A VACCINE CONSISTING OF RECOMBINANT *BORRELIA BURGDORFERI* OUTER-SURFACE PROTEIN A TO PREVENT LYME DISEASE

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ABSTRACT

Background Lyme disease is a multisystem inflammatory disease caused by infection with the tick-borne spirochete *Borrelia burgdorferi* and is the most common vector-borne infection in the United States. We assessed the efficacy of a recombinant vaccine consisting of outer-surface protein A (OspA) without adjuvant in subjects at risk for Lyme disease.

Methods For this double-blind trial, 10,305 subjects 18 years of age or older were recruited at 14 sites in areas of the United States where Lyme disease was endemic; the subjects were randomly assigned to receive either placebo (5149 subjects) or 30 μg of OspA vaccine (5156 subjects). The first two injections were administered 1 month apart, and 7515 subjects also received a booster dose at 12 months. The subjects were observed for two seasons during which the risk of transmission of Lyme disease was high. The primary end point was the number of new clinically and serologically confirmed cases of Lyme disease.

Results The efficacy of the vaccine was 68 percent in the first year of the study in the entire population and 92 percent in the second year among the 3745 subjects who received the third injection. The vaccine was well tolerated. There was a higher incidence of mild, self-limited local and systemic reactions in the vaccine group, but only during the seven days after vaccination. There was no significant increase in the frequency of arthritis or neurologic events in vaccine recipients.

Conclusions In this study, OspA vaccine was safe and effective in the prevention of Lyme disease. (N Engl J Med 1998;339:216-22.)

LYME disease is a multisystem inflammatory disease caused by *Borrelia burgdorferi*, transmitted by the bite of infected ixodes ticks. *B. burgdorferi* elicits an antibody response to a repertoire of borrelial proteins, including outer-surface protein C (OspC) and flagellin. In the early stage of the disease, free antibodies to OspC and flagellin are measurable, whereas only later are responses to outer-surface protein A (OspA) — a surface lipoprotein — demonstrable in standard assays.[1] The protective role of antibodies to *B. burgdorferi* has been explored in animal models of Lyme disease.[2] Findings indicated that antibodies against OspA were protective,[3-8] making this antigen a likely candidate for a vaccine.[9-13]

Early clinical trials demonstrated that recombinant OspA was immunogenic and well tolerated,[14,15] even in subjects with a history of Lyme disease.[16] A dose-ranging study showed that as compared with doses of 1, 5, and 10 μg, a dose of 30 μg of the OspA vaccine elicited an optimal antibody response without causing an increased rate of symptomatic reactions. We conducted a multicenter, randomized, double-blind, placebo-controlled study in areas of the United States in which Lyme disease is endemic to evaluate the protective efficacy of a 30-μg dose of vaccine in adults at risk for *B. burgdorferi* infection.

METHODS

Vaccine Preparation

The vaccine consists of purified OspA lipoprotein produced as described previously.[14,17] The lipoprotein was expressed in *Escherichia coli* and purified from the Triton X-114 detergent phase of chromatography with two ion-exchange resins: diethylaminoethyl–Sephacel and S-Sepharose. Sodium dodecyl sulfate–polyacrylamide-gel electrophoresis of the material purified with use of S-Sepharose indicated that OspA constitutes approximately 92 to 95 percent of the total protein. The material contained less than 0.05 endotoxin unit per microgram of OspA on testing with the limulus amebocyte lysate assay[18] and was nonpyrogenic in rabbits at a dose of 0.17 μg per kilogram of body weight (equivalent on the basis of weight to a 10-μg dose in a 60-kg human).

For the preparation of the final vaccine, the purified OspA was sterilized by passage through a 0.2-μm filter and diluted to vaccine strength in phosphate-buffered saline, with a final detergent concentration of less than 0.02 percent. The protective epitopes of the protein appear to be conserved after recombinant expression and purification: serum from vaccinated mice inhibited the growth of the spirochete in vitro.[19] The vaccine contained 30 μg of purified recombinant protein and was supplied in single-use vi-

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als. The identical-appearing placebo preparation consisted of phosphate-buffered saline without OspA and was also provided in single-use vials. Vaccine was administered under double-blind conditions.

Study Sample and Design

The study subjects were healthy men and women, 18 years of age or older, who were at risk for Lyme disease because they lived, worked, or spent their leisure time in areas in which Lyme disease was endemic as defined by the Centers for Disease Control and Prevention (CDC) and county and state health departments in Connecticut, Massachusetts, New Jersey, New York, and Wisconsin. Subjects were excluded from the study if they were receiving long-term antibiotic therapy, had received any vaccine for Lyme disease within the 18 months before the study began, or had a history consistent with the occurrence of Lyme disease within the two months preceding the study.

