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# Efficacy of an OspA Vaccine Preparation for Prevention of Lyme Disease in New York State

**Summary: A multicenter, double-blinded, placebo-controlled study was done comparing a 30-μg dose of a single protein recombinant OspA vaccine preparation with a saline placebo for efficacy in prevention of Lyme disease in humans. The OspA vaccine (30-μg dose) or saline placebo was given intramuscularly at day 0, 1 month later, and 12 months later. Cases of possible Lyme disease were evaluated clinically and using culture, polymerase chain reaction and immunoblot assays. Safety data are being analyzed separately. 1,634 adult volunteers were enrolled at a single center in New York State. Vaccine efficacy during the first year was 40% and during the second 37%. Compared with placebo, the OspA vaccine significantly reduced the frequency of Lyme disease during the 2-year study period ($P < 0.04$, one-tailed Fisher's exact test). Vaccine efficacy was restricted to volunteers under 60 years old (50% vs 9%, $P < 0.03$, two-tailed Fisher's exact test). A recombinant OspA vaccine preparation was found to have moderate activity in preventing Lyme disease in adults under 60 years old from New York State. Reasons for vaccine failure need to be addressed and a risk benefit ratio calculated.**

## Introduction

Lyme borreliosis is the most common vector-borne disease in the USA and parts of Eurasia [1]. Experimental studies in animals have suggested that recombinant single protein outer surface protein A (OspA) preparations are highly effective immunogens, particularly when the OspA protein of the challenge strain of *Borrelia burgdorferi* is closely related in amino acid structure to the OspA immunogen [2–4].

Recombinant OspA vaccine preparations have been studied in human volunteers in phase I/II trials [5–7], and a placebo-controlled double-blinded multicenter efficacy study was initiated in early 1994. In this paper the efficacy of an OspA vaccine preparation in volunteers recruited at a site in Westchester County, NY, is reported.

## Patients and Methods

*Patient population:* Adults at least 18 years old who were at risk for *Ixodes scapularis* tick bites were eligible to participate. Exclusions included: allergy to a vaccine component; impairment of the immune system due to disease or medications; a febrile illness at the time of vaccination; receipt of chronic antibiotic therapy; receipt of another Lyme disease vaccine within 18 months; pregnancy; a chronic medical condition which may impair interpretation of results (e.g. collagen vascular disease); history of Lyme disease within the past 2 months; and heart block.

*Vaccination:* After giving written informed consent, eligible volunteers in the spring of 1994 were vaccinated intramuscularly with two 30-μg doses of a proprietary recombinant OspA vaccine preparation (manufactured by Connaught Laboratories, Inc., Swiftwater PA [5, 8], given 1 month apart, or an identical appearing saline placebo. Volunteers were randomized according to a random number list in blocks of ten. One year later, consenting volunteers were vaccinated (boosted) with a single dose of the same OspA preparation (30 μg) or placebo given originally.

*Study procedures:* At baseline (prevaccination), a medical history was taken recording demographic data, medical illnesses and medications. For women of childbearing age, a negative pregnancy test on urine was demonstrated prior to each vaccine/placebo dose. Volunteers were asked to contact the study center for any symptoms of Lyme disease. In addition, volunteers were contacted by postcard and/or telephone monthly during the Lyme disease transmission seasons. Volunteers reporting symptoms compatible with Lyme disease were asked to return to the study center for medical interview, physical examination and laboratory testing.

*Lyme serology:* Volunteers who were suspected to have Lyme disease had acute and convalescent phase (2–6 weeks later) Lyme disease serology performed by separate IgM and IgG immunoblots (MarDx test kits, MarDx Diagnostics, Inc., Carlsbad, CA) according to the manufacturer's instructions. For purpose of this analysis, weak bands were not scored as positive. To maintain blinding, the band representing OspA antibodies (31 kDa), if present, was not reported.

Serologic evidence in support of a clinical diagnosis of Lyme disease was based on the development of either two new bands (IgM or IgG) in the convalescent phase serum sample in comparison to the acute phase sample results or two new bands in either the acute or convalescent phase serum samples, compared to the baseline (prevaccine) serum sample. Baseline, acute and convalescent phase serum samples were tested in parallel.

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*Volunteers with erythema migrans-like rashes:* Volunteers with rashes suggestive of erythema migrans (EM) [9] were asked to undergo a 2 mm punch biopsy. Part of the skin sample was cultured in Barbour-Stoenner-Kelly (BSK) medium in a manner similar to that previously reported [10–12] and part was processed for detection for *Borrelia burgdorferi* DNA using the polymerase chain reaction (PCR) technique. PCR was performed with primers IS1 and IS2 as previously described [10]. For selected volunteers with erythema migrans, whole blood or serum was cultured in BSK according to published methods [13].

*Data analysis:* Analysis of efficacy was based on the incidence of Lyme disease beginning over 30 days after the second dose of OspA/placebo vaccine. Patients with erythema migrans-like rashes were categorized hierarchically A through D based on the investigators' assessment of the strength of the supporting laboratory data.

