Efficacy of a Recombinant Glycoprotein D Subunit Vaccine on the Development of Primary and Recurrent Ocular Infection with Herpes Simplex Virus Type 1 in Mice

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The protective efficacy of a glycoprotein D subunit vaccine (gD2 SB AS4) was evaluated in a mouse model of human recurrent herpetic stromal keratitis (HSK). When administered before primary infection, gD2 SB AS4 protected mice against corneal pathology, mortality, and latency resulting from ocular viral challenge with herpes simplex virus type 1 (HSV-1) McKrae strain. In addition, gD2 SB AS4 significantly decreased postreactivation corneal disease. A control vaccine, gD2 alum, protected against acute ocular infection only. When administered after primary infection, gD2 SB AS4 vaccination decreased postreactivation ocular shedding but had no other significant effects. Vaccination with gD2 SB AS4 was associated with high anti-gD antibody responses and low delayed-type hypersensitivity responses. These results have identified a prophylactic vaccine, gD2 SB AS4, with activity against acute and recurrent HSK in mice and emphasize the need for vaccine evaluation in both primary and recurrent ocular herpetic disease models.

Herpes simplex virus (HSV) is a leading cause of virus-induced corneal disease in humans. The pathogenesis of the disease is complex and consists of a primary infection of the eye, transport of the virus to the sensory ganglia, where acute and latent infection occurs, and periodic reactivation of latent virus, leading to recurrent corneal disease. Prolonged or repeated inflammatory reactions in the corneal stroma can eventually lead to the development of irreversible tissue damage, scarring, neovascularization, and blindness. Thus, recurrent herpetic stromal keratitis (HSK) has the greatest potential to impair vision. At the present time, there are no prophylactic or therapeutic vaccines that are both safe and effective against recurrent ocular HSV infections in humans.

Glycoprotein D (gD), which is present on HSV envelope and infected cell membranes, is one of the prime targets for a variety of host immunologic responses and is a particularly good candidate for incorporation into herpesvirus vaccines.

Materials and Methods

Virus and Cells

The virus used was HSV-1 McKrae strain. A plaque-purified stock was grown and assayed on Vero cells in MEM with Earle’s balanced salts (MEM-EBS) containing 5% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Material from eye swabs was similarly cultured on Vero cells, which were then monitored for cytopathic effects. Cells were cultured at 37°C in a humidified incubator containing 5% CO2. To titrate the amount of virus present in tear films following acute infection, serial dilutions of eye swab material were plated onto Vero cells, and virus plaques were counted.
Mice

Female NIH inbred mice were obtained from Harlan Olac (Oxford, UK). The eyes of all mice were examined for corneal opacity before infection and irradiation, and only animals with clear corneas were used.

Vaccinations

Vaccines contained a truncated form of gD of HSV-2 (gD2). This protein was produced by recombinant DNA technology and expressed in CHO cells by Genentech (Emeryville, CA) [8]. Production and purification of gD2 were performed at SmithKline Beecham Biologicals (Rixensart, Belgium). The protein was purified to near-homogeneity under nondenaturing conditions from transfected CHO culture supernatants. Vaccines consisted of gD2 combined with aluminum salt (gD2 alum vaccine) or aluminum salt with monophosphoryl lipid A, referred to as SB AS4 adjuvant formulation (gD2 SB AS4 vaccine). Monophosphoryl lipid A was provided by Ribi ImmunoChem Research (Hamilton, MT). Prophylactic and therapeutic vaccines consisted of 5 and 10 μg of gD2, respectively, combined with 100 μg of aluminum salt (alum) or 100 μg of aluminum salt plus 25 μg of monophosphoryl lipid A (SB AS4). Vaccines were administered subcutaneously in two sites in a total volume of 0.2 mL. All vaccinations were repeated 3 weeks later.