A total of 10,305 subjects (age range, 18 to 92 years of age) were enrolled at 14 study sites in areas of the United States in which Lyme disease was endemic and were randomly assigned to receive two doses of either 30 μg of OspA vaccine or placebo, given one month apart, according to a preset randomization schedule. Randomization was balanced at each study center in treatment blocks of 10. The first injection was given between March 1, 1994, and April 30, 1994, and the second injection was given approximately one month later, between April 1, 1994, and May 31, 1994. At the request of the Food and Drug Administration, we obtained data on a third dose of vaccine given 12 months after the first injection. A total of 7515 subjects received a third (booster) injection of placebo or vaccine between March 1, 1995, and April 30, 1995. The subjects were observed during the two seasons in which the risk of disease transmission was greatest, irrespective of whether they had received the booster dose.

The sample size was selected to test the null hypothesis of 60 percent efficacy of the vaccine against the alternative hypothesis of superior efficacy. Assuming an attack rate of 0.8 percent (67 cases in the control group and 13 cases in the vaccine group), we calculated that a sample size of 10,000 (5000 in each group) was sufficient to provide the study with a power of 81 percent to reject the null hypothesis according to a one-sided hypothesis test with a type I error of 5 percent. The study design was based on a one-sided alternative, but two-sided results are given to conform with the standard practice of the *Journal*.

Vaccination Protocol

Before receiving the first injection, all subjects provided written informed consent by signing a document approved by the institutional review board at each participating site and were counseled on Lyme disease and its prevention. A single dose (0.5 ml) of vaccine or placebo was injected intramuscularly into the deltoid. A second dose was administered 30 days after the first dose. The timing of entry into the study was such that both inoculations occurred before the end of May 1994 — before the start of the "Lyme disease season" in the areas included in the study. Subjects who enrolled in the extension study received a single booster dose of vaccine or placebo approximately 12 months after the first dose. All these subjects received the booster dose before the end of May 1995.

Follow-up Procedures

Identification of Cases of Lyme Disease

Subjects were instructed to contact the study center immediately if they had any symptoms suggestive of Lyme disease. In addition, the subjects were also monitored monthly by mail (postcards), telephone, or both for adverse events during the first nine months after vaccination and then every three months for a total of two years (covering two Lyme disease seasons), the follow-up period of this study. Subjects who did not respond to any single postcard or who replied positively to any question on a postcard were contacted by study personnel.

Serologic Assessment

Subjects in whom Lyme disease was suspected returned to the study center, where a clinical evaluation was performed by the investigator and serum samples were obtained during the acute and convalescent phases of the illness. Western blot analyses (MarDx Western blot kit, MarDx Diagnostics, Carlsbad, Calif.) to determine whether IgG or IgM antibodies to *B. burgdorferi* were present were performed for all subjects with suspected cases of Lyme disease by the central laboratory (Division of Infectious Diseases, New York Medical College, Valhalla), according to the manufacturer's instructions. Results were considered positive if the serum sample contained IgM antibodies that reacted with at least 2 of the following 3 bands: 25 kd (corresponding to band 23, which indicates OspC), 39 kd, and 41 kd within the first 60 days after the onset of erythema migrans, symptoms, or both or with IgG to at least 5 of the following 10 bands: 21, 25 (23), 28, 30, 39, 41, 45, 58, 66, and 83 (corresponding to band 93) kd at any time after the onset of symptoms. The reports of the results of these tests were sent to the investigators and did not include specific information on reactivity to OspA bands.

Safety Assessment

Subjects reported any adverse effects that occurred within seven days after each vaccination. They were also asked to report any unexpected visits to physicians other than the investigators that took place in the first 30 days after each injection. Serious or unexpected adverse events were followed up throughout the study.

Case Definitions

Data on all subjects with symptoms that the local investigator decided were consistent with the presence of Lyme disease were reviewed by an independent data and safety monitoring board whose members were unaware of subjects' treatment assignments. The diagnosis was confirmed only if it met the strict CDC case definition,[20] which requires the presence of erythema migrans or later manifestations of Lyme disease, with laboratory confirmation in either case. Erythema migrans had to be documented by a photograph or a definitive descriptive clinical note clearly stating the size of the lesion. Laboratory confirmation consisted of proof of seroconversion (from seronegative results at base line to seropositive results at a subsequent analysis). In the absence of laboratory confirmation, the presence of erythema migrans, regardless of the size of the lesion, or of late manifestations of Lyme disease was insufficient for a diagnosis of Lyme disease.

