Recombinant Glycoprotein Vaccine for the Prevention of Genital HSV-2 Infection
Two Randomized Controlled Trials

Lawrence Corey, MD
Andria G. M. Langenberg, MD
Rhoda Ashley, PhD
Rose E. Sekulovich, PhD
Allen E. Izu, MS
John M. Douglas, Jr, MD
H. Hunter Handsfield, MD
Terri Warren, NP, RN
Lisa Marr, MD
Stephen Tyring, MD, PhD
Richard DiCarlo, MD
Adaora A. Adimora, MD, MPH
Peter Leone, MD
Cornelia L. Dekker, MD
Rae Lyn Burke, PhD
Wai Ping Leong, MS
for the Chiron HSV Vaccine Study Group

HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) seroprevalence has increased by 32% in the last decade.1 Overall, HSV-2 seroprevalence is 22% in the general US adult population, and in some populations, such as sexually transmitted disease (STD) clinic attendees, seroprevalence varies between 30% and 50%.1-3 Concomitant with this increase in genital herpes has been an increase in reported cases of neonatal herpes.6

Context In the last 3 decades, herpes simplex virus type 2 (HSV-2) infection seroprevalence and neonatal herpes have increased substantially. An effective vaccine for the prevention of genital herpes could help control this epidemic.

Objective To evaluate the efficacy of a vaccine for prevention of HSV-2 infection.

Design Two randomized, double-blind, placebo-controlled multicenter trials of a recombinant subunit vaccine containing 30 µg each of 2 major HSV-2 surface glycoproteins (gB2 and gD2) against which neutralizing antibodies are directed, administered at months 0, 1, and 6. Control subjects were given a citrate buffer vehicle. Participants were followed up for 1 year after the third immunization.

Setting and Participants We enrolled 2393 persons from December 10, 1993, to April 4, 1995, who were HSV-2 and human immunodeficiency virus seronegative. One trial with 18 centers enrolled 531 HSV-2-seronegative partners of HSV-2-infected persons; the other, with 22 centers, enrolled 1862 persons attending sexually transmitted disease clinics. A total of 2268 (94.8%) met inclusion criteria and were included in the analysis with 1135 randomized to placebo and 2012 to vaccine.

Main Outcome Measure Time to acquisition of HSV-2 infection, defined by seroconversion or isolation of HSV-2 in culture during the study period by randomization group.

Results Time-to-event curves indicated a 50% lower acquisition rate among vaccine vs placebo recipients during the initial 5 months of the trial; however, overall vaccine efficacy was 9% (95% confidence interval, −29% to 36%). Acquisition rates of HSV-2 were 4.6 and 4.2 per 100 patient-years in the placebo and vaccine recipients, respectively (P = .58). Follow-up of vaccine recipients acquiring HSV-2 infection showed vaccination had no significant influence on duration of clinical first genital HSV-2 episodes (vaccine, median of 7.1 days; placebo, 6.5 days; P > .10) or subsequent frequency of reactivation (median monthly reoccurrence rate with vaccine, 0.2; with placebo, 0.3; P > .10). The vaccine induced high levels of HSV-2-specific neutralizing antibodies in vaccinated persons who did and did not develop genital herpes.

Conclusions Efficient and sustained protection from sexual acquisition of HSV-2 infection will require more than high titers of specific neutralizing antibodies. Protection against sexually transmitted viruses involving exposure over a prolonged period will require a higher degree of vaccine efficacy than that achieved in this study.

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lished maternal infection.7 Thus, an effective method for control of genital HSV is of major public health importance. A reduction in HSV-2 prevalence would also reduce the influence of HSV-2 infection in human immunodeficiency virus (HIV) 1 acquisition and transmission.8-10

Several experimental and epidemiological factors indicate that development of an effective vaccine against HSV-2 should be possible. First, HSV-2–specific antibodies are associated with reduction of maternal-fetal transmission of HSV-2.5,11-13 Also, new infection with the same HSV-2 subtype is rare,14,15 and prior HSV-1 infection appears to ameliorate frequency and severity of subsequent HSV-2 disease.16-18 In experimental models, both humoral and cellular immune responses have been effective in preventing HSV in experimental challenge.19 Passive immunization has been achieved with typespecific neutralizing antibodies, and monoclonal antibodies directed at major neutralizing epitopes of HSV-2 glycoproteins B2 (gB2) and D2 (gD2) protected against experimental challenge in mice.20,21 Immunization with gD2 and gB2 proteins is similarly effective in a guinea pig model.22,23 Phase 1 and 2 clinical trials with these recombinant proteins have shown that humans develop postimmunization neutralizing antibodies to HSV-2 that are similar to those that arise following natural infection.24

