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EDITORIAL COMMENTARY

Searching for a Serological Correlate of Protection for a CMV Vaccine

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(See the Major Article by Baraniak et al, on pages 1907–17.)

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In the so-far elusive search for an effective cytomegalovirus (CMV) vaccine, a major emphasis has been placed on targeting the virally encoded envelope protein designated as gpUL55, more commonly referred to as glycoprotein B (gB). Interest in harnessing recombinant forms of gB as subunit vaccine candidates has been driven by longstanding observations of the key role that anti-gB antibodies play in the neutralization of CMV. Early studies demonstrated that antibodies to gB are invariantly present in CMV-seropositive individuals, and one key study showed that between 40% and 70% of the virus-neutralizing capacity (as assessed in fibroblast cell culture infections) of convalescent human sera following natural infection is specific for gB [1]. Studies of gB vaccines based on both virally vectored systems as well as adjuvanted, recombinant protein subunit immunogens have demonstrated efficacy in animal challenge models of congenital CMV infection [2, 3]. In light of this knowledge, several vaccine candidates employing diverse gB expression strategies have advanced to clinical trials [4]. In some vaccines, gB is expressed alone; in other iterations, it is expressed in combination with other antigen(s), most commonly the tegument phosphoprotein ppUL83 (also known as pp65).

The CMV gB vaccine that has been most extensively studied in clinical trials was developed in the late 1980s by Chiron corporation and was acquired by Sanofi-Pasteur in the 1990s. The gB protein is normally expressed as a 906 or 907 amino acid (aa) open reading frame (ORF) with a leader peptide that, following posttranslational modification, is cleaved at a furin recognition site and processed into a heavily glycosylated, approximately 90-kDa aminoterminal component and a less-heavily glycosylated, approximately 60-kDa carboxyterminal moiety, containing the transmembrane domain and the cytoplasmic tail of the protein. These 2 components are linked by disulfide bonds to form the mature gB expressed in a trimeric conformation on the viral envelope [5, 6]. The construct employed for expression of the Sanofi vaccine derives from the CMV Towne strain, and the gB ORF was rather extensively modified in the vaccine [7], such that the transmembrane domain and the furin cleavage site had been removed. The carboxyterminal cytoplasmic component downstream of the transmembrane domain was re-engineered as an in-frame fusion with the truncated gB ORF. As such, the vaccine was expressed as a truncated, secreted polypeptide, and the protein was subsequently purified by chromatography from tissue culture supernatants of Chinese hamster ovary (CHO) cells expressing the uncleaved, secreted gB polypeptide.

Clinical trials performed with the gB vaccine to date have targeted 2 groups of individuals in particular need of vaccination: namely, adolescents and women of child-bearing age, who need protection against the risk of congenital CMV transmission during their future pregnancies [8], and solid organ transplant (SOT) patients, who require strategies to protect against both CMV-associated disease and transplant-related complications triggered by CMV [9, 10]. Several phase II clinical trials utilizing the CHO-cell expressed, recombinant gB, admixed with microfluoridized adjuvant 59 (MF59), a proprietary oil-in-water emulsion from Novartis, have been completed [11–13]. Most studies have employed a 3-dose series of vaccine. Studies performed in adolescents and young women demonstrated a modest degree of protection against acquisition of a CMV infection conferred by the gB/MF59 vaccine, with efficacy rates ranging from 43% to 50% [11, 12]. In a study of gB/MF59 vaccine in SOT recipients, vaccination resulted in reduced duration of viremia in seronegative recipients with seropositive donors, which translated into a reduced number of days of antiviral therapy [13]. It was

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noted that the major benefit of vaccination seemed to be on new CMV infections acquired from the donor. Anti-gB antibody titer significantly increased 1 month after the second gB dose in both seropositive and seronegative subjects but, notably, there was no apparent induction of neutralizing antibody following gB immunization. Thus, the authors proposed that antibodies induced by gB vaccine might function through nonneutralizing mechanisms, perhaps by binding virus in the donated organ and, hence, preventing transmission to the recipient.