Efficacy Analysis

According to criteria established before the study began, for a case of Lyme disease to be included in the efficacy analysis, the signs and symptoms must have begun to appear at least one month after the second injection and the Western blotting criteria described above must have been met. The primary efficacy end point was based on the number of definite cases of Lyme disease, as determined by the data and safety monitoring board. The risk of Lyme disease in vaccine recipients as compared with placebo recipients was calculated by dividing the number of cases in each group by the number of subjects in each group. The relative risk is the ratio of the two risks. Vaccine efficacy and the associated 95 percent confidence interval were derived from the relative risk and its confidence interval with the following equation: vaccine efficacy = 1 − relative risk. We computed exact 95 percent confidence intervals according to the assumption that the number of cases in the vaccine group followed a binomial distribution (whereas the total number of cases was fixed), correcting the estimates of the upper and lower bounds for the number of subjects in each treatment group. The study had insufficient power to compare treatments within subgroups.

The data were analyzed in the following categories: all cases occurring during the first year per protocol, all cases occurring during the second year, all cases occurring in both years, cases occurring in the second year among subjects who did not receive the booster dose, cases occurring in the second year among subjects who did receive the booster dose, and cases occurring in both years among subjects who received three injections. No interim efficacy analyses were planned or performed. We used SAS software (version 6.12, SAS Institute, Cary, N.C.) for all analyses.

Statistical Analysis

Adverse events were classified according to the Medical Dictionary for Drug Regulatory Affairs.[21] The rates of adverse events in the vaccine and placebo groups were compared with Fisher's exact test among subjects who received only one injection during the original study phase, subjects who received two injections during the original study phase, and subjects who received two injections as well as the booster dose. All P values are two-sided.

RESULTS

Characteristics of the Subjects

A total of 5149 subjects were randomly assigned to receive placebo, and 5156 were assigned to receive OspA vaccine. The proportion of subjects enrolled at each of the 14 study sites ranged from 1.4 to 15.8 percent of the total population. There were no significant differences with respect to age, sex, race, or history of Lyme disease between groups; the predominance of whites in the study populations reflects the makeup of the communities from which subjects were recruited (Table 1).

In the first year of the study, 211 subjects in the vaccine group and 187 subjects in the placebo group withdrew or were withdrawn from the study or died. The respective numbers for the second year of the study (booster phase) were 43 and 17. Reasons for not completing the study are given in Figure 1.

All subjects received at least one dose of vaccine or placebo; 5050 (98.1 percent) subjects in the placebo group and 5034 (97.6 percent) subjects in the vaccine group received the first two inoculations. A total of 3770 (73.2 percent) subjects in the placebo group and 3745 (72.6 percent) in the vaccine group received two immunizations and a booster dose. Thus, 1280 (24.9 percent) subjects in the placebo group and 1289 (25.0 percent) subjects in the vaccine group received only the first two doses, but not the booster.

Confirmed Cases of Lyme Disease

During the two-year observation period, 1734 subjects reported clinical symptoms that were considered by the investigators to be consistent with a diagnosis of Lyme disease and required Western blot testing. These potential cases were reviewed to determine whether the clinical and laboratory results warranted further review by the data and safety monitoring board. A total of 499 were reviewed in depth by the board, and 86 cases of Lyme disease in 84 subjects were confirmed. Five subjects (6.0 percent) did not have erythema migrans as the presenting feature. In the first year of the study, there was one case of Lyme arthritis in a placebo recipient and one case of radiculoneuritis in a placebo recipient. In the second year of the study, there was one case of Lyme arthritis in a placebo recipient; one case of isolated facial-nerve palsy in a vaccine recipient, which occurred 11 days after the booster injection; and one case of arthritis in a placebo recipient that was followed, 6 months later, by tertiary neuroborreliosis, with a cerebrospinal fluid analysis revealing antibodies to *B. burgdorferi*.

TABLE 1. BASE-LINE CHARACTERISTICS OF THE SUBJECTS.

