A Subunit Cytomegalovirus Vaccine Based on Recombinant Envelope Glycoprotein B and a New Adjuvant

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A phase I randomized, double-blind, placebo-controlled trial was done with a cytomegalovirus (CMV) vaccine based on the envelope glycoprotein, gB, combined with a novel adjuvant, MF59. Participants received CMV gB vaccine with MF59 or CMV gB with alum or placebo at 0, 1, and 6 months. A fourth vaccine was given at 12 months to a subgroup. Levels of neutralizing antibody and antibody to gB 2 weeks after the third dose of vaccine exceeded those in seropositive control subjects. The formulation with MF59 was more immunogenic than that with alum. The optimal dose of gB appeared to be between 5 and 30 μg. The fourth dose produced a prompt rise in antibody level. There were no serious adverse events associated with vaccine. Local and systemic reactions were generally mild and, except for pain at the injection site, occurred with similar frequency in recipients of placebo and CMV vaccine.

Congenital cytomegalovirus (CMV) infection is the leading infectious cause of central nervous system (CNS) disease in children in the United States and therefore merits a high priority for prevention [1]. It has been estimated that a vaccine that could match the ability of immunity acquired from past infection to prevent fetal disease would reduce the morbidity due to congenital CMV infection by ~80% [2]. No CMV vaccine is currently available, and previous evaluations of live virus vaccines have not led to licensure [3, 4].

A CMV envelope glycoprotein commonly referred to as gB is the major target of neutralizing antibody (NA) induced by naturally acquired infection and therefore a logical choice for a recombinant vaccine [5–7]. A recombinant gB molecule was mutated to eliminate a cleavage site [8], and the transmembrane region was deleted to facilitate secretion of the glycosylated protein in Chinese hamster ovary cell culture. The mutated, recombinant CMV gB molecule was combined with a new adjuvant, MF59, which is based on an oil-in-water emulsion of squalene [9]. After initial open-label safety testing in 12 seronegative subjects, this new CMV vaccine was evaluated for safety and immunogenicity in a phase I trial with 46 seronegative healthy adults.

Subjects and Methods

Study population and enrollment criteria. Adults from 18 to 50 years of age who volunteered to participate and met enrollment criteria were entered into the study. To qualify for enrollment, volunteers could have no detectable serum antibody to CMV as determined by a widely available assay (ImX; Abbott Laboratories, North Chicago, IL), could not be at known increased risk for acquired CMV infection (i.e., no children <2 years of age in day care and no one with recent sexually transmitted diseases or attendance at a sexually transmitted disease clinic), and had to be in generally good health with no known chronic diseases. Exclusion criteria included receipt of steroids or other immunosuppressives, immunodeficiency or inflammatory disease or malignancy, or immunization within 1 month of study enrollment. Women who enrolled were asked to use an effective means of birth control and were tested for pregnancy prior to each dose of vaccine. Participants were asked to avoid immunization with other vaccines during the study if possible.

Vaccine formulation and administration. The CMV antigen that was used as a vaccine component contains the entire extracellular, glycosylated domain and the entire intracellular domain of envelope gB. The intervening membrane-spanning domain was deleted to facilitate secretion, and the two terminal regions were fused to generate a full-length transmembrane construct of Towne strain CMV gB. In addition, the gB gene was mutated to remove the single natural protease cleavage site in the extracellular domain. The recombinant gene was expressed in Chinese hamster ovary cell culture and secreted as a protein of 807 amino acids with 19 putative N-linked glycosylation sites. The vaccine was formulated with MF59 (10.75 mg/dose) or standard aluminum hydroxide gel (2.18 mg/dose) as adjuvant. MF59 is a proprietary oil-in-water emulsion of squalene (Chiron Vaccines, Emeryville, CA). The CMV antigen
Figure 1. Antibody response to cytomegalovirus (CMV) envelope glycoprotein (gB) vaccine administered at 0, 1, and 6 months by dose of gB and adjuvant. A. Antibody to gB by ELISA; geometric mean titer (GMT ± 1 SD) for 200 seropositive adults with naturally acquired infection was 3186 ± 1989. B. Neutralizing antibody response; GMT ± 1 SD) for 60 seropositive adults with naturally acquired infection was 60 ± 38 gB/adjuvants: 5/30/, and 100/MF59 = 5, 30, and 100 μg of CMV gB in novel adjuvant MF59; 100/alum = 100 μg of CMV gB in standard aluminum hydroxide gel.
Table 1. Percentage of vaccinated subjects, by vaccine group, with neutralizing antibody (titer >1: 10) to cytomegalovirus (CMV) envelope glycoprotein (gB).