Infection

Mice were infected as previously described [5]. Briefly, following corneal scarification, 10^4 or 10^5 pfu of HSV-1 McKrae strain in 5 μL of MEM-EBS was placed onto the eye. Mock-infected mice received virus-free MEM-EBS in the scarified eye. HSV model mice consisted of nonvaccinated mice infected as above with concurrent administration of 1 mL of pooled human serum (Chemicon, Temecula, CA; known to have anti-HSV reactivity with an effective dose for 50% virus neutralization of 1:800). Administration of anti-HSV antibodies at the time of ocular infection has been previously demonstrated to have a protective effect during primary infection, resulting in mice with clear corneas and the ability to undergo recurrent viral shedding (in 70% of latently infected mice) and herpetic ocular disease following UV-B irradiation (250 mJ/cm²) [5]. These antibodies are no longer detectable at the time of UV-B irradiation 5 weeks after primary infection [9].

UV-B Irradiation

The UV-B source used was a TM20 Chromato-Vu transilluminator (UVP, San Gabriel, CA), which emits UV-B at a peak wave length of 302 nm. At least 5 weeks after primary infection, the right eyes of latently infected and control mice were exposed to 250 mJ/cm² UV-B light. Before (day 0) and on days 1–7 after UV-B irradiation, the eyes of mice were swabbed with surgical sponges (WeckCel; Xomed-Treace, Jacksonville, FL) saturated with tissue culture medium. The swab material was cultured on Vero cells, as described above, to detect recurrent viral shedding from the cornea.

Clinical Observation

Eyes were observed through a binocular dissecting microscope by an observer with no knowledge of treatment groups. Stromal opacification was rated on a scale of 0–4, where 0 indicates clear stroma, 1 indicates mild stromal opacification, 2 indicates moderate opacity with discernible iris features, 3 indicates dense opacity with loss of defined iris detail except pupil margins, and 4 indicates total opacity with no posterior view.

Antibody Assays

ELISA procedure. Serial 3-fold dilutions of mice sera were incubated on ELISA plates coated with purified recombinant gD2 protein (1 μg/mL). The presence of specific antibodies was revealed by addition of anti-mouse immunoglobulin conjugated to biotin, followed by streptavidin-biotinylated peroxidase complex. ELISA titers were defined as the reciprocal of the serum dilution that produced an absorbance (optical density) measured at 492 nm equal to 50% of the maximal absorbance value (midpoint titer).

Neutralization procedure. Two-fold dilutions of mice sera were incubated on 96-well plates with 4000 pfu of HSV-2 strain HG52 and complement. BHK21 cells were then added to the wells. The plates were incubated for 3 days, and the appearance of virus plaques was monitored by microscopic examination. The neutralizing titer was defined as the reciprocal of the serum dilution giving 100% protection against virus-induced cytopathic effects.

Delayed-Type Hypersensitivity (DTH) Assays

In this procedure, 5 × 10⁵ pfu (before inactivation) of UV-inactivated HSV-1 McKrae strain in 30 μL of medium was injected into the right rear footpad of mice. The left rear footpad was injected with 30 μL of virus-free tissue culture medium. Footpad thickness was determined with a micrometer (Mitutoyo Manufacturing, Tokyo) immediately prior to and 48 h after injection. HSV-specific footpad swelling was determined by the formula (right footpad swelling at 48 hours – right footpad swelling before injection) – (left footpad swelling at 48 hours after injection – left footpad swelling before injection). Mice used for DTH assays were excluded from any further studies.

Acute Trigeminal Ganglia Virus Titers and Latency

To determine the titer of virus present in trigeminal ganglia 5 days after acute infection with 10⁶ pfu of HSV-1 McKrae, ganglia were harvested, disrupted, and plated onto Vero monolayers. Resulting virus plaques were quantitated by an observer with no knowledge of treatment groups. For determination of latency, the relevant trigeminal ganglion was removed from each mouse 3 weeks after UV-B exposure (8 weeks after primary infection). This interval provides adequate time for cessation of active viral replication and return to the latent state. Individual trigeminal ganglia were minced and incubated on Vero cell monolayers for 3 weeks with three media changes per week. Latency was confirmed by the appearance of typical virus plaques.
Table 1. No. of mice in prophylactic and therapeutic vaccine experiments.