In light of these observations, a critical and unresolved question about the gB vaccine is the issue of the precise immunologic correlate of protection induced by immunization. In an interesting and important article by Baraniak and colleagues in this issue of the *Journal of Infectious Diseases* [14], the analysis of the immune response in the previously reported SOT patient study is extended to the examination of epitope-specific responses to the gB protein. The humoral response to gB in seropositive individuals targets 5 major antigenic domains (ADs): AD1, which consists of approximately 80 aa spanning codons 560 and 640 (CMV strain AD169 sequence); AD2, which consists of two binding sites (aa 50–54 and 68–77); AD3, a linear epitope (aa 798–805 in the intraluminal region of gB); domain I (AD5), located between aa 133 and 343; and domain II (AD4), a discontinuous domain mapped to aa 121–132 and 344–438 [15]. In this study, Baraniak and colleagues investigated vaccine-induced, epitope-specific immune responses in those subjects in the vaccine trial who were CMV seropositive prior to transplantation. Using a well-characterized and previously validated panel of recombinant proteins (variably expressed either as fusion proteins in *Escherichia coli*, synthetic peptides, or domain-specific recombinant proteins expressed in HEK 293T cells), enzyme-linked immunosorbent assays (ELISAs) for AD1, 2, 4, and 5 were examined using sera obtained from subjects at multiple time points pre- and postvaccination. As expected, the vast majority of seropositive individuals had antibody to AD1, which demonstrated boosting following vaccination. Vaccination boosted responses to the AD4 and AD5 domains in seropositives, and de novo responses to AD5 were also observed in CMV-seropositive recipients who were negative for AD5 antibody prevaccination. The key question next addressed in this study was the issue of whether or not the induction or magnitude of gB vaccine-induced boosting of immune responses against these epitopes had any impact on protection against CMV disease. Despite clear evidence of a boost in responses to AD1, AD4, and AD5, there was no statistically significant correlation between vaccine-induced boosting and patterns of CMV viremia posttransplant. In contrast, AD2 antibody level was significantly lower in subjects who developed viremia following SOT, consistent with the hypothesis that an effector of protection against CMV disease following transplantation is the induction of anti-AD2 antibody. Notably, this protection was restricted to individuals with AD2 responses prior to vaccination, because gB/MF59 itself did not induce de novo anti-AD2 responses in CMV-seropositive subjects who lacked AD2 reactivity prior to immunization. A similar trend was observed for AD4, in which subjects who had higher levels of AD4-specific antibody responses appeared less likely to experience CMV viremia post-SOT, although this effect did not achieve statistical significance. Additionally, a 2-component analysis demonstrated no correlation between the AD2 and AD1 responses in seropositive SOT recipients postvaccination that correlated with viremia. This observation is important because of previous studies that demonstrated competition between nonneutralizing and neutralizing antibodies against AD1, an observation which prompted the hypothesis that AD1 antibody binding may provide an immune-evasive mechanism mediated by prevention of the binding of AD2 antibody to virus particles [16]. As Baraniak and colleagues comment, one solution to this proposed viral immune evasion strategy, if it were to be validated in vivo, could conceivably be the engineered deletion of the AD1 domain from a recombinant gB vaccine. Although more data are needed, the current findings in this new study suggest that this kind of modification to gB vaccine is unlikely to be required.

In summary, this report advances our understanding of the potential correlates of protection conferred by gB/MF59 vaccine in the SOT population. In CMV-seropositive patients, gB/MF59 vaccine boosted preexisting anti-AD2 responses, and the magnitude of the AD2-response correlated with reduced viremia posttransplantation. If antibody to AD2 emerges as a reproducible correlate of protection against CMV disease in the context of natural infection and/or following gB vaccination, strategies to optimize immune responses to this region of the protein will be highly desirable. In the Baraniak et al study, only approximately 50% of CMV-seropositive subjects had preexisting antibody to AD2, and gB/MF59 vaccine did not induce AD2 antibody in seropositive subjects who lacked it prior to immunization. These data are in keeping with previous observations about AD2. Although antibodies to AD2 are known to play an important role in protection, this epitope, paradoxically, is poorly immunogenic following natural infection and, as this current study demonstrates, following vaccination [17]. Antibodies to the AD2 epitope are predominantly encoded by genes derived from recombination events involving a single VH gene, *IGHV3-30*18*, and a single Vκ gene, *IGKV3-11*01*, in a process that has been proposed to be driven by long-standing evolutionary pressure exerted by the ubiquitous presence of this infection in the population throughout all of human history [18]. Moreover, there is little coding variation in the AD2 epitope among clinical isolates of CMV, likely reflecting a strict sequence

requirement for AD2 in ensuring the proper functionality of gB in the viral replication cycle. Thus, if strategies could be developed to improve AD2 immunogenicity, increased emphasis on this epitope could create exciting opportunities for future design of improved gB vaccines [17]. Although preclinical evaluations of AD2-peptide conjugate vaccines have yielded disappointing results in terms of induction of virus-neutralizing responses in mice [19], human monoclonal antibodies targeting AD2 are in development as potential therapeutic agents [20], with one agent, monoclonal TCN 202 (Theraclone), having progressed to phase II studies [21].

In spite of these encouraging developments, many challenges remain for gB-based vaccines. Although the Baraniak et al study provides important new information about potential correlates of protection in the SOT transplant population, it does not provide insight into mechanisms of protection. As previously noted by these investigators [13], virus neutralization did not appear to be the main effector of protection against viremia, although protection was correlated with the magnitude of the anti-gB titer. Examination of other potential, nonneutralizing functions of IgG, including antibody-dependent cellular cytotoxicity, should be examined in this as well as other [12, 13] gB/MF59 study cohorts. It should also be noted that this study focused just on seropositive recipients of gB/MF59 vaccine. The mechanisms of protection in gB/MF59 vaccinees who were *CMV-seronegative* pretransplantation requires examination. Because seropositives who were negative for anti-AD2 antibodies prevaccination failed to generate any de novo anti-AD2 responses following vaccination, it will be important to evaluate whether different correlates of protection (other than AD2 responses) were responsible for the improvement in outcomes attributable to gB vaccination in the seronegative group. Finally, it is very important that similar analyses be conducted using sera from studies performed in adolescents and young women [11, 12], in addition to SOT patients. Although a CMV vaccine is needed for both populations, it is by no means certain that the same immunological correlates that confer protection for transplant patients are relevant to young women of child-bearing age. Because the major driving force behind development of a CMV vaccine is to protect newborns against the disabling effects of congenital CMV infection [22], understanding mechanism(s) of protection in subjects from nontransplant trials is a high-priority area for future research.

Notes

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