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COMMENTARY

Congenital Cytomegalovirus: a "Now" Problem—No Really, Now

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ABSTRACT Despite the clear need, progress toward 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 to starting. In this issue of *Clinical and Vaccine Immunology*, Schleiss and colleagues show that a nonreplicating lymphocytic choriomeningitis virus (rLCMV)-vectored vaccine expressing CMV glycoprotein B (gB) and/or pp65 induces B and T cells and improves pup survival in a guinea pig model of congenital CMV infection (Clin Vaccine Immunol 24:e00300-16, 2017, https://doi.org/10.1128/CVI.00300-16). The combination vaccine appeared to be the most effective.

KEYWORDS CMV vaccine, congenital infections, cytomegalovirus, guinea pig

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In 1989, over 27 years ago, Martha Yow wrote an editorial in the *Journal of Infectious Diseases* entitled "Congenital cytomegalovirus disease: a NOW problem" (1). In her article, she described the burden of disease and called for the development of a vaccine. In the 27 years since publication of that article, almost 1 million newborns have been congenitally infected in the United States 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 (2). Yet, it is now 2016 and the availability of a CMV vaccine is at least 6 to 10 years away. It is clear that part of the problem lies in the public perception and lack of awareness of CMV (3, 4). Trying to shed light on this perception, some have asked "What if CMV caused a rash?" (5) or "What if CMV was transmitted by a mosquito?" (6).

In 2000, a U.S. Government-sponsored meeting proposed activities to support CMV vaccine development within the disciplines of virology, immunology, epidemiology, and clinical trials (7). 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 (8). 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. Nevertheless, trials will require more participants and more complexity than usual.

The other recent impetus to the development of a CMV vaccine for cCMV were the results of recent trials evaluating a glycoprotein B (gB) subunit 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% (9),

while the second showed about a 40% reduction in infection rate in young women but this difference was not significant (10). Both used infection of the participants identified by seroconversion and/or detection of virus as the endpoint. Other trials of different gB vaccines (GlaxoSmithKline and VBI) are also under way (registered at ClinicalTrials.gov under study numbers NCT00435396 and NCT02826798, respectively).

Other phase 1 safety and immunogenicity studies have evaluated gB and pp65 expressed in alphavirus replicons (11). The phosphoprotein, pp65, was selected because it is the principal target of CMV-specific $CD8^+$ cytotoxic T lymphocyte 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 (12). Most recently, another new vaccine, V160 (Merck Sharpe and Dohme), is being evaluated (ClinicalTrials.gov registration number NCT01986010), while other approaches include the use of the live attenuated Towne vaccine strain (13) and chimeras related to the Towne and Toledo strains (14).

In their most recent article, published in *Clinical and Vaccine Immunology*, Schleiss et al. (15) evaluated a somewhat different approach to a CMV vaccine, utilizing a nonreplicating 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 (16). As discussed above, gB was selected to induce neutralizing antibodies and pp65 was the major target for $CD8^+$ T cells. The Schleiss group initially evaluated the immunogenicity of gB and pp65 in mice and rabbits. The gB vaccine elicited high-titer binding and neutralizing antibodies, while the pp65 vector induced a robust T cell response. Because previous studies had shown interference between pp65 and gB (17, 18), these authors compared immunization with each vaccine to the bivalent preparation and, importantly, found no interference. They then performed a proof-of-principle evaluation using a guinea pig model of congenital CMV infection (19) 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 placenta and infect pups, causing morbidity, including hearing loss, and mortality depending on the challenge dose and timing of the challenge (20).

Similar rLCMV vaccines were initially evaluated by Cardin et al. (21). In that 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 in limiting congenital transmission than the control or the rLCMV-pp65 vaccine. In the study presented here, the bivalent vaccine of Schleiss et al. induced potent neutralizing and T cell responses in the guinea pigs without interference from the combination (15). 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 group that received the bivalent preparation, but it did not decrease infection rates (50% in the control group versus 53% in the bivalent vaccine group). 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 endpoint in phase 3 clinical trials (8), and sequelae from congenital infections can occur years after birth (22). As a comparison, the mortality in the gB subunit vaccine control was 20% and the infection rate was 55%. Vaccination also decreased the maternal virus load and improved the duration of 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 are encouraging and similar to other preclinical studies of subunit gB vaccines (23–25) and are comparable to those obtained in the moderately successful human trials (9, 10). Besides gB, there is considerable interest in adding proteins from the pentameric complex of CMV to enhance and extend neutralizing antibody (26). 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 nonhuman primates (27, 28) and mucosal administration of the challenge inoculum to better mimic natural infection.

Better times for CMV vaccines are here and it is hoped good times will follow.

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