Based on these preclinical and phase 1 and 2 studies, we conducted 2 large, randomized, double-blind, placebo-controlled trials with 27 participating study sites to evaluate the efficacy of a recombinant glycoprotein subunit vaccine for the prevention of genital HSV-2 infection. The vaccine contained 30 µg each of gD2 and gB2, in combination with the adjuvant MF59, a 5% squalene oil-in-water emulsion. Persons who were HSV-2–seronegative (either HSV–seronegative or seropositive for HSV-1 only) were eligible. The HSV serological status was determined using Western blot assay performed at the University of Washington, and HIV screening was performed using enzyme-linked immunosorbent assay at the individual sites.25,26 The studies were approved by the respective institutional review boards or ethics committees. One trial (involving 18 centers) enrolled 531 HSV-2–seronegative partners of HSV-2–infected persons who reported a minimum of 6 months of mutual monogamy. The second trial (involving 22 centers) enrolled 1862 persons attending STD clinics who reported 4 or more sexual partners in the year prior to enrollment or having had 1 of the following STDs: gonorrhea, chlamydial infection, pelvic inflammatory disease (women), first-episode nongonococcal urethritis (men), primary or secondary syphilis, or trichomoniasis. The demographic characteristics and history of HSV-1 disease of the 2 trial populations were expected to differ.

Written informed consent was obtained from all study participants prior to randomization. Informed consent was also obtained from the HSV-2–infected partners of the susceptible monogamous participants; these infected persons agreed not to use suppressive acyclovir therapy during the trial. All subjects received standardized counseling about safer sex, including the recommendation to use condoms with all sexual exposures. These counseling sessions occurred at each study visit. Subjects were also instructed about the signs and symptoms of genital herpes and were requested to present to the study clinic for all such signs and symptoms during the trial. At some sites, subjects were reimbursed for study participation. Reimbursement was subject to local customs and institutional review board approval. Median reimbursement for trial participation was $75 per subject (range, $50-$700).

At study entry, each participant was randomly assigned to receive either the gD2-gB2-MF59 vaccine or citrate buffer vehicle in MF59 (identical in appearance to the active vaccine). Injections were administered intramuscularly into the deltoid muscle at study entry, at 1 month, and at 6 months. Each vaccine dose contained 30 µg of gD2 and 30 µg of gB2. All subjects, investigators, and trial coordinators were blinded to treatment assignment. Subjects were randomly assigned to treatment according to a block randomization scheme stratified by study site, sex, and HSV-1 serostatus. Subjects were followed up for 12 months after the third injection for a total of 18 months of individual study participation. They also kept 2 separate diary cards throughout the study, 1 for a standard profile of adverse events and 1 to record episodes of genital-to-genital contact with partners. Eleven routine study visits were scheduled at 2-week to 3-month intervals throughout the 18-month study, including visits at 1, 1½, 4, 6, 6½, 8, 10, 12, 15, and 18 months after initial immunization. Blood was collected at every scheduled clinic visit and at the time of visits for genital symptoms. Subjects were asked to return to the clinic when experiencing genital symptoms. At these visits, study staff performed genital examinations to evaluate lesions. A separate form was designed for the recording of lesion number and area, and duration of symptoms and lesions. In the event of culture-confirmed acquisition of genital HSV-2 or HSV-1, the subject was withdrawn from the active portion of the trial (ie, no further injections, if applicable) and subsequently followed up for general vaccine safety and clinical recurrences of genital HSV.

Determinants of HSV Acquisition
To detect seroconversion to HSV, paired serum samples drawn at days 0 and 540 were sent to the laboratory along with information on the HSV serostatus of the subject at enrollment. Serum samples from HSV-seronegative subjects were preabsorbed using Sepharose 4B beads containing gB2 and gD2 to remove any antibodies against the 2 vaccine proteins. Serum samples from HSV-1–
seropositive subjects were first preabsorbed using Sepharose 4B beads containing HSV-1 proteins to remove HSV-1 antibodies and then with Sepharose 4B beads containing gB2 and gD2 to remove possible vaccine-induced antibodies. These steps ensured that laboratory staff reviewing Western blot profiles would remain blinded to subject vaccine status. Evaluation of specimens from phase 2 trial HSV-1-seropositive vaccine recipients showed that these procedures resulted in complete removal of vaccine-induced antibodies while reducing HSV antibody titers by a factor of 4 (median reduction, 36 to 9 µg/mL).

Preabsorbed serum specimens were added to a pair of standard Western blots, 1 containing HSV-1 proteins and 1, HSV-2 proteins. Immunostaining was performed as described using 3,3’5,5’-tetrachloro-1-naphthyl-diamine substrate. This method is about 10 times more sensitive than the standard staining method that uses 4-chloro-1-naphthol and was selected to compensate for loss of titer due to preabsorption. Blots were examined by 1 of the authors (R.A.) for changes in profile consistent with HSV-1 and/or HSV-2 seroconversion (in subjects who were HSV-seronegative at entry), or HSV-2 seroconversion (in subjects who were HSV-1-seropositive at entry). Results were coded in a blinded manner and transferred electronically to the study database.

