Effect of Previous or Simultaneous Immunization with Canarypox Expressing Cytomegalovirus (CMV) Glycoprotein B (gB) on Response to Subunit gB Vaccine plus MF59 in Healthy CMV-Seronegative Adults

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Development of a vaccine for prevention of congenital cytomegalovirus (CMV) disease is a priority. This study evaluated a “prime-boost” strategy by comparing the safety and immunogenicity of 3 doses of subunit CMV glycoprotein B (gB) vaccine plus MF59 (a squalene-in-water emulsion), 2 doses of a canarypox recombinant vaccine expressing CMVgB (ALVAC-CMVgB) followed by 2 doses of the subunit gB vaccine, 3 doses of both vaccines administered concomitantly, and placebo in 105 healthy, CMV-seronegative adults. Systemic adverse events were rare, but local reactions were common in all groups. After the first subunit vaccination, neutralizing antibody titers in the prime-boost group were comparable to those in subjects receiving 2 subunit vaccinations, indicating a priming effect of ALVAC-CMVgB. However, after the final dose, antibody and cell-mediated immune responses were not significantly different among the groups. All 3 vaccine regimens induced high-titer antibody and lymphoproliferative responses, but no benefit for priming or simultaneous vaccination was detected.

Cytomegalovirus (CMV) infection is the most common congenital infection in the United States [1] and is also an important cause of morbidity and occasional mortality in immunocompromised individuals [2, 3]. In view of the limitations of available measures for preventing CMV disease and the relatively severe consequences of infection, an effective vaccine against CMV would be highly desirable. Because CMV glycoprotein B (gB; gpUL55) is the major target for CMV neutralizing antibody [4], it is a prime candidate for a CMV vaccine. This study examined responses to 2 investigational gB-based vaccines, a canarypox vaccine (ALVAC-CMVgB) and a protein subunit vaccine (gB/MF59), which is a recombinant form of gB protein expressed in CHO cells and administered with the adjuvant MF59, a squalene-in-water emulsion (Chiron Vaccines) [5, 6].

In 2 recent clinical trials, the subunit vaccine was found to be safe and induced neutralizing titers similar to those induced by natural infection [5, 6]. The canarypox-vectored gB vaccine is based on an attenuated strain of canarypox virus that has been shown to be safe and immunogenic in humans [7, 8]. An ALVAC vector expressing human CMV gB was shown to be safe and immunogenic in mice and guinea pigs [9] but produced only a weak gB response in humans [10]. This vaccine was, however, able to prime for a booster response after immunization with the live attenuated CMV Towne vaccine [10]. Support for using ALVAC recombinants to prime for subsequent boosting with purified glycoprotein antigens comes from studies of human immunodeficiency virus–based immunogens [11]. These observations of a “prime-boost” effect provided the rationale for the present study.

Subjects and Methods

Vaccines. The subunit CMV gB vaccine is constituted of purified gB (derived from Towne strain CMV [5]) produced in a soluble form by recombinant CHO. The vaccine was formulated with MF59, a proprietary microfluidized oil-in-water emulsion of squalene (Chiron Vaccines). The dose of gB was 20 mg, and the dose of MF59 was 10.75 mg. ALVAC-CMVgB is a recombinant canarypox virus encoding the full-length gB gene of the Towne strain of CMV [9]. Each dose of ALVAC-CMVgB contained 10^8 TCID50 of virus.
after 5 days of incubation and were stored at 2°C.

For cytokine assays, supernatants from the cultures described above were collected after 3 days and those from separate wells were injected into the deltoid muscle.

Subjects. Healthy adult volunteers (18–45 years old) were enrolled if they were CMV antibody negative and healthy, as assessed by medical history, physical examination, and screening laboratory tests. Women were required to use an effective form of birth control and were tested for pregnancy prior to each dose of vaccine.

Vaccination and study design. A total of 105 healthy subjects were randomized to receive 1 of 3 possible regimens: 32 subjects received gB/MF59 at 0, 1, and 6 months (gB/MF59 group); 32 subjects received ALVAC-CMVgB at 0 and 1 month, followed by gB/MF59 at 3 and 6 months (prime-boost group); and 26 subjects received both gB/MF59 and ALVAC-CMVgB in different arms at 0, 1, and 6 months ("both" group). For each group of vaccine recipients, there was a corresponding group of 5 placebo recipients (i.e., a total of 15 placebo recipients).

A nurse who was not involved in any safety assessments administered vaccine or placebo according to the randomization code. Every evening for 7 days after each vaccination, clinical signs and symptoms were recorded by the volunteer on a diary card. The diary card included local reactions (erythema, induration, swelling, tenderness when palpated, pain with movement, and pruritus) and systemic adverse reactions (fever [oral temperatures were taken daily], rash, headache, nausea, vomiting, diarrhea, malaise, and fatigue). Subjects were called twice during that week to evaluate adverse effects. The severity of symptoms was graded (mild, moderate, or severe) by each subject.

