Protection against Congenital Cytomegalovirus Infection and Disease in Guinea Pigs, Conferring by a Purified Recombinant Glycoprotein B Vaccine

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Glycoprotein B (gB) has emerged as a subunit-vaccine candidate for congenital cytomegalovirus (CMV) infection, a major public health problem. The present study evaluated a cloned, recombinant gB vaccine in the guinea pig cytomegalovirus (GPCMV) model of congenital infection. Guinea pigs were immunized with gB, which was coadministered with either Freund's adjuvant or alum. All gB-immunized dams had enzyme-linked immunosorbent–assay and neutralizing-antibody responses, with significantly higher titers in the gB/Freund's group. Pregnant dams were challenged with GPCMV subcutaneously during the 3rd trimester. Maternal DNAemia on day 10 after infection trended lower in gB-immunized dams than in control animals, with significant reductions in the gB/Freund's group. Vaccination resulted in a highly significant reduction in pup mortality. For the gB-vaccine groups, pup mortality was significantly lower, and reduced rates of GPCMV transmission were noted, for dams immunized with gB and Freund's adjuvant, compared with dams immunized with gB and alum. These are the first data indicating that a recombinant gB vaccine protects against congenital CMV infection and disease.

Infection with human cytomegalovirus (HCMV) acquired in utero is common in developed countries and complicates an estimated 1%–2% of all pregnancies, annually resulting in ~40,000 cases of congenital infection in the United States and Europe [1]. Congenital HCMV infection causes particularly severe disease manifestations in the infants of women who acquire primary infection during pregnancy. Infected infants born to such women have a high incidence of neurodevelopmental sequelae, including sensorineural deafness [2]. Preconception maternal immunity to HCMV provides some degree of protection against vertical transmission of infection [3, 4], and, although infants infected in the face of transplacental immunity may still have sequelae, the incidence of severe HCMV disease is decreased. Therefore, there has been considerable interest in the development of HCMV vaccines for potential administration, prior to the establishment of pregnancy, to young women of childbearing age. Vaccines capable of protecting newborns from congenital HCMV infection and its attendant sequelae were recently identified as a "level 1" (most cost-effective category) priority by the Institute of Medicine [5].

Immunity to HCMV is complex and involves induction of both humoral (virus-neutralizing antibody) responses and cellular (cytotoxic T lymphocyte) responses [reviewed in 6]. Vaccine strategies in clinical trials include both live attenuated vaccines and subunit vaccines that target immunogenic HCMV proteins [7–9]. One of the most important candidates for a subunit vaccine against HCMV is the major envelope glycoprotein B (gB; gpUL55) [10]. All convalescent sera from HCMV-infected individuals contain anti-gB antibodies, and the majority of the virus-neutralizing antibody response after HCMV infection targets gB [11]. Recombinant HCMV gB expressed in CHO cells has been examined as a vaccine in clinical trials and has been...
found to be safe and well tolerated when administered intramuscularly [12, 13]. The gB protein has also been expressed in a recombinant canarypox (ALVAC) system, and this candidate vaccine has been found to be safe and immunogenic, both when administered alone and when administered, in a sequential prime-boost fashion, with either live attenuated Towne vaccine or purified gB expressed in CHO cells [14–16]. To date, however, no HCMV vaccine has been evaluated for its efficacy in the prevention of congenital infection or disease.

