

# A Novel Multivalent OspA Vaccine against Lyme Borreliosis Is Safe and Immunogenic in an Adult Population Previously Infected with *Borrelia burgdorferi Sensu Lato*

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**Lyme borreliosis (LB) patients who recover, as well as previously infected asymptomatic individuals, remain vulnerable to reinfection with *Borrelia burgdorferi sensu lato*. There is limited information available about the use of OspA vaccines in this population. In this study, a randomized double-blind phase I/II trial was performed to investigate the safety and immunogenicity of a novel multivalent OspA vaccine in healthy adults who were either seronegative or seropositive for previous *B. burgdorferi sensu lato* infection. The participants received three monthly priming immunizations with either 30 µg or 60 µg alum-adjuvanted OspA antigen and a booster vaccination either 6 months or 9 to 12 months after the first immunization. The antibody responses to the six OspA serotypes included in the vaccine were evaluated. Adverse events were predominantly mild and transient and were similar in the seronegative and seropositive populations. Substantial enzyme-linked immunosorbent assay (ELISA) and surface-binding antibody responses against all six OspA antigens were induced after the primary immunization schedule in both populations, and they were substantially increased with both booster schedules. The antibody responses induced by the two doses were similar in the seronegative population, but there was a significant dose response in the seropositive population. These data indicate that the novel multivalent OspA vaccine is well tolerated and immunogenic in individuals previously infected with *B. burgdorferi sensu lato*. (This study is registered at ClinicalTrials.gov under registration no. NCT01504347.)**

Lyme borreliosis (LB) is a major multiorgan infectious disease occurring in the United States, Europe, and Asia and can have serious health consequences if left untreated (1, 2). LB was the seventh most common notifiable disease in the United States in 2012 (2, 3). Moreover, recent CDC data estimate that the number of diagnosed cases in the United States is approximately 300,000 annually, which is 10-fold higher than the number of reported cases (4). There are about 85,000 cases reported annually in Europe; however, this number is largely underestimated, as case reporting is highly inconsistent in Europe, and many LB infections go undiagnosed (5). LB is caused by a number of bacterial species of the *Borrelia burgdorferi sensu lato* complex. Four species, *B. burgdorferi sensu stricto*, *Borrelia afzelii*, *Borrelia garinii*, and *Borrelia bavariensis* cause the majority of human disease in Europe, whereas only a single species, *B. burgdorferi sensu stricto*, causes LB in the United States (1, 2).

LB can usually be successfully treated with antibiotics (1, 2), but patients and health care providers may remain unaware of the infection until the onset of severe disease symptoms (6). Approximately 60% of the untreated infections in U.S. patients result in Lyme arthritis (1). Moreover, about 10% of patients in the United States do not respond clinically to antibiotic treatment and are said to have antibiotic-refractory Lyme arthritis (1, 7).

Not all individuals who are infected with *B. burgdorferi* develop symptoms. In the United States, epidemiological studies have reported the frequency of asymptomatic *B. burgdorferi sensu stricto* infection to be between 0% and 50% (8–11). In Europe, where more infections are caused by *B. afzelii*, *B. garinii*, and *B. bavari-*

*ensis* than by *B. burgdorferi sensu stricto*, studies have reported that the majority of seropositive individuals were asymptomatic (11–14). Importantly, both previously infected asymptomatic individuals and some LB patients who recover after antibiotic treatment remain vulnerable to reinfection (15, 16); thus, previously infected individuals represent a substantial proportion of those at high risk for LB. Vaccination would be the most effective intervention to prevent LB, but there is no vaccine currently available for human use.

Two monovalent recombinant vaccines based on outer surface protein A (OspA) serotype-1 derived from *B. burgdorferi sensu stricto* were developed in the 1990s and demonstrated to be safe and efficacious in placebo-controlled phase III studies in the United States (17, 18). However, a subsequently disproven hypothesis that a licensed monovalent OspA-1 vaccine might trigger arthritis in some vaccine recipients, mediated by T-cell cross-reactivity with an OspA-1 epitope (19, 20), was one of a number of factors that contributed to the limited acceptance and subsequent

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discontinuation of the vaccine in 2002 (6, 21). Irrespective of these disproven safety concerns, monovalent OspA vaccines were designed for use only in the United States and would not have been effective in preventing LB occurring in Europe or Asia. Because OspA-mediated protection is largely OspA serotype specific and the disease in Europe and Asia is caused by several *Borrelia* species that encode antigenically divergent OspA proteins (1, 2), a globally effective OspA vaccine requires the induction of antibodies against multiple OspA serotypes.

We have developed a novel multivalent OspA vaccine, which comprises three recombinant OspA antigens, each containing protective epitopes from two different OspA serotypes, i.e., OspA serotypes 1 and 2 (*B. burgdorferi sensu stricto* and *B. afzelii*), 5 and 3 (both *B. garinii*), and 6 and 4 (*B. garinii* and *B. bavariensis*) (22). The multivalent vaccine is designed to protect against all major disease-causing *Borrelia* species in the United States (OspA-1), Europe (OspA-1 to -6), and potentially globally. The hypothetical risk of T-cell cross-reactivity has been eliminated by replacing the putative cross-reactive OspA-1 epitope with the corresponding OspA-2 sequence (23). In a phase I/II study in healthy adults seronegative for *B. burgdorferi sensu lato* infection, the novel multivalent OspA vaccine was demonstrated to be well tolerated and to induce potent antibody responses against all major *Borrelia* species after three primary immunizations (22). In the present study (ClinicalTrials.gov registration no. NCT01504347), we extend our investigation to include a study population seropositive for *B. burgdorferi sensu lato* infection. We also investigated the seropersistence of the primary antibody responses up to 12 months after the first immunization, and we evaluated the effectiveness of a booster immunization at 6 or 9 to 12 months after the first immunization.

