Recombinant cytomegalovirus glycoprotein B vaccine: Rethinking the immunological basis of protection

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Development and licensure of a vaccine against human CMV infection is a major public health priority. Although the major driving force for a CMV vaccine is prevention of congenital infection—since CMV is the most common viral cause of permanent neurodevelopmental disability in infants globally (1)—other patient populations, in particular recipients of hematopoietic stem cells and solid organ transplant (SOT) patients, could benefit from a vaccine. A number of CMV vaccines have been evaluated in clinical trials in recent years (2). These have included live, attenuated vaccines; vectored vaccines expressing CMV-encoded immunogens; and purified recombinant protein vaccines coadministered with adjuvant. The most extensively studied vaccine to date is a subunit vaccine based on the viral envelope glycoprotein B (gB; gpUL55). Two important papers published in PNAS (3, 4) provide surprising and novel insights into the protecting mechanisms conferred by this gB vaccine.

How did the CMV gB emerge as a leading vaccine candidate? CMV gB is a type 1 envelope glycoprotein and class III viral fusogen (5). It is synthesized as a 906- or 907-aa polypeptide (depending upon the strain of CMV) that undergoes extensive posttranslational modification, including glycosylation at N- and O-linked sites, followed by cleavage by the ubiquitous cellular endoprotease, furin, into amino (gp90)- and carboxy (gp58)-terminal moieties (refs. 6 and 7 and Fig. 1). The gp90 and gp58 subunits are covalently connected by disulfide bonds and the mature, glycosylated gB assumes a trimeric configuration; the trimer subsequently dimerizes as the protein assumes its final envelope conformation (8). CMV gB interacts with the virally encoded glycoprotein H/L (gH/gL) complex as an essential component of the core fusion machinery required for infection and cell-to-cell spread of virus (9). Up to 70% of the virus-neutralizing capacity of CMV immune sera recognizes gB (10). Although recent studies of anti-CMV immune globulin suggest that virus-neutralizing responses targeting the CMV pentameric complex (gH/gL/UL128/UL130/UL131A) are essential for optimized protective immunity (11), gB remains a cornerstone component of all CMV vaccines currently in development. A subunit gB vaccine was developed in the late 1980s, composed of a CHO cell-derived protein admixed with an oil-in-water emulsion, MF59 (12). In the design of this vaccine, gB was truncated at the transmembrane domain, and the furin protease site was deleted, to facilitate purification from CHO cell supernatants. The vaccine was found to be safe and immunogenic in phase I studies (13) and elicited potent anti-gB responses in vaccinees (14).

Driven by these positive findings of safety and immunogenicity in phase I trials, the recombinant

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Author contributions: M.R.S. wrote the paper.

The author declares no conflict of interest.

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See companion articles on pages 6267 and 6273.

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Published online June 6, 2018.
gB/MF59 vaccine subsequently underwent a series of phase II studies. Three clinical trials have been reported to date. The first was in CMV-seronegative women, immunized in the immediate postpartum period (15). Since study subjects in this trial were known to be at high risk for acquisition of a primary CMV infection, this study offered an opportunity to evaluate gB/MF59 efficacy against acquisition of CMV in a key group of individuals targeted for vaccine development, namely, young women of child-bearing age. The gB/MF59 vaccine was demonstrated to have an efficacy of 50% for prevention of primary CMV infection. A second study in seronegative adolescent girls, using the same three-dose regimen (16), demonstrated similar efficacy (43%). A third study conducted in SOT patients employed endpoints different from those examined in postpartum women and adolescent girls, namely, reduction of CMV disease and decreased utilization of anti-CMV antivirals in the posttransplant period (17). In seronegative SOT recipients with seropositive organ donors, duration of CMV viremia and total days of antiviral treatment were both significantly reduced in vaccinees, compared with placebo recipients (P < 0.05).

The benefit of gB/MF59 vaccine was generally presumed, at least in the initial interpretation of study results, to derive primarily from induction of virus-neutralizing antibody responses. This presumption was apparently incorrect, as is now reported in two innovative and provocative papers in PNAS (3, 4). Intriguingly, using stored serum samples from vaccinees in both the SOT study (4) and the study in postpartum women (3), these new data reveal that although vaccination elicited IgG responses with gB-binding magnitude comparable to natural infection, minimal induction of virus-neutralizing responses was observed—despite the fact that protection against CMV infection/disease was conferred. These results suggest that the antiviral IgG response to gB/MF59 vaccine confers protection through nonneutralizing mechanisms that may be quite distinct from those occurring in the context of natural infection. If the correlate of antiviral immunity is not virus-neutralizing antibody, then what is it? Both studies examined whether antibody-dependent cellular cytotoxicity (ADCC), a nonneutralizing function of notable importance noted in the RV144 HIV vaccine trial (18), was responsible. However, ADCC was not induced by gB/MF59 vaccine in either study, although it was demonstrable in CMV-seropositive controls. An alternative nonneutralizing IgG-mediated function, antibody-dependent cellular phagocytosis (ADCP), was identified by Nelson et al. in the postpartum women vaccinees (3), and ADCP may emerge as a novel CMV immune correlate warranting additional attention in future studies.