To define time of seroconversion more precisely, sequential serum samples between days 0 and 540 were then assayed by standard Western blot without prior absorption for gB2 and gD2. Seroconversion was confirmed in all cases. In addition, for 28 of the HSV-2 seroconversions, day 260 and day 540 serum samples were rerun in a strip immunoblot assay using recombinant glycoprotein G2. Again, seroconversion was confirmed in all 28. Thus, all seroconversions detected by the original Western blot procedure were confirmed by subsequent assays. All serologic evaluations were done without knowledge of clinical data or vaccination status. For data analysis purposes, time of HSV-2 acquisition was determined as the earlier of 2 points, either time of HSV-2 isolation from genital lesions or midpoint between last negative and first HSV-2–positive serological test result.

Measurement of HSV Antibodies

Immune response to the vaccine was assessed by measuring neutralizing antibodies to HSV-2 and binding antibodies to gD2 and gB2. Neutralizing HSV-2 antibody titers were determined using a complement-dependent microneutralization assay as described and antibodies to HSV-2 glycoproteins gB2 and gD2 were measured using enzyme-linked immunosorbent assay. Neutralization titer was reported as reciprocal of the serum dilution that inhibited cytolysis of cell monolayers by 50%. Plates contained an HSV-positive human specimen of known neutralization titer as a standard for normalization between assays.

All serum antibody assays were run without knowledge of vaccination status; blinding was assured by the assignment of unique numeric identification to each serum sample. Blood was collected for these antibody determinations on study days 0 (preimmunization), 28 (1 month after first immunization [HSV-1–seropositive subjects only]), 194, 360, and 540.

HSV Culture

Cultures for HSV were done at local study sites using standard techniques.

Management of Protocol

Number of enrollees was based on prior epidemiological study findings showing an annual HSV-2 seroconversion rate of about 3% to 15%, based on sex (women > men) and HSV serologic status (HSV-seronegative > HSV-1-seropositive). Numbers were intended to account for an estimated dropout rate of 20% in the partner study and 40% in the STD clinic group.

A data safety monitoring board oversaw trial progress. The board was composed of 2 independent clinical infectious disease experts; an independent statistician; an independent medical ethicist; and a statistician employed by the sponsor, a nonvoting member not involved in study conduct or analysis (see “Acknowledgment” at end of the article). Neither participants nor personnel directly involved in conducting or monitoring the study or reviewing the data knew treatment assignments until the code was broken and the data were analyzed.

Statistical Analysis

Time to HSV-2 acquisition as determined by HSV Western blot seroconversion or a positive genital HSV-2 culture was the primary end point. For the STD clinic trial, a minimum enrollment of 1834 eligible subjects was planned, and for the partner trial, a minimum enrollment of 400. It was estimated that annual HSV-2 infection rates in the STD clinic and partner study populations for subjects receiving placebo would be 3% and 10%, respectively. The method of Lachin and Foulkes was used to determine power, stratifying by HSV 1+/2− and HSV 1–2– status. The sample sizes above ensured that a 2-sided stratified log-rank test with overall significance level of 0.05 would have a power of 93% in the STD clinic trial and 88% in the partner trial to detect a vaccine efficacy of 75%. The power calculations assumed a loss-to-follow-up rate of about 40% for the STD clinic trial (hazard ratio = 0.357), and about 20% for the partner trial (hazard ratio = 0.149). It was assumed for the analysis that the follow-up period started in the HSV 1+/2− group 2 weeks after the second injection and in the HSV 1–2– group 2 weeks after the third injection.

For the partner trial, an alternative calculation using a modified version of the Bernstein and Lagakos program was used for modeling constant hazard ratios but differential hazards for the 4 strata. For this calculation, we used an acquisition rate of 4% in each male strata, 10% in the female HSV 1+/2− stratum, and 30% in the female HSV 1–2– stratum, providing an overall infection rate of about 12%. Based on 75% vaccine efficacy and a 20% loss-to-
follow-up rate, power was in excess of 90%.