CHARACTERISTIC	INITIAL PHASE		BOOSTER PHASE	
	PLACEBO GROUP (N=5149)	VACCINE GROUP (N=5156)	PLACEBO GROUP (N=3770)	VACCINE GROUP (N=3745)
Age (yr)				
Mean	46.2	46.1	48.7	48.7
Range	18–88	18–92	19–86	19–94
Male sex (%)	59.4	59.4	59.2	59.6
Range at the 14 study centers	42.3–67.0	45.8–68.4	40.3–69.1	46.7–71.8
White race (%)	98.7	98.8	98.8	98.8
History of Lyme disease (%)	12.7	13.2	12.8	13.3
Range at the 14 study centers	1.4–32.4	5.6–30.7	1.6–34.3	6.7–32.9

Vaccine Efficacy

During the Lyme disease season in the first year of the study, all subjects were monitored for evidence of Lyme disease and there were only 49 with confirmed cases of disease: 37 in the placebo group and 12 in the vaccine group. The overall vaccine efficacy was 68 percent (95 percent confidence interval, 36 to 85 percent) (Table 2). In the second year of the study, 35 cases of confirmed Lyme disease were reported: 28 in the placebo group and 7 in the vaccine group. Vaccine efficacy among subjects who received the booster dose was 92 percent (95 percent confidence interval, 69 to 97 percent). In the absence of the booster dose, there was little demonstrable effect of vaccination during the Lyme disease season in the second year of the study; however, because there was such a wide confidence interval, these results do not rule out the possibility of a significant protective effect in this population.

The efficacy of the vaccine did not differ significantly between subjects who reported prior exposure to Lyme disease and those with no history of exposure (86.3 percent [95 percent confidence interval, 0 to 96.9 percent] vs. 68.1 percent [95 percent confidence interval, 27.0 to 84.9 percent] in the first year; 100 percent [95 percent confidence interval, 43.3 to

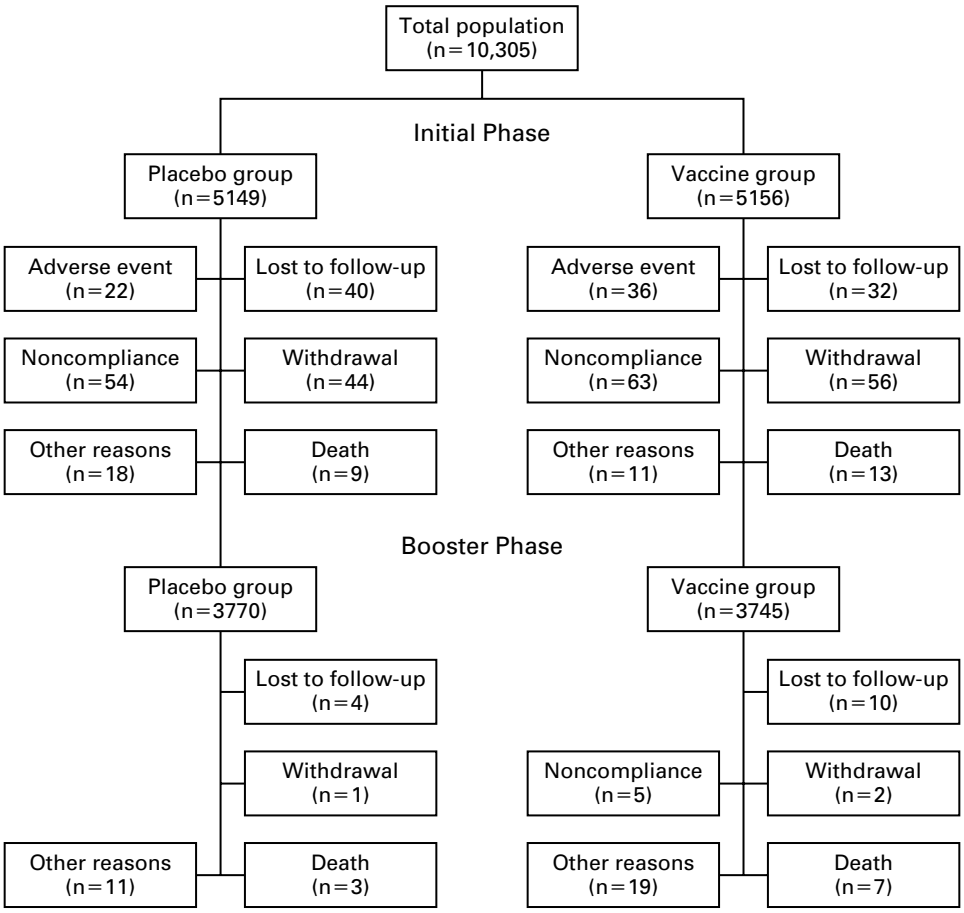

Figure 1. Reasons for Not Completing the Study.
Subjects were followed irrespective of the number of immunizations they received.

