Immunotherapy of Recurrent Genital Herpes with Recombinant Herpes Simplex Virus Type 2 Glycoproteins D and B: Results of a Placebo-Controlled Vaccine Trial

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To determine the safety, immunogenicity, and efficacy of a recombinant herpes simplex virus type 2 glycoprotein D and B vaccine in the treatment of recurrent genital herpes, a randomized, placebo-controlled trial was held at two referral centers. Healthy patients with 4–14 recurrences per year received injections of both glycoproteins in MF59 adjuvant or of MF59 alone at 0, 2, 12, and 14 months. For 18 study months, the rate and number of recurrences, the duration and severity of the first confirmed recurrence, vaccine immunogenicity, and rates of local and systemic reactions were determined. The monthly rate of recurrences was not significantly improved, but the duration and severity of the first study outbreak was reduced significantly by vaccination. Glycoprotein-specific and neutralizing antibodies were boosted by vaccination for the duration of the study. This vaccine is safe and immunogenic and ameliorated an observed first postvaccination genital recurrence, but it does not reduce recurrence frequency.

Genital herpes is a prevalent sexually transmitted disease that causes acute and recurrent symptoms and lesions [1, 2]. Symptomatic recurrences can be ameliorated and suppressed by episodic or maintained use of available nucleoside analogues, such as acyclovir, valaciclovir, and famciclovir [3–7]. Nonetheless, development of safe and effective prophylactic and immunotherapeutic vaccines would constitute a substantial public health advance [8].

In recent years, efforts at creating vaccines for genital herpes have concentrated on the administration to experimental animals and human subjects of recombinant herpes simplex virus type 2 (HSV-2) envelope glycoproteins D and B (gD2, gB2), which are known to elicit potent humoral and cellular immune responses [9–20]. These preliminary studies documented that such vaccines are useful candidates for fuller exploration in large-scale, controlled trials. The present report summarizes the results of a placebo-controlled trial of recombinant gD2 and gB2 in MF59, a novel oil-in-water adjuvant, for the treatment of established genital herpes.

Materials and Methods

Vaccine. The composition of the vaccine and the preparation of its components were described previously [20–23]. Briefly, the genes encoding gD2 and gB2 were cloned, truncated to remove their carboxy-terminal coding sequences, and stably expressed in Chinese hamster ovary cells. The proteins were secreted from the cells, purified from culture media under nondenaturing conditions, and formulated for injection in MF59 adjuvant emulsion, which contained squalene, polysorbate 80, and sorbitan trioleate. The placebo vaccine contained MF59 alone in citrate buffer.

Study design. This was a randomized double-blind, placebo-controlled trial conducted in two centers, the University of Washington (Seattle) and the National Institutes of Health Clinical Center (Bethesda, MD). Randomization was stratified by center and by sex. Participants were randomly assigned to receive deltoid injections of MF59 placebo alone or of 10 μg each of gD2 and gB2 (gD2/gB2) in MF59 at entry and again at months 2, 12, and 14. One group, termed the placebo-first or ''placebo'' group, received MF59 alone at months 0 and 2 but were crossed over to receive gD2/gB2 in MF59 at months 12 and 14. The other group of patients, the vaccine-first or ''vaccine'' group, received gD2/gB2 in MF59 at months 0, 2, and 12 and MF59 alone at month 14. Thus, all subjects knew they were to receive at least two doses of gD2/gB2, including one at month 12. This design was thought to permit subject retention through 18 months of study to allow accrual of safety data on repeated vaccination. The truly blinded phase of the study ended at month 12.

Patients were scheduled for at least 17 study visits each, including initial screening, vaccination, assessment of adverse reactions 7 days after each vaccination, and collection of serum for antibody determinations at months 0, 1, 3, 6, 8, 9, 12, 13, 15, and 18. Unscheduled visits were made to assess signs and symptoms of outbreaks and to recover virus from lesion swabs. Long-term suppressive use of antiviral drugs (primarily acyclovir, since famciclovir and valacyclovir were licensed only late in this study) was prohibited starting 3 months before the first immunization and for the entire duration of the 18-month trial. Episodic oral antiviral drug treatment was prohibited starting 2 weeks before the first

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immunization, but acyclovir was allowed (200-mg capsules, five times daily for 5 days) for clinically documented recurrences beginning with the fourth recurrence following the second vaccination until month 12 and again upon the fourth occurrence following the month 14 immunization.