Subjects found to be HSV-2–seropositive by Western blot prior to the first injection were excluded from HSV-2 efficacy analyses. Remaining subjects randomized to enter the study with available follow-up Western blot results were analyzed in the intent-to-treat analysis group. For this analysis, the follow-up time for each subject started from the first injection date. The protocol-stipulated analysis was designed to evaluate HSV-2 acquisition after maximal response to vaccination; follow-up time began 2 weeks after the third immunization for HSV-seronegative subjects and 2 weeks after the second immunization for HSV-1–seropositive subjects. These starting times were based on the phase 2 clinical trials, which showed that 100% of HSV-1–seropositive persons experienced postimmunization HSV-2 binding and neutralization titers equal to or higher than those with established HSV-2 infection after 2 injections (month 1/2) of the vaccine. Three injections (month 6/2) were needed to achieve such titers for the HSV-seronegative persons.24

The statistical methods used included the Pearson χ² test, analysis of variance, and the t test.33–34 The distribution of time to HSV-2 acquisition was compared between the treatment groups by means of the stratified log-rank test,35–38 for which both HSV-1 serostatus and sex defined the strata.

The Cox proportional hazards regression model was used to determine relative risk (RR) of HSV-2 acquisition and overall vaccine efficacy rates, and it was also used to analyze effects of covariates such as serologic status and sex on HSV-2 acquisition. Assumption of proportionality for the model was tested by 2 methods and was valid. Overall vaccine efficacy was estimated as \((1 - RR) \times 100\%\), where \(RR = e^\lambda\); \(\lambda\) was the estimated coefficient from the Cox model with treatment as the only independent variable, stratified for HSV-1 serostatus and sex. We calculated 95% confidence intervals (CIs) in a similar fashion, using the upper and lower 95% confidence limits for \(\lambda\). Curves showing cumulative proportion of patients acquiring HSV-2 infection were generated by the Kaplan-Meier method.38 Statistical analyses were computed using statistical analysis software.39 All P values reported for this trial are 2-tailed.

RESULTS

Demographic Characteristics of the Study Population

A total of 2393 persons were enrolled in both studies over a 16-month period (December 10, 1993, to April 4, 1995), 531 in the uninfected partner study and 1862 in the STD clinic study (FIGURE 1). We included 2268 (94.8%) of the enrolled participants in the analyses. Reasons for exclusion were HSV-2 seroconversion between dates of initial screening and first immunization (day 0, n = 29); no follow-up after the initial injection visit (n = 94); and HSV Western blots impossible to evaluate (n = 2). While it was requested that infected partners not receive long-term antiviral therapy, about 4% of the total partners in the monogamous partners study (2% per treatment group) took suppressive acyclovir at some time during the study. Their uninfected partners were not excluded from the analyses. The 2 treatment groups within each study population (STD and partners)
were comparable at enrollment in all demographic characteristics (Table 1). Subjects from STD clinics were younger (median age, 26 years), less often white, more often male, and more often HSV-1–seropositive prior to vaccination than those in the partner study. These differences were expected and contributed to the rationale for vaccine evaluation in 2 study populations.

**Acquisition of HSV-2 During Trial**
From initial immunization to study termination, 126 participants acquired HSV-2 infection (Table 2). Overall, 57 (45%) of the HSV-2 acquisitions were associated with signs and symptoms of genital herpes. During the trial, HSV-2 was isolated from 42 (74%) of these 57 patients during a symptomatic first episode; also, 51 (89%) of the 57 symptomatic participants seroconverted to HSV-2 by Western blot. There were no follow-up serum samples for 3 culture-positive nonseroconverters after the 1, 9, or 13 months, respectively. Eighteen (64%) of the 28 cases of HSV-2 acquisition in persons in the partner study were associated with a clinically symptomatic first episode of genital herpes, vs 39 (40%) of the 98 instances of HSV-2 seroconversion in the STD clinic trial (Table 2) (P < .05). This lower frequency of recognized symptomatic seroconversion in the STD clinic trial may reflect differences in awareness of genital symptoms or variations in protocol compliance between groups. Clinical acumen may have varied between STD clinic and partner trial sites. Variations in rates of symptomatic first episodes in the 2 trials may also have occurred due to higher rate of HSV-1 seropositivity at entry in the STD clinic cohort.

During the study, HSV-2 infection was acquired subclinically (defined as seroconversion without identified genital lesions) by 69 persons. Of these, 23 (33%) were female and 46 (67%) male; 22 (32%) were HSV-seronegative prior to entry and 47 (68%) had HSV-1 antibodies at entry. Of the 69 persons, 9 (13%) subsequently developed genital lesions clinically diagnosed as HSV during study follow-up.

**Effect of Vaccination on Acquisition of HSV-2 Infection**
Vaccination appeared to have a partial but transient effect on HSV-2 acquisition. As shown in Figure 3, vaccine recipients acquired HSV-2 infection at a lower rate during the initial 150 days of the trial than placebo recipients did. This is most apparent in women and in HSV-1–seropositive persons (Figure 3, A and D). However, over the entire study, acquisition rates were similar between vaccine and placebo groups.

