo et al. 1990). \(Cmv\)-1 encodes the NK cell activation receptor Ly49H (Dimasi and Biassoni 2005). Ly49H molecules on the surface of NK cells interact directly with the murine CMV-encoded protein m157 on the surface of infected cells. This interaction results in the production of interferon-gamma and perforin, which help to promote MCMV clearance and resistance to infection (Arase et al. 2002; Smith et al. 2002). Recently, NK cells have been shown to control MCMV infection through additional, \(Cmv\)-1-independent pathways, indicating that NK cells may utilize multiple antiviral mechanisms (Rodriguez et al. 2004). The variation in the host genetic susceptibility to MCMV infection and disease is an important factor for investigators to keep in mind when considering the experimental design and anticipated outcomes in vaccine/challenge studies performed in the murine model.

Vaccine Approaches

A variety of vaccine approaches have been evaluated extensively using the MCMV model. Based on a series of temperature-sensitive mutants of MCMV, a series of live attenuated vaccines have been shown to protect mice against wild-type MCMV challenge (Gill et al. 2000). In another study based on a defined MCMV deletion mutant, a live attenuated vaccine was effective in protecting against lethal MCMV challenge (MacDonald et al. 1998). Interestingly, the critical protective element in this study appeared to be the induction of CD8+ T-cells by vaccination because the vaccine was effective in B-cell-deficient mice as well as gamma-interferon receptor-deficient mice. This observation may have relevance to human vaccine design. Subunit vaccines, based on immunodominant, cloned MCMV proteins, have also been evaluated in mouse protection studies. A recombinant vaccinia virus expressing the cloned MCMV homolog of the gB glycoprotein protected against lethal

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**Table 1 Animal models of cytomegalovirus (CMV) infection**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Order</th>
<th>CMV</th>
<th>Viral Genome</th>
<th>Virus Crosses Placenta</th>
<th>Disease Models in Newborns</th>
<th>Status of Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimpanzee</td>
<td>Primates</td>
<td>CCMV(^a)</td>
<td>-241,000 bp</td>
<td>Uncertain</td>
<td>Yes</td>
<td>Unstudied</td>
</tr>
<tr>
<td>Rhesus macaque</td>
<td>Primates</td>
<td>RhCMV(^a)</td>
<td>-221,000 bp</td>
<td>Yes</td>
<td>Yes</td>
<td>Studies in progress: glycoprotein B, UL83 homolog</td>
</tr>
<tr>
<td>Tupaia Pig</td>
<td>Scandentia</td>
<td>TCMV(^a)</td>
<td>-195,000 bp</td>
<td>Unknown</td>
<td>Unstudied</td>
<td>Unstudied</td>
</tr>
<tr>
<td></td>
<td>Artiodactyla</td>
<td>PCMV(^a)</td>
<td>Incompletely Sequenced</td>
<td>Yes</td>
<td>Unstudied</td>
<td>Vaccines unstudied: antiviral therapies being studied for xenotransplantation</td>
</tr>
<tr>
<td>Rat Mouse</td>
<td>Rodentia</td>
<td>RCMV(^a)</td>
<td>-230,000 bp</td>
<td>Uncertain</td>
<td>No</td>
<td>Unstudied</td>
</tr>
<tr>
<td></td>
<td>Rodentia</td>
<td>MCMV(^a)</td>
<td>-230,000 bp</td>
<td>Yes</td>
<td>(Infection via direct viral inoculation)</td>
<td>Extensively studied: live attenuated, protein subunit, vectored vaccines, DNA vaccines</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Caviomorpha</td>
<td>GPCMV(^a)</td>
<td>-231,000 bp</td>
<td>Yes</td>
<td>Yes</td>
<td>Extensively studied: live attenuated, protein subunit, vectored vaccines, DNA vaccines</td>
</tr>
</tbody>
</table>

\(^a\)CCMV, chimpanzee CMV; GPCMV, guinea pig CMV; MCMV, murine CMV; PCMV, porcine CMV; RCMV, rat CMV; RhCMV, rhesus macaque CMV; TCMV, tupaia CMV.
challenge in BALB/c mice (Rapp et al. 1993), and a recombinant adenovirus vaccine expressing gB resulted in reduced lung titers following intranasal challenge of mice with MCMV (Shanley and Wu 2003).