Genital herpes recurrences were assessed by uniformly applied criteria [19]. A recurrence could be confirmed either or both by isolation of virus from lesions (culture-positive episode) and by examination in the clinic if one or more papular, vesicular, ulcerative, crusted, or healing lesions in a typical distribution were observed (clinically confirmed recurrence). Lesional outbreaks not observed in clinic and recorded on patient diary cards were analyzed as subject-reported recurrences. All recurrences were to be evaluated in clinic within 48 h of onset, and patient records ascertained the date of healing. The first postvaccination recurrence in each study year, however, was to be evaluated intensively. Within 48 h of onset, the patient would present to clinic, whereupon a history of the episode was recorded, lesions were located, their stage and size were determined, and a culture was taken. The process was repeated on days 2, 3, 4, 5 or 6, 7 or 8, 9 or 10, 11 or 12, 13 or 14 or 15, as required until healed.

Entry criteria and exclusion. Otherwise healthy men and women aged 18–55 years were enrolled if they had genital HSV-2 infections for at least 1 year, as documented by medical records, and a positive Western blot test for HSV-2–specific antibodies. Enrollment criteria included a patient history of 4–14 outbreaks per year in a year before enrollment when not taking suppressive acyclovir. Women of childbearing potential agreed to practice effective contraception for the duration of the study and were required to have negative pregnancy tests before vaccinations. Subjects could not have received other HSV vaccines; have a history of anaphylaxis, serious vaccine reactions, or HSV-associated erythema multiforme; or have medical, social, or psychiatric problems that would impair vaccine assessment or study participation.

Adverse reactions. Specific symptoms arising within 7 days of vaccination and all medical problems occurring after enrollment were sought and recorded. Mild adverse reactions were defined as those that were transient, did not limit activity, and required no treatment. Moderate reactions affected daily activity and may have necessitated treatment. Severe reactions prevented usual activities and required medical intervention.

Immunologic studies. Western blotting for documentation of HSV-2–specific antibodies, quantitation of gB2-specific and gD2-specific antibodies by ELISA, and determination of neutralizing antibody titers were done as described previously [20, 24, 25].

Statistical assumptions and analyses. The primary study end point was prospectively defined to be the monthly rate of recurrences between study months 0 and 8. A sample size of 100 subjects per study arm was estimated to be necessary to show a 40% reduction in monthly recurrence rate, assuming 0.5 outbreaks per month for placebo recipients, 90% power, and a 15% dropout rate. Secondary efficacy variables included the duration of viral shedding, symptoms, and healing events in the first documented study outbreak and the time to first recurrence. The data were analyzed according to the principle of intent-to-treat.

Differences in monthly recurrence rate between patient groups were compared by analysis of variance using a linear model with effects for treatment, center, sex, and associated interactions. The time to first recurrence was assessed by Kaplan-Meier time-to-event analyses, and treatment differences were assessed by log-rank tests. Differences in antibody titers before and after immunization were assessed by analyses of variance applied to the logarithm of titers. Rates of adverse reactions were compared by χ^2 or Fisher's tests, as appropriate.

Table 1. Demographics of patients randomized to receive herpes simplex virus (HSV) type 2 glycoprotein B– and D–containing or MF59 placebo vaccines.

	MF59 placebo recipients	gB2/gD2/MF59 recipients
No. of subjects	100	102
University of Washington	50	53
National Institutes of Health	50	49
Median age, years (range)	34 (21–55)	38 (21–54)
No. male/female	45/55	47/55
% white/African American/Hispanic	86/7/4	90/6/3
Median years of genital herpes (range)	6.4 (1.1–26.6)	6.1 (1.2–27.5)
Median no. of recurrences in prior year (range)	7.0 (4.0–14.0)	8.0 (4.0–14.0)
% HSV-1–seropositive	39	41
Prior acyclovir use (%)		
Episodic	68	62
Suppressive	29	27
Median days since last acyclovir use	230	130

Results

One hundred ten women and 92 men were enrolled in the study. The placebo-first group and the gD2/gB2-containing vaccine–first group were similar in all key demographic, clinical, and serologic characteristics (table 1). Ten patients in Seattle and 13 in Bethesda terminated the study prematurely, for a total of 12 in the placebo arm and 11 in the gD2/gB2 arm. Four members of each study arm withdrew because of adverse reactions. Among the placebo recipients, 7 others withdrew to resume acyclovir or because they moved from the study area; 8 gD2/gB2 recipients withdrew for one of these reasons.