Category A patients had to have either a positive blood or skin culture for *B. burgdorferi* or a positive PCR for *B. burgdorferi* DNA in a skin sample. Category B patients did not satisfy the laboratory criteria of category A patients but had at least two new bands on the convalescent phase serum immunoblots compared to the acute phase serum sample. Category C patients did not satisfy the laboratory criteria of categories A and B, but did demonstrate at least two new bands compared to the baseline serum sample (i.e. the sample obtained pre-vaccine and pre-illness). Category D patients had a skin lesion ≧5 cm in diameter consistent with erythema migrans but had no supporting laboratory test results.

Patients with objective extracutaneous signs of Lyme disease and those with only subjective complaints had to have supporting serologic test results to be considered as a possible Lyme disease case. The latter patients were further categorized based on the presence or absence of a documented febrile illness.

Cases of Lyme disease were evaluated based on date of occurrence. Volunteers with onset of symptoms within 12 months of the first dose of vaccine/placebo were considered year 1 or pre-booster cases. Volunteers with onset of symptoms from 12 months after receipt of the first vaccine/placebo dose until the close of the study in early 1996 were considered year 2 or post-booster cases, regardless of whether the volunteer agreed to receive the third dose of vaccine or placebo (intent-to-treat analysis).

*Statistical analysis;* Categorical variables were compared by the Fisher's exact test. Confidence intervals were calculated assuming a Gaussian distribution.

## Results

Over the approximately 2-year study period from the spring of 1994 to early 1996, 10,306 volunteers, 18–92 years old, were enrolled at 14 United States Study Centers [8]. 1,634 volunteers (16%), of whom 61% were male, were enrolled at the Westchester County, NY study site. 817 of whom received the OspA vaccine preparation. Of these 1,634 New York State volunteers, 1,339 (82%) received the booster vaccine/placebo dose 1 year later, including 665 who received vaccine and 674 who received placebo.

Sixty-three episodes of possible Lyme disease were identified in 61 volunteers over the study interval. The age range of these 61 volunteers was 21 to 79 years and 62% were male. For 32 (52%) of the 61 volunteers the onset of Lyme disease occurred during the first study year; i.e. before the time of the booster vaccine/placebo dose. Two volunteers had separate Lyme disease illnesses in successive study years. Although all patients were treated with antibiotics, the level of certainty of diagnosis varied considerably (Table 1). Diagnostic accuracy was highest for the 20 episodes involving an erythema migrans skin lesion and for which there was also culture or PCR laboratory evidence of *B. burgdorferi* infection. An additional 15 episodes of erythema migrans had serologic evidence in support of the diagnosis.

Vaccine efficacy for prevention of Lyme disease associated with erythema migrans during the first year was 65% (95% C.I. = 42% to 87%) ($P < 0.02$ one-tailed, Fisher's exact test, in comparison with placebo). The overall vaccine efficacy for prevention of Lyme disease over the 2-year study was 38% (95% C.I. 23% to 54%) ($P < 0.04$ one-tailed, Fisher's exact test, in comparison with placebo). The efficacy was 40% during the first year and 37% during the second; this difference was not significant. Efficacy for study year 2 was somewhat better (53%) if analysis was restricted to those volunteers who actually received the booster dose.

Over both study years, the most consistent degree of efficacy was found for category A erythema migrans cases: 50–67%. In addition, vaccine efficacy was greater in volunteers under 60 years of age compared to older subjects (50% vs 9%, $P < 0.03$ Fisher's exact test, two-tailed).

## Discussion

Compared with placebo, immunization with the OspA vaccine preparation evaluated in this study significantly reduced the frequency of Lyme disease associated with erythema migrans during the first study year ($P < 0.02$) (Table 1). The efficacy for prevention of erythema migrans was moderate (65%, 95% C.I. 42% to 87%). The vaccine was less successful in the second year of the study for the most rigorously defined patients with erythema migrans (category A) (50% vs 67% protection). The nearly 20% drop-out rate during the second year of the study may have introduced certain unknown biases that caused a lower assessment of vaccine efficacy. The high drop-out rate may be attributed in part to the fact that the study was not originally designed to extend into a second year or include a booster dose. Further, the intent-to-treat analysis contributed to the lower vaccine efficacy rate observed in year 2, since several of the volunteers who developed Lyme disease that year had not received the booster dose of OspA vaccine.

Why the vaccine failures occurred is not known, but reasons may include significant OspA protein heterogeneity among *B. burgdorferi* isolates in our region, lack of development of a protective antibody response by some volunteers, or incorrect Lyme disease diagnosis.

The OspA immunogen used in this study was expressed from the gene of *B. burgdorferi sensu stricto* B31 [5, 14].