## MATERIALS AND METHODS

**Study design and participants.** A randomized double-blind phase I/II study was conducted between 1 March 2011 and 4 March 2013 at eight study sites in Austria and Germany, in accordance with the International Conference on Harmonisation guideline for good clinical practice. Prior institutional review board (IRB) approval was obtained from each institution that participated in the research. An independent data monitoring committee consisting of three external medical experts reviewed the data.

Healthy adults age 18 to 70 years who provided written informed consent were eligible for inclusion in the study. All subjects were recruited in centers located in regions endemic for LB and following screening were assigned to the seronegative or seropositive cohort based on a screening assay using a commercially available C6-enzyme-linked immunosorbent assay (ELISA) (Immunitics, Boston, MA, USA); this C6-ELISA is based on a synthetic C6 peptide antigen derived from the VIsE protein shown to be conserved among *B. burgdorferi sensu lato* species. Asymptomatic seropositive individuals were identified by a medical history absent for previous LB. A detailed medical history of the symptomatic individuals with respect to LB manifestations, such as Lyme arthritis, Lyme neuroborreliosis, or acrodermatitis chronica atrophicans, was not systematically captured. Additional C6 antibody screening was undertaken before the booster immunization.

The exclusion criteria were active LB, LB-related chronic illness, treatment for LB with antibiotics within 3 months, tick bite within 3 weeks, and receipt of live or inactivated vaccine within 4 or 2 weeks of enrollment, respectively.

**Procedures.** The design and manufacture of the multivalent recombinant OspA vaccine has been described in detail (22). Approximately 350 subjects were to be recruited into one of two parallel cohorts (subjects who were either seronegative or seropositive for *B. burgdorferi sensu lato* anti-

bodies) and randomized at a 1:1 ratio to receive either 30 or 60  $\mu$ g OspA antigen with aluminum hydroxide adjuvant. The dose selection was based on the results of a dose- and formulation-finding study in seronegative adults (22). Randomization was performed centrally via an electronic data capture system. The subjects and investigators were blinded to treatment allocation.

The subjects received three intramuscular immunizations, 28 days apart, and a booster immunization either 6 months or 9 to 12 months after the first immunization. Blood was drawn prior to the first immunization, 28 days after each immunization, and prior to the booster immunizations. The primary safety endpoint was the frequency and severity of injection site and systemic reactions within 7 days after each vaccination. Subject diaries were used to collect daily oral body temperature, solicited injection site and systemic reactions, and other adverse events (AEs), which were assessed using the FDA toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials (24) as guidance.

The primary immunogenicity endpoint was the antibody response to OspA serotypes 1 to 6, 28 days after the third vaccination, as determined by endpoint ELISA using affinity-purified recombinant OspA antigens representing OspA serotypes 1 to 6. In this assay, ELISA plates are coated with OspA antigen (recombinant glutathione S-transferase -fusion proteins), and serial dilutions of the test sera are applied. Bound antibodies are detected by enzyme-labeled anti-human IgG antibodies. We defined the titer as the highest serum dilution giving an optical density  $\geq 3$ -fold higher than background (22). The secondary immunogenicity endpoints were the antibody responses at baseline, 28 days after each vaccination, and prior to the booster vaccinations. The ability of vaccine-induced functional antibodies to bind to *Borrelia in vitro* was determined by surface-binding assays. Briefly, flow cytometry was used to quantify antibody binding to *Borrelia* via phycoerythrin-labeled anti-IgG antibodies and a DNA-specific dye. We defined the surface-binding titer as the highest dilution at which fluorescence was at least three times higher than that of the negative control (22).

**Statistical analyses.** The sample size of the study was chosen so that the combined study arms (350 subjects) enabled the detection of an AE having a true underlying incidence of 1:100, with a probability of 97%. The risks of injection site and systemic reactions after vaccination, as well as the risk ratios of moderate or severe AEs, were calculated as previously described (22). Assuming a dropout rate of around 10%, approximately 135 seronegative and 67 seropositive subjects per study group would be available for immunogenicity evaluation, such that the 90% confidence interval (CI) of the seroconversion rates would extend no more than 5.7% or 8.7%, respectively, from the observed rates if these are approximately 90%. A longitudinal analysis was performed for the log-transformed antibody titers against the 6 OspA serotypes within a repeated mixed-model analysis of covariance (ANCOVA) framework, accounting for the effect of vaccine dose, adjuvantation, time, age in years, and baseline titer as a covariate and for the random subject effect.

## RESULTS

**Study population.** Table 1 shows the demographic and clinical characteristics of the participants at baseline. The dose groups were balanced with respect to all demographic characteristics. Of the 151 participants who were seropositive for *B. burgdorferi sensu lato* antibodies as assessed by C6-ELISA (range of Lyme index, 1.10 to 11.47 for seropositive subjects and 0.03 to 0.90 for seronegative subjects), 89 (58.9%) reported previously having LB symptoms, indicating that a substantial proportion of seropositive subjects had had asymptomatic infection with *B. burgdorferi sensu lato*.