**Table 1. Demographic Characteristics of the Study Population by Study Group and Treatment Assignment**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partners Trial</th>
<th>STD Clinic Trial</th>
<th>Total (N = 2268)</th>
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<tr>
<td>Age, median (range), y</td>
<td></td>
<td></td>
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<tr>
<td>Subtotal</td>
<td>69 (35-35)</td>
<td>70 (35-35)</td>
<td>70 (35-35)</td>
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<td>Total</td>
<td>139 (69-69)</td>
<td>139 (69-69)</td>
<td>139 (69-69)</td>
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<tr>
<td>Sex, %</td>
<td></td>
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<tr>
<td>Male</td>
<td>50</td>
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<tr>
<td>Female</td>
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<td>50</td>
<td>50</td>
</tr>
<tr>
<td>HSV serostatus, %</td>
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<tr>
<td>HSV-1+/2−</td>
<td>41</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>HSV-1+/2−</td>
<td>59 (95)</td>
<td>59 (95)</td>
<td>59 (95)</td>
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<tr>
<td>Race, %</td>
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</tr>
<tr>
<td>White</td>
<td>95</td>
<td>93</td>
<td>94</td>
</tr>
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<td>2</td>
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<td>1</td>
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<tr>
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<td>0</td>
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<td>Sexual orientation, %</td>
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<tr>
<td>Female heterosexual</td>
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<td>&lt;1</td>
</tr>
<tr>
<td>Female bisexual</td>
<td>2</td>
<td>&lt;1</td>
<td>1</td>
</tr>
<tr>
<td>Male bisexual</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duration of monogamous relationship with same partner, mo, mean (range)</td>
<td>18 (2-300)</td>
<td>18 (2-300)</td>
<td>NA</td>
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</table>

*HSV-2 indicates herpes simplex virus type 2; STD, sexually transmitted disease; and NA, not applicable. Percentages may not add to 100% due to rounding.*
TABLE 3 displays acquisition rate by treatment group over the 18-month study. Both in the intent-to-treat analysis and the protocol-stipulated analysis (designed to measure acquisition after expected peak responses to immunization), frequency of HSV-2 acquisition was similar in the vehicle vs vaccine groups. Overall, the acquisition rate was 4.6 cases of HSV-2 per 100 person-years of follow-up in the placebo group vs 4.2 in the vaccine group (66 vs 60 cases of HSV-2 acquired, respectively [P = .38]). These data yield an overall calculated vaccine efficacy of 9% (95% CI, –29% to 36%). For the partners study, calculated vaccine efficacy was 29% in the intent-to-treat analysis and 13% in the protocol-stipulated analysis. For the STD clinic trial, vaccine efficacy was less than 5% in both the intent-to-treat analysis and the protocol-stipulated analysis. Similar results were obtained when such correlates as frequency of sexual intercourse and condom use were evaluated. An additional 20 subjects seronegative for HSV-1 and HSV-2 at entry seroconverted to HSV-1 during the trial; 12 received placebo and 8, vaccine. The HSV-1 acquisition rates per 100 person-years of follow-up were 2.1 and 1.4 in placebo and vaccine groups, respectively (P = .37).

Effect of Vaccine on Disease Modification

To assess whether newly acquired HSV-2 infections were milder in vaccine than placebo recipients, we evaluated relative frequency of acquisition of symptomatic vs subclinical HSV-2 in each group. As shown in TABLE 4, there were no differences between vaccine and placebo groups in rates of symptomatic HSV-2 acquisition. Also, there was no significant difference in time of acquisition of symptomatic vs subclinical infection by Kaplan-Meier analysis. Among the total 57 subjects with symptomatic acquisition of genital herpes, 1 (2%) had received 1 injection and 56 (98%) had received 2 or more injections of vaccine prior to disease. The median number of days to healing for recurrent genital lesions reported by persons acquiring symptomatic HSV-2 infection during the study was 7.1 days for vaccine recipients and 6.5 days for vehicle recipients. Among the 57 cases of symptomatic HSV-2, 34 (60%) recurred during the study. The median monthly recurrence rate was 0.21 for those who received vaccine vs 0.34 for placebo recipients. These data show no discernible differences in subsequent course of disease between those receiving vaccine vs placebo prior to HSV-2 acquisition, and no sex differences were identified.

Vaccine Immunogenicity

We measured HSV-2 neutralizing and gB3 and gD3 binding antibodies at peak
time periods (day 194 in seronegative and day 28 in HSV-1–seropositive persons). Titers were similar to those seen in phase 2 studies and exceeded those of persons with sexually acquired HSV-2.24 No sex differences in immunogenicity were identified.

