

GLYCOPROTEIN-D-ADJUVANT VACCINE TO PREVENT GENITAL HERPES

LAWRENCE R. STANBERRY, M.D., PH.D., SPOTSWOOD L. SPRUANCE, M.D., ANTHONY L. CUNNINGHAM, M.D.,
DAVID I. BERNSTEIN, M.D., ADRIAN MINDEL, M.D., STEPHEN SACKS, M.D., STEPHEN TYRING, M.D., PH.D.,
FRED. Y. AOKI, M.D., MONCEF SLAOUI, PH.D., MARTINE DENIS, PH.D., PIERRE VANDEPELIERE, M.D.,
AND GARY DUBIN, M.D., FOR THE GLAXOSMITHKLINE HERPES VACCINE EFFICACY STUDY GROUP*

ABSTRACT

Background An effective prophylactic vaccine would help control the spread of genital herpes.

Methods We conducted two double-blind, randomized trials of a herpes simplex virus type 2 (HSV-2) glycoprotein-D-subunit vaccine with alum and 3-*O*-deacylated-monophosphoryl lipid A in subjects whose regular sexual partners had a history of genital herpes. In Study 1, subjects were seronegative for herpes simplex virus type 1 (HSV-1) and HSV-2; in Study 2, subjects were of any HSV serologic status. At months 0, 1, and 6, subjects received either vaccine or a control injection and were evaluated for 19 months. The primary end point was the occurrence of genital herpes disease in all subjects in Study 1 and in HSV-2-seronegative female subjects in Study 2.

Results A total of 847 subjects who were seronegative for both HSV-1 and HSV-2 (268 of them women, in Study 1) and 1867 subjects who were seronegative for HSV-2 (710 of them women, in Study 2) underwent randomization and received injections. Vaccination was well tolerated and elicited humoral and cellular responses. Overall, the efficacy of the vaccine was 38 percent in Study 1 (95 percent confidence interval, -18 to 68 percent; 15 cases occurred in the vaccine group and 24 in the control group), and efficacy in female subjects was 42 percent in Study 2 (95 percent confidence interval, -31 to 74 percent; 9 cases occurred in the vaccine group and 16 in the control group). In both studies, further analysis showed that the vaccine was efficacious in women who were seronegative for both HSV-1 and HSV-2: efficacy in Study 1 was 73 percent (95 percent confidence interval, 19 to 91 percent; $P=0.01$), and efficacy in Study 2 was 74 percent (95 percent confidence interval, 9 to 93 percent; $P=0.02$). It was not efficacious in women who were seropositive for HSV-1 and seronegative for HSV-2 at base line or in men.

Conclusions These studies suggest that the glycoprotein D vaccine has efficacy against genital herpes in women who are seronegative for both HSV-1 and HSV-2 at base line but not in those who are seropositive for HSV-1 and seronegative for HSV-2. It had no efficacy in men, regardless of their HSV serologic status. (N Engl J Med 2002;347:1652-61.)

Copyright © 2002 Massachusetts Medical Society.

GENITAL infection caused by herpes simplex virus type 1 (HSV-1) or herpes simplex virus type 2 (HSV-2) may be asymptomatic, mild, and unrecognized as herpes or severe with painful skin lesions and complications including urinary retention and meningitis, as well as substantial psychological illness.¹⁻⁸ Genital HSV infection occurs worldwide and appears to be epidemic in some populations despite the availability of condoms and chemoprophylaxis.⁹⁻¹¹ Evidence suggests that only the widespread use of an effective vaccine might control this epidemic.¹²

We describe the results of two multicenter, double-blind, randomized, controlled studies of an HSV-2 glycoprotein-D-subunit vaccine formulated with a new adjuvant (AS04) containing aluminum hydroxide (alum) and 3-*O*-deacylated monophosphoryl lipid A (MPL)¹³⁻¹⁵ to prevent acquisition of genital herpes disease.

METHODS

Study 1

Study 1 was a phase 3, double-blind, randomized efficacy trial involving subjects who were seronegative for HSV-1 and HSV-2. In 1995 and 1996, 2486 adults 18 to 45 years of age were screened, and 847 of them (268 women) underwent randomization and were vaccinated at 57 centers in Australia, Canada, the United Kingdom, and the United States. The primary end point was the occurrence of genital herpes disease. The sample size was calculated on the basis of the following assumptions: an annual attack rate of genital herpes disease in recipients of control injections of 10 percent among female subjects and 5 percent among male subjects, a dropout rate of 20 percent, vaccine efficacy of 70 percent, a two-tailed type I error of 0.05, and a power of 80 percent.

Study 2

Study 2 was a phase 3, double-blind, randomized trial that was initially designed to evaluate the safety of the vaccine in subjects of any HSV serologic status. In 1996 and 1997, 2834 adults 18 years

From the University of Texas Medical Branch, Galveston (L.R.S., S.T.); the University of Utah, Salt Lake City (S.L.S.); the Westmead Millennium Institute (A.L.C.) and the Sexually Transmitted Infections Research Centre (A.M.), Westmead Hospital and University of Sydney, Westmead, Australia; the Cincinnati Children's Hospital Medical Center, Cincinnati (D.I.B.); Viridae Clinical Sciences, Vancouver, B.C., Canada (S.S.); the University of Manitoba, Winnipeg, Canada (F.Y.A.); and GlaxoSmithKline Biologicals, Rixensart, Belgium (M.S., M.D., P.V., G.D.). Address reprint requests to Dr. Stanberry at the Department of Pediatrics and Sealy Center for Vaccine Development, University of Texas Medical Branch, 3.300 Children's Hospital, 301 University Blvd., Galveston, TX 77555-0351, or at l.stanberry@utmb.edu.

*The members of the study group are listed in the Appendix.

of age or older were screened, and 2491 of them (1867 of them seronegative for HSV-2, 710 of them HSV-2-negative women) underwent randomization and were vaccinated at 61 centers in Australia, Canada, Italy, and the United States. In 1998, when the results from Study 1 became available and before the results from Study 2 had been examined, the prevention of genital herpes disease during months 0 through 19 was added as a primary efficacy end point in female subjects who were seronegative for HSV-2 at base line and as a secondary end point in female subjects who were seronegative for both HSV-1 and HSV-2 at base line. Sample size was calculated on the basis of the following assumptions: an attack rate of genital herpes disease of 7 percent in female recipients of control injections who were seronegative for HSV-2, a dropout rate of 20 percent, vaccine efficacy of 70 percent, a type I error of 0.05, and a power of 80 percent. Data from each study were examined in intention-to-treat analyses.

Vaccine and Control Preparations

The glycoprotein-D-alum-MPL vaccine contained a truncated form of a recombinant HSV-2 glycoprotein D molecule, purified from Chinese-hamster-ovary cells transfected with a plasmid containing a glycoprotein D DNA fragment from the HSV-2 strain G. The antigen was adsorbed with 3-*O*-deacylated MPL onto alum. Each dose of vaccine contained 20 μ g of glycoprotein D, 50 μ g of MPL, and 500 μ g of alum. Control preparations were alum-MPL (in Study 1) and alum (in Study 2).