Figure 1 shows the trial profile. A total of 199 seronegative and 151 seropositive subjects were randomized to receive three priming immunizations and a booster immunization with either 30  $\mu$ g

TABLE 1 Demographics of the study population at baseline

Characteristic	Data by dose for:			
	Seronegative population		Seropositive population	
	30 µg	60 µg	30 µg	60 µg
<i>n</i>	101	98	75	76
Mean age (range) (yr)	40.5 (18–69)	43.0 (20–70)	48.8 (19–69)	46.9 (20–70)
Gender (no. [%])				
Male	48 (47.5)	53 (54.1)	41 (54.7)	51 (67.1)
Female	53 (52.5)	45 (45.9)	34 (45.3)	25 (32.9)
Race (no. [%])				
White	101 (100.0)	98 (100.0)	75 (100.0)	74 (97.4)
Black or African-American	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.6)
Ethnicity (no. [%])				
Hispanic or Latino	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Not Hispanic or Latino	100 (99.0)	98 (100.0)	75 (100.0)	76 (100.0)
Mean wt (range) (kg)	73.5 (47–115)	74.1 (48–102)	76.2 (50–113)	78.5 (51–115)
Mean ht (range) (cm)	172 (136–196)	173 (150–193)	174 (152–194)	175 (156–195)
Mean body mass index (range) (kg/m <sup>2</sup> )	24.6 (18.5–34.5)	24.7 (18.3–31.8)	24.9 (18.4–34.0)	25.5 (18.6–33.9)
Previous symptomatic infection (no. [%])	NA <sup>a</sup>	NA	30 (40.0)	44 (57.9)
Previous asymptomatic infection (no. [%])	NA	NA	45 (60.0)	32 (42.1)

<sup>a</sup> NA, not applicable.

or 60 µg of OspA antigen. The safety and immunogenicity data sets contain all subjects who were vaccinated at least once and had baseline and at least one postvaccination titer measurement.

**Safety and tolerability.** Figure 2 shows the rates of systemic and injection site reactions within 7 days of each immunization. The majority of the AEs occurred within 24 h of immunization, were predominantly mild in severity, and resolved spontaneously within 72 h. Successive vaccinations were generally associated with a decrease in systemic reaction rates. There were no statistically significant differences in the rates of systemic (relative risk [RR], 1.09 to 1.13;  $P > 0.5862$ ) or injection site (RR, 1.02 to 1.16;  $P > 0.2261$ ) reactions in the seronegative and seropositive subjects.

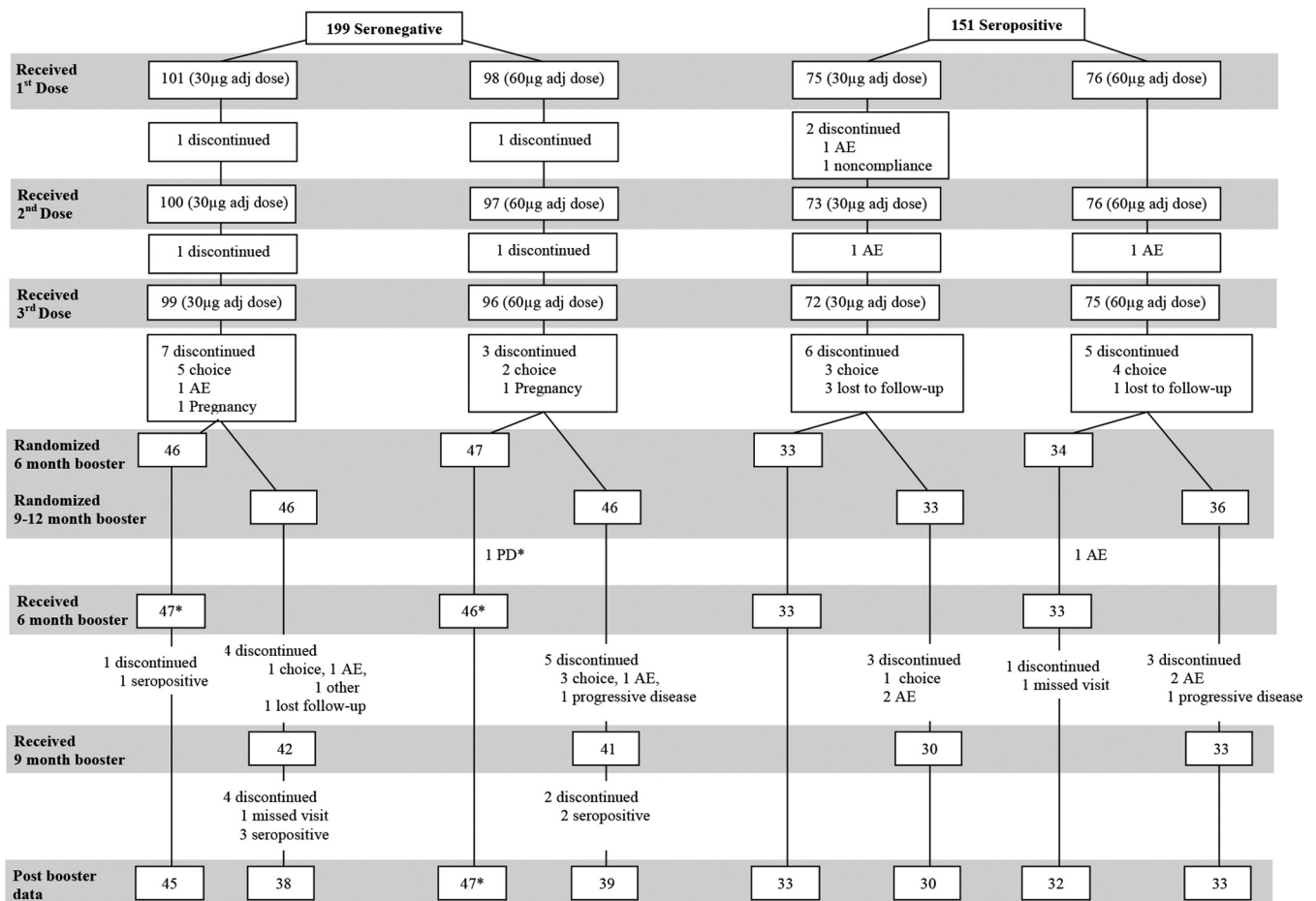
The rates of individual solicited systemic and injection site reactions after the first immunization are shown in Table 2. The most common injection site reactions in both the seronegative and seropositive populations were pain (36.7 to 48.7%) and tenderness (30.3 to 49.5%). Most solicited systemic reactions occurred very infrequently, irrespective of dose or serological status, and the vast majority were rated as mild; only headache (4.0 to 11.9%), myalgia (5.3 to 13.3%), and fatigue (5.1 to 7.9%) occurred at frequencies of  $>6\%$ . The frequencies of local and systemic reactions in the seronegative and seropositive subjects receiving the 30-µg or 60-µg dose were very similar (RR, 0.88 to 1.05;  $P = 0.3370$  to 0.9511). The risk of moderate or severe systemic reactions for the 30-µg and 60-µg dose was very similar in both populations (RR, 0.97 to 1.11;  $P = 0.9054$  to 0.9651).