<table>
<thead>
<tr>
<th>Time after 3d doce</th>
<th>Vaccine group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/MF59</td>
</tr>
<tr>
<td>2 weeks-3d</td>
<td>70</td>
</tr>
<tr>
<td>6 months-3d</td>
<td>78a</td>
</tr>
</tbody>
</table>

NOTE. Vaccine groups: 5/, 30/, and 100/MF59 = 5, 30, and 100 µg of CMV gB formulated with novel adjuvant MF59 (10.75 mg/dose); 100/alum = 100 µg of CMV bg formulated with standard aluminum hydroxide gel (2.18 mg/dose).

One subject in 5/MF59 group and 2 subjects in 30/MF59 group had insufficient serum for assay at this time point.

b n = 9. One subject in the alum group acquired CMV between the first and second doses of vaccine and was excluded from immunogenicity analysis.

was stored in 0.25 mL of citrate buffer at −70°C, and the adjuvants (0.25 mL/vial) were stored at 4°C. One hour before use, antigen was thawed and mixed 1 : 1 (v : v) with adjuvant. Placebo (citrate buffer) was stored at 4°C. Vaccine (0.5 mL) was administered intramuscularly in the left deltoid.

Study design. For this phase I trial, we used a double-blind, randomized, placebo-controlled design. Volunteers were randomly assigned to receive one of three doses (5, 30, or 100 µg; 10 subjects/group) of CMV gB formulated with MF59 (gB/MF59) or 100 µg of CMV gB with alum (gb/alum; 10 subjects) or placebo (6 subjects). Immunizations were given at enrollment and 1 and 6 months later. A fourth dose of vaccine was given 12 months after the first immunization to subjects who consented to participate in an extension with 12 months of follow-up after the fourth immunization. Participants in the extension received the same vaccine formulation (or placebo) that they had been given for the first three immunizations.

Reactogenicity and adverse events. Local and systemic reactogenicity of vaccines was assessed by examinations, phone calls (24 h after immunization), and diary cards covering the 7 days after immunization. Participants were assessed by history and examination prior to each vaccine and 30 min and 48 h after immunization. Adverse events were ascertained by review of interval history with the patient at each visit and by phone contact. Women were screened for pregnancy at enrollment and prior to each dose of vaccine. Safety laboratory tests (complete blood cell count, platelet count, serum levels of alanine aminotransferase [ALT] and creatinine, and urinalysis) were obtained at enrollment, prior to each immunization, and at 1.5, 6.5, and 12 months.

Assessment of immune response. An ELISA assay developed and performed at Chiron Vaccines was used to measure IgG antibody to CMV gB; the CMV gB used in the vaccine was used as antigen in this assay. NA was measured by use of a plaque-reduction technique; the end point was calculated as the dilution of serum that produced 50% reduction in plaque count with the stock pool of Towne CMV. Sera from healthy adults who were seronegative for CMV and from those with naturally acquired CMV infection were used for negative and positive controls with each antibody assay.

To determine whether any study participants acquired CMV during the course of the trial, the sera for days 0 and 360 from each participant were tested for antibody to recombinant CMV proteins pp65, pp150, and pp50 by use of a Western blot assay. Purified proteins were electrophoresed on separate gels along with colored blot markers (α-2-macroglobulin, 180 kDa, and triosephosphate isomerase, 28 kDa; Sigma, St. Louis) under reducing conditions by SDS-polyacrylamide gel electrophoresis on 8% polyacrylamide two-dimensional–well gels. Blots on nitrocellulose were cut into 4-cm strips and separately probed with patient sera, monoclonal antibody to each antigen, and CMV-positive and -negative control sera. Alkaline phosphatase-conjugated goat anti-human IgG and anti-mouse IgG second antibodies were used, respectively, for the human sera and mouse monoclonal antibodies; nitroblue tetrazolium–BCIP was used as a detection reagent.