Because CMVs are species specific, evaluation of the preclinical efficacy of HCMV vaccines cannot be performed in animal models [17]. This necessitates the study of animal CMVs to help illuminate potential strategies for the development of vaccines for humans. In contrast with the CMVs of most small animals, the guinea pig CMV (GPCMV) crosses the placenta and causes infection in utero [18], thus making the GPCMV model uniquely useful for the evaluation of potential vaccines. Studies using this model support the hypothesis that antibody responses that target envelope CMV glycoproteins are important in the protection of the fetus. In several studies, the immunization of guinea pigs with native virion glycoproteins prior to the establishment of pregnancy has proven to be an effective intervention, after viral challenge of pregnant dams, against GPCMV-induced pup mortality and infection [19–21]. To date, however, the lack of detailed molecular characterization of GPCMV proteins has precluded any detailed subunit-vaccine studies using this model with recombinant/cloning technologies. Recently, the GPCMV homolog of the immunodominant gB has been cloned and has been successfully expressed in a recombinant baculovirus [22, 23]. These studies were undertaken to test the protective efficacy of recombinant baculovirus-expressed gB used as a vaccine against congenital GPCMV infection and disease.

MATERIALS AND METHODS

Animal studies. Young female and proven-breeder male Hartley guinea pigs were obtained from Harlan Laboratories and were used for vaccination and pregnancy challenge studies. Inbred adult strain-2 guinea pigs were purchased from the Children’s Hospital Research Foundation (Cincinnati) and were used as a source of salivary-gland viral stock. Guinea pig breeding and viral-challenge studies were conducted as described elsewhere [24]. Animals were housed under conditions approved by the American Association of Accreditation of Laboratory Animal Care, in accordance with the policy established by the animal-use committee of the institution.

Virus and cells. GPCMV (strain 22122, ATCC VR682) was propagated on guinea pig lung fibroblasts (GPLs) (ATCC CCL 158) and was maintained in F-12 medium supplemented with 10% fetal calf serum (Hyclone Laboratories), 10,000 IU/L of penicillin, 10 mg/L of streptomycin (Gibco BRL), and 7.5% NaHCO3, (Gibco BRL). Trichoplusia ni (TN-5) and Spodoptera frugiperda (Sf9) cells were obtained from Invitrogen. Sf9 cells were maintained in Grace’s insect medium (Gibco BRL) supplemented with 10% fetal calf serum. TN-5 cells were maintained in serum-free conditions in Ex-Cell 401 (JRH Biosciences) medium. For the detection of congenital GPCMV infection, viral culture was performed. Liveborn pups were dissected within 72 h of delivery, and the livers and spleens were homogenized (10% wt/vol) for tissue culture. A total of 100 μL of homogenate was cultured in quadruplicate. The limit of detection, based on spiking experiments with viral stocks of known titer, was 1–10 pfu.

Recombinant baculovirus, vaccine preparation, and experimental design. Details of cloning used for the generation of a recombinant baculovirus expressing GPCMV gB are described elsewhere [23]. In brief, a truncated, secreted form of gB was engineered, with a modified, insect cell–specific leader peptide. The native gB leader peptide was replaced in the baculovirus shuttle-plasmid construct by the honeybee mellitin leader peptide (vector pMelBac C), to optimize expression in insect cells. The GPCMV gB open-reading frame was also truncated immediately upstream of the transmembrane domain, at Pro692. Recombinant gB expressed in insect cells was purified by lentil-lectin column chromatography, as described elsewhere [21, 23]. In brief, confluent flasks of TiNi cells were infected with recombinant baculovirus expressing gB. Supernatant and TiNi-cell lysates were combined 96 h after infection, then were briefly sonicated on ice and were solubilized in a sodium chloride deoxycholate Nonidet P-40 buffer. After a clarifying step, the solubilized proteins were loaded onto a lentil-lectin sepharose 4B column and were washed extensively. Bound protein was eluted by an elution buffer (200 mmol/L α-methyl mannoside/200 mmol/L α-methyl glucoside mixture) and was collected in 1-mL fractions. The protein concentrations of eluted fractions were determined by a commercial Lowry assay (Gibco BRL), according to the manufacturer’s specifications, and fractions containing gB were pooled and the integrity of the eluted protein was confirmed by PAGE analysis with Coomassie blue staining.