No deaths or vaccine-related serious AEs were reported during the entire study, and no symptoms reminiscent of LB or chronic arthritis were reported. Three cases of transient arthritis were reported in three subjects immunized with the 60-µg vaccine dose. In one case, a seronegative subject reported an inflamed left toe joint, which was mild and resolved within 4 days. This subject had a history of joint pain with the same localization prior to the study.

In the second case, a seropositive subject with a history of LB presented with acute arthritis of the proximal interphalangeal finger joints of two fingers of the right hand, of moderate severity; however, an X-ray examination gave no indication of arthritis or arthrosis, and although the subject experienced tenderness, swelling, and pain, all rheumatologic and laboratory assessments (e.g., rheumatoid factor, anti-citrullinated protein antibody, and C-reactive protein) were negative. In the third case, a seronegative subject reported arthritis in both hands with an onset of 36 days after the third vaccination, which resolved within 11 days. Ten severe adverse effects (SAEs) were reported in 8 subjects, all between 6 months after the first immunization and the booster immunization, i.e., during a period when no immunizations took place; all were considered to be unrelated to vaccination.

**Total IgG antibody response after primary immunizations.** Figure 3A shows the total IgG ELISA antibody responses to each OspA antigen after the primary immunization schedule. At baseline, the geometric mean titers (GMTs) against all six OspA antigens included in the vaccine were very low, and there was no difference in the OspA antibody titers between the C6-seronegative and -seropositive populations. Substantial GMTs against all of the six OspA antigens were induced after the primary immunization schedule in the seronegative and seropositive populations (range, 3,799 to 8,543 and 2,413 to 9,435, respectively, for the two different formulations).

In the seronegative participants, the ELISA antibody responses induced against each OspA serotype were comparable for the 30-µg (range, 3,799 to 6,937) and 60-µg (range, 4,575 to 8,543) doses ( $P \geq 0.062$ ). However, in the seropositive subjects, the 60-µg dose (range, 4,895 to 9,435) resulted in significantly higher GMTs than those for the 30-µg dose (range, 2,413 to 4,371) ( $P < 0.0001$ ). There was a trend toward lower antibody titers in the seropositive population compared to those of the seronegative population for the 30-µg dose; however, this was not statistically



**FIG 1** Trial profile. Asterisks (\*) indicate a protocol deviation (PD), in which the subject was randomized to receive the 60-µg dose but was mistakenly administered the 30-µg dose (the subject was included in the immunogenicity data set for the 60-µg dose). Discontinuation by choice denotes discontinuation at the request of the patient, unrelated to adverse events. Of the 10 participants who discontinued due to an AE, three were judged to be related or possibly related to the vaccination: one reported fatigue, one arthralgia, headache, fatigue, and myalgia, and one alopecia areata (also reported in the medical history) and moderate arthritis (acute arthritis of the proximal interphalangeal finger joints of two fingers of the right hand; an X-ray examination gave no indication of arthritis or arthrosis). Of the two participants who discontinued due to progressive disease, one discontinued due to symptoms of hypothyroidism and the other one due to symptoms associated with type 2 diabetes. Of six participants who discontinued between the primary and booster immunizations due to being assessed as seropositive by C6-ELISA, 1 was subsequently confirmed by line blot, and five were seronegative by line blot. adj, adjuvanted.

significant. The antibody titers against all six serotypes were significantly affected by time in both the seronegative and seropositive subjects ( $P < 0.0001$  and  $< 0.0001$ , respectively), logarithmic titer at baseline ( $P \leq 0.0001$  and  $\leq 0.0045$ , respectively), and age ( $P \leq 0.0067$  and  $\leq 0.0536$ , respectively).

