Could Therapeutic Vaccination of Cytomegalovirus-Seropositive Persons Prevent Reinfection and Congenital Virus Transmission?

Mark R. Schleiss

Center for Infectious Diseases and Microbiology Translational Research, Division of Pediatric Infectious Diseases and Department of Pediatrics, University of Minnesota Medical School, Minneapolis

(See the article by Sabbaj et al, on pages 1534–41.)

In the developed world, cytomegalovirus (CMV) is the most common congenital viral infection, with an overall birth prevalence of ~0.6% [1]. Approximately 10% of congenitally infected infants have signs and symptoms of disease at birth, and these symptomatic infants have been reported to have a 40%–90% risk of subsequent neurologic sequelae, including mental retardation, microcephaly, development delay, seizure disorders, and cerebral palsy [2–4]. Seven percent –to 20% of asymptomatically infected newborns will also demonstrate sequelae, particularly sensorineural hearing loss [5–7]. The public health impact of congenital CMV infection is substantial and underrecognized; although more children suffer from long-term neurodevelopmental handicaps as a result of congenital CMV infection than either Down syndrome or fetal alcohol syndrome [8], awareness unfortunately remains low, particularly among women of childbearing age [9, 10]. An effective vaccine could, by preventing neurological sequelae and other disabilities, provide a newborn with a lifetime of benefit. For that reason, a report from the Institute of Medicine (IOM) of the National Academy of Sciences placed CMV in its highest priority category for vaccine development, concluding that a vaccine would be strongly cost saving [11, 12].

Among the various CMV vaccine candidates currently in clinical trials [13], the most encouraging results to date have been observed in studies of a vaccine based on the immunodominant envelope glycoprotein B (gB). Several clinical trials have been performed using a recombinant form of this protein expressed in Chinese hamster ovary cells, purified and combined with an oil-in-water adjuvant known as MF59 [14–17]. Pass et al recently reported the results of a seminal phase II efficacy trial of the gB-MF59 vaccine conducted at the University of Alabama, Birmingham. This trial was undertaken in adolescent and young adult women. The study population was remarkable for being at a particularly high risk for acquisition of a primary CMV infection, with an annualized seroconversion rate noted in previous studies of 7.8% [18]. This study was a randomized, double-blind, placebo-controlled clinical trial in seronegative women, recruited from post-partum units. A substantial proportion (>20%) had a toddler (age, 13–36 months) at home, who could potentially serve as a vector for acquisition of CMV in at least some instances of primary infection. Vaccine (20 micrograms of gB admixed with MF59 adjuvant) or placebo was administered according to a 0-, 1-, and 6-month schedule [19]. The primary endpoint reported in this study was the time to primary CMV infection, documented by seroconversion to non-gB CMV antigens, using an IgG assay from which the gB-specific antibodies had been removed [20]. An overall efficacy of 50% (95% confidence interval, 7%–73%) for prevention of CMV infection was observed. This exciting result suggested that a gB vaccine may be able to prevent primary infection and, by definition, congenital CMV transmission in young women.

Given the encouraging results observed in this phase II study, is a solution

Received 18 February 2011; accepted 22 February 2011.

Potential conflicts of interest: M. R. S. has received funding from GSK to study preclinical models of CMV vaccines and has served as a consultant to Merck on the topic of CMV vaccines.

Correspondence: Mark R. Schleiss, MD, Center for Infectious Diseases and Microbiology Translational Research, Div of Pediatric Infectious Diseases, Dept of Pediatrics, University of Minnesota Medical School, 2001 6th St SE, Minneapolis, MN, 55455 (schleiss@umn.edu).

The Journal of Infectious Diseases 2011;203:1513–6

© The Author 2011. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com

0022-1899 (print)/1537-6613 (online)/2011/20311-0003$14.00
DOI: 10.1093/infdis/jir144