<table>
<thead>
<tr>
<th>Vaccine or control</th>
<th>Prophylactic</th>
<th>Therapeutic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10⁵</td>
</tr>
<tr>
<td>gD2 alum</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>gD2 SB AS4</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Alum</td>
<td>—</td>
<td>50</td>
</tr>
<tr>
<td>SB AS4</td>
<td>40</td>
<td>90</td>
</tr>
<tr>
<td>HSV model</td>
<td>—</td>
<td>30</td>
</tr>
<tr>
<td>Nonvaccinated</td>
<td>—</td>
<td>170</td>
</tr>
<tr>
<td>PBS</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

NOTE. Data are total no. of vaccinated or control mice before infection with 0, 10⁵, or 10⁶ pfu of HSV-1 McKrae in prophylactic experiments and total no. of latent (10⁶)– or mock (0)–infected mice before vaccination or control treatment in therapeutic experiments.

Experimental Protocols

**Prophylactic vaccination.** At 3 weeks of age, groups of mice (table 1) were vaccinated with gD2 alum, gD2 SB AS4, SB AS4, and alum. Two weeks after the last vaccination (8 weeks of age), mice were infected as described. Age-matched nonvaccinated mice were similarly infected with (HSV model) or without (nonvaccinated control) concurrent administration of protective human serum. Five weeks after primary infection, the eyes of latently infected and control mice were UV-B–irradiated. Post–UV-B eye swabs confirmed recurrent viral shedding. Clinical observation of the eyes of mice was performed on days 0, 3, 7, 14, and 35 relative to infection and on days 0, 7, 14, and 21 relative to UV-B irradiation. Antibody and DTH responses were measured before vaccination and 2 weeks after vaccination, infection, and UV-B exposure for random samples of mice in each treatment group.

**Therapeutic vaccination.** Mice were infected at 4 weeks of age with concurrent administration of protective human serum (HSV model). Five weeks after primary infection, latently infected mice were vaccinated with gD2 SB AS4, SB AS4 alone, or PBS. Mock-infected mice received gD2 SB AS4 or PBS (table 1). Two weeks after the last vaccination, the inoculated eyes of latently infected and control mice were UV-B–irradiated, and recurrent shedding was confirmed from positive eye swabs. Clinical observation of the eyes of mice was performed on days 0, 7, 14, and 21 after UV-B exposure.

Statistical Methods

Opacity scores were analyzed by using Student’s t test (Blossom, release 2.01; Lotus, Bethesda, MD). Mortality, reactivation, and latency data were examined by using Fisher’s exact test (SAS Institute, Cary, NC). Comparisons within DTH, antibody, and virus titer data sets were performed with the aid of the Wilcoxon rank sum test (as implemented in NPAR1WAY Procedure of SAS).

Results

**Effects of Prophylactic gD2 Vaccination on Acute Corneal Disease and Death**

Corneal pathology and mortality were examined in groups of vaccinated and nonvaccinated mice following acute corneal infection with 10⁵ or 10⁶ pfu of HSV-1 McKrae. Data from the 10⁵-pfu infection are shown in figure 1. Corneal opacity observed in gD2 alum, gD2 SB AS4, and HSV model mice was mild and transient compared with the severe disease displayed by nonvaccinated and adjuvant-vaccinated controls (P < .01) at 7, 14, and 35 days after infection. Opacity in the alum control group was reduced (P < .05) compared with that in nonvaccinated mice on days 3, 7, and 14 after infection for 10⁵-pfu but not 10⁶-pfu infections.