For vaccine study, young, female Hartley guinea pigs were immunized subcutaneously with either gB and Freund’s adjuvant (gB/Freund’s) or gB and alum (gB/alum) (Alhydrogel; Accurate Scientific and Biochemical). Three doses (50 μg/dose) were administered at ~2-month intervals. Animals in the gB/Freund’s group received complete Freund’s adjuvant for the 1st dose and incomplete Freund’s adjuvant for the subsequent 2 doses. All animals were then placed with breeder males and were examined weekly for evidence of pregnancy. In the 3rd trimester of pregnancy, dams were inoculated subcutaneous-
ly with $1.5 \times 10^3$ pfu of salivary gland–passaged GPCMV and were examined daily thereafter. Pregnancy outcomes were monitored, with daily assessment of maternal health, ascertainment of date of delivery, size of litter, pups’ weights, and number of liveborn pups and of dead pups.

**Immunological assessment.** Immune responses in vaccinated dams were monitored by ELISA and neutralization assay. Serum was obtained ~1 month after the completion of the immunization series, for the determination of the ELISA titer and the neutralizing titer. ELISA was performed as described elsewhere [24], by consistent use of a coating antigen of sonicated, clarified GPCMV-infected GPLs. ELISA titer was defined as the reciprocal of the highest dilution that produced an absorbance of ≥0.10 and twice the absorbance of a negative-control antigen prepared from uninfected GPLs. Neutralization assays were performed as described elsewhere [25], except that 5% rabbit serum was used as a source of exogenous complement. Neutralization assays were performed with an isolate of GPCMV tagged with green fluorescent protein, and plaques were enumerated by fluorescence microscopy [26]. The neutralizing titer was defined as the dilution of serum that resulted in a ≥50% reduction in plaques, compared with control (preimmune) sera.

**Maternal quantitative competitive PCR (qcPCR) analyses.** On day 10 after subcutaneous inoculation with GPCMV, maternal blood was obtained for DNAemia analysis, by qcPCR. DNA from whole blood (200 μL) was extracted by use of the QIAamp DNA Mini Kit DNA extraction system (Qiagen), according to the manufacturer’s specifications. Eluted DNA (1% of sample) was subjected to qcPCR analysis, as described elsewhere [27]. In brief, the primer pair UL83F6 (5′-CGACGACGACGATGACGAAAAC-3′) and UL83B11 (5′-TCCTCGGTCTC-AACGAAGGGTC-3′) amplifies a 225-bp region, corresponding to Asp402 through Ser473 of GP83. This plasmid was modified by engineering a 68-bp internal deletion. The resultant clone served as an internal standard for qcPCR. A standard curve was generated by measuring the ratio of the relative signal intensity of amplification products on ethidium bromide–stained gels, for increasing amounts of full-length plasmid with the internal standard. The signal intensity of the experimental standard was compared with this standard curve, to quantify the total copy number (i.e., GPCMV genome equivalents) per milligram of tissue extracted.

**Statistical analyses.** Incidence data were compared by Fisher’s exact test. Nonparametric variables were compared by the Mann-Whitney U test. All comparisons were 2-tailed.

**RESULTS**

**Immune response to recombinant gB vaccination.** Previous analyses of native-glycoprotein vaccine suggested that 3 doses of vaccine were required for optimal immunogenicity [21]. Therefore, 3 doses of recombinant gB was administered, at ~6–8 week intervals, by use of either Freund’s adjuvant or alum. The results of ELISA showed that all vaccinated guinea pigs seroconverted to GPCMV after the 3-dose series, with titers of 2.2 log<sub>10</sub>—4.6 log<sub>10</sub> (figure 1A). The ELISA titers were significantly higher in the gB/Freund’s group (mean, 4.9 log<sub>10</sub>) than in the gB/alum group (mean, 4.5 log<sub>10</sub>) ($P<.05$, Mann-Whitney U test). Similarly, complement-dependent virus-neutralizing titers were significantly higher in the gB/Freund’s group (mean, 2.9 log<sub>10</sub>) than in the gB/alum group (mean, 2.6 log<sub>10</sub>) ($P<.05$, Mann-Whitney U test) (figure 1B).