Vaccination and Design of the Studies

The studies were approved by human-investigations review committees at all centers, and subjects provided written informed consent. All subjects had a regular sexual partner (the "source partner") with clinically confirmed genital herpes, were randomly assigned to receive either vaccine or a control preparation by intramuscular injection in the deltoid area at months 0, 1, and 6, and were followed for a total of 19 months. The primary efficacy end point, the occurrence of genital herpes disease, was defined as genital signs or symptoms (e.g., pain, itching, swelling, papules, vesicles, ulcers, or crusts) with either a positive HSV culture or detection of HSV DNA by polymerase chain reaction (PCR) and HSV seroconversion. A secondary efficacy end point, HSV infection, was defined as genital herpes disease or asymptomatic seroconversion to HSV antigens not contained in the vaccine.

Visits were scheduled at months 0, 1, 6, 7, 13, and 19 and, in Study 1, also at months 4, 10, and 16. Blood samples for serologic analysis were obtained at all visits in Study 1 and at months 0, 7, and 19 in Study 2. Source partners agreed (after giving written informed consent) not to use suppressive antiviral therapy during Study 1; in Study 2, they were allowed to use such therapy.

Assessments of Efficacy

Subjects were advised regarding signs and symptoms of genital herpes and reduction in the risk of infection, including recommended use of condoms. Subjects recorded details of suspected episodes of herpes on diary cards and visited a clinic within 48 hours after the onset of signs or symptoms for a genital examination, collection of swab samples from lesions or the site of symptoms, and a blood sample for serologic testing. Swabs were tested by HSV culture; if the results were negative and the subject subsequently had seroconversion, a second swab was evaluated by PCR. Treatment with antiviral therapy was allowed if the diagnosis of genital herpes was confirmed by physical examination.

Assessments of Safety

Diary cards documented symptoms at the site of the injection and general symptoms that occurred during the three days after each injection. Subjects reported adverse events for 30 days after each injection and serious adverse events that occurred at any time during the trial.

Trial Design, Data Management, and Data and Safety Monitoring Board

The trial designs were developed by protocol teams that included academic investigators as well as clinical-trial specialists and scientists at GlaxoSmithKline Biologicals. Data were gathered by the study investigators and transmitted to the sponsor, who was responsible for maintaining the data base and performing analyses according to a prespecified plan. Supervision of data management and analyses was a responsibility shared by a panel of investigators, the data and safety monitoring board, and the sponsor. The board was also responsible for oversight of the studies and for final categorization of all cases before unblinding. On the advice of the data and safety monitoring board, no interim analyses were performed in either study. The manuscript was written by a committee consisting of the academic investigators and a single representative of the sponsor; all investigators involved in writing the paper had full and unfettered access to the data.

Laboratory Methods

Base-line HSV serologic status and seroconversion to HSV antigens not contained in the vaccine were evaluated by validated Western blot assays (Study 1) and enzyme-linked immunosorbent assays (ELISAs) for anti-glycoprotein-G1 and anti-glycoprotein-G2 antibodies (Study 2). These assays were similar to standard type-specific HSV serologic assays.¹⁶ For the Western blot assays, a blot was considered to be positive if two of three bands (for glycoprotein B, virion polypeptide 5, and infected cell protein-35) were visualized. The locations of the bands for anti-glycoprotein-D antibodies were obscured in order to maintain blinding.

Viral cultures were performed at qualified laboratories with the use of standard techniques. PCR analysis of HSV DNA was performed according to the method of Kimura et al.¹⁷ The presence or absence of a humoral response to the vaccine was determined by ELISA for anti-glycoprotein-D antibodies and standard HSV-2 neutralization assays. Peripheral-blood lymphocytes were collected before and after vaccination, frozen in liquid nitrogen, incubated with various concentrations of glycoprotein-D antigen, and assayed to assess the incorporation of tritium-labeled thymidine or secretion of interferon- γ into cell supernatants.

Statistical Analysis

The primary end point was the occurrence of genital HSV disease in all subjects in Study 1 and in HSV-2-seronegative female subjects in Study 2. The log-rank test was used to compare Kaplan-Meier survival curves for the time to occurrence of genital herpes disease (for months 0 through 19). Data on time to events were censored at the time of the subject's last known status. Analyses were performed according to sex and initial HSV serologic status. Vaccine efficacy, with two-sided 95 percent confidence intervals, was estimated by Cox regression.

Fisher's exact tests were used to compare the treatment groups in terms of the attack rates of infection. Vaccine efficacy was defined as the percentage reduction in the frequency of the end point among recipients of vaccine as compared with the frequency among recipients of control injections; it was calculated as $1 - (\text{the attack rate among recipients of vaccine} \div \text{the attack rate among recipients of control injections})$, with two-tailed 95 percent confidence intervals, according to the Cochran-Mantel-Haenszel method. All reported P values are two-sided. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Demographic Characteristics

A total of 847 subjects who were seronegative for HSV-1 and HSV-2 (in Study 1) and 1867 subjects who were seronegative for HSV-2 (in Study 2) underwent

randomization and received injections. The groups were similar at randomization in terms of all demographic characteristics (Table 1). In Study 2, 200 female subjects (96 in the vaccine group and 104 in the control group) and 354 male subjects (187 in the vaccine group and 167 in the control group) were seronegative for both HSV-1 and HSV-2 at base line. The demographic characteristics of these subjects were similar to those of the entire cohort of HSV-2-seronegative subjects, except that they had a shorter mean duration of relationship with source partners (26 months among male subjects and 23 months among female subjects).

Compliance and Follow-up

The rate of compliance with study procedures was similar in the two treatment groups in each study (Table 2), as was the dropout rate, with about 80 percent of randomized subjects receiving all three doses and completing the final study visit. Data collected through the dates on which the data bases were closed (April

1998 for Study 1 and April 2000 for Study 2) are included in the analyses.

Vaccine Efficacy

The attack rates of newly acquired genital herpes disease and HSV infection are shown in Table 3. In Study 1, we did not observe significant efficacy of the vaccine against the acquisition of genital herpes in subjects who were seronegative for HSV-1 and HSV-2 at base line (efficacy, 38 percent [95 percent confidence interval, -18 to 68]; P=0.14). Cox regression analysis revealed a statistically significant interaction between sex and treatment group (P=0.04) for the efficacy analysis. Time-to-event analyses indicated that the vaccine was efficacious against genital herpes disease in female subjects (efficacy, 73 percent; 95 percent confidence interval, 19 to 91; P=0.01) but not in male subjects (efficacy, -11 percent; 95 percent confidence interval, -161 to 53; P=0.81). Survival curves showing the time to genital herpes disease are presented in Figure 1.