Figure 2 presents mortality data following acute infection. Compared with adjuvant controls and nonvaccinated mice, gD2 vaccinees were completely protected against death at 10⁵ and 10⁶ pfu, while only 1 fatality was observed in the HSV model group at 10⁵ pfu (P = .015 to P < .001). Fewer deaths (P < .01) occurred in adjuvant groups at 10⁵ but not 10⁶ compared with nonvaccinated mice. Thus, while adjuvant effects were observed for opacity and mortality data at 10⁵ pfu, the effect was lost at higher infection levels, and the protective effects of gD2 vaccines were always much greater.

**Prophylactic Vaccination and Virus Titters in Tear Films and Trigeminal Ganglia following Acute Infection**

Following infection of the eye with 10⁶ pfu of HSV-1, the virus content of tear films was quantitated. Table 2 presents data obtained from swabs taken 1, 3, and 5 days after infection.

![Figure 1](image-url)
While both gD2 vaccine groups displayed virus titers that were strikingly lower than that seen in control groups on day 1 (P < .001), the gD2 SB AS4 group demonstrated significantly lower titers than did the gD2 alum group (P = .0016). In addition, SB AS4 (P < .001) and HSV model (P = .004) groups had significantly lower titers than did nonvaccinated animals.

Previous studies indicated that maximal virus titers in trigeminal ganglia occurred at 4–5 days after acute corneal infection in nonvaccinated mice [10, 11]. Therefore, 5 days after acute infection, mice were sacrificed, and the presence of virus in the trigeminal ganglia was assessed (table 2). Ganglia from HSV model and gD2 alum– and gD2 SB AS4–vaccinated mice all contained significantly less (P < .001) active virus than did ganglia from adjuvant and nonvaccinated groups, with >120-fold reductions in virus titer for these groups.

Effects of Prophylactic gD2 SB AS4 Vaccination on Latency and Recurrent Ocular HSV-1 Infection

Effects on latency. Figure 3 presents latency data for each vaccination or control group. The latency rate for 10^5- and 10^6-pfu infections in the gD2 SB AS4 group (30% and 26%) was significantly lower than in the gD2 alum (60% and 65%), the HSV model (71% and 78%), and the control groups (65%–83%) (P = .025 to P < .001). No other differences were found.

Effects on reactivation and postreactivation disease. Reactivation was defined as the finding of any HSV-positive eye swab from latently infected mice during days 1–7 after exposure to 250 mJ/cm^2 UV-B irradiation, with day 0 swabs serving as a control. Although lower reactivation rates relative to the HSV model were noted in all groups at 10^5 and 10^6 pfu, a significant decrease (P = .05 to .004) was observed only in mice in the gD2 SB AS4, adjuvant, and nonvaccinated groups (figure 4). Reactivation rates of gD2 SB AS4 and nonvaccinated mice were less (P = .015 and .041) than that of gD2 alum mice at the 10^6-pfu infection. There was no difference between treatment groups in the number of days virus was shed after reactivation (data not shown).

Table 3 presents corneal opacity data for reactivated (virus-shedding) mice in each combined 10^5/10^6 pfu treatment group following UV-B exposure. To control for UV-B effects, corneal opacity of each group was compared with that of mock-infected animals that experienced the same UV-B exposure. Corneal opacity above this background level is considered to be virus-induced. Both vaccinated and nonvaccinated mock-infected groups were included. Opacity scores for reactivating mice in alum-vaccinated and nonvaccinated groups have been combined (3 mice total). Notably, opacity scores for gD2 SB AS4–vaccinated mice were statistically equal to both UV control groups on all days after reactivation, indicating an absence of virus-induced pathology, and significantly less than that for gD2 alum (P < .01), nonvaccinated/alum-vaccinated (P < .01), and HSV model mice (P < .05) on days 14 and 21. Corneal opacity in gD2 alum, HSV model, and nonvaccinated/alum vaccinated groups exceeded that of either or both UV

Table 2. Effect of prophylactic vaccination on virus titers in tear films and trigeminal ganglia following primary infection.