close at hand for the prevention of congenital CMV infection? Unfortunately, the answer right now appears to be "no." A growing body of evidence has identified previously unanticipated challenges in conceptualizing the ideal patient population for implementation of a vaccine against congenital CMV infection. This is because it has become clear that congenital CMV infection can occur not only as the result of a primary or reactivated maternal infection, but also as a result of reinfection with a new strain of virus in a woman who has preexisting immunity. Although the IOM modeled a hypothetical CMV vaccine that would target seronegative adolescents, based on the premise that most symptomatic and disabling disease occurred in the infant infected in utero in the context of a *primary* maternal infection, it is now clear that *nonprimary* maternal infections account for most of the disease burden associated with congenital CMV infection. Indeed, a number of recent studies have described fetal CMV transmission in women with preconception immunity, occurring as the result of reinfection with new strains of CMV [21–26]. Such infections can produce sequelae identical to those observed in congenitally infected infants born to women who acquire a primary CMV infection during pregnancy. The inability of "natural" infection to prevent reinfection has been modeled in revealing fashion in an experimental rhesus macaque system. The lack of protective long-term immunity has been attributed in the rhesus macaque model to the expression of virally encoded genes that facilitate evasion of the protective T-cell response, through interference with the MHC class I pathway of antigen presentation [27]. These observations have greatly complicated CMV vaccine design and suggest that (1) for full protection, a CMV vaccine may need to enhance responses superior to those conferred by natural immunity, and (2) there may be a strong rationale for vaccinating women of childbearing age who are already CMV seropositive, toward the goal of preventing reinfection with subsequent transmission of the new strain.

To deal with the problem of CMV reinfection, it is reasonable to ask, given the success of gB vaccine trials performed to date, whether therapeutic vaccination of seropositive individuals can augment the imperfect immunity conferred by natural infection. An innovative and intriguing study by Sabbaj et al [28] published in this issue of the *Journal* addresses this issue. Sabbaj and colleagues discovered that the gB-MF59 vaccine does indeed have the potential to boost immune responses in individuals who were already CMV seropositive. In this study, 120 CMV-seropositive women (age, 14–40 years) received a 3-dose series of gB-MF59 vaccine, with use of the same 0-, 1-, and 6-month schedule described by Pass et al [19] in the efficacy study in seronegative persons. Notably, both CMV-specific antibody (enzyme-linked immunosorbent assay [ELISA] and neutralizing titer) and $CD4^+$ T-cell responses were significantly boosted. Remarkably, after a single dose of vaccine, the geometric mean gB ELISA titers were boosted by 5-fold and neutralizing titers augmented by nearly 4-fold. Intracellular cytokine staining for IFN-γ also demonstrated significant boosting of this response. In addition, the population of gB-specific cells induced by vaccination was shown to be of a long-lived memory phenotype, based on analysis of CD127 high expression. Although waning immunity over time appeared to be a concern (as it was in the prior efficacy study in seronegatives), differences between vaccine and placebo recipients were still noted as far out as 360 days of follow-up evaluation. That the boosting was attributable to gB vaccination was convincingly demonstrated by the absence of any boost in response to another nonvaccine CMV protein, pp65.

The demonstration that antibody and T-cell responses could be augmented in an individual already chronically infected with CMV may have implications for the ultimate utilization of a CMV vaccine in the clinic. The commonly held view among primary care providers that only those women who contract a primary CMV infection during pregnancy will give birth to a baby that suffers CMV-related injury is no longer correct in light of the emerging data on reinfection and transmission in pregnancy. It may prove that a CMV vaccine may be valuable, and should be offered, to all women considering pregnancy, irrespective of prepregnancy serostatus. Because ~75% of congenital CMV infections in the United States result from recurrent infections among pregnant women [29], the data presented by Sabbaj and colleagues in this study may have important implications for preventing CMV reinfection and subsequent congenital transmission in both nonimmune and immune women. Even in CMV-seropositive young women, recent evidence indicates that viruria and viremia are common [30], and the reservoir for reinfection appears extensive, potentiated not only by exposure to infectious secretions of young children, but also through sexual transmission. The fact that preexisting immunity to CMV may not alter shedding patterns or prevent reinfection after exposure to new strains is sobering for those who would seek to ultimately control congenital CMV infection through immunization. In this context, the report of Sabbaj et al is of potentially great significance. If recombinant gB vaccine prevents reinfection, perhaps by augmenting neutralizing titers and/or $CD4^+$ responses above levels observed after primary or recurrent infection, the majority of cases of disabling congenital CMV infections could possibly be prevented by a universal vaccination policy in adolescence. It will be of tremendous interest to examine whether serum samples from persons vaccinated with gB broadly cross-neutralize diverse CMV isolates or whether vaccination

engenders a selective advantage for transmission of certain genotypes in vaccinated individuals [31]. Based on these encouraging preliminary data, a protection study examining the efficacy of gB/MF59 vaccine in preventing CMV reinfection in seropositive women (and men) seems to be warranted, and hopefully will be forthcoming.

