

Congenital Cytomegalovirus: A Now Problem, No, Really Now

David I. Bernstein MD, MA

Cincinnati Children's Hospital Medical Center, University of Cincinnati

3333 Burnet Ave

Cincinnati, OH 45229

513.636.7625

david.bernstein@cchmc.org

*The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.*

#### Abstract

Despite the clear need, progress towards a vaccine for congenital cytomegalovirus (CMV) has been slow. However, recent events have provided new interest and several vaccine candidates are either in clinical trials or the trials are close. In this issue of the journal, Dr. Schleiss and colleagues show that a non-replicating lymphocytic choriomeningitis virus (rLCMV) vectored vaccine expressing CMV gB and/or pp65 induced B and T cells and improved pup survival in the guinea pig model of congenital CMV. The combination vaccine appeared to be the most effective.

In 1989, over 27 years ago, Dr. Martha Yow wrote an editorial in the Journal of Infectious Diseases entitled Congenital Cytomegalovirus Disease a NOW Problem. In this article she described the burden of disease and called for the development of a

vaccine. In the 27 years since publication almost 1 million newborns were congenitally infected in the USA and over 100,000 were significantly affected by their infection including neurologic disabilities, developmental delays and most commonly sensorial hearing deficits. In 2000 the Institute of Medicine reported that a cytomegalovirus (CMV) vaccine had the highest priority based on cost savings (1). Yet, it is now 2016 and the availability of a CMV vaccine is at least 6-10 years away. It is clear that part of the problem lies in the public perception and lack of awareness of CMV (2, 3). Trying to shed light on this perception some have asked; What if CMV caused a rash (4) or What if CMV was transmitted by a mosquito (5).

In 2000, a U.S. Government-sponsored meeting proposed activities to support CMV vaccine development within the disciplines of virology, immunology, epidemiology, and clinical trial (6). More recently, in 2012, representatives from government, industry, academia, patient advocacy groups, and professional societies met and provided further impetus to the field by identifying and beginning to address challenges to CMV vaccine development (7). Participants discussed optimal uses of a CMV vaccine, aspects of clinical study design, and the need for additional research in specified areas. It was suggested that clinical trials of CMV vaccines in women should evaluate protection against congenital CMV (cCMV) infection, an essential precursor of cCMV disease. Thus, although the goal of a cCMV vaccine is to prevent disease, the infection endpoint was felt to be a more practical and acceptable endpoint for assessing vaccine effects on maternal-fetal transmission. Never the less trials will require more participants and more complexity than usual.

The other recent impetus to the development of a CMV vaccine for cCMV was the result of recent trials evaluating a glycoprotein B (gB) sub unit vaccine adjuvanted with MF-59 (Sanofi Pasteur). gB, the major surface glycoprotein, was chosen as it is a major target for CMV neutralizing antibodies and MF-59 was selected as the adjuvant because it boosts antibody levels. The first trial showed a significant reduction in the infection rate of vaccinated women compared to the placebo group of about 50% (8) while the second showed about a 40% reduction in young women that was not significant (9). Both used infection of the participants identified by seroconversion and/or detection of virus as the endpoint. Other trials of a different gB vaccines (GlaxoSmithKline and VBI) are also underway (clinical trials.gov, NCT00435396, NCT02826798, respectively).

Other phase 1 safety and immunogenicity studies have evaluated gB and pp 65 expressed in alpha virus replicons (10). The phosphoprotein, PP65, was selected because it is the principal target of CMV-specific CD8<sup>+</sup> CTL responses. These studies showed this approach was safe and immunogenic inducing both antibody and T cells. DNA vaccines expressing gB and pp65 have also been reported (11). Most recently another new vaccine, V160 (Merck Sharpe and Dohme) is being evaluated (clinicalTrials.gov, NCT01986010) while other approaches include the use of the live attenuated Towne vaccine strain (12) and chimeras related to the Towne and Toledo strains (13).

In the article published in this issue of Clinical and Vaccine Immunology Schleiss et al evaluated a somewhat different approach to a CMV vaccine, a non -replicating

Lymphocytic Choriomeningitis Virus (rLCMV) vectored vaccine expressing gB and/or pp65. LCMV and Arenaviruses in general, have been known to elicit strong and long lasting humoral and especially cellular immune responses (14). As discussed above gB was selected to induce neutralizing antibodies and pp65 as the major target for CD8+ T cells. They initially evaluated the immunogenicity of gB and pp65 in mice and rabbits. The gB vaccine elicited high titered binding and neutralizing antibodies while the pp65 vector induced a robust T cell response. Because previous studies had shown interference between the pp65 and gB (15, 16) they compared immunization with each vaccine to the bivalent preparation and importantly found no interference. They then performed a proof of principal evaluation using the guinea pig model of congenital CMV (17) and the guinea pig homologs of gB and pp65. As CMV is highly species specific only the corresponding CMV strain can be used in animal evaluations. The guinea pig is considered the best small animal model because the guinea pig has a hemomonochorial placenta similar to humans that allows CMV to cross the placental and infect the pups causing morbidity including hearing loss and mortality depending on the challenge dose and timing of the challenge (18).