<table>
<thead>
<tr>
<th>Site, group (n)</th>
<th>Days after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Eye</strong></td>
<td></td>
</tr>
<tr>
<td>gD2 alum (13)</td>
<td>13.8 ± 4.1*</td>
</tr>
<tr>
<td>gD2 SB AS4 (14)</td>
<td>4.1 ± 5.1*</td>
</tr>
<tr>
<td>SB AS4 (14)</td>
<td>133.9 ± 34.6</td>
</tr>
<tr>
<td>Nonvaccinated (16)</td>
<td>476.0 ± 35.1</td>
</tr>
<tr>
<td>HSV model (15)</td>
<td>259.7 ± 42.1</td>
</tr>
<tr>
<td><strong>Trigeminal ganglia</strong></td>
<td></td>
</tr>
<tr>
<td>gD2 alum (13)</td>
<td>—</td>
</tr>
<tr>
<td>gD2 SB AS4 (14)</td>
<td>—</td>
</tr>
<tr>
<td>SB AS4 (14)</td>
<td>—</td>
</tr>
<tr>
<td>Nonvaccinated (16)</td>
<td>—</td>
</tr>
<tr>
<td>HSV model (15)</td>
<td>—</td>
</tr>
</tbody>
</table>

* P < .001, compared with SB AS4, nonvaccinated, and HSV model groups for day 1.
1 P = .0016, compared with gD2 alum for day 1.
2 P < .001, compared with gD2 SB AS4 and gD2 alum on day 5.
3 P < .001 and P = .0041, compared with SB AS4 and HSV model groups for day 1.
4 P = .011, 019, and .027 compared with nonvaccinated, gD2 SB AS4, SB AS4, and gD2 alum on day 3.
5 P < .001, compared with SB AS4 and nonvaccinated groups.
control groups on all days after irradiation (table 3). The latter findings demonstrate the presence of virus-induced corneal pathology in these groups. Opacity scores in gD2 alum, HSV model, and nonvaccinated/alum-vaccinated groups were equivalent on all days.

Previous work in our laboratory has shown that non-virus-shedding mice may nevertheless harbor reactivated HSV in swab-inaccessible sites, such as deep stromal layers of the cornea. Therefore, latently infected mice that do not shed virus in their tear film after UV-B irradiation cannot be considered reactivation-negative. The data for ‘nonreactivated’ mice are not presented.

DTH and Antibody Responses in Prophylactically Vaccinated Mice

Table 4 depicts DTH responses in samples of vaccinated and nonvaccinated mice 2 weeks following ocular infection with HSV-1. DTH responses in adjuvant controls, nonvaccinated mice, and HSV model mice were 4- to 30-fold greater (P = .001 to .015) than those of gD2-vaccinated animals after infection with 10^6 pfu of HSV-1. No significant differences in DTH responses in vaccinated, uninfected mice were demonstrable. Post–UV-B results were similar, with no differences between reactivated and nonreactivated mice.

Table 4 also shows postinfection anti-gD2 antibody titers as determined by ELISA for mice in the study. Mice prophylactically vaccinated with gD vaccines produced high anti-gD2 ELISA titers that were much greater (up to 600-fold) than those in controls (P < .001 to .0012). gD2 SB AS4 vaccination elicited greater (P = .0021 for mock, P < .001 for 10^6 pfu) anti-gD antibody levels than did gD2 alum vaccination. After infection, control groups experienced an increase in antibody titer, while titers of gD2 vaccinees remained stable, indicating that maximal antibody response was elicited by gD2 vaccina-

Table 3. Effect of prophylactic vaccination on corneal opacity in recurrent herpetic stromal keratitis.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Days after reactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>gD2 alum (12)</td>
<td>3.25 ± 0.37*</td>
</tr>
<tr>
<td>gD2 SB AS4 (5)</td>
<td>2.40 ± 0.36</td>
</tr>
<tr>
<td>Nonvaccinated/alum (3)</td>
<td>3.00 ± 0*</td>
</tr>
<tr>
<td>HSV model (18)</td>
<td>2.83 ± 0.13*</td>
</tr>
<tr>
<td>Vaccinated/mock (54)</td>
<td>2.57 ± 0.13</td>
</tr>
<tr>
<td>Nonvaccinated/mock (15)</td>
<td>1.80 ± 0.17</td>
</tr>
</tbody>
</table>

NOTE. Data are mean opacity scores ± SE.