**Pregnancy outcomes in viral-challenge study.** To assess the protective efficacy of gB vaccine against adverse pregnancy outcomes associated with GPCMV infection, a challenge experiment was performed. Twelve dams in the gB/Freund’s group and 14 dams in the gB/alum group became pregnant. A group of 14 unimmunized dams served as controls. Pregnancy was established after the 3rd dose of gB vaccine, and, after 3rd-

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**Figure 1.** ELISA (A) and neutralizing-antibody (B) responses in guinea pigs immunized with glycoprotein B (gB) and either Freund’s adjuvant (gB/Freund’s) or alum (gB/alum). ELISA was performed to determine response to guinea pig cytomegalovirus antigen [25]. Animals were bled after the 3rd dose of vaccine. Data shown are mean titers ± SEM. *$P<.05$ vs. gB/alum (Mann-Whitney U test).
trimester GPCMV challenge, outcomes were compared among animals for which there was an elapsed period of ≥7 days between infection and delivery. Among control dams, the pup mortality rate was 76% (31/41); in contrast, among pups born to gB-immunized dams, the pup mortality rate was 25% (23/91) (P<.0001) (table 1). There was a significant reduction in mortality rates both for the gB/Freund’s group and the gB/alum group. Among pups born to dams in the gB/alum group, the pup mortality rate was 25% (17/68) compared with 35% (9/26) in the gB/Freund’s group (P<.0001 vs. the control group); among pups born to dams in the gB/Freund’s group, the mortality rate was reduced even further, to 14% (6/42; P<.0001 vs. the control group and P<.05 vs. the gB/alum group) (table 1). There also were significant differences, between the vaccine group and the control group, in the number of litters that contained ≥1 dead pup: 50% (13/26) of the litters in the vaccine group had ≥1 dead pup (64% [9/14] in the gB/alum group and 25% [4/16] in the gB/Freund’s group), compared with 86% (12/14) of the litters in the control group (P<.05; Fisher’s exact test).

Viral-load comparisons in immunized and unimmunized dams after GPCMV challenge. To assess the effect that gB vaccination had on maternal viral load after GPCMV challenge, qcPCR was used to compare the magnitude of DNAemia. A subset of dams (8 dams each from the gB/Freund’s and control groups and 9 dams from the gB/alum group) were bled on day 10 after viral challenge, for DNAemia analysis by qcPCR. The qcPCR assay indicated that gB vaccine when administered with Freund’s adjuvant resulted in a significant reduction in maternal viral load (figure 2). The mean viral load in control dams 10 days after viral infection was 4.6 log10 genomes/mL. Among dams in the gB/Freund’s group, the mean viral load was 3.7 log10 genomes/mL (P=.05 vs. the control group; Mann-Whitney U test), and dams in the gB/alum also showed a trend toward reduced maternal DNAemia (4.2 log10 genomes/mL; P=NS vs. the control group). Interestingly, a threshold level of maternal DNAemia, 3.5 log10 genomes/mL, was identified that appeared to be predictive of adverse pregnancy outcomes (table 2). The mortality rate for pups born to dams with systemic viral load greater than this level was 64% (34/53), compared with 8% (3/36) for pups born to dams with a systemic viral load less than or equal to this level (P<.00001; Fisher’s exact test).

Impact of adjuvant on congenital GPCMV infection rates among liveborn pups. To analyze the impact that gB vaccination had on congenital GPCMV infection, liveborn pups were dissected within 72 h of delivery, and livers and spleens were homogenized for tissue culture. The 34% (23/68) vertical transmission rate among liveborn pups born to dams that had been immunized with gB was comparable to the 50% (5/10) congenital infection rate among liveborn pups born to unimmunized dams; however, it should be noted that there was a paucity of liveborn pups (10) in the control group (table 3). The higher survival rate in the vaccine group allowed for a more robust analysis in the liveborn subset of pups, and choice of adjuvant was shown to have a significant impact on the congenital infection rate. Among liveborn pups in the gB/Freund’s group, only 22% (8/36) had congenital GPCMV infection, compared with 47% (15/32) of pups in the gB/alum group (P<.05).