Table 1: Results of OspA Lyme vaccine trial.

| Lyme disease-category | Study year 1 | | Study year 2 | | | | |
|---|---|---|---|---|---|---|---|
| | No. who received vaccine | No. who received placebo | No. who received vaccine | No. who received placebo | Protection rate year 1 | Protection rate year 2 | Overall protection rate* |
| EM – A** | 2 | 6 | 4 | 8 | 67% | 50% | 57% |
| EM – B** | 2 | 3 | 0 | 1 | 33% | 100% | 50% |
| EM – C** | 1 | 3 | 3 | 2 | 67% | None | 20% |
| EM – D** | 1 | 5 | 4 | 2 | 80% | None | 29% |
| Total EM | 6 | 17 | 11 | 13 | 65% | 15% | 43% |
| Non EM – objective | 0 | 0 | 0 | 1 | – | 100% | 100% |
| Febrile illness | 3 | 1 | 1 | 4 | None | 75% | 20% |
| Subjective illness | 3 | 2 | 0 | 1 | None | 100% | None |
| Total episodes | 12 | 20 | 12 | 19 | 40% | 37% | 38% |

* for both study years combined; ** category A patients had to have either a positive blood or skin culture for *Borrelia burgdorferi* or a positive PCR for *B. burgdorferi* DNA in a skin sample; category B patients did not satisfy the laboratory criteria of category A patients but had at least two new bands on the convalescent phase serum immunoblot compared to the acute phase serum sample; category C patients did not satisfy the laboratory criteria of categories A and B, but did demonstrate at least two new bands compared to the baseline serum sample (i.e. the sample obtained pre-vaccine and pre-illness); category D patients had a skin lesion ≥ 5 cm in diameter consistent with erythema migrans (EM) but had no supporting laboratory test results.

Since all known human isolates of *B. burgdorferi* from North America belong to the *sensu stricto* genospecies, only limited variability of the OspA epitope was anticipated among the strains of *B. burgdorferi* likely to infect our volunteers [15]. However, depending on the experimental conditions, laboratory animals immunized with a *sensu stricto* OspA vaccine may [16] or may not [17] be protected against challenge with heterologous strains of *B. burgdorferi sensu stricto*. In Europe the heterogeneity of OspA is much greater due to the existence of at least two additional genospecies, *Borrelia garinii* and *Borrelia afzelii* [18]. Consequently, a monovalent OspA vaccine is much less likely to be successful outside the United States.

The significantly greater efficacy rate observed in volunteers under 60 years of age is unexplained but is consistent with the hypothesis that lack of immunogenicity was an important factor in vaccine failure. Markedly reduced immunogenicity in adults over 60 years of age has been observed with other vaccine preparations, such as the hepatitis B vaccine [19]. Against this hypothesis was the observation that an IgG immune response to OspA was evident on immunoblot at the time of acute illness in all six of the vaccine recipients who developed category A erythema migrans (data not shown). Immunoblot detection of OspA antibody, however, is non-quantitative and does not indicate whether the antibody detected is protective [17].

Incorrect diagnosis may explain the lack of vaccine efficacy, especially among patients without erythema migrans who had non-specific febrile illnesses or who presented with only subjective complaints. In this regard, six of the seven volunteers with febrile illnesses (and serologic evidence of Lyme disease), who were also evaluated for human granulocytic ehrlichiosis (HGE), had laboratory evidence of HGE. HGE is another disease transmitted by *Ixodes scapularis* in our area, and has been suspected to cause false-positive immunoblots for Lyme disease [20–22]. Nevertheless, for purposes of this study, such patients with HGE were counted as Lyme disease cases if they met the Lyme disease serologic criteria specified for this study.

An argument could be made that the serologic criteria of at least two new bands on immunoblot may not have been sufficiently stringent and may have resulted in the inclusion of patients who did not have Lyme disease. Unfortunately, there is no accepted standard for Lyme disease immunoblot "seroconversion." If evaluable cases had been limited to those for whom there were more than four new bands, more than four new IgM bands, or more than four new IgG bands compared to baseline, the protective efficacy rates over the 2-year study were little changed at 35%, 33%, and 40%, respectively.

Adverse effects were not evaluated in this analysis. There was, however, no suggestion that receipt of the OspA vaccine either enhanced or reduced the likelihood of developing disseminated Lyme disease (20% of the placebo group with erythema migrans had multiple lesions vs 19% of the vaccine recipients), extra-cutaneous manifestations or atypical presentations (Table 1).

Although this was a multicenter study, an in-depth evaluation of our single center's experience is important for sev-

eral reasons. First, the effect on vaccine efficacy of OspA heterogeneity may be more evident in certain geographic areas compared to others (see above) [18, 23–28]. Second, a special effort was made at our site to evaluate a broad range of complaints. At least 11% of volunteers in year 1 and 17% in year 2 underwent diagnostic testing for Lyme disease at our site. Furthermore, our center used additional specific diagnostic modalities such as PCR and blood cultures for *B. burgdorferi*, which were not offered at other sites.

In conclusion, a recombinant OspA vaccine preparation in the dosage and schedule administered in this study had biologic activity in preventing cases of Lyme disease in New York State in adults under 60 years of age. A risk benefit ratio and the duration of protection will need to be determined. In addition, the reasons for vaccine failure must be addressed before the preparation's full potential as a vaccine to prevent Lyme disease in humans can be realized.

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