Data management and statistical analysis. Clinical data were collected on standardized case report forms; clinical and laboratory data were entered into a computer data set at Chiron Vaccines. Group antibody results were expressed as geometric mean titers (GMTs). Antibody response was compared between groups by use of a one-way analysis of variance linear model with a single term for vaccine group followed by simple pairwise t test. The frequency of local and systemic reactions was compared by use of Pearson’s χ² test for vaccine group differences.

Results

Characteristics of the study population. Study volunteers ranged in age from 21 to 50 years. Seventeen were men (37%), and 29 were women (63%). Five participants (11%) were black, and the remainder were white.Weights ranged from 44.1 to 138.0 kg, and heights ranged from 144 to 200 cm. There was not a statistically significant difference across groups in age, sex, race, height, or weight.

Antibody response to CMV gB. Antibody response to CMV gB as measured by ELISA is shown in figure 1. Four of the five vaccine groups developed antibody to CMV gB during the study. Peak antibody levels were achieved 2 weeks after the third dose of vaccine, all vaccinees had antibody to gB (ELISA titer >1: 50); from 2 weeks after the third dose of vaccine to

Table 2. Percentage of subjects showing local reactions, by cytomegalovirus envelope glycoprotein (gB) vaccine group.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>5/MF59</th>
<th>30/MF59</th>
<th>100/MF59</th>
<th>100/alum</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>100</td>
<td>80</td>
<td>100</td>
<td>90</td>
<td>17</td>
</tr>
<tr>
<td>Warmth</td>
<td>40</td>
<td>20</td>
<td>30</td>
<td>50</td>
<td>33</td>
</tr>
<tr>
<td>Erythema (&gt;3 mm)</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>Induration (&gt;3 mm)</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>17</td>
</tr>
</tbody>
</table>

NOTE. Vaccine groups: 5/, 30/, and 100/MF59 = 5, 30, and 100 µg of CMV gB formulated with novel adjuvant MF59 (10.75 mg/dose); 100/alum = 100 µg of CMV bg formulated with standard aluminum hydroxide gel (2.18 mg/dose).

a P < .001, Pearson’s χ² test for vaccine group differences.

b P > .5
study termination, all vaccinees had ELISA antibody to CMV gB at each time point. Pairwise comparison of each of the vaccine groups at 2 weeks and 6 months after the third dose of vaccine revealed that differences between each of the MF59 groups compared with the alum group were statistically significant; \( P < .001 \) at 2 weeks and \( P = .002, .018, \) and \( .009 \) at 6 months, respectively, for \( 5, 30, \) and \( 100 \mu g \) of gB in MF59 versus alum \( (P < .001) \). There were no statistically significant differences in antibody level between MF59 groups.

NA levels in each group up to 6 months after the third dose of vaccine are shown in figure 1A. These antibody levels paralleled gB ELISA results in that peak levels were achieved 2 weeks after the third dose of vaccine. The highest peak GMT for NA \( (55) \) was also observed in recipients of the \( 30-\mu g \) gB dose. In comparison, 77 healthy adults with naturally acquired CMV infection had a GMT of \( 60 \pm 58 \). The percentage of subjects with NA \( (titer > 1:10) \) is shown in table 1. Two weeks after the third dose of vaccine, \( 24 \) (80%) of 30 recipients of CMV gB in MF59 had detectable NA; however, only \( 4 \) (44%) of 9 recipients of gB/alum had detectable NA. Although NA levels had declined by 6 months after the third dose of vaccine, NA was still detectable in the serum of 74% of those who received gB/MF59, compared with 22% of the gB/alum recipients.

Two weeks after the third dose of vaccine, NA GMTs were higher in those who received gB/MF59 instead of gB/alum \( (P = .038, .001, \) and .15, respectively, for \( 5, 30, \) and \( 100 \mu g \) in MF59 vs. \( 100 \mu g \) gB in alum). NA responses were also higher 6 months after the third dose of vaccine for each of the CMV gB/MF59 groups compared with the gB/alum group \( (P = .008, .027, \) and .039, respectively, for \( 5, 30, \) and \( 100 \mu g \) gB in MF59 vs. \( 100 \mu g \) gB in alum). There were no statistically significant differences between the GMT of NA at either time point for the 3 gB/MF59 groups.