**Total IgG antibody seropersistence and booster response.** Six months after the first immunization, the ELISA antibody titers were maintained at levels substantially and significantly above baseline in the seronegative ( $P < 0.0001$ ) and seropositive ( $P < 0.0001$ ) subjects (Fig. 3B). Analogous to the situation 1 month after the third immunization, no differences were observed in the seronegative subjects (GMT range, 933 to 1,641 and 1,213 to 2,036 for the 30-µg and 60-µg doses, respectively), but the seropositive subjects receiving the 60-µg dose maintained higher antibody titers than those receiving the 30-µg dose (range, 1,342 to 2,306 and 680 to 1,103, respectively).

In both the seronegative and seropositive subjects receiving a booster 6 months after the first immunization, the ELISA antibody responses against all six OspA serotypes contained in the

vaccine were substantially higher than those measured after the primary immunization schedule (Fig. 3B). The postbooster antibody GMTs were comparable for the subjects administered the 30-µg (seronegative range, 9,927 to 14,591; seropositive range, 10,419 to 15,896) and 60-µg (seronegative range, 11,545 to 18,102; seropositive range, 8,064 to 11,167) doses, with no statistically significant dose effect.

Nine to 12 months after the first immunization, the antibody titers were maintained at levels substantially and significantly above baseline in the seronegative ( $P < 0.0001$ ) (GMT range, 339 to 558 and 590 to 1,054 for the 30-µg and 60-µg doses, respectively) and seropositive ( $P \leq 0.0141$ ) (GMT range, 300 to 613 and 531 to 870 for the 30-µg and 60-µg doses, respectively) subjects (Fig. 3C). In the subjects receiving a booster 9 to 12 months after the first vaccination, increases in the antibody GMTs against all six OspA serotypes were observed for both vaccine doses in both populations (Fig. 3C), which were higher than those observed after the 6-month booster. In the seronegative subjects, the postbooster antibody GMTs were comparable for subjects across the two doses

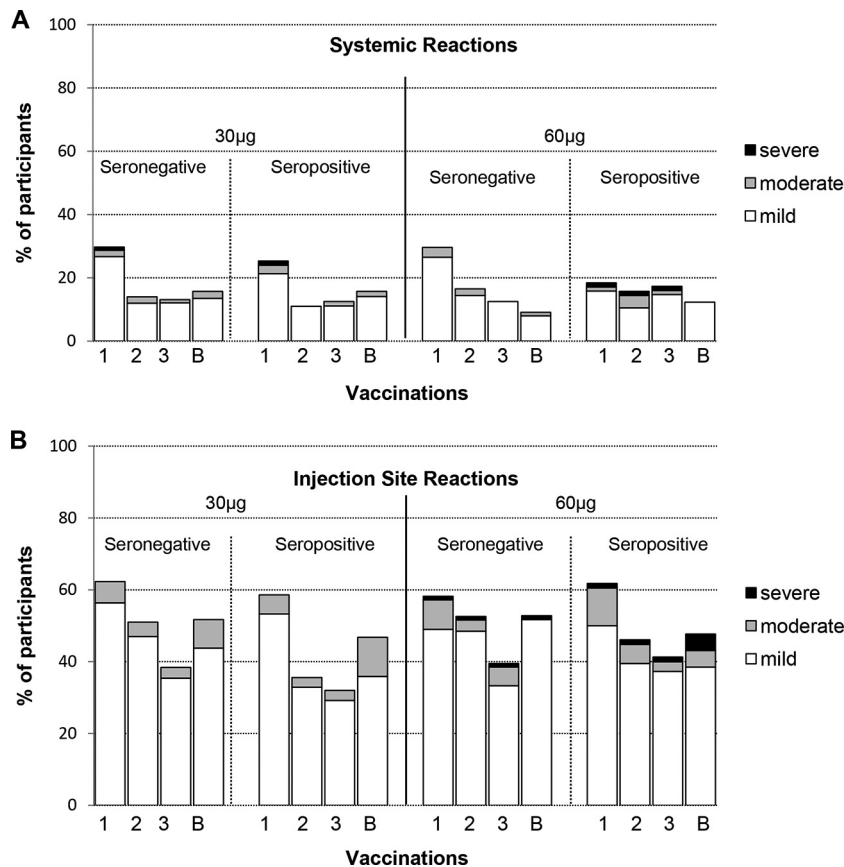


FIG 2 Tolerability profile. Shown are solicited systemic (A) and injection site (B) reactions within 7 days of first (1), second (2), third (3), and booster (B) vaccination with the 30- $\mu$ g and 60- $\mu$ g doses in seronegative and seropositive participants. The data are the % participants with mild (white), moderate (gray), and severe (black) reactions. The data for the 6-month and 9- to 12-month booster immunizations are combined.