### Table 1. Pup mortality after cytomegalovirus challenge following maternal immunization with recombinant glycoprotein B (gB).

<table>
<thead>
<tr>
<th>Group</th>
<th>Litters</th>
<th>With ≥1 Dead pup</th>
<th>Total</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (unvaccinated)</td>
<td>14</td>
<td>12 (86)</td>
<td>41</td>
<td>31</td>
</tr>
<tr>
<td>gB vaccine</td>
<td>26</td>
<td>13 (50)</td>
<td>91</td>
<td>23 (25)</td>
</tr>
<tr>
<td>With Freund’s adjuvant</td>
<td>12</td>
<td>4 (25)</td>
<td>42</td>
<td>6 (14)</td>
</tr>
<tr>
<td>With alum</td>
<td>14</td>
<td>9 (64)</td>
<td>49</td>
<td>17 (35)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%).

a P<.05 vs. control group.
b P<.00001 vs. control group.
c P<.05 vs. the group immunized with gB and alum.

DISCUSSION

A number of studies have examined, by use of animal models, the protective efficacy of vaccines consisting of CMV proteins. Cloned subunit vaccines, in particular, have been evaluated in detail in the murine CMV (MCMV) model. In one study, a recombinant vaccinia-virus vaccine expressing MCMV gB provided protection against lethal MCMV challenge in BALB/c mice [28]. Interestingly, the protection of mice against MCMV challenge could be achieved with gB vaccine but not with a vaccine targeting glycoprotein H. Studies of subunit vaccines against MCMV have also examined protection conferred by DNA vaccines, using MCMV homologs of the immediate early protein and of the UL84 protein [29–32]. Although useful, are of limited relevance to the problem of the prevention of congenital CMV infection, because MCMV does not cross the placenta and cause disease in utero [33]. Although the rhesus macaque CMV provides a useful model for the study of the pathogenesis of fetal CMV infection, the expense of primate studies limits extensive research in this system [34]. Insights from the GPCMV model are, therefore, uniquely useful in the analysis of vaccine strategies for the prevention of congenital CMV, both because of greater technical ease relative to primate studies and because, in contrast to what is seen in other small-animal models, infection and disease occur in utero.

Previous vaccine studies with the GPCMV model of congenital infection have chiefly focused on native-protein im-
Freund’s adjuvant (3.7 log10 genomes/mL), compared with that in the
maternal DNAemia was reduced in the group immunized with gB and
viral load in each group is indicated by a dashed line. The magnitude of
B (gB) and either Freund’s adjuvant ( ) or alum ( ). The mean
log10 genomes/mL) compared with that in the control group, but the
Immunization with gB and alum also reduced maternal DNAemia (4.2
* (Mann-Whitney
difference only approached—but did not reach—statistical significance.

Figure 2. Effect of baculovirus glycoprotein B (gB) vaccine, after chal-
lenge with guinea pig cytomegalovirus (GPCMV). Shown is a summary
of the magnitude of maternal DNAemia (day 10 after inoculation), de-
determined by a sensitive quantitative competitive PCR assay, in control
(unvaccinated) dams (n = 8) and in dams immunized with glycoprotein
B (gB) and either Freund’s adjuvant (n = 8) or alum (n = 9). The mean
viral load in each group is indicated by a dashed line. The magnitude of
maternal DNAemia was reduced in the group immunized with gB and
Freund’s adjuvant (3.7 log10 genomes/mL), compared with that in the
control group (4.6 log10 genomes/mL) ( , Mann-Whitney
U-test).

