

Development of a Guinea Pig Model for Congenital CMV Infection

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The most common infectious cause of birth defects is human cytomegalovirus (HCMV). Each year over 5,500 congenital HCMV infections result in permanent neurological disabilities or death in the United States. Consequently, the National Vaccine Program Office of the U.S. Department of Health and Human Services has prioritized the development of a vaccine for HCMV, with an emphasis on preventing prenatal infections. In a recent paper published in the journal *Virology*, graduate student Craig Bierle and senior author Dr. Adam Geballe (both of the Human Biology Division) report new findings from a model for congenital CMV infection that promises to speed the eventual design of an effective HCMV vaccine. Their collaborator on this work is Dr. Mark Schleiss, who once worked in the Geballe Lab as a Pediatrics and Infectious Diseases Fellow and is now the Director of the Division of Pediatric Infectious Diseases and Immunology at the University of Minnesota Medical School.

Antiviral responses to CMV and scores of other viruses include global repression of protein production, upon which viral replication depends. Host attenuation of protein synthesis is triggered, for example, when protein kinase R (PKR) or 2'-5' oligoadenylate synthetase (OAS) binds to double-stranded viral RNA (dsRNA), a common byproduct of viral infection. Viral countermeasures to evade these kinds of host defenses include the production of proteins that sequester dsRNA before it can activate PKR or OAS, as well as proteins that can inhibit PKR activation. Two such proteins expressed by HCMV, TRS1 and IRS1, are members of the β -herpesvirus US22 gene family. Previously, researchers in Dr. Geballe's laboratory showed that HCMV replication in human cell culture was severely disrupted when TRS1 and IRS1 were simultaneously deleted from the virus ([Marshall et al., 2009](#)). This finding suggested that HCMV vaccine design would benefit greatly from a better understanding of how TRS1 and IRS1 facilitate HCMV replication in living cells. Unfortunately, HCMV cannot be studied directly in non-human animal models due to the high species-specificity that has evolved during the arms race between different CMV species and their hosts.

Guinea pig cytomegalovirus (GPCMV) promises to be a powerful tool in the quest to better understand congenital CMV infection in humans. GPCMV can cross the placenta and infect guinea

pig fetuses *in utero* (see Figure), like HCMV but unlike mouse CMV (MCMV). In addition, the recently sequenced GPCMV genome appears, in many ways, to be more similar to primate CMVs than to rodent CMVs ([Schleiss *et al.*, 2008](#)).

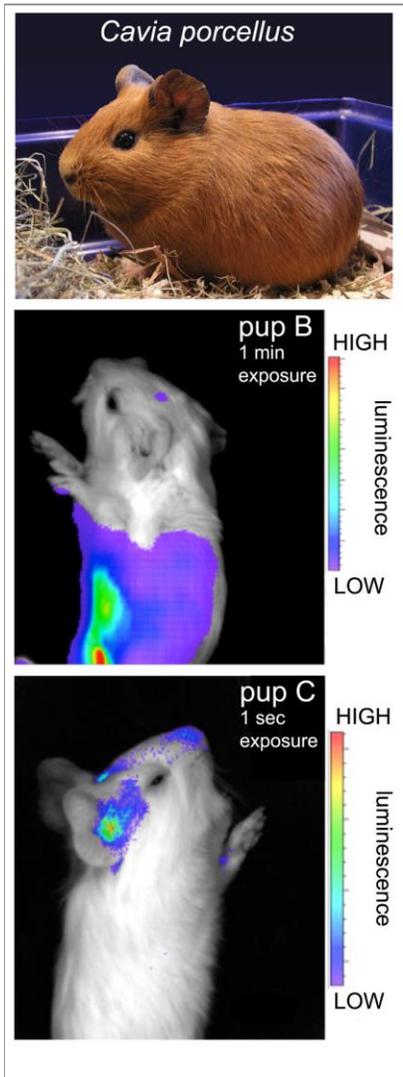
Garnering the greatest returns from research on the emerging GPCMV model will require a better understanding of the similarities and differences in how HCMV and GPCMV evade their host defenses. With this goal in mind, Bierle *et al.* conducted a proteomic screen for dsRNA-binding proteins produced by GPCMV. Testing lysates of infected guinea pig cells with the immunostimulant poly I:C, which mimics the structure of viral dsRNA, the authors identified a number of dsRNA-binding proteins. However, they found that only one of the identified viral proteins, gp145, rescued replication of a vaccinia virus strain lacking the interferon resistance gene *E3L* that counteracts PKR. The outcome of this assay established that gp145, made in living guinea pig cells, binds to dsRNA, and it further suggested that gp145 acts as an antagonist of the PKR pathway. To firmly establish that gp145 directly antagonizes PKR, the authors demonstrated that gp145 counteracted the inhibitory effects of PKR activation on the expression of a co-transfected SEAP reporter gene.

Prior to the work of Bierle *et al.*, PKR antagonists had only been characterized for one rodent CMV (MCMV) and two primate CMVs (HCMV and Rhesus macaque CMV, or RhCMV). Like the PKR antagonists of MCMV, RhCMV and HCMV, gp145 also belongs to the US22 gene family. In fact, Bierle *et al.* mapped the dsRNA-binding ability of gp145 to the same domain that serves this function in HCMV TRS1, though their work also identified differences in structure and function between the GPCMV and HCMV homologs. For example, unlike PKR antagonists encoded by HCMV, gp145 broadly antagonizes the PKR pathway in both rodent and primate cells. In light of the severe replication defect that the Geballe Lab previously found for HCMV Δ *IRS1*/ Δ *TRS1*, their latest discovery of PKR antagonism by gp145 reveals that GPCMV Δ *gp145* will likely be a profitable model for HCMV vaccine development.

[Bierle CJ, Schleiss MR, Geballe AP](#). 2012. Antagonism of the protein kinase R pathway by the guinea pig cytomegalovirus US22-family gene *gp145*. *Virology* 433:157-166.

Also see: [Marshall EE, Bierle CJ, Brune W, Geballe AP](#). 2009. Essential role for either *TRS1* or *IRS1* in human cytomegalovirus replication. *J. Virol.* 83:4112-4120.

[Schleiss MR, McGregor A, Choi KY, Date SV, Cui X, McVoy MA](#). 2012. Analysis of the nucleotide sequence of the guinea pig cytomegalovirus (GPCMV) genome. *Viol. J.* 5:139.



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Examples of vertical transmission of guinea pig cytomegalovirus (GPCMV) from mother to pups. Above: Adult guinea pig (*Cavia porcellus*). Below: Light emission from pups infected in utero with a GPCMV strain engineered to contain a luciferase reporter gene. As with human CMV, GPCMV can be found in many different body regions of infected hosts. For more details about this technique, see Chapter II.5 (The Guinea Pig Model of Congenital Cytomegalovirus Infection) by Alistair McGregor, Michael A. McVoy and Mark R. Schleiss in *Cytomegaloviruses: From Molecular Pathogenesis to Intervention* (Reddehase MJ, Ed.; Caister Academic Press; to be published in April 2013).