(range, 23,799 to 41,735), with no statistically significant differences across all OspA serotypes. However, in the seropositive subjects, the postbooster antibody GMTs were significantly higher against 5 of the 6 OspA serotypes ( $P \leq 0.0359$ ) in the subjects administered the 60- $\mu$ g adjuvanted dose (range, 28,735 to 42,381) than those in the subjects who received the 30- $\mu$ g dose (range, 12,653 to 17,485). Reverse cumulative distributions of the ELISA antibody titers induced by the multivalent OspA vaccine are shown in Fig. 4. After the third primary immunization,  $\geq 93\%$  of the seronegative and  $\geq 94\%$  of the seropositive participants had ELISA antibody titers of  $\geq 1,000$  against the individual OspA serotypes;  $\geq 57\%$  and  $\geq 61\%$ , respectively, achieved titers of  $\geq 5,000$ . After the booster immunization,  $\geq 95\%$  of the seronegative and  $\geq 97\%$  of the seropositive participants had ELISA antibody titers of  $\geq 5,000$ ;  $\geq 87\%$  and  $\geq 79\%$ , respectively, achieved titers of  $\geq 10,000$ .

**Functional antibody responses.** In addition to the high titers of the total IgG OspA antibodies induced by the multivalent OspA vaccine, potent functional antibody responses were generated that bound to the surface of *B. burgdorferi sensu stricto* (OspA-1), *B. afzelii*, (OspA-2), *B. bavariensis* (OspA-4), and *B. garinii* (OspA-3, -5, and -6) (Fig. 5). It should be noted that the differences in surface-binding antibody titers measured in the different OspA types reflect differences in the expression levels of OspA on the different *Borrelia* strains used rather than differences in the poten-

cies of the functional antibody responses. Thus, the comparisons are valid within the same dose and population across time points but not between different OspA serotypes.

At baseline, the seropositive population had significantly higher surface-binding antibody titers against 5 of the 6 *Borrelia* strains used in the surface-binding assay ( $P \leq 0.0486$ ). After the primary immunization schedule, the surface-binding antibody GMTs among the different OspA serotypes ranged from 10.3 to 1,092.7 in the seronegative subjects and from 9.4 to 1,193.8 in the seropositive subjects. After the 9- to 12-month booster immunization, the GMTs among the different serotypes ranged from 108.4 to 3,744.8 in the seronegative subjects and from 79.3 to 4,093.6 in the seropositive subjects.

The antibody titers measured by the surface-binding assay were highly significantly correlated with those measured by ELISA (Spearman correlation coefficient, 0.786 to 0.943 for the six different OspA serotypes). In agreement with the ELISA data, there were no differences in the GMTs induced by the 30- $\mu$ g and 60- $\mu$ g doses in the seronegative subjects, but there was a dose response in the seropositive subjects, with higher GMTs induced by the 60- $\mu$ g dose than by the 30- $\mu$ g dose. Also in agreement with the ELISA data, higher surface-binding antibody titers were induced by the 9- to 12-month booster than those by the 6-month booster (Fig. 5).

TABLE 2 Participants with solicited injection site and systemic reactions within 7 days of first immunization

Reaction	No. (%; 90% CI) for <sup>a</sup> :			
	Seronegative population		Seropositive population	
	30 µg (n = 101)	60 µg (n = 98)	30 µg (n = 75)	60 µg (n = 76)
<b>Local reactions</b>				
Any	63 (62.4, 53.8–70.4)	57 (58.2, 49.4–66.6)	44 (58.7, 48.5–68.3)	48 (63.2, 53.1–72.4)
Swelling	3 (3, 0.8–7.5)	5 (5.1, 2–10.4)	6 (8, 3.5–15.2)	5 (6.6, 2.6–13.3)
Induration	4 (4, 1.4–8.8)	4 (4.1, 1.4–9.1)	4 (5.3, 1.8–11.8)	6 (7.9, 3.5–15)
Redness	3 (3, 0.8–7.5)	5 (5.1, 2–10.4)	2 (2.7, 0.5–8.2)	6 (7.9, 3.5–15)
Injection site pain	42 (41.6, 33.3–50.3)	36 (36.7, 28.6–45.5)	32 (42.7, 33–52.8)	37 (48.7, 38.8–58.7)
Tenderness	50 (49.5, 40.9–58.1)	40 (40.8, 32.4–49.6)	31 (41.3, 31.7–51.5)	23 (30.3, 21.6–40.1)
<b>Systemic reactions</b>				
Any	30 (29.7, 22.3–38.1)	29 (29.6, 22–38.1)	19 (25.3, 17.3–34.9)	14 (18.4, 11.5–27.3)
Malaise	5 (5, 2–10.1)	2 (2, 0.4–6.3)	2 (2.7, 0.5–8.2)	2 (2.6, 0.5–8.1)
Fatigue	8 (7.9, 4–13.8)	5 (5.1, 2–10.4)	4 (5.3, 1.8–11.8)	6 (7.9, 3.5–15)
Headache	12 (11.9, 7–18.5)	6 (6.1, 2.7–11.7)	3 (4, 1.1–10)	4 (5.3, 1.8–11.6)
Nausea	1 (1, 0.1–4.6)	0 (0, 0–3)	2 (2.7, 0.5–8.2)	1 (1.3, 0.1–6.1)
Vomiting	0 (0, 0–2.9)	0 (0, 0–3)	0 (0, 0–3.9)	1 (1.3, 0.1–6.1)
Myalgia	12 (11.9, 7–18.5)	12 (12.2, 7.2–19.1)	10 (13.3, 7.4–21.6)	4 (5.3, 1.8–11.6)
Arthralgia	2 (2, 0.4–6.1)	4 (4.1, 1.4–9.1)	4 (5.3, 1.8–11.8)	1 (1.3, 0.1–6.1)
Fever (>38.0°C)	0 (0, 0–2.9)	0 (0, 0–3)	0 (0, 0–3.9)	0 (0, 0–3.9)

<sup>a</sup> CI, confidence interval.