Immunization with gB and alum also reduced maternal DNAemia (4.2
log10 genomes/mL) compared with that in the control group, but the
difference only approached—but did not reach—statistical significance.

*P = .05 (Mann-Whitney U-test).

munogens, largely because of the lack of cloned recombinant
vaccine candidates. GPCMV proteins have been purified by
immunofinity-column chromatography, lectin-column chro-
matography, or detergent solubilization of virus and dense-
body fractions and have been administered as vaccines with
Freund’s adjuvant [19–21]. All of these strategies have dem-
onstrated protection against congenital GPCMV infection and
disease, with the extent and nature of protection being depen-
dent on the strain of guinea pigs used, the timing of viral
challenge, and the study endpoints examined. To date, however,
there have been no subunit-vaccine studies reported that have
used recombinant-expression strategies. The recent successful
cloning and expression of the GPCMV gB protein has enabled
such investigations, and the present study was therefore un-
dertaken to evaluate, by use of a vaccine derived by expression
and purification in recombinant baculovirus, the gB protein’s
protective efficacy against congenital GPCMV infection and
disease. The evaluation of the use of Freund’s adjuvant and of
alum, the most commonly used adjuvants in human vaccines,
was undertaken to compare their relative efficacy. The choice
of baculovirus for gB expression was based, in part, on the
successful expression of HCMV gB in insect cells, and was
fostered by the observation that HCMV gB expressed in this
system retained immunoreactivity with monoclonal anti-gB an-
tibodies [35, 36] as well as with convalescent antisera both from
CMV-seropositive individuals and from gB-vaccine recipients
[37]. Previous studies, using other recombinant herpesvirus
glycoproteins expressed in insect cells, also suggested that this
expression system was suitable for vaccine production and test-
ing [38–40]. Consistent with these reports, the present study
demonstrated that gB expressed in baculovirus is highly im-
munogenic and protective in guinea pigs. Although the present
study used unimmunized guinea-pig dams as a negative control
group, previous studies using this model, with glycoprotein
from uninfected cells and administered with Freund’s adjuvant,
did not demonstrate any protection against CMV-associated
disease [21]. This observation, coupled with the lack of im-
munoreactivity of GPCMV-immune antisera against insect-cell
proteins [23], supports the conclusion that the protection ob-
served in the present study is attributable to immune responses
to recombinant gB. The present study demonstrates, for the
first time in an animal model of congenital CMV infection,
that preconception maternal vaccination with a gB protein gen-
erated by recombinant/cloning technology is capable of con-
ferring protection against CMV-associated disease in newborns.

An observation of interest in gB-immunized dams was the
apparent influence that maternal postvaccine antibody re-
sponse—particularly the neutralizing antibody response—had
on GPCMV-induced pup mortality. Statistically significant im-
provements in pup-mortality rates and in congenital-infection
rates were observed in pups born to dams immunized with gB/
Freund’s, compared with pups born to dams immunized with
gB/alum. Because Freund’s adjuvant conferred higher neutral-
ization titers than did alum (P < .015), it is likely that maternal
and pup protection depend on the magnitude of the neutral-
izing antibody response conferred by vaccination. This hy-
thesis is consistent with previous reports of results with the
guinea pig model, reports in which passive-transfer studies sug-
Suggest that neutralizing antibody alone is sufficient to provide
protection against congenital infection and disease [24, 41].
The role played by neutralizing antibody has also been confirmed
in studies of a DNA vaccine that targets the GPCMV gB homolog
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Table 2. Comparison of mortality rates among pups born to
dams with DNAemia above and below threshold level of 3.5 log10

<table>
<thead>
<tr>
<th>Level of DNAemia</th>
<th>Live</th>
<th>Dead</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;3.5 log10 genomes/mL</td>
<td>19</td>
<td>34</td>
<td>53</td>
</tr>
<tr>
<td>≤3.5 log10 genomes/mL</td>
<td>30</td>
<td>3a</td>
<td>33</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of pups.