Nested Case-Control Analyses
A case-control study was performed separately for each trial population (STD clinic group and partners at risk) after unblinding and completion of all efficacy analyses. This was done to determine whether there was a difference in postimmunization antibody titers between vaccinees who did and did not acquire HSV-2. For this study, each vaccinated subject who acquired HSV-2 was matched with 3 randomly selected persons of the same sex, age, ethnicity, and baseline HSV-1 serostatus. In addition, subjects were matched for potential sexual exposure to HSV-2. Sexual intercourse frequency in the 2 visit intervals immediately prior to HSV-2 infection was classified into 1 of 3 categories: intercourse 1 to 2, 3 to 5, or more than 5 times per week. When possible, controls were selected with intercourse frequencies similar to those of the same visit intervals. Sex, HSV serologic status at entry, age (<35 and ≥35 years), and race were matched in all cases and controls. Intercourse frequency was matched in 98% of instances. We then compared levels of HSV-2 neutralizing and gB₂ and gD₂ binding antibodies at the time of expected peak responses to vaccination and in the 2 most recent blood samples in the period immediately prior to HSV-2 acquisition. A similar match was performed in subjects who were HSV-1–seropositive at entry who received placebo to determine if level of cross-reactive neutralizing antibodies predicted HSV-2 acquisition. The serum samples from cases and controls were assayed together to minimize effects of interassay variability.

Examination of the case-control immunogenicity plots in Figure 4 shows no association between lower HSV-2 neutralizing antibody titers and increased risk of HSV-2. Similar findings were seen in levels of anti-gB₂ and anti-gD₂ antibodies. Among HSV-1–seropositive persons who received placebo, higher titers of cross-reactive antibodies to HSV-2 did not protect against disease (Figure 5). Therefore, neither levels of HSV-2–specific neutralizing antibodies nor gB₂ or gD₂ binding antibodies predicted protection of HSV-1–seropositive persons from HSV-2 (P > .10 for all 3 types of antibodies).

Safety
The vaccine was safe and well tolerated. Frequencies of local and systemic reactions to the vaccine were similar to those noted previously.24

COMMENT
These trials represent the largest efficacy studies ever conducted of a vaccine for STD prevention. Preclinical, clinical, and epidemiologic data predicted that a vaccine producing high levels of HSV-2–specific neutralizing antibodies would effectively prevent acquisition of genital HSV-2 infection.

Table 3. Acquisition Rates of HSV-2*

<table>
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<tr>
<th>Partners Trial</th>
<th>STD Clinic Trial</th>
<th>Total</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>Vaccine</td>
<td>Vehicle</td>
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<td>No. of person-years</td>
<td>345</td>
<td>351</td>
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Intent-to-Treat Analysis

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<tr>
<th>Overall</th>
<th>4.6 (16)</th>
<th>3.4 (12)</th>
<th>4.6 (50)</th>
<th>4.4 (48)</th>
<th>4.6 (66)</th>
<th>4.2 (60)</th>
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<tr>
<td>Male</td>
<td>1.1 (2)</td>
<td>2.8 (5)</td>
<td>4.3 (55)</td>
<td>4.1 (33)</td>
<td>3.7 (37)</td>
<td>3.8 (38)</td>
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<tr>
<td>Female</td>
<td>8.8 (14)</td>
<td>4.0 (7)</td>
<td>5.4 (15)</td>
<td>5.5 (15)</td>
<td>6.7 (20)</td>
<td>4.9 (22)</td>
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<td>HSV-seronegative</td>
<td>6.3 (9)</td>
<td>3.5 (5)</td>
<td>4.4 (18)</td>
<td>5.1 (20)</td>
<td>4.9 (21)</td>
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<td>HSV-1–seropositive</td>
<td>3.5 (7)</td>
<td>3.4 (7)</td>
<td>4.7 (22)</td>
<td>4.1 (28)</td>
<td>4.4 (39)</td>
<td>3.9 (35)</td>
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Protocol-Stipulated Analysis

<table>
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<tr>
<th>Overall</th>
<th>3.7 (10)</th>
<th>3.3 (9)</th>
<th>3.8 (33)</th>
<th>4.2 (36)</th>
<th>3.8 (43)</th>
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<td>Male</td>
<td>1.4 (2)</td>
<td>2.1 (3)</td>
<td>3.7 (24)</td>
<td>3.6 (23)</td>
<td>3.3 (26)</td>
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<td>Female</td>
<td>6.3 (8)</td>
<td>4.4 (6)</td>
<td>4.2 (8)</td>
<td>6.1 (13)</td>
<td>5.0 (17)</td>
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<td>HSV-seronegative</td>
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<td>HSV-1–seropositive</td>
<td>3.3 (6)</td>
<td>3.7 (7)</td>
<td>4.1 (25)</td>
<td>4.3 (27)</td>
<td>3.9 (31)</td>
<td>4.2 (34)</td>
</tr>
</tbody>
</table>

*HSV-2 indicates herpes simplex virus type 2; STD, sexually transmitted disease. Data are presented as HSV-2 rate per 100 person-years (number of cases).