100 percent] vs. 86.6 percent [95 percent confidence interval, 42.2 to 95.6 percent] in the second year among subjects who received the booster dose).

In the first year of the study, the efficacy rate was highest among subjects less than 60 years old. In the second year of the study, among subjects who received the booster dose, the rate was 100 percent in all women and in all men less than 60 years old.

Overall, cases of Lyme disease were confirmed at 11 of the 14 study sites. The percentage of subjects with definite Lyme disease was significantly higher in the placebo group than in the vaccine group (1.3 percent vs. 0.4 percent, $P<0.001$). In addition, the percentage reporting symptoms of Lyme disease was significantly higher in the placebo group than in the vaccine group (5.5 percent vs. 4.2 percent, $P=0.002$), as was the percentage with seroconversion (1.5 percent vs. 0.6 percent, $P<0.001$).

Vaccine Safety

As expected, adverse effects were more common in the subjects who received the vaccine than in those who received placebo. In the first year of the study, 2857 events were reported by 1661 of the 5156 subjects in the vaccine group (32 percent), whereas 2313 events were reported by 1431 of the 5149 placebo recipients (28 percent). In the second year of the study, among those who received the booster dose, 2818 events were reported by 1361 of 3745 vaccine recipients (36 percent) and 2370 events were reported by 1211 of 3770 placebo recipients (32 percent). The difference in the incidence of adverse effects between groups was significant only during the first seven days after vaccination (Table 3). The most frequently reported vaccine-related effects were pain or tenderness at the injection site and local muscle pain. The effects were typically mild and self-limited.

There was no significant difference between groups in the overall incidence of adverse effects reported as having occurred more than seven days after the first or second vaccination ($P=0.10$), although the differences in some of the individual effects were significant (data not shown). After the first and second

TABLE 2. VACCINE EFFICACY.

GROUP	PLACEBO GROUP		VACCINE GROUP		VACCINE EFFICACY (95% CI)*
	NO. OF SUBJECTS	NO. OF CASES	NO. OF SUBJECTS	NO. OF CASES	
					%
First year of study					
Two injections, per protocol	5149	37	5156	12	68 (36–85)
Second year of study					
Two injections and booster	3770	26	3745	2	92 (69–97)
Two injections, no booster	1379	2	1411	5	0 (0–60)
Both years of study					
Three injections, per protocol	5050	60	5034	14	77 (58–88)

*CI denotes confidence interval.

TABLE 3. INCIDENCE OF ADVERSE EFFECTS WITHIN SEVEN DAYS AFTER INJECTION.

VARIABLE	VACCINE GROUP	PLACEBO GROUP
First injection		
No. of subjects	5156	5149
Adverse effect (%)		
Any	9.8	4.1
Musculoskeletal	6.4	1.3
Myalgia	5.5	0.6
General	1.8	0.9
Pain at injection site	0.3	0.04
Second injection		
No. of subjects	5050	5034
Adverse effect (%)		
Any	6.1	3.1
Musculoskeletal	3.3	1.1
Myalgia	2.5	0.4
General	1.7	0.8
Pain at injection site	0.8	0.1
Third injection		
No. of subjects	3745	3770
Adverse effect (%)		
Any	11.2	5.5
General	7.3	2.6
Tenderness	2.3	0.2
Pain at injection site	1.5	0.2
Unspecified pain	1.0	0.1
Reaction at injection site	0.8	0.2
Swelling	0.6	0.1
Pain in limb	0.5	0.02
Edema at injection site	0.5	0
Rigors	0.2	0
Skin or subcutaneous tissue	2.1	0.2
Erythematous rash	1.9	0.1

immunizations, the placebo group had a higher incidence of viral infection (25 subjects, as compared with 9 subjects in the vaccine group; $P=0.01$) and hypoesthesia (9 subjects, as compared with 2 subjects; $P=0.04$). After the booster dose, more subjects in the placebo group than in the vaccine group reported pneumonia (27 subjects vs. 13 subjects, $P=0.04$), colonic polyps (8 vs. 1, $P=0.04$), and coronary artery disease (12 vs. 3, $P=0.04$). The vaccine group had a higher incidence of otitis externa (7 subjects vs. 1 subject, $P=0.04$), pain in the injected arm (21 vs. 10, $P=0.05$), and headache (40 vs. 23, $P=0.02$). There was no significant difference ($P=0.94$) in the occurrence of neurologic events between the vaccine group (77 subjects) and the placebo group (76 subjects).