TABLE 1. BASE-LINE DEMOGRAPHIC CHARACTERISTICS OF THE INTENTION-TO-TREAT POPULATION.*

VARIABLE	WOMEN IN STUDY 1		WOMEN IN STUDY 2		MEN IN STUDY 1		MEN IN STUDY 2	
	VACCINE GROUP (N=137)	CONTROL GROUP (N=131)	VACCINE GROUP (N=348)	CONTROL GROUP (N=362)	VACCINE GROUP (N=288)	CONTROL GROUP (N=291)	VACCINE GROUP (N=575)	CONTROL GROUP (N=582)
Age (yr)								
Mean	30	30	32	32	33	32	34	34
Range	18-45	18-45	18-59	18-47	18-45	18-46	18-45	18-52
Race (%)								
White	92	94	91	93	94	97	93	94
Black	1	1	1	1	3	1	2	3
Other	7	5	8	7	3	2	5	3
Sexual orientation (%)†								
Heterosexual	97	93	98	97	98	99	98	97
Homosexual or bisexual	3	7	2	3	2	1	2	3
Mean duration of relationship with source partner (mo)‡	24	24	32	31	34	31	34	34
Condom use (%)§								
Never	27	32	36	33	40	38	41	42
Sometimes (<50% of the time)	31	29	31	33	24	31	26	26
Usually (≥50% of the time)	17	15	13	15	15	15	12	13
Always	26	24	20	19	21	16	21	19
Use of antiviral therapy by partner (%)¶								
Yes	9	12	50	50	15	14	63	58
No	91	88	50	50	85	86	37	42

*All subjects in Study 1 were seronegative for herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2). Data for Study 2 include all subjects who were seronegative for HSV-2 at entry. For some variables, percentages may not add up to 100 because of rounding.

†Data were missing for two men in the vaccine group in Study 1 and two men in the control group in Study 1.

‡Data were missing for 15 women in the vaccine group in Study 2, 8 women in the control group in Study 2, 1 man in the vaccine group in Study 1, 14 men in the vaccine group in Study 2, and 13 men in the control group in Study 2.

§Data were missing for one woman in the vaccine group in Study 2 and one man in the control group in Study 2.

¶Data were missing for 1 woman in the control group in Study 1, 11 women in the vaccine group in Study 2, 11 women in the control group in Study 2, 2 men in the vaccine group in Study 1, 2 men in the control group in Study 1, 19 men in the vaccine group in Study 2, and 18 men in the control group in Study 2.

TABLE 2. RATES OF IMMUNIZATION AND STUDY COMPLETION.*

VARIABLE	STUDY 1		STUDY 2	
	VACCINE GROUP	CONTROL GROUP	VACCINE GROUP	CONTROL GROUP
	number (percent)			
Women				
Dose 1	137 (100.0)	131 (100.0)	348 (100.0)	362 (100.0)
Dose 2	133 (97.1)	126 (96.2)	338 (97.1)	351 (97.0)
Dose 3	121 (88.3)	113 (86.3)	316 (90.8)	333 (92.0)
Completed final study visit	107 (78.1)	106 (80.9)	280 (80.5)	285 (78.7)
Dropped out	30 (21.9)	25 (19.1)	68 (19.5)	77 (21.3)
Adverse event	1 (0.7)	0	4 (1.1)	5 (1.4)
Withdrawal of consent	5 (3.6)	7 (5.3)	16 (4.6)	7 (1.9)
Lost to follow-up or moved away	17 (12.4)	15 (11.5)	40 (11.5)	57 (15.7)
Other	7 (5.1)	3 (2.3)	8 (2.3)	8 (2.2)
Men				
Dose 1	288 (100.0)	291 (100.0)	575 (100.0)	582 (100.0)
Dose 2	279 (96.9)	281 (96.6)	558 (97.0)	564 (96.9)
Dose 3	262 (91.0)	266 (91.4)	527 (91.7)	533 (91.6)
Completed final study visit	237 (82.3)	247 (84.9)	455 (79.1)	470 (80.8)
Dropped out	51 (17.7)	44 (15.1)	120 (20.9)	112 (19.2)
Adverse event	2 (0.7)	1 (0.3)	3 (0.5)	2 (0.3)
Withdrawal of consent	12 (4.2)	8 (2.7)	13 (2.3)	15 (2.6)
Lost to follow-up or moved away	34 (11.8)	30 (10.3)	102 (17.7)	94 (16.2)
Other	3 (1.0)	5 (1.7)	2 (0.3)	1 (0.2)

*All subjects in Study 1 were seronegative for HSV-1 and HSV-2 at entry. Data for Study 2 include all subjects who were seronegative for HSV-2 at entry. In Study 1, mean follow-up was 16.4 months among women in the vaccine group, 16.6 months among women in the control group, 16.9 months among men in the vaccine group, and 17.2 months among men in the control group. In Study 2, mean follow-up was 16.7 months among women in the vaccine group, 16.9 months among women in the control group, 16.8 months among men in the vaccine group, and 16.9 months among men in the control group.

In Study 2, we did not observe significant efficacy of the vaccine against the acquisition of genital herpes disease in HSV-2-seronegative female subjects (efficacy, 42 percent; 95 percent confidence interval, -31 to 74; $P=0.19$). However, subgroup analysis indicated that the vaccine had significant efficacy in female subjects seronegative for both HSV-1 and HSV-2 (efficacy, 74 percent; 95 percent confidence interval, 9 to 93; $P=0.02$) but not in female subjects who were seropositive for HSV-1 and seronegative for HSV-2 at base line (efficacy, -106 percent; 95 percent confidence interval, -723 to 49; $P=0.30$) or in HSV-2-seronegative male subjects (efficacy, -10 percent; 95 percent confidence interval, -127 to 47; $P=0.80$). Survival curves showing the time to genital herpes disease are presented in Figure 2.

Although they were not statistically significant, both studies showed trends toward protection against HSV infection in female subjects who were seronegative for HSV-1 and HSV-2. Vaccine efficacy against HSV infection in Study 1 was 46 percent (95 percent confidence interval, -2 to 71; $P=0.08$) among female subjects, as compared with -7 percent (95 percent

confidence interval, -108 to 45; $P=0.86$) among male subjects; efficacy against infection in Study 2 was 39 percent (95 percent confidence interval, -6 to 65; $P=0.08$) among female subjects who were seronegative for HSV-1 and HSV-2, as compared with -19 percent (95 percent confidence interval, -128 to 38; $P=0.70$) among male subjects who were seronegative for HSV-1 and HSV-2.

The glycoprotein-D-alum-MPL vaccine elicited binding and neutralizing antibodies against HSV and glycoprotein-D-specific responses in the form of lymphoproliferation and interferon- γ secretion (data not shown). Results were similar among male subjects and female subjects.

Adverse Events

The vaccine was generally well tolerated. Although the majority of doses of vaccine were followed by soreness at the site of the injection, most symptoms were mild to moderate. The frequency of soreness at the injection site severe enough to prevent subjects from engaging in normal activities was higher among recipients of the vaccine (5 percent in both studies) than

TABLE 3. ATTACK RATES OF AND VACCINE EFFICACY AGAINST NEWLY ACQUIRED GENITAL HERPES DISEASE AND HERPES SIMPLEX VIRUS INFECTION.*