Table 4. Clinical Course of Acquired HSV-2 Infection*

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n = 66)</th>
<th>Vaccine (n = 60)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median duration of initial lesions, d (range)</td>
<td>6.5 (2-20)</td>
<td>7.1 (1.6-17)</td>
<td>.45</td>
</tr>
<tr>
<td>Persons with recurring lesions postacquisition of HSV-2, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic HSV-2 seroconversion</td>
<td>21/33 (64)</td>
<td>13/24 (54)</td>
<td>.47</td>
</tr>
<tr>
<td>Subclinical HSV-2 seroconversion</td>
<td>1/33 (3)</td>
<td>6/36 (17)</td>
<td>.11</td>
</tr>
<tr>
<td>Median monthly rate of recurrence in those with recognized symptomatic recurrence (range)</td>
<td>0.34 (0.06-0.81)</td>
<td>0.21 (0.07-1.09)</td>
<td>.21</td>
</tr>
</tbody>
</table>

*HSV-2 indicates herpes simplex virus type 2.
However, these trials showed that this glycoprotein subunit vaccine produced only partial and transient protection despite levels of HSV neutralizing and binding antibodies in the range of those seen in natural HSV-2 infection. The vaccine proved ineffective in reducing HSV-2 acquisition rate in both men and women, independent of baseline HSV-1 serostatus.

Despite this outcome, several important issues pertinent to vaccine development for HSV-2 (and potentially for HIV) emerged. First, there is evidence that, in the early period of the trial, there was a consistent reduction of acquisition rates in vaccine vs placebo recipients. This was seen in male and female and HSV-1-seropositive subjects, and, to a lesser extent, HSV-seronegative subjects. Acquisition rate during the first 150 days in the vaccine group was 3.7 per 100 person-years of follow-up vs 7.6 in the placebo group, a 50% lower rate. While not part of the formal vaccine efficacy analysis, exploratory analyses suggested this observation was not serendipitous.

Reasons for this partial and transient protection are unclear. Subject sexual behavior, including number of sexual exposures and use of condoms (which did not increase in either group during the trial), or use of acyclovir by sexual partners, did not differ between vaccine and vehicle groups. One hypothesis for our finding is that a transient protective immune response was elicited, the precise composition of which is unknown. Identification and elicitation of such a response with a subsequent increase in its magnitude and duration might be associated with greater persistence. Another hypothesis, perhaps uniquely related to STDs, suggests that the initial protection afforded by the vaccine was lost with frequent exposure to the HSV-2 virus. Transmission of neonatal herpes probably entails only a single exposure of the infant to virus shedding, which may be easier to prevent than genital HSV-2 transmission, which may involve multiple exposures to virus. Recent data show that infectious HSV-2 is shed in the genital tract up to 15% to 20% of days. Thus, persons exposed to HSV-2 during unprotected sexual intercourse at a frequency of 3 to 4 times per week would, over a 6-month period, potentially be challenged 80 to 100 separate times. Maintaining protective immunity in such a setting is difficult. Thus, a modest level of vaccine failure per exposure would result in increasing ineffectiveness over time. This leads to the hypothesis that a clinically useful vaccine for chronic latent viral STDs that persist, are commonly reactivated, and in which the virus appears in genital secretions (such as HSV-2, HIV, or human papillomavirus) must elicit high levels of protection for each exposure.

The successful conduct of these multicenter trials is an important accomplishment in its own right. These tri-
als may serve as a model for other vaccine trials for viral STDs, especially HIV. The recruitment, retention, follow-up, and counseling provided each person at each study site were stringent and successful. Counseling about STD reduction was outlined uniformly and continuously monitored during the trial. Although cases of genital herpes did occur, frequencies of sexual exposure and number of new partners decreased during the study (A.G.M.L., unpublished data, November 1996). Moreover, the retention rate among these high-risk populations was higher than expected in both studies (92% vs anticipated 60%–80%), and compliance with providing follow-up information in persons who acquired HSV-2 infection was excellent. This level of compliance should be ascribed to intensive communication efforts by study staff.