Adverse reactions. The vaccinations were generally well-tolerated, but most subjects experienced transient mild or moderate local or systemic reactions following each dose. Rates of injection site pain, erythema, induration, maximum oral temperature, malaise, myalgias, and headache were statistically higher ($P < .01$ for each) after one or more injections of gD2/gB2 in MF59 than after MF59 alone (figure 1). There were trends suggesting increasing rates of local adverse reactions with repeated antigen administration.

Recurrence rate and number. The primary efficacy end point of the study, the monthly recurrence rate for the first 8 months, was similar in the 2 study groups. Specifically, the monthly rates of culture-positive, clinically confirmed, or any recorded lesional outbreaks were only 10%–19% lower for

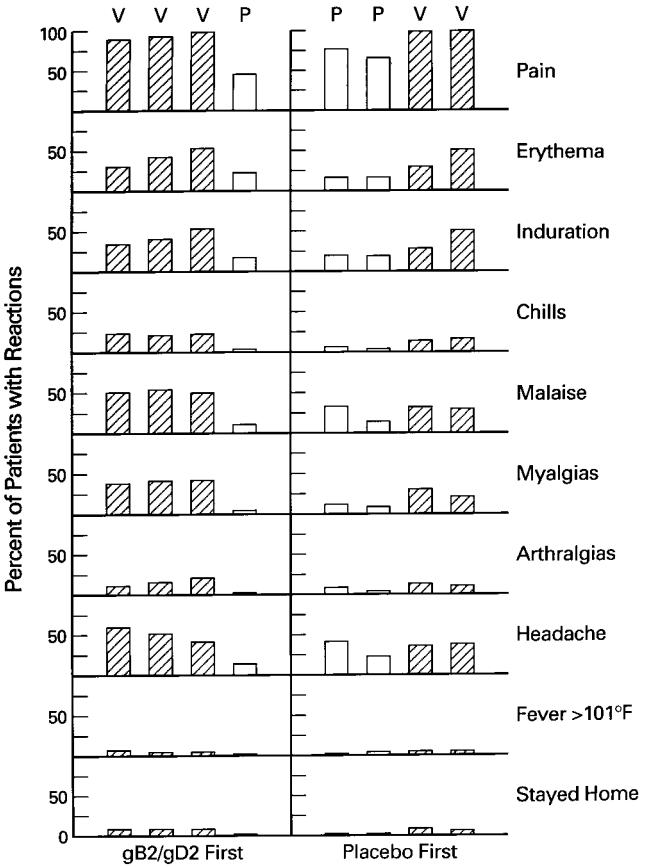

Figure 1. Percentages of patients recording local or systemic reactions to each sequential dose of MF59 (open bars) or herpes simplex virus glycoproteins gD2/gB2 with MF59 (hatched bars). Those initially assigned to receive gD2/gB2/MF59 received 3 doses of vaccine (V) followed by 1 dose of MF59 placebo (P). MF59 placebo-first recipients received 2 doses of placebo (P) followed by 2 containing glycoproteins (V).

gD2/gB2 recipients than for placebo recipients, all statistically insignificant differences (table 2). In the first 8 months of the study, there were 464 lesional recurrences reported by placebo recipients, of which 372 were clinically confirmed; 214 of these were culture-positive. Among gD2/gB2 recipients, there were 418 reported recurrences (10% fewer); 342 were clinically confirmed recurrences (8% fewer), and 175 of these were culture-positive (18% fewer). None of these differences was statistically significant. For the entire first year, the gD2/gB2 recipients had 5%–15% fewer recurrences than MF59 recipients—again, statistically insignificant reductions. In the absence of substantial differences in the rate of recurrences in the blinded study phase, it was not possible to discern or comment on trends in recurrence rates in months 12–18.