Despite the higher overall incidence of adverse effects in the vaccine group, there were no significant differences between the groups in the frequency of severe effects or serious or unexpected effects. During the two years of observation, severe effects were reported by 2 to 3 percent of vaccine recipients and 4 percent of placebo recipients and serious or unexpected effects were reported by 4 to 5 percent in each group. There was no significant difference between the groups in the number of deaths (20 in the vaccine group and 12 in the placebo group, $P=0.21$ by Fisher's exact test). All deaths were reviewed by the data and safety monitoring board, and none were considered to have been related to vaccination.

There were only three cases of Lyme arthritis in the study population during the clinical follow-up. There was no evidence that the vaccine predisposed subjects with prior self-reported Lyme arthritis to an exacerbation of arthritis. There was no increase in the frequency of erythma migrans in subjects with a history of Lyme disease or Lyme arthritis in either group. There was no evidence of a vaccine-associated

exacerbation of neurologic events or conditions among the few subjects with a history of such conditions. Early in the first year, there were two cases of transverse myelitis in each study group, with no additional events occurring in the remainder of the study. In all cases, the data and safety monitoring board determined that the transverse myelitis represented neither a case of Lyme disease nor an adverse effect related to vaccination.

DISCUSSION

We evaluated the efficacy and safety of an OspA vaccine without adjuvant, which contained 30 μg of purified recombinant protein, in more than 10,000 adults. Although the total number of confirmed cases of Lyme disease reported during the two-year surveillance period was somewhat lower than anticipated, largely because of the very stringent case definition used, vaccine efficacy was high.

Two doses of vaccine administered one month apart reduced the incidence of confirmed Lyme disease during the first year of the study, with an efficacy of 68 percent for all enrolled subjects. However, the vaccine was more effective when a booster dose was administered 12 months after the first immunization, achieving an efficacy of 92 percent among the group of subjects who received the booster dose. Subjects who did not receive the booster dose appeared to be minimally protected against disease during the second year of observation.

Although there has been some concern about the efficacy of a vaccine that includes only a single OspA protein against different strains of *B. burgdorferi*,[22,23] in our study, such a vaccine prevented Lyme disease at locations in five different states in the United States, suggesting that it provides coverage against a wide range of strains. Nevertheless, it may be necessary to use different OspA proteins in the development of vaccines intended for use in Europe and North America.[24] No inference can be drawn regarding the ability of this vaccine to protect against the two other genospecies implicated in so-called European Lyme disease, *B. garinii* and *B. afzelii*.

The OspA vaccine was well tolerated. Not unexpectedly, there was a higher incidence of acute vaccine-related side effects in the vaccine group within 7 days after injection and, frequently, within 24 hours. These side effects consisted mostly of pain or tenderness at the injection site, were mild and self-limited, and were similar to those seen after the administration of other vaccines.[25]

Previous studies have suggested that there is an autoimmune component to Lyme disease,[26-30] although the specific molecule underlying the presumed molecular mimicry implicit in the proposed OspA-based autoimmune model has not been identified.[31] Nonetheless, analysis of the safety data from our trial revealed no evidence that the vaccine exacerbated prior Lyme arthritis, or caused arthritis in either subjects with a history of Lyme disease or those without such a history. Similarly, the vaccine did not cause neurologic disease in recipients.

Finally, there has been some concern that an OspA vaccine might alter the natural course of infection, rather than preventing the infection,[2,9] a possibility for which there may be a precedent in other infectious diseases.[32] If vaccination prevented erythema migrans or other early (and more easily treated) manifestations of Lyme disease, then vaccine recipients would probably have no signs or symptoms related to early infection[33] (latent Lyme disease) and would seek medical attention only after the onset of later-stage manifestations of *B. burgdorferi* infection. Approximately 60 percent of our subjects have now been followed up for four years, and no cases of late Lyme disease have been recognized, indicating that there has been no progression of subclinical infection to clinical disease.

Further studies will be needed to evaluate the vaccine in children and adolescents. In addition, although the booster dose clearly increased the efficacy of the vaccine in the second year of the study, the optimal regimen remains to be determined. Whether booster doses are required beyond the second year is as yet unresolved. Nevertheless, our results indicate that OspA vaccine is a successful and safe antispirochetal vaccine.

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APPENDIX

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