**Immune response to fourth dose of vaccine.** ELISA antibody to CMV gB and NA levels immediately prior to and up to 12 months after a fourth dose of vaccine are shown in figure 2 for the subjects who received \( 30 \mu g \) of gB in MF59. Antibody to gB increased by 7-fold \( (14,201) \) within 2 weeks of receipt of the fourth dose of vaccine and declined over the subsequent 12 months to a level \((2398) \) similar to that observed prior to the fourth vaccination. NA also increased promptly from a GMT of \( 26 \) to 113 2 weeks after the fourth dose of vaccine. Twelve months after the fourth vaccination, the GMT of NA was \( 57 \), a level similar to that observed in controls with naturally acquired infection \((GMT, 60)\).

**CMV infection in vaccine recipients.** At day 360, 6 months after the third dose of vaccine, 1 of the 46 trial participants had serologic evidence of CMV infection \((antibodies to pp50, pp65, \) and pp150) by Western blot assay. All seral sera from this subject were run together in the Western blot assay in a blinded fashion with purified recombinant CMV proteins. Pre-vaccine serum had no reactivity in the Western blot assay; by day 28, faint bands with pp50 and a combined pp50-65 antigen were present. The day-42 serum had strong bands with pp50, pp65, pp150, and gB. The subject had been randomized to the gB/alum group. Remarkably, after her second dose of vaccine, the subject developed a urticarial rash. The rash cleared spontaneously within 3 h of appearance, and the subject developed no other systemic signs of reaction to vaccine. This subject had no other symptoms associated with acquisition of CMV. She did not receive the third dose of vaccine, and she was not included in the analysis of vaccine immunogenicity. None of the other trial participants developed serologic evidence of CMV infection during the course of the trial.

**Vaccine reactogenicity and safety.** The frequency of postimmunization reactions was compared across groups for each im-

**Table 3.** Percentage of subjects showing systemic reactions, by cytomegalovirus envelope glycoprotein (gB) vaccine group.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>5/MF59</th>
<th>30/MF59</th>
<th>100/MF59</th>
<th>100/alum</th>
<th>Placebo</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chills</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>.11</td>
</tr>
<tr>
<td>Nausea</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td>20</td>
<td>0</td>
<td>.099</td>
</tr>
<tr>
<td>Malaise</td>
<td>20</td>
<td>50</td>
<td>0</td>
<td>10</td>
<td>17</td>
<td>.063</td>
</tr>
<tr>
<td>Myalgia</td>
<td>30</td>
<td>40</td>
<td>20</td>
<td>30</td>
<td>50</td>
<td>.76</td>
</tr>
<tr>
<td>Rash</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>.61</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>.15</td>
</tr>
<tr>
<td>Headache</td>
<td>60</td>
<td>40</td>
<td>40</td>
<td>60</td>
<td>83</td>
<td>.41</td>
</tr>
<tr>
<td>Fever (&gt;38°C)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>.29</td>
</tr>
</tbody>
</table>

NOTE: Vaccine groups: 5, 30, \( \), and \( 100/MF59 = 5, 30, \) and \( 100 \mu g \) of CMV gB formulated with novel adjuvant MF59 \((10.75 \text{ mg/dose})\); \( 100/alum = 100 \mu g \) of CMV gB formulated with standard aluminum hydroxide gel \((2.18 \text{ mg/dose})\). \( ^a \) Pearson's \( \chi^2 \) test for vaccine group differences.
munization. The frequency of local reactions across all four doses of vaccine is summarized in table 2. Among local reactions (pain, warmth, erythema, and induration), only pain at the injection site occurred more frequently among recipients of CMV gB than among placebo recipients. There was no difference in frequency of local reactions by quantity of gB administered or by adjuvant, and there was no significant difference in rates of local reactions by immunization number.

The frequency of systemic reactions (fever, chills, malaise, headache, nausea, arthralgia, and myalgia) was likewise compared across groups for each immunization. There was a statistically significant difference in the frequency of malaise among vaccine groups but only with the third immunization. After the third immunization, malaise was reported by 1 recipient (10%) of 5 μg of gB in MF59, by 4 recipients (40%) of 30 μg of gB in MF59, and by none of the other vaccine or placebo recipients (P = .021). There was no difference in the frequency of systemic reactions with increasing immunization number. The frequency of systemic reactions across all immunizations is shown in table 3; in general, systemic reactions were infrequent and mild when they did occur. No serious adverse events occurred, and no vaccine-related abnormalities or changes in hematologic values, serum ALT or creatinine, or urinalysis results were observed.