The basis for testing the glycoprotein vaccine efficacy in this large phase 3 trial was demonstrated by the elicitation of high levels of type-specific neutralizing antibodies following immunization. Also, these antibodies were capable of neutralizing "wild type" (circulating strains) HSV-2.41 We hypothesized that there would be a threshold level of HSV-2–specific neutralizing antibodies above which protection against disease would be evident, an observation based on maternal-fetal HSV-2 transmission studies.11,13,42 However, the case-control studies performed indicated no differences in peak titer after vaccination for HSV-2 antibodies prior to infection between "vaccinated infected" and "vaccinated uninfected" persons. Also, HSV-1–seropositive participants who acquired HSV-2 had levels of HSV-2 binding and neutralizing antibodies similar to those of HSV-1–seropositive persons who did not acquire HSV-2. Also, prior HSV-1 infection did not appear to protect against HSV-2 infection. These findings suggest more information about the cellular and mucosal immune responses to HSV-2 infection is needed before we can attempt to identify laboratory-based correlates of immune protection. The objective of the trials was to reduce the rate of HSV-2 infection as defined by HSV-2 seroconversion. The rationale for setting this standard was that previous epidemiologic studies showed that people who acquire HSV-2 asymptptomatically still develop latency of the virus in sacral ganglia and subsequently have clinically recognized recurrences as well as episodes of subclinical shedding.11–13 Retrospective studies show that these people are the most frequent transmitters of HSV-2 infection.15,44 Nine (13%) of the 69 persons who acquired HSV-2 asymptptomatically as detected by seroconversion subsequently developed recognized genital lesions during the trial, and this number would likely be increased with more follow-up. Since subclinical reactivation in a person with unrecognized or undiagnosed genital herpes is a major factor in the epidemiology of HSV-2 transmission, a vaccine that does not affect acquisition of genital HSV-2 but does limit subsequent clinical disease manifestations could paradoxically increase the prevalence of subclinical infection. Such a vaccine would require careful evaluation regarding its ability to decrease subclinical transmission and hence subsequent acquisition of disease among future sexual partners for it to be useful in having an impact on the current epidemic of genital herpes. Such a trial would require the major logistic difficulty of daily sampling of the genitalia in individuals who acquire HSV-2 to measure the frequency of HSV-2 reactivation.45 Finally, to evaluate any potential influence of vaccination on subsequent clinical disease manifestations, duration of clinical first episode and subsequent recurrence rate of genital ulcerations after acquisition of HSV-2 were also monitored. No evidence of vaccine effect on disease modification was noted.

In summary, this recombinant HSV-2 glycoprotein vaccine proved to be only transiently successful in reducing the rate of genital HSV-2 acquisition and lacked overall efficacy. Despite the described association between HSV-2–specific antibodies and reduction of maternal-fetal HSV-2 transmission, it appears that high levels of HSV-2–specific neutralizing antibodies induced by immunization with gD1 and gB2 alone are insufficient for protection against genital HSV-2 infection or disease in adults. An effective HSV-2 vaccine that reduces the continued epidemic spread of this infection worldwide remains elusive.

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Ms Leong were investigators for, received research funding from, owned stock in, or were consultants for Chiron, Emeryville, Calif. Dr Burke was a consultant and had stock options for Antigenics, New York, NY. Dr Corey was on the speaker bureau of and received a lecture honorarium from Merck, West Point, Pa; Dr Strauss received research funding from Merck. Ms Warren and Drs Tyring and Strauss received research funding or honoraria from were consultants or advisors to, or received lecture scholarships from Bristol-Myers Squibb, Princeton, NJ. Drs Handsfield, Tyring, and Leone received research funding, honoraria, or lecture sponsorships from or were consultants and advisors to 3M, Duluth, Minn. Dr Corey was a consultant for Wyeth Lederle, Malvern, Pa, and was a scientific advisor to Diagnost, Belfast, Ireland. Dr Ashley was a consultant for MRL, Cypress, Calif, and Viridea, Vancouver, British Columbia. Dr Tyring received research funding from Gilead Sciences, Foster City, Calif, and VaxGen, Berkeley, Calif. Drs Tyring and Strauss received research funding for Abbott Pharmaceuticals, Abbott Park, Ill, and Pharmacia & Upjohn, Kalamazoo, Mich, and received lecture support from Wellcome Co. Funding/Support: This study was supported by Chiron Corporation and NIH grant AI-30731 (Dr Corey and Ashley).


Acknowledgments: We thank all the investigators and study staff for their tremendous contribution to this large collaborative effort. We also thank Chiron clinical research associates Astrid Ramans, Catherine Schellenger, and Cheryl S. and Adrian Hirsch for their excellent monitoring and Dino Din, MD, and Judith Zeh, PhD, for their input into protocol design. We appreciate the time and efforts of data safety monitoring board members Richard Lockley, MD; King K. Holmes, MD, PhD; William Blackwelder, PhD; Bernard Lo, MD; and Gregory Schwemer, PhD; and the support that William R. Rutter, PhD, and Ed Penhoet, PhD, of Chiron Corporation gave to this program.

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340 JAMA, July 28, 1999—Vol 281, No. 4
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