## DISCUSSION

A novel multivalent OspA vaccine is safe and immunogenic in healthy seronegative and seropositive adults and induces a significant increase in total ELISA and functional surface-binding antibody titers against all six OspA serotypes after vaccination with either a 30-µg or 60-µg OspA antigen dose adjuvanted with alum. The antibody responses induced after a three-dose primary immunization schedule declined up to 12 months after the first immunization but remained significantly above baseline and were effectively boosted by a fourth dose either 6 months or 9 to 12 months after the first immunization. The booster response was higher when the booster immunization was administered 9 to 12 months than at 6 months after the first immunization. In the seronegative subjects, the antibody responses induced by the 30-µg and 60-µg doses were similar, in agreement with a previous study of the multivalent OspA vaccine in a seronegative population (22). However, in the seropositive subjects, the 60-µg dose induced significantly higher antibody titers than did the 30-µg dose. The vaccine was well tolerated, with similar tolerability profiles across the two doses in both populations.

As expected, because OspA is downregulated upon transmission to the infected host (25), such that OspA antibodies are not usually detectable in infected individuals, there was no difference in the baseline OspA antibody titers in the seropositive and seronegative populations. In contrast, because the surface-binding assay detects all antibodies that bind to *Borrelia*, the baseline surface-binding antibody titers were significantly higher against 5 of the 6 *Borrelia* strains used in the surface-binding assay in the seropositive population than those in the seronegative population.

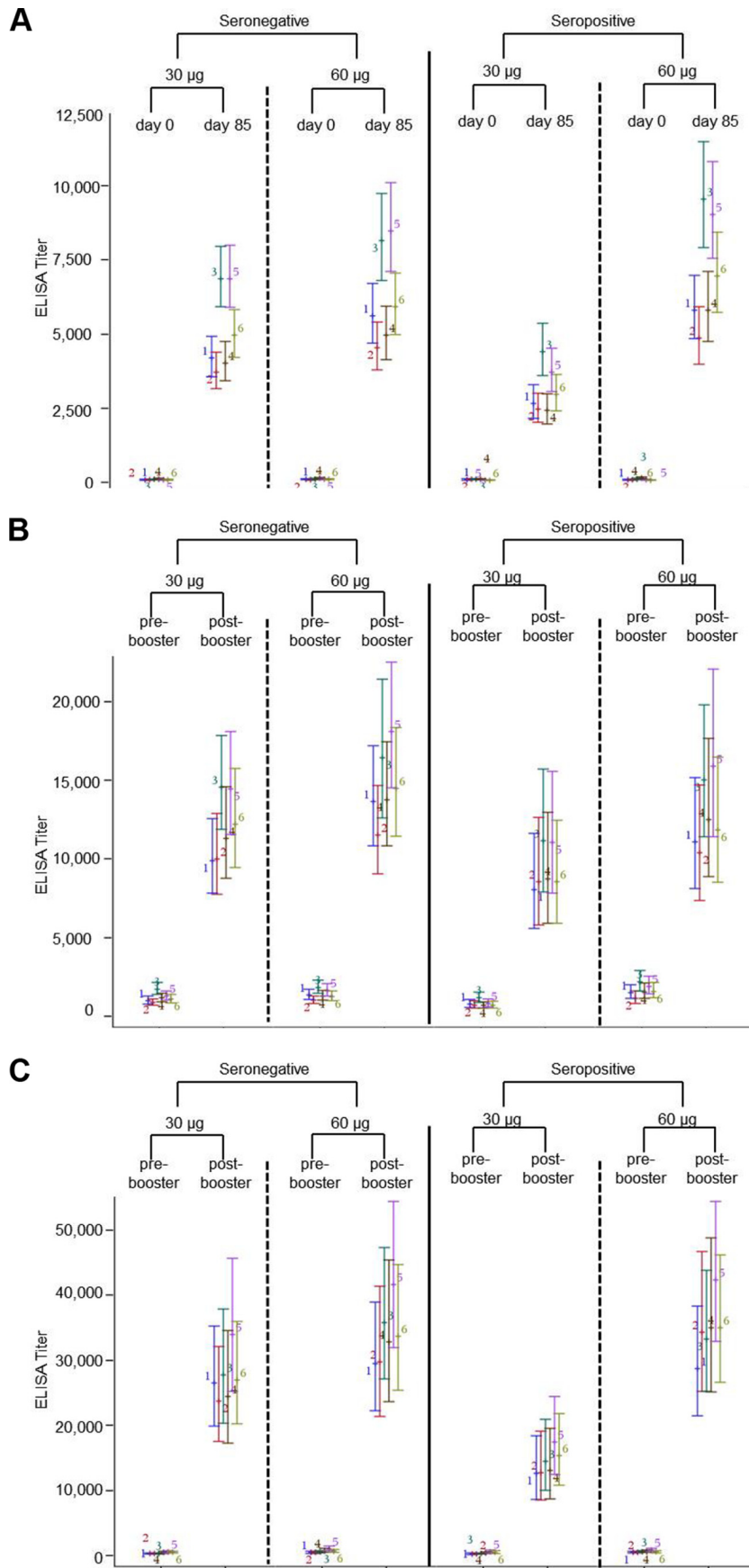
It is not clear why a dose response was observed in the seropositive but not the seronegative population, such that the 30-µg dose induced lower antibody titers in the seropositive population than those in the seronegative population. It has been suggested that prior infection with *B. burgdorferi sensu lato* might blunt the immune response, resulting in a lower ability of seropositive subjects