Laboratory immunology measurements or assays. The micro-neutralization [12] and ELISA [5, 6] assays were done as described in the just-cited reports. Lymphocyte proliferation and cytokine assays (interferon [IFN]–γ and interleukin [IL]–4) were done using mononuclear cells that were purified by ficoll-hypaque (BioWhitaker) density centrifugation and stored frozen in liquid nitrogen. Cryopreserved peripheral blood mononuclear cells were stimulated with purified gB (Chiron) in quadruplicate at final concentrations of 2 and 0.5 μg/mL. Plates were incubated for 5 or 6 days, and [3H]-thymidine (Amersham) was added to each well for the last 6 h of incubation. A positive control was included in each assay. A stimulation index ≥3 and a difference in experimental counts per minute (counts per minute in the presence of gB antigen – counts per minute in control wells) of 500 were considered to be positive. For cytokine assays, supernatants from the cultures described above were collected after 3 days and those from separate wells after 5 days of incubation and were stored at −70°C. The concentrations of IFN-γ and IL-4 were determined by use of immunoassay kits (Endogen) according to the manufacturer’s instructions.

Statistical analysis and sample size calculations. Analysis of variance was used to compare mean log titers among the 4 groups. Scheffe’s test was then used, as a conservative test for differences between groups, for comparisons of antibody levels. A similar approach was used to compare lymphoproliferative and IFN-γ responses. The incidence of all local and systemic reactions and the incidence of moderate-to-severe reactions were compared by use of Fisher’s exact test.

Results

Safety. Two hundred eighty-three volunteers were screened for the study, and 105 subjects who met entry criteria were enrolled and received a first dose of vaccine or placebo. One hundred four subjects received a second vaccination. Ninety-nine subjects received the third vaccination, and 34 of 35 scheduled subjects received a fourth vaccination. A total of 6 subjects were dropped or terminated from the study. No subject discontinued for reasons related to the vaccines.

Systemic adverse events were rare, and none significantly increased on any day or over the 7 days during which placebo and vaccine recipients were compared. Fever, for example, was not seen in the week after the first vaccination and occurred only in 4 subjects receiving the last vaccine. Symptoms such as head-

Figure 1. Cytomegalovirus (CMV) ELISA and neutralizing antibody levels in subjects receiving CMV vaccine or placebo. Subjects received a subunit glycoprotein B (gB) vaccine with MF59 (gB/MF59), a prime-boost regimen of 2 doses of canarypox vaccine (ALVAC-CMVgB) followed by 2 doses of gB/MF59 (prime-boost), or both gB/MF59 and ALVAC-CMVgB simultaneously (both). A, Geometric mean titers (±SD) of ELISA antibody to CMV gB. B, CMV neutralizing antibody titers (±SD). a, Significantly less than either of the other 2 vaccine groups; b, significantly less than the gB/MF59 group; c, significantly less than the prime-boost group; m, month.
ache, malaise and fatigue were more common than other systemic symptoms in both vaccine and placebo recipients but were not significantly increased in any vaccine group, compared with the placebo group.

Local symptoms, especially pain and tenderness, were common, whereas erythema, swelling, and induration were rare. Pain developed at the local site in 78%—85% of vaccine recipients during the week after the first immunization, compared with 10% of placebo recipients ($P < .001$ for each vaccine group vs. placebo group). Pain lasted 2–3 days. This pain was felt to be moderate to severe in 23%—46% of vaccine recipients versus 5% of placebo recipients ($P < .05$ for each vaccine group compared with the placebo group) and lasted 1–2 days at this intensity. After the last vaccine dose, pain was even more common, occurring in 67%—100% of vaccine recipients, compared with none of the placebo recipients. In a similar manner, moderate-to-severe pain developed in 27%—75% of vaccine recipients. Pain was not more severe in the prime-boost group than in the other vaccine groups.

**Antibody response.** None of the placebo recipients developed CMV antibody at any time. All titers in the immunized groups were significantly greater than those in placebo recipients, except for neutralizing titers at 2 months in the prime-boost group (figure 1). After 2 immunizations, neutralizing and gB ELISA antibodies were induced in all subjects receiving gB/MF59 or both vaccines. In contrast, none of the subjects receiving ALVAC-CMVgB alone developed detectable neutralizing antibody, and only 20 of 32 developed detectable gB ELISA antibody. Antibody titers after ALVAC-CMVgB alone were also significantly lower than titers in subjects receiving gB/MF59 or both vaccines. Of interest, neutralizing titers were significantly lower in the group receiving both vaccines, compared with the group receiving gB/MF59 alone. This may represent a slight inhibitory effect of ALVAC-CMVgB on gB/MF59.

After the first dose of gB/MF59 in the prime-boost group, both ELISA and neutralizing titers increased to levels similar to those found after immunization with 2 doses of gB/MF59. This could represent a priming effect of the 2 ALVAC-CMVgB immunizations, but, since antibody titers were not obtained after only 1 gB/MF59 immunization, no direct comparisons are available.