Similar rLCMV vaccines were initially evaluated by Cardin et al (19). In their study the rLCMV-gB vaccine significantly improved pup survival and also increased pup weights and gestation time. The gB vaccine was also more effective at decreasing viral load in dams and pups and limiting congenital transmission than the control or the rLCMV-pp65 vaccine. In the study presented here, the bivalent vaccine induced potent neutralizing and T cell responses in the guinea pigs without interference from the

combination. Vaccination of the dam improved pup mortality from 93% in the control group (a higher than usual outcome suggesting a more stringent test of efficacy) to 8% in the bivalent preparation but did not decrease infection rates (50% in control group vs. 53% in bivalent). Perhaps the lack of decrease in infection can be partially explained by the differences in the rate of identification of CMV in live and dead pups that were available from the groups to determine infection. Decreasing the infection rate is important as this may be the end point in phase 3 clinical trials (7) and sequelae from congenital infections can occur years after birth (20). As a comparison, the mortality in the gB subunit vaccine control was 20% and the infection rate 55%. Vaccination also decreased the maternal virus load and improved the duration of the pregnancy. In most comparisons the bivalent vaccine was superior to either of the monovalent vaccines.

Thus, the results from 2 preclinical studies of the rLCMV vectored approach for cCMV were encouraging and similar to other preclinical studies of subunit gB vaccines (21-23), that are comparable to those used in the moderately successful human trials (8, 9). Besides gB, there is considerable interest in adding proteins from the pentameric complex of CMV to enhance and extend neutralizing antibody (24). Because preventing infection is so important it is not clear whether vaccines tested to date in this model should proceed to human trials or whether further evaluations are necessary. Perhaps future refinements in the evaluation of CMV vaccines could include the use of non-human primates (25, 26) and mucosal administration of the challenge inoculum to better mimic natural infection.

Better times for CMV vaccines are here hopefully good times to follow.

**REFERENCES**

1. **Development IoMUCtSPfV.** 2000. Vaccines for the 21st Century: A Tool for Decisionmaking. National Academic Press, Washington, DC.
2. **Price SM, Bonilla E, Zador P, Levis DM, Kilgo CL, Cannon MJ.** 2014. Educating women about congenital cytomegalovirus: assessment of health education materials through a web-based survey. *BMC Womens Health* **14**:144.
3. **Cannon MJ, Westbrook K, Levis D, Schleiss MR, Thackeray R, Pass RF.** 2012. Awareness of and behaviors related to child-to-mother transmission of cytomegalovirus. *Prev Med* **54**:351-357.
4. **Adler SP.** 2008. Human CMV vaccine trials: what if CMV caused a rash? *J Clin Virol* **41**:231-236.
5. **Schleiss MR.** 2016. Does public perception of exposure risks and transmission mechanisms drive antiviral vaccine awareness? What if cytomegalovirus was transmitted by mosquitoes? *Curr Opin Virol* **17**:126-129.
6. **Arvin AM, Fast P, Myers M, Plotkin S, Rabinovich R.** 2004. Vaccine development to prevent cytomegalovirus disease: report from the National Vaccine Advisory Committee. *Clin Infect Dis* **39**:233-239.
7. **Krause PR, Bialek SR, Boppana SB, Griffiths PD, Laughlin CA, Ljungman P, Mocarski ES, Pass RF, Read JS, Schleiss MR, Plotkin SA.** 2013. Priorities for CMV vaccine development. *Vaccine* **32**:4-10.
8. **Pass RF, Zhang C, Evans A, Simpson T, Andrews W, Huang ML, Corey L, Hill J, Davis E, Flanigan C, Cloud G.** 2009. Vaccine prevention of maternal cytomegalovirus infection. *N Engl J Med* **360**:1191-1199.

9. **Bernstein DI, Munoz FM, Callahan ST, Rupp R, Wootton SH, Edwards KM, Turley CB, Stanberry LR, Patel SM, McNeal MM, Pichon S, Amegashie C, Bellamy AR.** 2016. Safety and efficacy of a cytomegalovirus glycoprotein B (gB) vaccine in adolescent girls: A randomized clinical trial. *Vaccine* **34**:313-319.
10. **Bernstein DI, Reap EA, Katen K, Watson A, Smith K, Norberg P, Olmsted RA, Hoepfer A, Morris J, Negri S, Maughan MF, Chulay JD.** 2009. Randomized, double-blind, Phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers. *Vaccine* **28**:484-493.
11. **Wloch MK, Smith LR, Boutsaboualoy S, Reyes L, Han C, Kehler J, Smith HD, Selk L, Nakamura R, Brown JM, Marbury T, Wald A, Rolland A, Kaslow D, Evans T, Boeckh M.** 2008. Safety and immunogenicity of a bivalent cytomegalovirus DNA vaccine in healthy adult subjects. *J Infect Dis* **197**:1634-1642.
12. **Jacobson MA, Sinclair E, Brecht B, Agrillo L, Black D, Epling CL, Carvidi A, Ho T, Bains R, Girling V, Adler SP.** 2006. Safety and immunogenicity of Towne cytomegalovirus vaccine with or without adjuvant recombinant interleukin-12. *Vaccine* **24**:5311-5319.
13. **Adler SP, Manganello AM, Lee R, McVoy MA, Nixon DE, Plotkin S, Mocarski E, Cox JH, Fast PE, Nesterenko PA, Murray SE, Hill AB, Kemble G.** 2016. A Phase 1 Study of Four Live, Recombinant Human Cytomegalovirus Towne/Toledo Chimera Vaccines in CMV Seronegative Men. *J Infect Dis* doi:10.1093/infdis/jiw365.