Both studies also examined immune responses to specific domains of the gB polypeptide engendered by gB/MF59. The humoral response to gB in CMV-seropositive individuals targets five major antigenic domains (ADs) schematically represented in Fig. 1: AD-1, a domain of 80 aa spanning codons 560 and 640 (AD169 strain sequence); AD-2, which consists of two binding sites (amino acids 50–54 and 68–77); AD-3, a linear epitope (amino acids 798–805 in the intraluminal region of gB); AD-5, located between amino acids 133 and 343 (also known as domain I); and AD-4, a discontinuous domain mapping to amino acids 121–132 and 344–438 (19). In a previous paper by Baraniak et al. (20), in gB/MF59-vaccinated CMV SOT patients the AD-2 domain appeared to be of particular importance in mediating protection against CMV disease in seropositives, since antibody response to this epitope was significantly lower in seropositive subjects who developed CMV viremia following SOT, compared to seropositives who remained free of viremia. However, in the current PNAS study reported by this same group of investigators (4), no responses to AD-2 were detectable in CMV-seronegative SOT vaccinees. Thus, for seronegative SOT subjects no direct correlate of protection relative to AD-2 could be established for recipients of gB/MF59 vaccine.

Similar analyses of gB epitope responses reported in ref. 3 also demonstrated that, although vaccination elicited high levels of gB-binding antibody, there was limited targeting of key neutralizing epitopes. There was one novel and striking exception: induction of antibody responses to the gB AD-3 domain. In gB/MF59 vaccinees, an average of 76% of the total anti-gB response was directed against this region, whereas in CMV-seropositive controls only 32% of the response targeted this domain. As the authors point out, it is difficult to envision a proposed mechanism of protection, given the nature of the AD-3 epitope. The AD-3 region is intracellular—and presumably not recognized by the immune system when gB is expressed on a cell membrane—and, hence, anti–AD-3 antibodies would not be predicted to bind to or neutralize infectious virus during the initial phase of infection (Fig. 1). An as-yet-unexplained contribution to vaccine-mediated protection, possibly mediated by ADCP, could be at play. Nelson et al. (3) also provocatively suggest that anti–AD-3 responses may paradoxically compromise the efficacy of gB/MF59 by diverting responses away from neutralization epitopes, and they propose that an improved gB vaccine could be contemplated in which the AD-3 coding region was removed from the polypeptide sequence. A similar suggestion has been made with respect to the AD-1 domain, since it has been proposed that induction of nonneutralizing responses to AD-1 may promote antibody binding to gB that, in turn, may block access to the more potent virus-neutralizing AD-2 epitope (21, 22).

A key question in the study by Nelson et al. (3) is whether a serological correlate of protection could be identified, in light of the 50% efficacy against acquisition of CMV infection demonstrated in the trial (15). Although the limited number of pre- and postvaccine sera from vaccinated subjects in the trial precluded a statistically robust assessment of precisely which parameters were associated with protective immunity against acquisition of infection, interestingly there was evidence (table S2 in ref. 3) of a statistically significant increase in binding to linear peptides corresponding to the AD-2 region in women who remained CMV-uninfected during the period of follow-up. This difference was only significant for AD-2 site 2, which is a nonneutralizing epitope, but the findings are nonetheless of considerable interest in light of the protective AD-2 vaccine responses noted in CMV-seropositive SOT subjects (20). Preclinical development of both AD-2–peptide conjugate vaccines (23) and AD-2–specific therapeutere monoclonal antibodies (24) supports continued experimental evaluation of the AD-2 region of gB.

In summary, the reports by Nelson et al. and Baraniak et al. indicate that we must rethink our understanding of the protective mechanisms of gB/MF59 vaccine response and reconsider our presumptions about vaccine-induced correlates of protection against acquisition of CMV infection.
our presumptions about vaccine-induced correlates of protection against acquisition of CMV infection. It will be important to examine responses in patient populations other than transplant recipients and postpartum women, since the altered immune responses associated with pregnancy (25) may not fully predict vaccine responses that could be anticipated in healthy, nonpregnant women. Another area for more detailed analysis should be resolving the issue of whether the gB/MF59 vaccine—which is based on the sequence of the Towne strain of CMV—can be expected to confer cross-protective immunity against diverse strains with divergent coding sequences that result in polymorphisms in gB epitopes (26, 27), since the data from Nelson et al. (3) suggest that there is minimal cross-species neutralization by vaccinee sera of heterologous clinical isolates of CMV. This is particularly important in light of the emerging data indicating that reinfection of CMV-immune women with novel (previously unencountered) strain variants can occur during pregnancy and that such reinfections can lead to congenital transmission (28). Put more simply, immunity to one strain of CMV clearly does not mean immunity to all strains of CMV. Another issue to consider in future gB vaccine modification is the fact that in the virion, CMV gB is heavily glycosylated, which may limit access of neutralizing antibodies to the key functional domains. The ability of vaccination to induce antibody responses that overcome the glycan shielding of gB epitopes warrants further study. Finally, the question of whether vaccine-induced neutralizing responses should target epitopes in the native trimeric, prefusion form versus the postfusion form (29, 30) of the gB protein must be considered (29). Isolating a stable prefusion form of CMV gB, as well as antibodies specific to the prefusion form, would be a boon to future vaccine design. These and other experimental approaches to understanding the immunological basis of protection conferred by the most successful CMV vaccine tested to date, gB/MF59, will help inform and direct development of improved, “next-generation” gB vaccines for this important public health problem.

Acknowledgments

The authors’ research is supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health Grant R01 HD079918, and by March of Dimes Birth Defects Foundation Grant FY17-849.

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