Plasmid-derived DNA vaccines have also shown efficacy in mouse models. A “prime-boost” strategy entailing immunization with a cocktail of 13 MCMV-containing plasmids and formalin-inactivated alum-adjuvanted MCMV particles elicited high levels of neutralizing antibodies as well as CD8+ T cells specific for the virion-associated antigens (Morello et al. 2002). Another approach to DNA vaccination utilizes a cloned viral genome maintained as bacterial artificial chromosome (BAC1) in Escherichia coli (McGregor and Schleiss 2001). Using this approach in mice, investigators have generated MCMV BAC-derived vaccines that elicit immunity not only to MCMV antigens but also to cloned heterologous antigen expressed in the BAC genome (Cicin-Sain et al. 2003; Redwood et al. 2005).

Pathogenesis

Although the observations made in the course of MCMV vaccine studies have advanced our understanding of mechanisms of protective immunity to CMV, there is one major limitation of this model. Unfortunately, MCMV does not appear to infect either the mouse placenta or the fetus (Medearis 1964). For this reason, it is difficult to evaluate the protective efficacy of vaccines against congenital MCMV transmission in the mouse model. However, Dr. Yoshihiro Tsutsui and colleagues have made progress in establishing a model to study fetal infection. These investigators have developed model systems for studying brain abnormalities induced by infection of mouse embryos with MCMV. Brain abnormalities, such as microphthalmia and brain atrophy, can be induced in mouse embryo by injection of MCMV directly into the conceptus at mid-gestation, thereby bypassing the need for transplacental transmission (Li and Tsutsui 2000; Naruse and Tsutsui 1989; Tsutsui et al. 1991, 2005). Viral antigens in neuronal cells have been observed for a prolonged time following infection in this model, and lytic infection has been induced in glial cell in the developing brain (Shimamura et al. 1997). Disturbance of neuronal migration and loss of neurons were observed in the brains of mice infected postnatally with MCMV, similar to the neuropathology that is observed in congenitally infected infants.

Similar insights into the pathogenesis of fetal infection have been made by use of histopathological, virological, and morphological analyses (including scanning electron microscopy) of mouse embryos following MCMV injection into the endometrial lumina of pregnant mice at the time of embryo implantation (Baskar et al. 1983, 1987). These studies have revealed a significant increase in the incidence of abnormal fetuses among MCMV-infected animals.

Mechanisms of fetal injury in the MCMV model have also been explored using transgenic mouse technologies. In these studies, a transgenic mouse model has been utilized for analysis of the regulation of the CMV major immediately early (IE1) promoter. These studies revealed that the sites of IE promoter activity during murine embryogenesis correlate with known target tissues of congenital HCMV infection in human fetuses (Koedood et al. 1995). Immunohistochemical double-labeling analyses identified specific cell types with promoter activity that were restricted to specific endothelial cells, ependymal cells, choroid plexus epithelia, and neurons at discrete locations in the forebrain, brainstem, and cerebellum (Fritschy et al. 1996). Similar restriction of distribution of transgene expression was observed in another study in which the enhancer domain of the CMV IE promoter was found to determine cell type-specific expression in transgenic mice (Baskar et al. 1996a,b).

Finally, insights into the relationship between CMV infection of the mouse central nervous system (CNS1) and host immune responses have been made in the laboratory of Dr. James Lokensgard, who has demonstrated that adoptive transfer of total splenocytes from major histocompatibility complex-matched MCMV-primed animals restricts intracerebral viral infection (Cheeran et al. 2004). Additional research using these models could provide insights into the in utero pathogenesis of CMV in the mouse model, as well as mechanisms of immune-mediated protection of the CNS. Ultimately, however, murine models at best provide only indirect evidence of the mechanisms by which transplacentally acquired CMV infection injures the developing fetus.

Guinea Pig Cytomegalovirus

Among the small animal models of congenital CMV infection, the guinea pig cytomegalovirus (GPCMV1) offers some unique advantages compared with rodent models. Chief among these advantages is the fact that GPCMV crosses the guinea pig placenta, causing infection in utero. For this reason, the guinea pig is well suited to the study of vaccines designed to interrupt vertical virus transmission. The unique biology of the guinea pig and the use of this model to study congenital CMV infection are summarized below.