VARIABLE	ALL SUBJECTS IN STUDY 1		ALL SUBJECTS IN STUDY 2		SUBJECTS SERONEGATIVE FOR HSV-1 AND HSV-2 AT ENTRY IN STUDY 2	
	VACCINE GROUP	CONTROL GROUP	VACCINE GROUP	CONTROL GROUP	VACCINE GROUP	CONTROL GROUP
Overall						
Total no.	425	422	923	944	283	271
No. with disease	15	24	24	30	9	20
Attack rate of disease — % (95% CI)	3.5 (1.7 to 5.3)	6.0 (3.7 to 8.5)	3.0 (1.8 to 4.2)	3.6 (2.3 to 4.9)	3.4 (1.2 to 5.6)	8.3 (4.8 to 11.8)
Vaccine efficacy against disease — % (95% CI)	38 (-18 to 68)	—	18 (-41 to 52)	—	57 (6 to 81)	—
No. with infection	30	39	71	84	34	43
Attack rate of infection — % (95% CI)	7.1 (4.8 to 9.9)	9.2 (6.6 to 12.4)	8.6 (6.8 to 10.7)	9.7 (7.8 to 11.8)	13.7 (9.6 to 18.6)	17.7 (13.1 to 23.1)
Vaccine efficacy against infection — % (95% CI)	24 (-21 to 52)	—	11 (-20 to 34)	—	23 (-17 to 49)	—
Women						
Total no.	137	131	348	362	96	104
No. with disease	4	14	9	16	3	12
Attack rate of disease — % (95% CI)	3.1 (0.1 to 6.1)	11.6 (5.9 to 17.4)	3.3 (1.1 to 5.4)	5.0 (2.6 to 7.5)	3.5 (0 to 7.4)	13.3 (6.3 to 20.3)
Vaccine efficacy against disease — % (95% CI)	73 (19 to 91)	—	42 (-31 to 74)	—	74 (9 to 93)	—
No. with infection	13	23	38	55	15	29
Attack rate of infection — % (95% CI)	9.5 (5.2 to 15.7)	17.6 (11.5 to 25.2)	12.3 (8.9 to 16.5)	16.3 (12.5 to 20.7)	18.3 (10.6 to 28.4)	29.9 (21.0 to 40.0)
Vaccine efficacy against infection — % (95% CI)	46 (-2 to 71)	—	25 (-11 to 49)	—	39 (-6 to 65)	—
Men						
Total no.	288	291	575	582	187	167
No. with disease	11	10	15	14	6	8
Attack rate of disease — % (95% CI)	3.7 (1.5 to 6.0)	3.7 (1.4 to 5.9)	2.8 (1.4 to 4.2)	2.7 (1.3 to 4.1)	3.3 (0.7 to 6)	5.3 (1.7 to 8.9)
Vaccine efficacy against disease — % (95% CI)	-11 (-161 to 53)	—	-10 (-127 to 47)	—	32 (-95 to 76)	—
No. with infection	17	16	33	29	19	14
Attack rate of infection — % (95% CI)	5.9 (3.5 to 9.3)	5.5 (3.2 to 8.8)	6.4 (4.5 to 8.9)	5.5 (3.7 to 7.7)	11.4 (7.0 to 17.2)	9.6 (5.3 to 15.6)
Vaccine efficacy against infection — % (95% CI)	-7 (-108 to 45)	—	-17 (-91 to 27)	—	-19 (-128 to 38)	—

*Data are for months 0 through 19 in the intention-to-treat population. All subjects in Study 1 were seronegative for herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) at entry. Data for Study 2 include all subjects who were seronegative for HSV-2 at entry. For newly acquired genital herpes disease, estimates of the attack rates and confidence intervals (CIs) were calculated with the Kaplan-Meier estimator, and estimates of vaccine efficacy and confidence intervals were determined by Cox regression. For HSV infection, attack rates were estimated as the proportion of subjects acquiring HSV infection between the month 0 study visit and the month 19 study visit, with exact 95 percent confidence intervals, and estimates of vaccine efficacy and confidence intervals were determined by the Cochran-Mantel-Haenszel method. Given the long duration of follow-up in Study 2 and the fact that blood samples were obtained at only two times after vaccination, some subjects with missing blood samples were excluded from the analysis, since infection could not be assessed. Therefore, the calculations of attack rates and vaccine efficacy for HSV infection are based on the following numbers of subjects who could be evaluated: subjects seronegative for HSV-1 and HSV-2 at entry, 249 recipients of the vaccine (167 men and 82 women) and 243 recipients of the control injections (146 men and 97 women); all subjects seronegative for HSV-2 at entry, 824 recipients of the vaccine (515 men and 309 women) and 869 recipients of the control injections (532 men and 337 women). Of the subjects who were seronegative for HSV-1 and HSV-2 at base line, three in the vaccine group in Study 1, three in the control group in Study 1, two in the vaccine group in Study 2, and two in the control group in Study 2 acquired HSV-1 disease during the course of the study; three subjects in the vaccine group in Study 1, three in the control group in Study 1, nine in the vaccine group in Study 2, and eight in the control group in Study 2 acquired HSV-1 infection during the course of the study.