* P < .00001, Fisher’s exact test.
typically induces a Th2 cytokine profile [43]. These differences, in turn, may have contributed to the adjuvant-specific differences in protection that were observed in the present study. Future studies, which focus on more-detailed analyses of guinea pig cellular immune responses, will be necessary to ascertain the relative contributions, in this model, of humoral and cell-mediated immunity against CMV-associated disease.

An apparently important effect of gB vaccination was the attendant reduction of systemic maternal viral load after GPCMV challenge. Vaccination with gB resulted in reduction of the magnitude of maternal DNAemia at day 10 after viral challenge. Although vaccination resulted in mean viral-load reduction in both the gB/Freund’s group and the gB/alum group, the magnitude of the reduction appeared to be greatest in dams that had received Freund’s adjuvant (mean, 0.9 log10 genomes/mL; \( P < .05 \)). A threshold level of maternal DNAemia of 3.5 log10 genomes/mL was predictive of the most-adverse pregnancy outcomes: mortality for pups born to dams with DNAemia above this level was 64% (34/53), compared with 8% (3/36) for pups born to dams with DNAemia below this level (\( P < .00001 \)).

Future studies with the GPCMV model, which examine, in pregnant guinea pig dams with DNAemia, the cumulative viral load over a longer time period, should provide insights into the protective effect of CMV vaccines. The establishment of a threshold level of maternal DNAemia associated with poor outcome may ultimately be of great relevance to HCMV vaccine studies, because the observations of the present study imply that “sterilizing immunity” against CMV will not be required for a vaccine to be beneficial in protecting the fetus. Studies that suggest that the viral load present in a congenitally infected infant may predict neurodevelopmental sequelae further support the concept that a CMV vaccine that reduces the magnitude of fetal CMV infection may be of benefit, even if infection per se is not prevented [44].

In summary, the present study provides evidence for a protective effect against fetal infection and disease in a small-animal (guinea pig) model of congenital CMV infection. Protection depends on the adjuvant, the magnitude of the neutralizing antibody response, and the magnitude of the reduction of maternal DNAemia. The usefulness of the guinea pig model in the study of vertically transmitted infections has been demonstrated for a variety of pathogens, including CMV, toxoplasmosis, syphilis, and listeria monocytogenes [45–48]; to date, however, there have been few evaluations of subunit–vaccine strategies against these diseases based on recombinant-expression technologies. The results of the present study indicate that, in the guinea pig, gB expressed in baculovirus is an effective preconception subunit vaccine against congenital CMV infection and disease, an observation that provides support for the continued development of gB-based vaccines for human use. Continued investigation of subunit vaccines in the GPCMV model should focus on improving the expression strategies, to effect greater reductions in maternal DNAemia after viral challenge, and on better elucidating the role that cell-mediated immune responses (in addition to neutralizing antibody responses) play in protection against CMV disease. In addition, the extent of protection conferred by gB when administered with clinically relevant adjuvants will require further exploration. The critical role of adjuvant has been demonstrated in studies using a guinea pig model of recurrent genital herpes simplex infection, where the choice of adjuvant has a significant impact on the recurrence phenotype [49]. Insights derived from future GPCMV studies may help to prioritize which adjuvants and subunit-expression strategies merit investigation in HCMV-vaccine clinical trials. Future studies should also help to define the correlates of protective maternal immunity. Although transmission of maternal antibody appears to be integral to the protection of the fetus in utero, cell-mediated immune responses, which are not necessarily transmitted to the fetus, are still likely to be integral to protection, perhaps by contributing to the reduction of maternal viral load. In addition to further research on envelope-glycoprotein vaccines, future vaccine studies using immunogens that predominately elicit cytotoxic T lymphocyte responses are warranted in the GPCMV model.

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