Biology and Reproduction of the Guinea Pig

The biology of the guinea pig has been the subject of several reviews (Donnelly and Brown 2004; Noonan 1994; Queenenberg 1994). The guinea pig (Cavia porcellus) has traditionally been considered a member of the order Rodentia, but recent molecular phylogenetic analyses suggest that it is not a rodent but is instead a member of the order Caviomorpha (D’Erchia et al. 1996). Guinea pigs originated in the Andes Mountains of South America, where they are still consumed as a source of meat. Like humans, guinea pigs require a dietary source of vitamin C. The three common
breeds of guinea pigs include the English (short-haired) breed, the Angora/Peruvian (long-haired) breed, and the Abyssinian breed, which has a rosette hair pattern. Of the strains used in biomedical research, derivatives of the Dunkin-Hartley line of short-haired guinea pigs are most commonly utilized. These guinea pigs are albinoid outbred derivatives of the English (short-haired) breed. Several inbred guinea pig strains are also available, including strain 2 and strain 13 animals. The comparative immunogenetic features of the human lymphocyte antigen loci of inbred and outbred strains have been characterized in a number of studies (Chiba et al. 1978; Geczy and de Weck 1976; Geczy et al. 1975). Interestingly, differences among guinea pig strains exist in susceptibility and disease expression for several infectious pathogens. For example, strain 13 guinea pigs have increased replication of respiratory syncytial virus in the lower airway after experimental challenge compared with strain 2 animals (Bramley et al. 2003). In addition, strain 13 animals also have the greatest susceptibility to experimental Treponema pallidum infection in a guinea pig model of syphilis (Wicher et al. 1985).

Guinea pigs have relatively lengthy gestational periods that range from 65 to 70 days. The average litter size is three newborn pups/pregnant animal; approximately 8.5% of all pups are stillborn (Noonan 1994). One aspect of guinea pig reproductive biology that makes this model particularly well suited to the study of congenital infection is the structure and histology of the guinea pig placenta, which is hemomonochorial. Because it contains a single trophoblast layer that separates maternal and fetal circulation, it is very similar to the human placenta histologically (Kaufmann and Davidoff 1977; Leiser and Kaufmann 1994). This feature of the guinea pig has enabled useful experimental evaluation of a number of transplacentally acquired infections, including syphilis (Wicher et al. 1985) and listeriosis (Bakardjiev et al. 2004, 2005).

Guinea Pig CMV Model: Vaccines for Prevention of Congenital Infection

Colleagues and I are using the guinea pig to evaluate the pathogenesis and prevention of congenital CMV infection, utilizing the species-specific cytomegalovirus indigenous to guinea pigs, the GPCMV. The history and biology of GPCMV have been reviewed recently (Schleiss and Lacayo 2005). Virtually all studies of GPCMV pathogenesis have been conducted with the strain originally isolated by Hartley in 1957 from infected guinea pig salivary glands. The strain was provided to the American Type Culture Collection as strain 22122 (Hartley et al. 1957). Although it causes a latent persistent infection in the salivary gland, GPCMV does not commonly cause serious disease in animals in the vivarium, although there have been reports of indigenous disseminated infection, including pneumonia, in guinea pigs that were not being manipulated experimentally (Van Hoosier et al. 1985).

A summary of the features that make the GPCMV model valuable for the study of vaccines and pathogenesis is shown in Table 2. Like congenitally infected infants, congenitally GPCMV-infected guinea pigs exhibit a variety of forms of end-organ disease and injury, including injury to the CNS and injury to the inner ear that can result in viral labyrinthitis and deafness (Griffith et al. 1982; Woolf et al. 1989). Based on the ability of GPCMV to cross the placenta and infect the pup in utero, the guinea pig provides an ideal small animal model for the study of CMV vaccines.

Previous investigations that evaluated both a live attenuated GPCMV vaccine and a partially purified soluble envelope vaccine administered with Freund’s adjuvant were able to show protection against acute viremia and death, and vaccination resulted in a reduced incidence of generalized maternal and fetal infection (Bia et al. 1980). Immunofinity-purified native glycoprotein vaccines administered with Freund’s adjuvant have also been shown to protect newborn pups against congenital infection and disease (Bourne et al. 2001; Harrison et al. 1995). More recently, colleagues and I have applied molecular cloning techniques, based on an improved understanding of the GPCMV genome, to generate recombinant subunit vaccine candidates in a number of expression systems. Subunit vaccines based on the GPCMV homolog of the immunodominant envelope glycoprotein gB are capable of inducing neutralizing antibody responses when administered either as DNA vaccines or as an adjuvant.