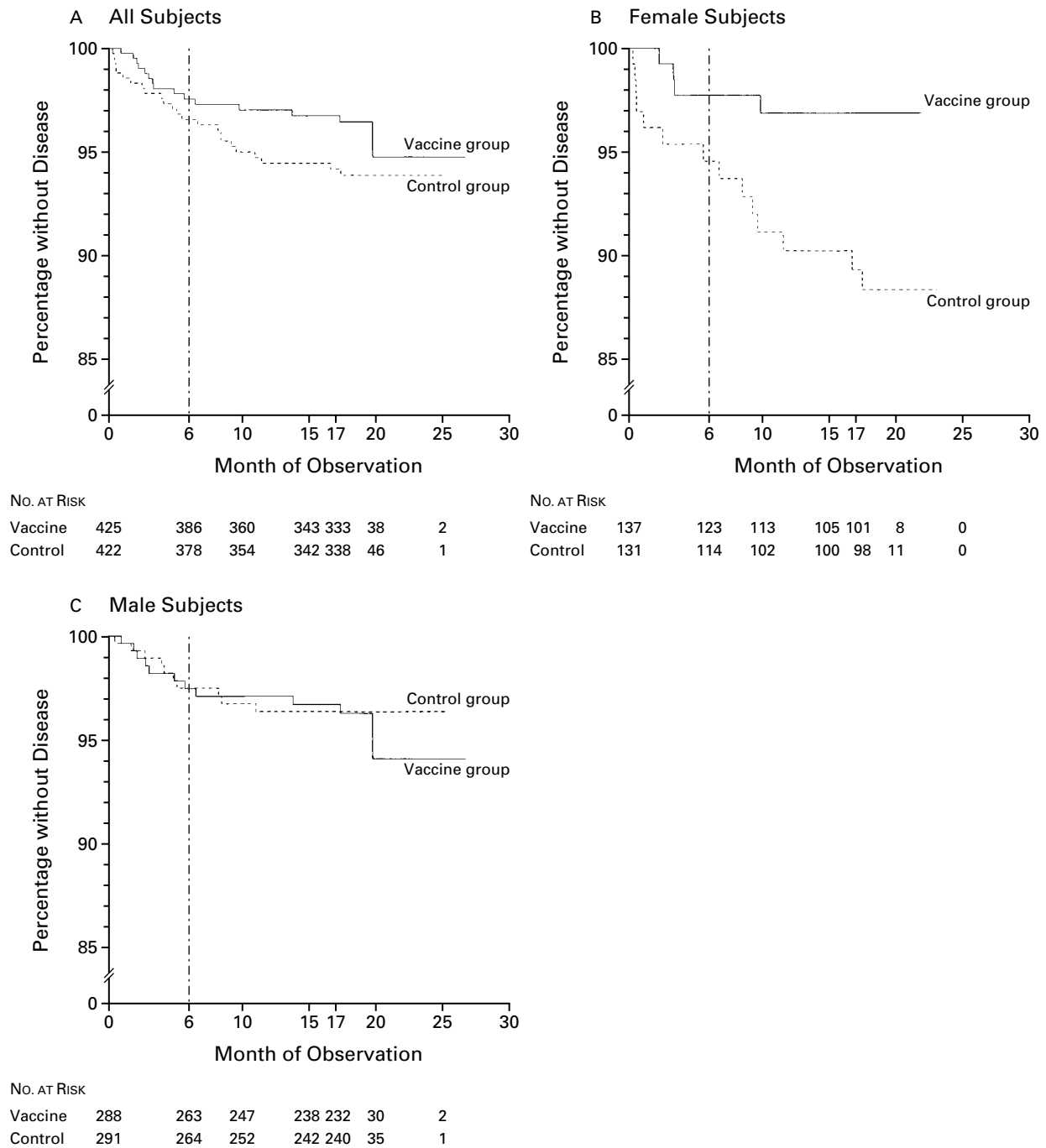


Figure 1. Kaplan–Meier Plots for Study 1, Showing Time to Occurrence of Genital Herpes Disease in Subjects Who Were Seronegative for Herpes Simplex Virus Type 1 (HSV-1) and Herpes Simplex Virus Type 2 (HSV-2) at Base Line. Broken vertical lines represent the scheduled time of the third and final vaccine or control injection.

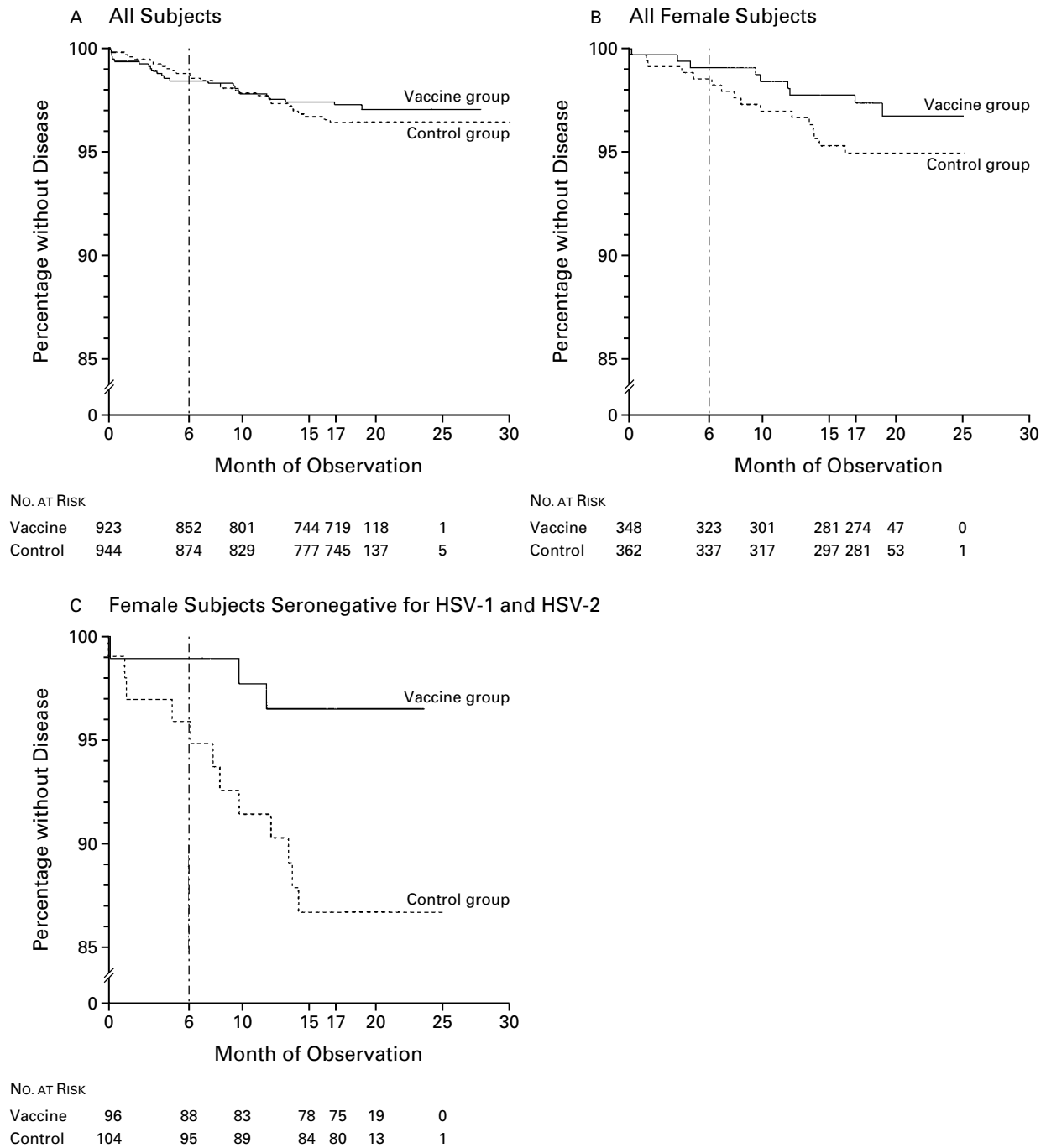


Figure 2. Kaplan–Meier Plots for Study 2, Showing Time to Occurrence of Genital Herpes Disease. Panels A and B are for subjects in Study 2 who were seronegative for herpes simplex virus type 2 (HSV-2) at base line; Panel C is for the subgroup of female subjects who were seronegative for herpes simplex virus type 1 (HSV-1) as well as for HSV-2 at base line. Broken vertical lines represent the scheduled time of the third and final vaccine or control injection.

among recipients of control injections (3 percent in Study 1 and 1 percent in Study 2). Other than local and general symptoms indicated on diary cards, there were no major differences between recipients of vaccine and recipients of control injections in the frequency and type of reported symptoms, and the dropout rates were similar in the two treatment groups. HSV-2-seropositive recipients of vaccine in Study 2 had a pattern of local and general symptoms similar to that in HSV-2-seronegative recipients of vaccine.

DISCUSSION

The glycoprotein-D-alum-MPL vaccine was immunogenic, safe, and well tolerated. The primary efficacy end point in Study 1 was occurrence of genital herpes disease in subjects seronegative for HSV-1 and HSV-2 at base line. Intention-to-treat analysis for months 0 through 19 demonstrated that the vaccine did not provide significant protection to the overall cohort. However, a post hoc subgroup analysis indicated that there was significant protection in female subjects but not in male subjects. These results led us to change the primary end point of Study 2 to efficacy of the vaccine in HSV-2-seronegative female subjects and to add the efficacy of the vaccine in HSV-1-seronegative and HSV-2-seronegative female subjects as a secondary end point. This decision was made before we examined the results of Study 2. Analysis of the intention-to-treat population for months 0 through 19 showed that the vaccine did not afford significant protection to all HSV-2-seronegative female subjects. However, the analysis according to baseline HSV serologic status indicated that there was significant protection in female subjects who were seronegative for both HSV-1 and HSV-2 at base line but not in female subjects who were seropositive for HSV-1 and seronegative for HSV-2 at base line. Similar results were obtained for both studies in a per-protocol analysis. Although the finding that the vaccine protected women who were seronegative for both types of HSV from acquiring genital herpes disease was not a prespecified outcome in Study 1, it was a prespecified outcome in Study 2. Although our findings are not definitive, the fact that similar results were obtained in both studies clearly suggests that the glycoprotein-D-alum-MPL vaccine can protect some women against symptomatic genital herpes.