Many important questions remain about the correlates of protective immunity to CMV and the virologic and immunologic correlates of reinfection. Is there a critical threshold of strain diversity required, perhaps in key hypervariable regions of the viral genome encoding proteins that are targets of the host immune response [32] or that play a role in immune modulation [33], before reinfection can occur? Previous studies in which live, attenuated CMV vaccines were administered to CMV-immune subjects stand in contrast to the Sabbaj study because these vaccines, surprisingly, failed to boost immunity in the already-seropositive individual [34]. Can this apparent shortcoming be corrected, toward the goal of improved live virus vaccines that might be useful in preventing reinfection in seropositives? Or, in a broader sense, can the viral and host factors that contribute to the suboptimal immune response to CMV be elucidated, toward the goal of designing more effective live virus vaccines that protect against both primary infection and reinfection? It is conceivable that this goal might be achieved by deletion of viral immune evasion genes from a live virus vaccine candidate, and there is evidence from animal models of CMV infection that this is feasible [35, 36]. Until such live vaccines pass the safety hurdles required for phase I studies, trials of gB/MF59 in CMV-seropositives should continue. In addition, other studies of "therapeutic vaccination" for chronic, latent and/or persistent infection merit consideration. Theses could include vaccines targeting HIV, human papillomavirus, herpes simplex virus, and the viral hepatitides B and C [37, 38]. Until an effective CMV vaccine is licensed, it will also be important to educate young women as well as primary care practitioners who care for young women, that a prepregnancy serology that demonstrates CMV seropositivity should not be misinterpreted as evidence of complete protection. Seropositive women should practice the same scrupulous hygienic precautions that seronegative women perform, because reinfection can result in vertical transmission, with neurodevelopmental sequelae. Handwashing, avoidance of infected secretions, and education will continue, for at least the near future, as the cornerstones of protection for the developing fetus.

Funding

Laboratory support from the National Institutes of Health (HD044864 and HD038416) is acknowledged.

References

1. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol **2007**; 17:253–76.
2. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol **2007**; 17:355–63.
3. Bale JF, Miner L, Petheram SJ. Congenital cytomegalovirus infection. Curr Treat Options Neurol **2002**; 4:225–30.
4. Cheeran MC, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. Clin Microbiol Rev **2009**; 22:99–126.
5. Williamson WD, Demmler GJ, Percy AK, Catlin FI. Progressive hearing loss in infants with asymptomatic congenital cytomegalovirus infection. Pediatrics **1992**; 90:862–6.
6. Fowler KB, McCollister FP, Dahle AJ, et al. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. J Pediatr **1997**; 130:624–30.
7. Foulon I, Naessens A, Foulon W, et al. A 10-year prospective study of sensorineural hearing loss in children with congenital cytomegalovirus infection. J Pediatr **2008**; 153:84–8.
8. Cannon MJ, Davis KF. Washing our hands of the congenital cytomegalovirus disease epidemic. BMC Public Health **2005**; 5:70.
9. Jeon J, Victor M, Adler SP, et al. Knowledge and awareness of congenital cytomegalovirus among women. Infect Dis Obstet Gynecol **2006**; 2006:80383.
10. Demmler-Harrison GJ. Congenital cytomegalovirus: public health action towards awareness, prevention, and treatment. J Clin Virol **2009**; 46:S1–S5.
11. Arvin AM, Fast P, Myers M, et al. Vaccine development to prevent cytomegalovirus disease: report from the National Vaccine Advisory Committee. Clin Infect Dis **2004**; 39:233–9.
12. Stratton KR, Durch JS, Lawrence RS. (eds). Vaccines for the 21st century: A tool for decisionmaking, Committee to Study Priorities for Vaccine Development, Division of Health Promotion and Disease Prevention. Washington, DC: Natl Acad Press, **1999.**
13. Sung H, Schleiss MR. Update on the current status of cytomegalovirus vaccines. Expert Rev Vaccines **2010**; 9:1303–14.
14. Spaete RR. A recombinant subunit vaccine approach to HCMV vaccine development. Transplant Proc **1991**; 23(Suppl 3):90–6.
15. Pass RF, Duliege AM, Boppana S, et al. A subunit cytomegalovirus vaccine based on recombinant envelope glycoprotein B and a new adjuvant. J Infect Dis **1999**; 180:970–5.
16. Frey SE, Harrison C, Pass RF, et al. Effects of antigen dose and immunization regimens on antibody responses to a cytomegalovirus glycoprotein B subunit vaccine. J Infect Dis **1999**; 180:1700–3.
17. Mitchell DK, Holmes SJ, Burke RL, et al. Immunogenicity of a recombinant human cytomegalovirus gB vaccine in seronegative toddlers. Pediatr Infect Dis J **2002**; 21:133–8.
18. Fowler KB, Stagno S, Pass RF. Maternal immunity and prevention of congenital cytomegalovirus infection. JAMA **2003**; 289:1008–11.
19. Pass RF, Zhang C, Evans A, et al. Vaccine prevention of maternal cytomegalovirus infection. N Engl J Med **2009**; 360:1191–9.
20. Zhang C, Buchanan H, Andrews W, et al. Detection of cytomegalovirus infection during a vaccine clinical trial in healthy young women: seroconversion and viral shedding. J Clin Virol **2006**; 35:338–42.
21. Ahlfors K, Ivarsson SA, Harris S. Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden. Review of prospective studies available in the literature. Scand J Infect Dis **1999**; 31:443–57.
22. Boppana SB, Rivera LB, Fowler KB, et al. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. N Engl J Med **2001**; 344:1366–71.
23. Gaytant MA, Rours GI, Steegers EA, et al. Congenital cytomegalovirus infection after recurrent infection: case reports and review