* P < .01, compared with nonvaccinated/mock-infected controls.
* P < .05, compared with vaccinated/mock-infected controls.
SB AS4, and DTH responses were of the same magnitude as SB AS4 vaccination, anti-gD antibody responses were comparable indicating a lack of virus-induced pathology. Because transient shedding was observed in gD2 SB AS4 vaccinees (gD2 SB AS4 vaccination decreased HSV induction), this effect was elicited in mice that survive acute infection with the highly neurovirulent McKrae strain of HSV-1.

Interestingly, prophylactic gD2 SB AS4 vaccination was found to decrease HSV-induced recurrent corneal disease (table 3), while gD2 alum vaccination did not. Thus, in contrast to what was seen in gD2 alum, HSV model, and nonvaccinated/alum groups, stromal opacification in gD2 SB AS4 vaccinees was equivalent to that of both UV-B controls on all days, and PBS-treated mice.

### Discussion

The vaccine tested in our studies consisted of gD2 combined with the adjuvant SB AS4, which contains alum and a nontoxic derivative of lipid A [4]. gD was selected because it is a target for the large majority of HSV-1– and 2–neutralizing antibodies and of cell-mediated immunity in humans [12, 13]. gD1 and gD2 have been shown to elicit cross-protective immune responses [14, 15]. While alum is known for its ability to induce humoral immune responses, SB AS4 has been shown to induce high neutralizing antibody and specific T-cell–mediated immune responses [1–4]. In contrast to many of the adjuvants used in animal studies of gD vaccines, SB AS4 has been demonstrated to be safe for human use [3, 4, 16, 17].

As demonstrated here, both gD2 SB AS4 and gD2 alum vaccine preparations had positive effects on the acute disease process. Both vaccines protected mice from acute corneal disease (figure 1) and death (figure 2) following ocular infection with HSV-1. In addition, gD2 SB AS4 vaccination significantly reduced the establishment of latency (figure 3). Findings such as these have been reported by other researchers using gD vaccines in murine and rabbit models of primary ocular HSV infection [17–22]. One study, using HSV-1 (McKrae strain) as the challenge virus, found that prophylactic vaccination with gD1 in complete Freund’s adjuvant, a potent and potentially toxic preparation, resulted in acute disease and latency protection levels in mice that were very similar to those following vaccination with gD2 SB AS4 [17].

Because in humans with ocular HSV infections, permanent corneal scarring and blindness is most often associated with virus recrudescence, the most important findings of our studies pertain to the effects of gD2 SB AS4 vaccination on recurrent ocular HSV shedding and HSK. As figure 4 indicates, gD2 SB AS4 vaccination decreased the number of mice with UV-B–induced recurrent HSV shedding compared with gD2 alum–treated and HSV model mice. This effect may be explained, however, by low latency rates in this group (figure 3). Low reactivation rates also occurred in adjuvant and nonvaccinated mouse groups (despite high latency levels), possibly due to corneal nerve damage [23] or protective immune responses elicited in mice that survive acute infection with the highly neurovirulent McKrae strain of HSV-1.

### Table 4. Antibody and delayed-type hypersensitivity responses in prophylactically vaccinated mice 2 weeks after ocular infection with HSV-1.