14. **Ring S, Flatz L.** 2016. Generation of Lymphocytic Choriomeningitis Virus Based Vaccine Vectors. *Methods Mol Biol* **1404**:351-364.
15. **Swanson EC, Gillis P, Hernandez-Alvarado N, Fernandez-Alarcon C, Schmit M, Zabeli JC, Wussow F, Diamond DJ, Schleiss MR.** 2015. Comparison of monovalent glycoprotein B with bivalent gB/pp65 (GP83) vaccine for congenital cytomegalovirus infection in a guinea pig model: Inclusion of GP83 reduces gB antibody response but both vaccine approaches provide equivalent protection against pup mortality. *Vaccine* **33**:4013-4018.
16. **Selinsky C, Luke C, Wloch M, Geall A, Hermanson G, Kaslow D, Evans T.** 2005. A DNA-based vaccine for the prevention of human cytomegalovirus-associated diseases. *Hum Vaccin* **1**:16-23.
17. **Kern ER.** 2006. Pivotal role of animal models in the development of new therapies for cytomegalovirus infections. *Antiviral Res* **71**:164-171.
18. **Schleiss MR, McVoy MA.** 2010. Guinea Pig Cytomegalovirus (GPCMV): A Model for the Study of the Prevention and Treatment of Maternal-Fetal Transmission. *Future Virol* **5**:207-217.
19. **Cardin RD, Bravo FJ, Pullum DA, Orlinger K, Watson EM, Aspoeck A, Fuhrmann G, Guirakhoo F, Monath T, Bernstein DI.** 2016. Replication-defective lymphocytic choriomeningitis virus vectors expressing guinea pig cytomegalovirus gB and pp65 homologs are protective against congenital guinea pig cytomegalovirus infection. *Vaccine* **34**:1993-1999.

20. **Fowler KB, McCollister FP, Dahle AJ, Boppana S, Britt WJ, Pass RF.** 1997. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. *J Pediatr* **130**:624-630.
21. **Schleiss MR, Bourne N, Stroup G, Bravo FJ, Jensen NJ, Bernstein DI.** 2004. Protection against congenital cytomegalovirus infection and disease in guinea pigs, conferred by a purified recombinant glycoprotein B vaccine. *J Infect Dis* **189**:1374-1381.
22. **Bourne N, Schleiss MR, Bravo FJ, Bernstein DI.** 2001. Preconception immunization with a cytomegalovirus (CMV) glycoprotein vaccine improves pregnancy outcome in a guinea pig model of congenital CMV infection. *J Infect Dis* **183**:59-64.
23. **Schleiss MR, Choi KY, Anderson J, Mash JG, Wettendorff M, Mossman S, Van Damme M.** 2014. Glycoprotein B (gB) vaccines adjuvanted with AS01 or AS02 protect female guinea pigs against cytomegalovirus (CMV) viremia and offspring mortality in a CMV-challenge model. *Vaccine* **32**:2756-2762.
24. **McVoy MA, Lee R, Saccoccio FM, Hartikka J, Smith LR, Mahajan R, Wang JB, Cui X, Adler SP.** 2015. A cytomegalovirus DNA vaccine induces antibodies that block viral entry into fibroblasts and epithelial cells. *Vaccine* **33**:7328-7336.
25. **Bialas KM, Tanaka T, Tran D, Varner V, Cisneros De La Rosa E, Chiuppesi F, Wussow F, Kattenhorn L, Macri S, Kunz EL, Estroff JA, Kirchherr J, Yue Y, Fan Q, Lauck M, O'Connor DH, Hall AH, Xavier A, Diamond DJ, Barry PA, Kaur A, Permar SR.** 2015. Maternal CD4+ T cells protect against severe congenital cytomegalovirus disease in a novel nonhuman primate model of

placental cytomegalovirus transmission. Proc Natl Acad Sci U S A **112**:13645-13650.

26. **Deere JD, Barry PA.** 2014. Using the nonhuman primate model of HCMV to guide vaccine development. *Viruses* **6**:1483-1501.