<table>
<thead>
<tr>
<th>Table 2 Useful features of guinea pig cytomegalovirus (CMV) congenital infection model</th>
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<tbody>
<tr>
<td>• Guinea pig CMV</td>
</tr>
<tr>
<td>• Completely sequenced</td>
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<tr>
<td>• Strong conservation of vaccine target and pathogenesis genes with human CMV</td>
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<tr>
<td>•Envelope glycoprotein genes</td>
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<tr>
<td>• Tegument phosphoprotein genes</td>
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<tr>
<td>• Immune modulation genes</td>
</tr>
<tr>
<td>• Cloned as bacterial artificial chromosome</td>
</tr>
<tr>
<td>• Maintain viral genome in <em>Escherichia coli</em></td>
</tr>
<tr>
<td>• Readily generated mutants to study pathogenesis</td>
</tr>
<tr>
<td>• Cloned recombinant expression of subunit vaccines</td>
</tr>
<tr>
<td>• DNA vaccines</td>
</tr>
<tr>
<td>• Adjuvanted protein vaccines</td>
</tr>
<tr>
<td>• Vectored vaccines</td>
</tr>
<tr>
<td>• Guinea pig reproductive biology</td>
</tr>
<tr>
<td>• Hemomonochorial placenta</td>
</tr>
<tr>
<td>• CMV infects syncytiotrophoblast</td>
</tr>
<tr>
<td>• Maternal viremia, transplacental infection of pup</td>
</tr>
<tr>
<td>• Vaccine study endpoints</td>
</tr>
<tr>
<td>• Maternal viremia and disease</td>
</tr>
<tr>
<td>• Pup mortality and infection</td>
</tr>
<tr>
<td>• Visceral and brain involvement in pup</td>
</tr>
<tr>
<td>• Labyrinthitis and sensorineural deafness</td>
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<td>• Viral load in pup</td>
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vanted purified protein vaccine expressed in baculovirus (Schleiss and Jensen 2003; Schleiss et al. 2000). These vaccines have been evaluated for protective efficacy against congenital GPCMV infection and disease using the experimental approach outlined in Figure 1. A series of preconceptual vaccines are administered to young outbred female Hartley guinea pigs, followed by establishment of pregnancy and GPCMV challenge in the early third trimester. Experimental outcomes monitored in these studies include maternal immune responses, maternal incidence and magnitude of viremia (DNAemia), pup mortality rates, and pup infection rates.

Both DNA and protein-based gB vaccines have been tested for efficacy in the guinea pig model. In a DNA vaccine study, gB subunit vaccine had a strong effect on the congenital GPCMV infection rate following a sublethal viral challenge in the early third trimester. Among 26 live-born pups in the control group, the total congenital infection rate was 77% (20/26). In contrast, in the gB group the total congenital infection rate was 41% (11/27; \( p < 0.05 \) vs. control group). These data were the first to demonstrate that a DNA vaccine was capable of providing protection against congenital CMV infection (Schleiss et al. 2003), and provided support for the continued development and testing of CMV DNA vaccines for human clinical trials. As noted, a purified recombinant protein-based gB formulation was also examined in the guinea pig model. In this study, purified recombinant gB was administered in a three-dose series before pregnancy was established, using either Freund’s adjuvant or alum, which is the most commonly used adjuvant used in human vaccines (Schleiss et al. 2004). Overall, gB vaccine had a significant effect on pup mortality and congenital infection although, interestingly, there was a major advantage associated with the use of Freund’s adjuvant.