Discussion

This study demonstrates that immunization of healthy adults with a subunit CMV vaccine that combines recombinant envelope glycoprotein gB with MF59, a novel adjuvant, induces antibody to gB and CMV NA. Antibody responses were greater in magnitude when gB was formulated with MF59 adjuvant than with alum, the current standard adjuvant for licensed vaccines. Peak levels of antibody to gB were higher in vaccine recipients than in seropositive adults. Although antibody levels declined steadily after immunization, the prompt increase in antibody to gB and NA after the fourth dose of vaccine suggests that vaccinees will respond similarly when they encounter virus. Although there is evidence that higher levels of NA are associated with lower rates of CMV reinfection [10], levels of antibody to gB or NA that can prevent infection or disease have not been defined in humans.

Three doses of vaccine antigen (5, 30, and 100 μg) were compared for the groups who received vaccine formulated with MF59 adjuvant. There were no statistically significant differences in antibody responses among these dose groups; however, the groups were small. Considering both anti-gB and CMV NA responses, it appeared that the optimal dose of CMV gB is probably between 5 and 30 μg; larger sample sizes should be evaluated to define an optimum dose of gB. CMV gB/MF59 was well tolerated. Considering both local and systemic reactions, the study vaccine was certainly no more reactogenic than many currently licensed vaccines.

In the United States, congenital CMV infection is the leading cause of sensorineural hearing loss and the leading infectious cause of brain damage in children [1, 11]. It has been estimated that congenital CMV infection causes more CNS damage than either congenital rubella or Haemophilus influenzae type b meningitis did prior to the routine use of vaccines against these infections [1]. Although the significance of congenital CMV infection as a cause of CNS impairment in children is indisputable, progress in vaccine development has been slow, perhaps because naturally acquired CMV infection does not produce immunity that prevents reinfection or transmission of CMV to the fetus [12–15]. Reinfection has been documented by recovery of different strains (by restriction fragment comparisons) of CMV from the same person, although it should be noted that reinfection has been observed mostly in immunocompromised hosts or persons with many intimate contacts [12–14, 16].

In addition, a recent trial with a live attenuated vaccine (Towne CMV vaccine) in parents of children in day care showed that vaccine-induced immunity did not decrease the rate of acquisition of CMV [10]. However, in that trial, the magnitude of the vaccine-induced CMV-specific immune response was 10-fold lower than that afforded by infection. Despite the uncertainty about the ability of immunity to prevent reinfection or to prevent transplacental transmission of CMV, there is evidence that it can prevent most fetal disease [2, 17]. It was estimated that a vaccine that could mimic naturally acquired infection in protecting the fetus could reduce the morbidity from congenital CMV infection by ~80% with no change in the overall rate of congenital CMV infection [2].

The subunit CMV vaccine evaluated in the present study was safe and immunogenic and merits further evaluation; additional phase I and II trials have been undertaken. If the safety and immunogenicity are confirmed in additional trials, it will be necessary to carefully consider how to assess vaccine efficacy. Although this study demonstrates the ability of a subunit vaccine based on a single protein, gB, to elicit an NA response, prevention of infection or disease may well require induction of immunity to other CMV proteins that are targets of NA or of cytotoxic T lymphocytes [18–20]. Although the CMV gB vaccine used in this study elicits serum antibody levels similar to those from infection and produces significant levels of antibody at mucosal surfaces [21], it is quite possible that vaccine-induced immunity will not prevent CMV infection. It may be unrealistic to base efficacy assessment of any CMV vaccine on the ability to prevent maternal infection, given the uncertainty about the ability of naturally acquired immunity to do so. However, it is quite possible that vaccine-induced immunity could lower the rate of fetal infection and the rate of fetal disease without changing the rate of maternal infection. Assessment of the efficacy of CMV vaccines should therefore focus on the ability of the vaccines to prevent fetal infection and disease.
References