Table 2. Monthly rate of genital herpes recurrences in gB2 and gD2 of MF59 placebo vaccine recipients.

	MF59 placebo recipients	gB2/gD2/ MF59 recipients	% difference	*P*
Culture-positive episodes	0.26 ± 0.02	0.21 ± 0.02	19	0.12
Clinically confirmed episodes	0.44 ± 0.03	0.40 ± 0.03	10	0.34
All lesional episodes (reported and confirmed)	0.55 ± 0.04	0.49 ± 0.04	12	0.22

NOTE. Data are for patients completing first 8 study months, primary study end point; data are mean ± SE.

Table 3. Duration and severity of first clinically confirmed genital herpes outbreak after vaccination with recombinant gB2 and gD2 or MF59 placebo.

Clinical end point	MF59 placebo recipients	gB2/gD2/ MF59 recipients	*P*
Virus shedding			
All subjects	3.8 ± 0.5	2.7 ± 0.6	.16
Men	3.3 ± 0.7	2.4 ± 0.9	.41
Women	4.2 ± 0.6	3.1 ± 0.7	.23
New lesion formation			
All subjects	6.9 ± 0.9	4.1 ± 1.0	.04
Men	5.8 ± 1.2	3.5 ± 1.5	.25
Women	7.9 ± 1.2	4.7 ± 1.1	.06
Itching and pain			
All subjects	6.8 ± 0.4	4.9 ± 0.5	.003
Men	5.4 ± 0.5	4.2 ± 0.5	.12
Women	8.2 ± 0.7	5.5 ± 0.6	.005
Complete healing			
All subjects	9.3 ± 0.5	7.0 ± 0.5	.002
Men	9.1 ± 0.7	6.9 ± 0.7	.04
Women	9.5 ± 0.8	7.1 ± 0.7	.02

NOTE. Data are days to resolution (mean ± SD).

During the first 8 months after vaccination, 81% of placebo recipients experienced a culture-proven recurrence; only 70% of gB2/gD2 recipients recurred. The relative risk of having a culture-positive recurrence for gB2/gD2 recipients was 0.64 (90% confidence interval, 0.46–0.89; $P = .007$), or 36% lower than that of placebo recipients. For the first 12 months of the study, the relative risk of having a culture-positive recurrence was 0.68 (95% confidence interval, 0.50–0.93; $P = .02$) for vaccine recipients.

Recurrence duration and severity. Recipients of gD2 and gB2 experienced consistent and significant improvements in the duration and severity of the first confirmed study recurrence after initial vaccination (table 3). Vaccine subjects showed reductions in the mean number of days new lesions continued to appear ($P = .04$) and symptoms persisted ($P = .003$); they also showed quicker lesion healing ($P = .02$) and a lower frequency of culture-positive lesional episodes. Significant reductions or, in some instances, similar trends were observed in these clinical end points for both men and women individually (table 3) and for participants at each study site (data not