In Study 2, recipients of control injections who were seropositive for HSV-1 and seronegative for HSV-2 at base line had a lower attack rate of genital HSV-2 disease than subjects who were seronegative for both types of HSV at base line, suggesting that previous infection with HSV-1 confers protection against acquisition of genital HSV-2 disease. This difference was more pronounced among female subjects (HSV-2 attack rate, 1.2 percent among HSV-1-seropositive fe-

male subjects and 11.9 percent among HSV-1-seronegative female subjects; $P < 0.001$ by Fisher's exact test) than among male subjects (attack rate, 1.5 percent among HSV-1-seropositive male subjects and 4.2 percent among HSV-1-seronegative male subjects; $P = 0.07$). However, a test (performed by logistic regression) of interaction between serologic status and sex in terms of the occurrence of genital HSV-2 disease did not reveal a significant interaction ($P = 0.12$).

Vaccines generally protect against disease, not infection.¹² In the case of genital herpes, protection against disease without a simultaneous reduction in the risk of latent infection and subsequent recurrent infections might benefit vaccinated women without reducing the epidemic spread of the virus. Administration of the glycoprotein-D-alum-MPL vaccine might result in protection against infection, symptomatic illness, and risk of transmission or it might result in protection against the development of signs and symptoms of genital herpes disease, with fewer recurrences (if it reduced the risk of or prevented latent infection) and thus a reduced risk of transmission; conversely, the vaccine might prevent symptoms without preventing the underlying infection, causing more episodes of unrecognized or asymptomatic infection, possibly resulting in an increased risk of transmission; or it might protect against neither disease nor infection. Modeling of the results of these trials suggests that widespread administration of this vaccine to women who are seronegative for both HSV-1 and HSV-2 could result in decreased spread of HSV-2 in the general population, including among men (unpublished data).

Although these trials were not initially designed to examine differences in the efficacy of the vaccine according to sex, we found marked differences between the efficacy in men and that in women. An understanding of the mechanism for this sex-specific protection could have implications for the development of vaccines against other sexually transmitted pathogens.

The biologic explanations for the finding are not clear. Possibly, sex differences in the pathogenesis of genital herpes — for example, differences in the portal of entry — could affect the effectiveness of the vaccine. For both men and women, an intact stratum corneum is a highly effective barrier against penetration by HSV. The presence of an intact stratum corneum over the external genitalia of circumcised men may explain the lower rate of HSV-2 seropositivity among men than among women with the same number of sexual partners.² Abrasions disrupting this layer may provide the principal portal of entry for HSV in men.

In women, acquisition of HSV is likely to occur through the vaginal-cervical mucous membrane, which has no stratum corneum. Secretions containing antibodies and migratory white cells constantly

bathe this membrane. HSV-specific responses induced by vaccination could act locally to provide an immunologic barrier to acquisition of infection at this mucosal site that is not applicable to men.

There may also be sex-specific differences in the induction of immune responses that are important for protection against HSV infection. Although no sex-specific differences in the measured immune responses were noted in our studies, there is growing evidence that with some infections, vaccinations, and autoimmune disorders, female subjects, human and animal, have enhanced immune responses by type 1 helper T (Th1) cells as compared with male subjects.¹⁸⁻²⁴ Induction of Th1-type responses, especially interferon- γ secretion, may be important for the control of HSV infection.^{12,25} Therefore, enhanced Th1 responses in women might account for the sex-specific differences observed in this study. Studies using a wider range of cytokines, especially interleukin-4, will be required in order to clearly differentiate a Th1 response to this vaccine (secretion of interferon- γ but not interleukin-4) from a response by precursors of Th1 and type 2 helper T (Th2) cells (designated as Th0) (secretion of interferon- γ and interleukin-4).

In Study 2, an apparent lack of protection among HSV-1-positive women was observed. This apparent lack may be due to immunity that results from protection against HSV-2 genital herpes disease provided by previous HSV-1 infection,^{26,27} which is not enhanced by the glycoprotein-D-alum-MPL vaccine.

The effectiveness of this vaccine differs from that of another vaccine containing two recombinant HSV-2 glycoproteins, glycoprotein B and glycoprotein D, combined with the adjuvant MF59.²⁶ Although the glycoprotein-B-glycoprotein-D vaccine induced high titers of neutralizing antibodies, it was ineffective in protecting subjects from acquiring HSV-2 infection. Differences in the adjuvant composition might have contributed to the differences in the effectiveness of the vaccines. MF59 has been shown to induce a more Th2-type response, biased toward the production of neutralizing antibody, in vaccinated mice,²⁸ whereas alum-MPL has been reported to induce a more Th1-type response in vaccinated animals and humans.²⁸⁻³² Studies in animals and analysis of human responses to recurrent infections suggest that Th1 responses involving CD4 and CD8 lymphocyte function may be more important than neutralizing antibody alone in the control of initial HSV infection.^{25,33,34}

Supported by GlaxoSmithKline Biologicals, Rixensart, Belgium, and GlaxoSmithKline Pharmaceuticals, Collegeville, Pa.

Drs. Slaoui, Denis, Vandepapeliere, and Dubin are employees of GlaxoSmithKline. Dr. Stanberry reports having consulted for or received lecture fees or research support from GlaxoSmithKline, Novartis, Starpharma, Eli Lilly, and Cantab Pharmaceuticals. Dr. Spruance reports having consulted for or received lecture fees or research support from GlaxoSmithKline, 3M, Berlin-Chemie, Novactyl, Biophoretics, Redox, and RxKinetics. Dr. Cun-

ingham reports having consulted for GlaxoSmithKline and 3M. Dr. Bernstein reports having consulted for or received lecture fees or research support from GlaxoSmithKline, 3M, Viridae, Wyeth-Ayerst, Aviron, Aventis, MedImmune, and American Home Products. Dr. Mindel reports having received research support from GlaxoSmithKline. Dr. Sacks reports having consulted for or received lecture fees or research support from GlaxoSmithKline, Novartis, 3M, Eli Lilly, Epicyte, Schering, and Starpharma. Dr. Tyring reports having received lecture fees and research support from GlaxoSmithKline. Dr. Aoki reports having consulted for or received lecture fees or research support from GlaxoSmithKline, 3M, and Roche. Dr. Dubin reports having received royalty payments from Wyeth Vaccines on a patent covering a candidate HSV vaccine in preclinical development.

We are indebted to all the members of the data and safety monitoring board for their review of the safety data and approval of final case assignments: Gun-Britt Löwhagen, Sahlgrenska Sjukhuset, Göteborg, Sweden; Kathy Edwards, Vanderbilt University Medical Center, Nashville; Theodore Eickhoff, University of Colorado Health Science Center, Denver; Allan Ronald, University of Manitoba, Winnipeg, Canada; and Ronald Marks, University of Florida, Gainesville. We are also indebted to Charles Lacey (Department of Genito-Urinary Medicine, Imperial College School of Medicine, Jefferiss Research Trust Laboratories, London) and Laurie Scott (Fort Lauderdale Perinatal Associates, Plantation, Fla.) for their assistance with the study design and interpretation of data; to Martin Myers (University of Texas Medical Branch, Galveston) for his contributions to the early stages of the project; to the research coordinators and study nurses for their support of these studies; and to Dr. Carys Calvert and Dr. Michelle Derbyshire for their assistance in the preparation of the manuscript.