of the literature. Eur J Pediatr **2003**; 162:248–53.
24. Novak Z, Ross SA, Patro RK, et al. Cytomegalovirus strain diversity in seropositive women. J Clin Microbiol **2008**; 46:882–6.
25. Yamamoto AY, Mussi-Pinhata MM, Boppana SB, et al. Human cytomegalovirus reinfection is associated with intrauterine transmission in a highly cytomegalovirus-immune maternal population. Am J Obstet Gynecol **2010**; 202:297 e1–e8.
26. Ross SA, Arora N, Novak Z, et al. Cytomegalovirus reinfections in healthy seroimmune women. J Infect Dis **2010**; 201:386–9.
27. Hansen SG, Powers CJ, Richards R, et al. Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. Science **2010**; 328:102–6.
28. Sabbaj S, Pass RF, Pichon S, Goepfert PA. CMV Glycoprotein B vaccine is capable of boosting both antibody and CD4 T cell responses to CMV in chronically infected women. J Infect Dis. **2011**; 203. doi: 10.1093/infdis/jir138.
29. Wang C, Zhang X, Bialek S, Cannon MJ. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clin Infect Dis **2011**; 52:e11–e13.
30. Arora N, Novak Z, Fowler KB, Boppana SB, Ross SA. Cytomegalovirus viruria and DNAemia in healthy seropositive women. J Infect Dis **2010**; 202:1800–3.
31. Murthy S, Hayward GS, Wheelan S, et al. Detection of a single identical cytomegalovirus (CMV) strain in recently seroconverted young women. PLoS One **2011**; 6:e15949.
32. Pignatelli S, Lazzarotto T, Gatto MR, et al. Cytomegalovirus gN genotypes distribution among congenitally infected newborns and their relationship with symptoms at birth and sequelae. Clin Infect Dis **2010**; 51:33–41.
33. Arav-Boger R, Battaglia CA, Lazzarotto T, et al. Cytomegalovirus (CMV)-encoded UL144 (truncated tumor necrosis factor receptor) and outcome of congenital CMV infection. J Infect Dis **2006**; 194:464–73.
34. Heineman TC, Schleiss M, Bernstein DI, et al. A phase 1 study of 4 live, recombinant human cytomegalovirus Towne/Toledo chimeric vaccines. J Infect Dis **2006**; 193:1350–60.
35. Slavuljica I, Busche A, Babić M, et al. Recombinant mouse cytomegalovirus expressing a ligand for the NKG2D receptor is attenuated and has improved vaccine properties. J Clin Invest **2010**; 120:4532–45.
36. Schleiss MR. Can we build it better? Using BAC genetics to engineer more effective cytomegalovirus vaccines. J Clin Invest **2010**; 120:4192–7.
37. Autran B, Carcelain G, Combadiere B, Debre P. Therapeutic vaccines for chronic infections. Science **2004**; 305: 205–8.
38. Berzofsky JA, Ahlers JD, Janik J, et al. Progress on new vaccine strategies against chronic viral infections. J Clin Invest **2004**; 114:450–62.