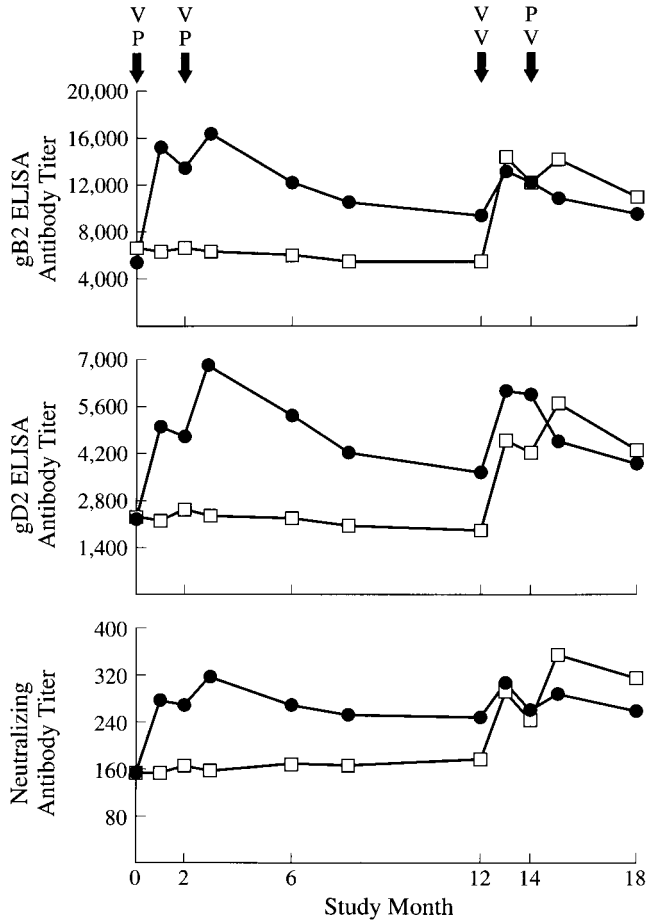

Figure 2. Geometric mean antibody responses to sequential doses of herpes simplex virus glycoprotein gD2/gB2 adjuvanted in MF59 or MF59 placebo alone. Top, gB2-specific ELISA titers; middle, gD2-specific ELISA titers; bottom, HSV-2 neutralizing antibody titers. ●, gB2/gD2-first recipients; □, MF59 placebo-first recipients. Those initially assigned to receive gD2/gB2/MF59 received 3 doses of vaccine (V) followed by 1 dose of MF59 placebo (P). MF59 placebo-first recipients received 2 doses of placebo (P) followed by 2 containing glycoproteins (V).

shown). Significant amelioration of the clinical end points of these first study outbreaks was also evident if only culture-proven recurrences were analyzed and if all lesional outbreaks were analyzed, whether clinically confirmed or not (data not shown). Although there were consistent trends through all of these analyses for shorter durations of viral shedding among gD2/gB2 recipients, none of the differences was statistically significant.

Antibody responses. Vaccination led to prompt and sustained boosts in preexisting antigen-specific and neutralizing antibody responses, in accord with earlier data (figure 2). Specifically, geometric mean antibody titers to HSV-2 glycoprotein B were boosted an average of 2.5-fold within 1 month of the second immunization with the glycoprotein-containing vaccine both in those who received it in the first study year and in those who were finally given it during the second, open phase of the study (the placebo-first group). Fifty-nine percent of subjects experienced a >4-fold geometric mean rise in gB2-specific antibody titers; although the titers fell somewhat by month 12, the geometric mean gB2-specific titers remained nearly twice what they were at study entry (figure 2, top). Comparable boosting of gD2-specific antibodies was also seen, with ~56% of recipients achieving a >4-fold augmentation in titer (figure 2, middle). Neutralizing antibodies to HSV-2 rose by a geometric mean of nearly 2-fold with glycoprotein vaccination: 46% and 17% of subjects had >2-fold and >4-fold boosts in neutralizing antibodies, respectively (figure 2, bottom). Again, these titers remained increased for the entire duration of the study.

It is important to note that initial, peak, and final antigen-specific and neutralizing antibody titers did not correlate with outbreak frequency or the duration of virus shedding, lesional symptoms, or healing.

Discussion

Several decades of clinical anecdote and casual study suggested that vaccines may ameliorate recurrent HSV disease [26–35]. A critical test of this possibility involved the administration of purified and recombinant glycoproteins D or B (or both) to guinea pigs who had been previously infected genitally with HSV-2. Depending on the adjuvant used and the vaccine composition, Stanberry and colleagues [12, 15], Ho et al. [17], and Burke [23] reported reductions in outbreak frequency of 25%–70% in infected guinea pigs. Clinical trials leading up to and including the present study verify that administration of recombinant HSV-2 glycoproteins can influence the features of genital herpes disease [18–20].

Initial trials in infected patients involved recombinant gD2 alone in the traditional alum adjuvant. Three doses given over 12 months were well-tolerated and enhanced both humoral and cellular immune responses to HSV-2 [18]. gD2-specific titers were boosted 8- to 25-fold, while neutralizing responses rose 4- to 6-fold. There were concomitant increases in the proportion of peripheral blood mononuclear cells that proliferated in vitro in response to HSV antigens (Tigges M, unpublished data).