APPENDIX

In addition to the authors, the following persons are members of the GlaxoSmithKline Herpes Vaccine Efficacy Study Group. *Australia and New Zealand:* J. Chuah, Gold Coast Sexual Health Clinic, Miami, Queensland; I. Denham, Melbourne Sexual Health Centre, Melbourne; S. Garland, Royal Woman's Hospital, Carlton, Victoria; R. Waddell, Royal Adelaide Hospital, Adelaide; M. Reid, Auckland Sexual Health Service, Grafton, Auckland, New Zealand. (Laboratory support: J. Taylor, Westmead Millennium Institute, Westmead. GlaxoSmithKline International, Dandenong, Victoria: G. Fearn, T. Ruff, S. Waite, D. Cramer.) *Italy:* A. Alinovi, Università degli Studi, Parma; R. Caputo, Clinica Dermatologica Policlinico, Milan; M. Negosanti, Ospedale S. Orsola-Malpighi, Bologna; A. Rebor, Università degli Studi di Genova, Genoa; G. Zucatti, Università degli Studi di Firenze, Florence. (GlaxoSmithKline Pharmaceuticals, Milan: R. Soncini and O. Frisca.) *United Kingdom:* R.J.C. Gilson, Academic Department of Genito-Urinary Medicine, Mortimer Market, London; T. McManus, Genito-Urinary Medicine Clinic, Camberwell, London. (GlaxoSmithKline Pharmaceuticals, United Kingdom Medical Department, Mundells, Welwyn Garden City: S. Baxendale, H. Tyrrell.) *United States:* K. Beutner, Solano Dermatology Associates, Vallejo, Calif.; M. Blatter, Pittsburgh Pediatric Research, Pittsburgh; M. Cabezas-Mijuste and R. Ortiz-Nalasco, San Juan Clinical Institute, Carolina, P.R.; D. Campbell, Family Medicine Research Center, St. Louis; R. Codario and D. Ginsberg, Concord Clinical Research, Philadelphia; D. Crosby, Medical College of Wisconsin Clinic at Froedtert, Milwaukee; M. Dreihobl, Scripps Clinic, San Diego, Calif.; R. Ettinger and K. Harless, Bend Medical Clinic, Bend, Oreg.; K. Fife, Indiana University, Indianapolis; A. Fisher, Omega Medical Research, Providence, R.I.; S. Fowler, Medical University of South Carolina, Charleston; R.G. Free, Scirex, Hartford, Conn.; H. Friedman, University of Pennsylvania, Philadelphia; H.H. Handsfield, Harborview Medical Center, Seattle; C. Harrison, S. Chartrand, and A. Chatterjee, Creighton University Medical Center, Omaha, Neb.; D. Havlicek and G. Stein, Michigan State University, East Lansing; D. Herman, Philadelphia Health Associates, Philadelphia; J.A. Holmes and F.E. Bustillo, Heart of America Research Institute, Mission, Kans.; A.K. Huang, University of Louisville, Louisville, Ky.; A.P. Korn, San Francisco General Hospital, San Francisco; R. Koup and J. Radolf, University of Texas Southwestern Health Science Center, Dallas; T. Kurtz, L.M. Leonard Research Institute, Des Moines, Iowa; W. Lang, ViRx, San Francisco; P. Lee, University of Texas Medical Branch Center for Clinical Studies, Nassau Bay; M. Levin, University Hospital, Denver; F. Maggiacomo, Silver Lake Medical Center, Providence, R.I.; G. Mertz, University of New Mexico, Albuquerque; L. Marr, C. Thoming, and T. Warren, Westover Heights Clinic, Portland, Oreg.; C.A. Patterson-Barnett, InSite Clinical Trials, Atlanta; D. Picquadio and S. Smith, Therapeutics, La Jolla, Calif.; J. Price, Family Medicine

of St. Louis, St. Louis; H. Resnick, R/D Clinical Research, Lake Jackson, Tex.; M. Sack, Center for Clinical Research, Austin, Tex.; B. Sha, Rush Medical College, Chicago; J. Shanley, University of Connecticut Health Center, Farmington; V. Sikand, Charter Oak Medical Center, East Lyme, Conn.; M. Spellman, Veterans Affairs Medical Center, San Diego, Calif.; J. Stern, Riverview Medical Associates, New York; R. Tucker, Wenatchee Valley Clinic, Wenatchee, Wash.; M. Weinstein, VIP Research, San Antonio, Tex.; R. Wolff, Radiant Research, St. Louis; B. Schwartz, J. Baggish, and A. Yataco, Innovative Medical Research, Towson, Md. (Laboratory support: R. Pyles, A. Zalar, Children's Hospital Medical Center, Cincinnati. GlaxoSmithKline, Collegeville, Pa.: O. Crayne, T. Mayewski, D. Parenti, K. Seddon, D. Sullivan, D. Verity, P. Goldberg, and D. Krause; IBIS: F. Rose, B. Innis, K. McCann). *Canada*: F. Diaz-Mitoma, Herridge Community Health Clinic, Ottawa, Ont.; A. Haase, Centre for Clinical Research, Halifax, N.S.; G.W. Hammond, D. Iwanoczek, P.H. Orr, S. Sanche, and J.N. Simonsen, University of Manitoba, Winnipeg; M. Lassonde, Hôpital Notre-Dame, Montreal; E. Lefebvre, Clinique Medicale L'actuel, Montreal; J. Mendelson, Jewish General Hospital, Montreal; K. Papp, Probit Medical Research, Waterloo, Ont.; B. Romanowski, University of Alberta, Edmonton; G. Sibbald, Psoriasis Education and Research Centre, Toronto; C. St. Pierre, Novabyss, Sherbrooke, Que.; S. Trotter, Centre Hospitalier Universitaire de Quebec, Ste. Foy; P.F. Whitsitt, Optimum Clinical Research, Oshawa, Ont.; K. Williams, Royal University Hospital, Saskatoon, Sask. (GlaxoSmithKline Pharmaceuticals: R. Anstey, G. Grande, J. Parsons, T. Russell, and S. Sobrian.) *Belgium*: GlaxoSmithKline Biologicals, Rixensart: K. Ahrens, K. Amfo, A. Bollaerts, G. Van den Bossche, J.-L. Bredart, C. Calvert, M. Derbyshire, C. Barnich, F. Collard, C. Carton, T. Darcis, J. Deman, P. Gerard, D. Gheysen, V. Grek, M. Koutsoukos, M.-T. Martin, M. Meaders, V. Melot, F. Meurice, T. Neven, V. Noiret, B. Tyrell, F. Van der Schueren; Medisearch International, Mechelen: M. Mergaerts and B. Van der Taelen. *Germany*: Kendle, Munich: J. Barthel, F. Beier, K. Honig-Blum, S. Laukamp, E. Sennewald, G. Stiegler, and T. Wagner.