<table>
<thead>
<tr>
<th>Inoculum (pfu/eye)</th>
<th>Response, group (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Antibody</td>
<td>gD2 alum (15)</td>
</tr>
<tr>
<td></td>
<td>gD2 SB AS4 (15)</td>
</tr>
<tr>
<td></td>
<td>SB AS4 (15)</td>
</tr>
<tr>
<td></td>
<td>Nonvaccinated (5)</td>
</tr>
<tr>
<td></td>
<td>HSV model (5)</td>
</tr>
<tr>
<td>Delayed-type hypersensitivity</td>
<td>gD2 alum (9)</td>
</tr>
<tr>
<td></td>
<td>gD2 SB AS4 (9)</td>
</tr>
<tr>
<td></td>
<td>SB AS4 (9)</td>
</tr>
<tr>
<td></td>
<td>Nonvaccinated (5)</td>
</tr>
<tr>
<td></td>
<td>HSV model (5)</td>
</tr>
</tbody>
</table>

NOTE. Data are geometric mean midpoint antibody titers or footpad swelling in mm (×10^-2) ± SE.

* P < .001 to .0012, compared with SB AS4, nonvaccinated, and HSV model groups for 0 and 10^6 pfu.

† P = .0021, compared with gD2 alum, 0 pfu.

‡ P < .001, compared with gD2 alum, 10^6 pfu.

§ P = .001 to .015, compared with SB AS4, nonvaccinated, and HSV model groups for 10^6 pfu.

Significantly higher neutralizing antibody levels were also detected in gD2 SB AS4 vaccinees compared with gD2 alum vaccinees (gD2 SB AS4 = 837.83 ± 371 vs. gD2 alum = 459.48 ± 197; P = .025). No differences were seen between reactivated and nonreactivated mice (data not shown).

### HSV-1—Induced Recurrent Disease after Therapeutic Vaccination with gD2 SB AS4

Because of its positive effects on recurrent HSK when administered prophylactically, vaccination with gD2 SB AS4 after primary ocular infection with HSV-1 was investigated. Accordingly, 2 weeks after vaccination of latently infected mice with gD2 SB AS4, SB AS4 alone, or PBS, eyes were UV-B–irradiated and monitored for recurrent viral shedding and corneal disease. Although there was a tendency for gD2 SB AS4 to decrease the reactivation frequency, this effect was not significant. In addition, gD2 SB AS4 vaccination had no effect on recurrent disease (data not shown). A significant decrease in the average number of days of post–UV-B viral shedding was observed in gD2 SB AS4 vaccinees (P = .0105 and .0024) compared with adjuvant- and PBS-treated mice (gD2 SB AS4 = 1.58 ± 0.17 days, SB AS4 = 2.63 ± 0.27 days, and PBS = 2.81 ± 0.28 days). After therapeutic gD2 SB AS4 vaccination, anti-gD antibody responses were comparable to those arising from prophylactic vaccination with gD2 SB AS4, and DTH responses were of the same magnitude as those of HSV model mice of the prophylactic study (data not shown).
tions), a significant difference in effects between the gD2 SB AS4 group and gD2 alum, HSV model, and nonvaccinated/ alum groups occurred only on days 14 and 21.

While only 5 mice in the gD2 SB AS4 group shed virus after UV-B irradiation, several factors suggest that the protective effect of this vaccine on recurrent HSV-induced corneal opacity is real. First, corneal opacity scores for gD2 SB AS4 vaccinated were equivalent to those of two different mock-infected, UV-B control groups on all days after irradiation. Second, like UV-B control groups, in the gD2 SB AS4 group, >70% of the opacity scores were ≤2 on days 14 and 21. Third, the variance of opacity scores in the gD2 SB AS4 group as measured by standard deviation was small on all days. Fourth, corneal opacity in at least 2 separate groups of mice (gD2 alum and HSV model) exhibiting virus-related corneal pathology was significantly greater than that of gD2 SB AS4 vaccinees on days 14 and 21.