A placebo-controlled trial was then conducted in which 100 μg of recombinant gD2 in alum was given at 0 and 2 months to 98 patients with recurrent genital herpes [19]. With just these two immunizations, gD2-specific titers were boosted an average of 7-fold, and neutralizing titers increased nearly 4-fold. Notably, vaccination was associated with statistically significant but modest decreases in the median and total number of recurrences. The monthly rates of recurrences were reduced by 24% and 36% for clinically confirmed and culture-proven episodes, respectively. Although that study was not designed to accurately assess the effect of vaccination on the duration of outbreaks, patient diaries indicated a significant benefit (P = .003; unpublished data).

In parallel with the controlled trial of recombinant gD2 in alum, recombinant gD2 plus gB2 and a novel oil-in-water emulsion, MF59, entered clinical assessment. Studies showed that in previously uninfected persons, HSV-2–specific immune responses are induced more rapidly and to higher levels with the addition of gB2 and with substitution of MF59 for alum [20]. Previously infected subjects sustained significant boosts in gB2-specific antibodies, but neutralizing responses did not rise farther than they did with the alum-containing gD2 vaccine (unpublished data). It was evident, however, that the rates and severity of local and systemic reactions to the gD2/gB2/MF59 vaccines were greater than those seen earlier with the alum vaccine [18, 19], especially in HSV-infected patients, and these findings were borne out in the present study (figure 1). On the basis of prior data, recombinant antigen concentrations of only 10 μg each were thought to lead to adequate immune responses in HSV-2–seropositive subjects with acceptable reactogenicity. Side effects were even more common with higher antigen doses; thus, the 10-μg dose of each antigen was chosen for the present trial.

Here, in 202 patients with frequently recurring genital herpes, we documented that immunization had a consistent and highly significant impact on the first carefully studied postvaccination recurrence. The duration of new lesion formation, symptoms, and time to healing were shortened to an extent comparable with—or possibly greater than—that achieved in controlled trials of episodic antiviral treatment [5, 6]. Obviously, a direct comparative trial would be needed to quantitate this issue. Although the risk of experiencing a culture-positive recurrence was reduced by vaccination, the lack of statistically significant shortening of the duration of viral shedding might be indicative of fundamental differences in the mechanism of action of antiviral drugs and therapeutic vaccines. Acyclovir inhibits viral replication and subsequent microbially induced injury. A therapeutic vaccine might alter host immune responses that speed viral clearance or, alternatively, that inflict tissue damage, the so-called immunopathologic response. That reduction in symptoms and time to healing were more impressive in the present trial than reduction in viral shedding argues for the existence of immunopathologic mechanisms in the genesis of herpetic lesions and for the possibility of favorably ameliorating those responses by vaccination.

The present study was not designed to discern whether benefits of vaccination on the duration and severity of outbreaks would be sustained through a year of study. The crossover to an open vaccination phase did not yield meaningful additional efficacy data. Unfortunately, the vaccine did not reduce the recurrence rate significantly. Some patients may accept a vaccine that with initial or periodic use merely attenuates outbreaks, but a more valuable therapy would reduce the frequency of herpetic outbreaks.

It is unclear why the gD2/gB2/MF59 vaccine had a lesser and statistically insignificant effect on recurrence rate than did the gD2 in alum vaccine in its preliminary analysis [19]. Among the possible reasons was the choice of a reduced and possibly suboptimal antigen dose (10 μg each of gD2 and gB2 in MF59 versus 100 μg of gD2 in alum) that would accommodate the greater reactogenicity of the MF59 adjuvant. In accord with this possibility was the lesser augmentation of HSV-2–specific humoral responses in the present study than in the gD2 in alum trial and, perhaps, also the more critical but unmeasured and still-undefined immune determinants of disease phenotype [18, 19]. This underscores, perhaps, the greater importance of antigen selection and dose than adjuvant in the design of therapeutic vaccines.

In conclusion, a vaccine composed of recombinant HSV-2 glycoproteins D and B in an oil-in-water emulsion adjuvant led to significant amelioration of the first observed genital recurrences, in further support of the concept of therapeutic vaccination for chronic viral diseases. A practical vaccine for disease modification would also affect recurrence frequency. Perhaps newer technologies involving injections of viral DNA sequences, immunostimulating cytokines, or genetically attenuated or replication-incompetent viruses would yield a practical immunotherapeutic vaccine for genital herpes [36–39].

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