**Figure 1** Overview of approach to vaccine studies using the guinea pig CMV model. Subunit vaccine candidate of interest (e.g., DNA vaccine, protein subunit vaccine, or vectored subunit vaccine) is administered at 4- to 6-wk intervals to nonpregnant guinea pigs, and immunological assays are performed after each vaccination. Animals are then bred for pregnancy and challenged with GPCMV in early 3rd trimester of pregnancy. A subset of animals is typically sacrificed for detailed immunological analyses, including CD4 and CD8 assays, and delayed type hypersensitivity (DTH) assay. Following viral infection, delivery commences within 15-30 days, with maternal viral load being monitored prior to pup delivery. If desired, a subset of animals can be sacrificed before delivery to obtain placentas for detailed virological and histological analyses. Study endpoints at time of delivery include pup survival, pup infection rate, tissue histopathology, and quantitative viral load analysis. ELISA, enzyme-linked immunosorbent assay; GPCMV, guinea pig cytomegalovirus; PCR, polymerase chain reaction.
Among 36 live-born pups in the Freund’s adjuvant group, only eight (22%) had congenital GPCMV infection, compared with 15/32 (47%) pups with congenital CMV infection in the alum group ($p < 0.05$ vs. Freund’s adjuvant). Animals immunized with gB/Freund’s adjuvant had significantly higher virus-neutralizing titers, which likely represented the basis for the improved protection in this group. These observations are of particular interest in light of the current efficacy trial of purified gB protein subunit being performed by Dr. Robert Pass at the University of Alabama/Birmingham (Zhang and Pass, 2004). Our studies in the guinea pig model provide strong evidence for the importance of gB in protective immunity against congenital CMV infection and disease, and support the continued optimization of adjuvants in gB vaccine clinical trials.

Future work in the guinea pig model should focus on evaluation of other subunit vaccine candidates for protective efficacy, such as the GPCMV homolog of the major CMV cytotoxic-T-cell target, GP83 (UL83). It is also possible to test other homologs of CMV envelope glycoproteins in this model (Britt and Boppana 2004), such as the gM/gN complex and the gH/gO/gL complex. In addition to further study of the correlates of protective maternal immunity in the guinea pig model, the opportunity now exists to study the role of viral genes in the pathogenesis of fetal infection and injury through the generation of recombinant GPCMV using BAC-based mutagenesis approaches (McGregor et al. 2004). Targeted deletion of putative pathogenesis genes, including genes potentially involved in the immunomodulation of the host immune response to infection (Haggerty and Schleiss 2002; Penfold et al. 2003), may provide insights into mechanisms of infection in utero and may enable the experimental evaluation of rationally designed live attenuated vaccines in this model. An increased availability of guinea pig immunological reagents will also be a priority in moving this and other guinea pig infectious diseases models forward.

**Other Animal Cytomegaloviruses**

As noted in Table 1, numerous other animal CMVs have been described and characterized in animal models of infection and disease to varying degrees. The chimpanzee cytomegalovirus genome (CCMV) has recently been sequenced and found to have the highest degree of homology of any animal CMV to the human CMV genome (Davison et al. 2003). Future in vivo studies of CCMV would likely be of great relevance to human health. The rat CMV (RMCV) is a well-characterized CMV at both the molecular and the biological level. The RCMV genome has been sequenced (Vink et al. 2000) and has proven amenable to mutagenesis strategies for pathogenesis analysis in rat models of disease (Streblow et al. 2005). The rat model has emerged as a particularly interesting and valuable model of transplant-associated vascular disease. However, there is little information about the use of RCMV for study of perinatal infection. The recent isolation of a novel strain of RCMV from placental tissue (Loh et al. 2003) raises the intriguing possibility that RCMV might be transmitted to the fetus, a question that merits further study. The porcine cytomegalovirus (PCMV) has been shown to cross the placenta in pregnant sows and to infect the fetus (Edington et al. 1977), but this model has not been explored further in the study of congenital CMV infection. The study of PCMV is important for xenotransplantation because porcine grafts are being used with increased frequency in human transplantation (Fryer et al. 2004).

**Summary**

Although congenital infection with CMV is a major public health crisis in the developed world, few strategies are available to prevent or treat CMV infection effectively, and progress in vaccine development has been frustratingly slow. Because human CMV will not infect laboratory animals, animal research with species-specific CMVs is needed. The rhesus macaque model has the greatest relevance to human health, given the genetic similarities at the virus and host levels and the similarities in the clinical manifestations of infection. Future characterization of the biology of CMV in other primate models, particularly chimpanzees, may be warranted. Mouse models of fetal pathogenesis are also available but are complicated by the fact that MCMV does not cross the placenta. Among the small animal models, the GPCMV model is uniquely valuable because the virus produces congenital infection and disease. Vaccine studies in the guinea pig model using cloned recombinant antigens may help prioritize which kinds of CMV vaccines should move forward in clinical trials. The ability to manipulate the viral genome via targeted mutagenesis should help shed light on the role or roles of specific viral genes in the in utero pathogenesis of infection in this model.

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