REFERENCES

- Reeves WC, Corey L, Adams HG, Vontver LA, Holmes KK. Risk of recurrence after first episodes of genital herpes: relation to HSV type and antibody response. *N Engl J Med* 1981;305:315-9.
- Langenberg A, Benedetti J, Jenkins J, Ashley R, Winter C, Corey L. Development of clinically recognizable genital lesions among women previously identified as having "asymptomatic" herpes simplex virus type 2 infection. *Ann Intern Med* 1989;110:822-7.
- Koutsky LA, Stevens CE, Holmes KK, et al. Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. *N Engl J Med* 1992;326:1533-9.
- Skoldenberg B, Jeansson S, Wolontis S. Herpes simplex virus type 2 and acute aseptic meningitis: atypical features of cases with isolation of herpes simplex virus from cerebrospinal fluids. *Scand J Infect Dis* 1975;7:227-32.
- Corey L, Spear PG. Infections with herpes simplex viruses. *N Engl J Med* 1986;314:749-57.
- Carney O, Ross E, Bunker C, Ikkos G, Mindel A. A prospective study of the psychological impact on patients with a first episode of genital herpes. *Genitourin Med* 1994;70:40-5.
- Mindel A. Psychological and psychosexual implications of herpes simplex virus infections. *Scand J Infect Dis Suppl* 1996;100:27-32.
- Patel R, Tyring S, Strand A, Price MJ, Grant DM. Impact of suppressive antiviral therapy on the health related quality of life of patients with recurrent genital herpes infection. *Sex Transm Infect* 1999;75:398-402.
- Fleming DT, McQuillan GM, Johnson RE, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med* 1997;337:1105-11.
- Cunningham AL, Mindel A, Dwyer DE. Epidemiology of sexually transmitted diseases. In: Stanberry LR, Bernstein DI, eds. Sexually transmitted diseases: vaccines, prevention and control. San Diego, Calif.: Academic Press, 2000:3-42.
- af Geijerstam V, Wang Z, Lewensohn-Fuchs I, et al. Trends in seroprevalence of human papillomavirus type 16 among pregnant women in Stockholm, Sweden, during 1969-1989. *Int J Cancer* 1998;76:341-4.
- Stanberry LR, Cunningham AL, Mindel A, et al. Prospects for control of herpes simplex virus disease through immunization. *Clin Infect Dis* 2000;30:549-66.
- Thoenen S, De Clercq N, Tornieporth N. A prophylactic hepatitis B vaccine with a novel adjuvant system. *Vaccine* 2001;19:2400-3.
- Leroux-Roels G, Moreau E, Desombere I, et al. Persistence of humoral and cellular immune response and booster effect following vaccination with herpes simplex (gD2t) candidate vaccine with MPL. In: Program and abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., October 4-7, 1994. Washington, D.C.: American Society for Microbiology, 1994:205. abstract.
- Francotte M, Pala P, Van Opstal O, et al. Comparative safety and immunogenicity of two adjuvant formulations of an HSV-2 glycoprotein D based subunit vaccine in man. In: Abstracts of the 18th International Herpesvirus Workshop, Pittsburgh, July 25-30, 1993. Pittsburgh: University of Pittsburgh, 1993:C38. abstract.
- Ashley RL, Wald A. Genital herpes: review of the epidemic and potential use of type-specific serology. *Clin Microbiol Rev* 1999;12:1-8.
- Kimura H, Shibata M, Kuzushima K, Nishikawa K, Nishiyama Y, Morishima T. Detection and direct typing of herpes simplex virus by polymerase chain reaction. *Med Microbiol Immunol (Berl)* 1990;179:177-84.
- Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. *Science* 1999;283:1277-8.
- Su Z, Stevenson MM. Central role of endogenous gamma interferon in protective immunity against blood-stage Plasmodium chabaudi AS infection. *Infect Immun* 2000;68:4399-406.
- Wilcoxon SC, Kirkman E, Dowdell KC, Stohlman SA. Gender-dependent IL-12 secretion by APC is regulated by IL-10. *J Immunol* 2000;164:6237-43.
- Kim S, Voskuhl RR. Decreased IL-12 production underlies the decreased ability of male lymph node cells to induce experimental autoimmune encephalomyelitis. *J Immunol* 1999;162:5561-8.
- Han X, Lundberg P, Tanamachi B, Openshaw H, Longmate J, Cantin E. Gender influences herpes simplex virus type 1 infection in normal and gamma interferon-mutant mice. *J Virol* 2001;75:3048-52. [Erratum, *J Virol* 2001;75:7789.]
- Barna M, Komatsu T, Bi Z, Reiss CS. Sex differences in susceptibility to viral infection of the central nervous system. *J Neuroimmunol* 1996;67:31-9.
- Wunderlich F, Maurin W, Benten WP, Schmitt-Wrede HP. Testosterone impairs efficacy of protective vaccination against P. chabaudi malaria. *Vaccine* 1993;11:1097-9.
- Deshpande SP, Kumaraguru U, Rouse BT. Why do we lack an effective vaccine against herpes simplex virus infections? *Microbes Infect* 2000;2:973-8.
- Corey L, Langenberg AG, Ashley R, et al. Recombinant glycoprotein vaccine for the prevention of genital HSV-2 infection: two randomized controlled trials. *JAMA* 1999;282:331-40.
- Langenberg AG, Corey L, Ashley RL, Leong WP, Straus SE. A prospective study of new infections with herpes simplex virus type 1 and type 2. *N Engl J Med* 1999;341:1432-8.
- Singh M, Carlson JR, Briones M, et al. A comparison of biodegradable microparticles and MF59 as systemic adjuvants for recombinant gD from HSV-2. *Vaccine* 1998;16:1822-7.
- Hermant P, Fabry L, Garcon N, et al. Effect of adjuvants on the immunogenicity of a recombinant herpes simplex virus glycoprotein D vaccine in the guinea pig model of HSV-2 infection. In: Abstracts of the 17th International Herpesvirus Workshop, Edinburgh, Scotland, August 1-7, 1992:376. abstract.
- Hermant P, Fabry L, Garcon N, et al. Comparison of adjuvant efficacy of a recombinant herpes simplex virus glycoprotein D vaccine in primates. In: Abstracts of the 17th International Herpesvirus Workshop, Edinburgh, Scotland, August 1-7, 1992:388. abstract.
- Bastin C, Hermant P, Francotte M, Garcon N, Slaoui M, Pala P. Synergistic association of adjuvants QS21 and MPL for induction of cytolytic T-lymphocytes and T-helper responses to recombinant protein antigens. In: Program and abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., October 4-7, 1994. Washington, D.C.: American Society for Microbiology, 1994:26. abstract.
- Moore A, McCarthy L, Mills KH. The adjuvant combination monophosphoryl lipid A and QS21 switches T-cell responses induced with a soluble recombinant HIV protein from Th2 to Th1. *Vaccine* 1999;17:2517-27.
- Cunningham AL, Mikloska Z. The Holy Grail: immune control of human herpes simplex virus infection and disease. *Herpes* 2001;8:Suppl 1:6A-10A.
- Simmons A, Nash AA. Zosteriform spread of herpes simplex virus as a model of recrudescence and its use to investigate the role of immune cells in prevention of recurrent disease. *J Virol* 1984;52:816-21.

Copyright © 2002 Massachusetts Medical Society.