This may be the first report of a recombinant gD2 vaccine with efficacy against recurrent HSK when administered before primary infection. Nonetheless, in the well-studied guinea pig model of vaginal HSV-2 infection, prophylactic and therapeutic gD vaccination has been shown to decrease both the severity and frequency of recurrent disease [8, 24–28]. Also in the HSV-2 genital disease model, prophylactically administered gD2 SB AS4 provided significant protection against recurrent genital disease compared with gD alum [2, 29].

In this report, we found that systemic vaccination of latently infected mice with gD2 SB AS4 decreased the number of days on which virus was shed from the cornea after UV-B irradiation but had no effect on recurrent HSK. This is in agreement with findings in a rabbit model of spontaneous ocular HSV recurrence, in which therapeutic periocular vaccination of latently infected animals with gB2/gD2 proteins and adjuvant resulted in decreased viral shedding and little effect on corneal scarring [30]. In contrast, periocular vaccination with gD1 or gD2 in this same system decreased the number of recurrent dendritic lesion readings [31]. There may be several explanations as to why therapeutic gD2 SB AS4 vaccination was not clinically effective. Factors shown to influence the outcome of vaccine therapy include the immunogen, dose, route of administration, timing of treatment relative to primary infection, adjuvant formulation, and animal model [32].

It is possible to attribute all of the effects of prophylactic gD2 SB AS4 vaccination to the presence of high antibody levels at the time of acute infection. In this regard, it is known that during primary ocular infection with HSV, antibodies can preserve corneal clarity and innervation while permitting the establishment of latency [5, 9, 21, 33, 34]. Thus, by enhancing virus clearance (table 2) from the cornea and restricting virus spread within the nervous system, antibodies induced by gD2 SB AS4 vaccination would serve to maintain the reactivation pathway after ocular challenge with HSV. Higher antibody levels or differences in antibody activity in gD2 SB AS4 vaccinees could account for a decrease in the number of mice with latent infections and the latent virus load in individual ganglia compared with gD2 alum—vaccinated and HSV model mice. After UV-B stimulation, lowered latency levels could in turn produce observed decreases in reactivation rate and postreactivation disease.

Although not demonstrated by our studies, gD2 SB AS4 and monophosphoryl lipid A vaccine has been associated with protective cell-mediated immune responses in other systems [1–4, 29]. It is possible that as-yet-uncharacterized cell- mediated immune responses were elicited by vaccination with gD2 SB AS4 in our studies and contributed to lower latency rates, reactivation rates, and postreactivation disease in this group. Relevant to this, mice vaccinated with gD and complete Freund’s adjuvant before primary ocular HSV infection were partially protected from the establishment of latency in association with significant antibody and NK cell activity [20].

Following therapeutic vaccination with gD2 SB AS4, latently infected mice exhibited high antibody and high DTH responses (data not shown) but were not protected from recurrent disease. As in other systems, antibody levels in animals with established latent ocular infections do not correlate with protection from recurrent shedding [30]. Therefore, failure of therapeutic vaccination with gD2 SB AS4 may be related to the inability of humoral immune responses to modulate recurrent corneal disease once infection has been established. In addition, high DTH responses present in these animals may have exacerbated corneal disease.

Three results from our work suggest that primary ocular HSV infection models are not sufficient for determining vaccine efficacy against recurrent HSK. First, prophylactic gD2 alum vaccination clearly protected against primary ocular infection with HSV-1 but had no effect on recurrent disease. Second, nonvaccinated and adjuvant-vaccinated control mice had high latency levels yet did not reactivate, indicating that latency is not necessarily predictive of true reactivation frequency and postreactivation disease. Third, gD2 SB AS4 vaccination had positive effects on recurrent disease when given before primary infection but not when given after. Together, these data emphasize the need for vaccine evaluation in both acute and recurrent models of HSK.

Acknowledgment

We thank Mae Gordon and staff for their valuable assistance with statistical analysis.

References