Antibody titers had decreased at 4 and 6 months in the groups not immunized at 3 months. After the final immunization at 6 months, titers were maximal in all 3 groups, significantly exceeding the previously demonstrated maximum titers. Most important, peak antibody titers were similar among the groups and were highest in the group receiving gB/MF59 alone. Twelve months after immunization, antibody titers had decreased significantly in all groups, compared with the peak titers at 7 months ($P < .001$). Antibody titers, however, remained similar among the groups, except for a significantly lower titer for ELISA gB antibody in the prime-boost group, compared with the gB/MF59 group.

**Cell-mediated immunity.** As shown in table 1, after 2 immunizations (month 2), 68% of gB/MF59 recipients and 79% of recipients of both vaccines developed a positive lymphocyte proliferation response, compared with only 6% of ALVAC-CMVgB recipients ($P < .001$ for each, compared with ALVAC-CMVgB). After 2 booster doses of gB/MF59 (month 7), the number of prime-boost recipients with a positive response increased to 80%, which was similar to the percentage in the other 2 groups. Thus, after all immunizations, 80%—90% of recipients developed a positive lymphocyte proliferation response, with no significant differences between groups. Similarly, no significant differences were found when the magnitude of the response was compared between any 2 vaccine groups, although the large SD would preclude detection of small differences.

In the analysis of cytokine responses, no measurable IL-4 was detected, but IFN-γ was produced after 3 or 5 days of culture. As shown in table 2, after 2 immunizations, 63% of gB/MF59 recipients and 58% of recipients of both vaccines had detectable IFN-γ in supernatants of peripheral blood mononuclear cells incubated with gB for either 3 or 5 days, compared with 25% of prime-boost recipients ($P < .05$). At 7 months,
46%–78% of vaccine recipients produced detectable levels of IFN-γ, with no significant difference between groups.

Discussion

All 3 vaccine regimens studied induced high levels of neutralizing and anti-gB ELISA antibodies, as well as cell-mediated immunity, and all were tolerated fairly well. However, priming with ALVAC-CMVgB (compared with immunization with subunit gB/MF59 vaccine) did not appear to benefit antibody and lymphoproliferative anti-CMV responses. Earlier experiments using the same ALVAC-CMVgB vaccine to prime for immunization with the live attenuated CMV Towne vaccine showed a definite priming effect for antibody responses [12]. The most likely explanation for this discrepancy would appear to be that the combination of gB with the potent adjuvant MF59 can induce higher levels of CMV antibody than can Towne vaccine and thus obscures the clear identification of a prominent priming effect.

There was, however, some evidence that ALVAC-CMVgB did prime for a more rapid antibody response after gB/MF59 immunization. Thus, after the first gB/MF59 vaccination in the group primed with 2 ALVAC-CMVgB immunizations, both neutralizing and ELISA antibody titers were similar to those seen after 2 immunizations with gB/MF59 alone. Because antibody levels were not measured after the first immunization with gB/MF59 alone, it cannot be stated with certainty that priming occurred. However, this would seem to be a reasonable assumption, given the poor antibody response following the first dose of gB/MF59 vaccine reported by Pass et al. and the increased levels seen by them following the second dose [7]. Similar to the findings reported by Adler et al. [10], we found that ALVAC-CMVgB induced only a minimal lymphoproliferative response and did not prime for a booster lymphoproliferative response by gB/MF59. Furthermore, no evidence of priming for the production of IFN-γ by gB-stimulated mononuclear cells was found in the present study. Another interesting observation was that administering the 2 vaccines simultaneously produced some significant reductions in antibody titers, compared with administration of gB/MF59 alone. This may indicate possible interference of ALVAC-CMVgB with the antibody response to gB/MF59, although it is unclear whether such interference would be of any clinical relevance.

The safety of the gB/MF59 vaccine was similar to that reported earlier [5, 6], although the intensity of pain appeared to be somewhat higher. Vaccination produced few systemic symptoms and little swelling or induration but frequently induced pain at the injection site (67%–100% of volunteers). There was, however, no evidence that priming with 2 doses of ALVAC-CMVgB increased the reactogenicity of the first dose or subsequent doses of gB/MF59.

In summary, immunization with gB/MF59 was safe and induced high levels of CMV antibody and cell-mediated immunity, although there was no obvious advantage to priming with ALVAC-CMVgB. ALVAC, however, remains an interesting vaccine strategy because of the vaccine’s ability to induce cytolytic T lymphocytes. Thus, one possible vaccine strategy would be a combination of gB/MF59, to induce neutralizing antibody, and an ALVAC construct expressing CMV pp65 (a UL83 gene product), the dominant cytolytic T lymphocyte target [13, 14]. Because of the potential utility of this strategy, the possible inhibition of ALVAC on the antibody response to gB should be further evaluated. The need for a CMV vaccine remains a high priority. Further evaluation of a subunit gB vaccine, with or without an ALVAC vector expressing pp65, is warranted.

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References