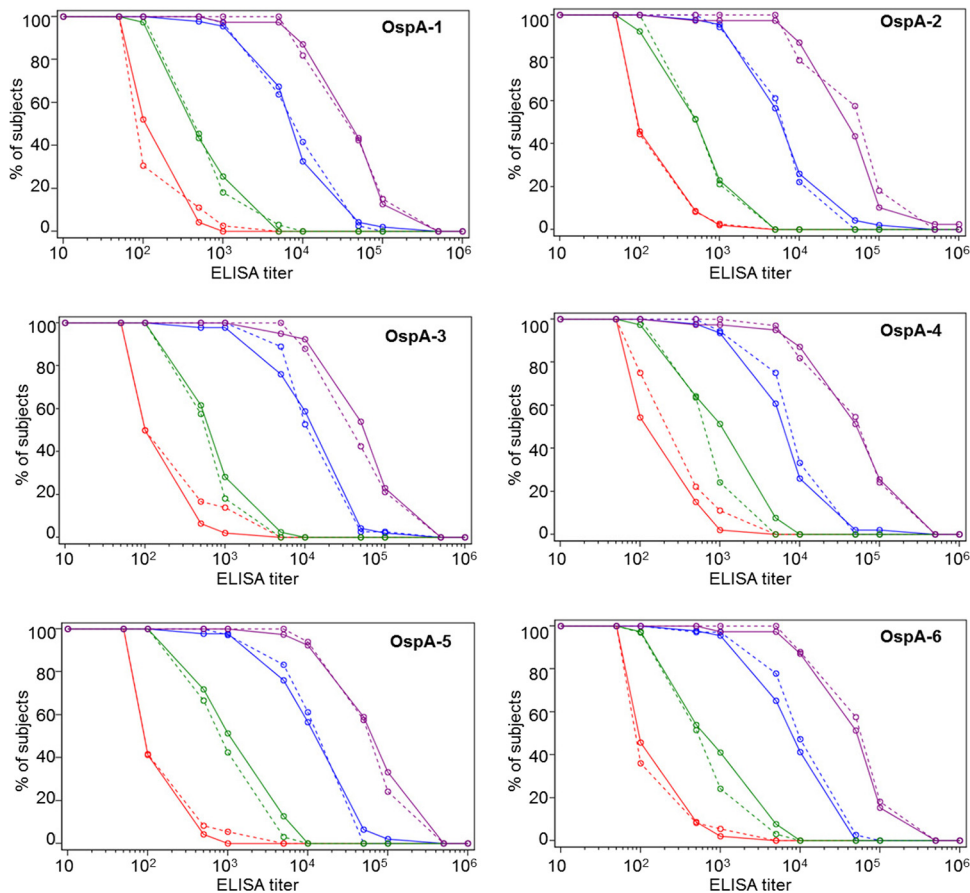
to mount an immune response to vaccination with OspA (26, 27). Alternatively, because of the lipidated nature of the OspA vaccine and the high expression of lipoproteins by *B. burgdorferi sensu lato*, it is possible that the anti-lipoprotein antibodies induced in seropositive subjects by previous infection interfere with the induction of antibody responses against the OspA vaccine, such that higher antigen doses are required in the seropositive population.

One previous study of a monovalent OspA-1 vaccine investigated the antibody responses to vaccination in seropositive subjects, but this study did not include a head-to-head seronegative cohort (27). Because of the lack of a direct comparator group in this previous study and the use of a different assay format than those in studies on the same vaccine in seronegative populations (28–33), it is not clear whether the previous monovalent OspA-1 vaccine induced similar antibody titers in seronegative and seropositive populations.

The higher antibody responses to the 9- to 12-month booster than to the 6-month booster is a known phenomenon reported in other studies of inactivated vaccines, in which later booster vaccinations were found to induce higher antibody titers (34). Residual antibodies present after priming can result in the formation and subsequent rapid clearance of antigen-antibody complexes (35, 36). Antibody secreting B or plasma cells may also negatively regulate cognate T-helper-cell functions essential for antigen-specific secondary B-cell responses (37, 38). In a previous study of a monovalent OspA-1 vaccine that compared the antibody responses induced by a two-dose primary immunization regimen followed by either a 6-month or 12-month booster, higher antibody responses were also induced after the later booster than after the earlier booster, although it was concluded that the two vaccination schedules elicited equivalent immune responses (33).

A considerable drop in antibody titers was observed prior to the booster immunizations. Despite this, the antibody levels for all OspA serotypes were substantially and significantly above baseline at these time points. Although a correlate of protection can be





**FIG 4** Reverse cumulative distribution of ELISA antibody titers against OspA serotypes 1 to 6 (marked by different color bars and labeled by number) in seronegative and seropositive participants receiving three priming immunizations with the 60- $\mu$ g dose and a booster at 9 to 12 months after the first immunization. The seronegative participants are represented by solid lines, and the seropositive participants are represented by dashed lines. The data are at baseline (red lines), 28 days after the third priming immunization (blue lines), before the 9- to 12-month booster (green lines), and 28 days after the 9- to 12-month booster (purple lines).

determined only in phase III efficacy studies, it is likely that repeat booster immunizations will be necessary to maintain high levels of circulating antibodies required for protection.

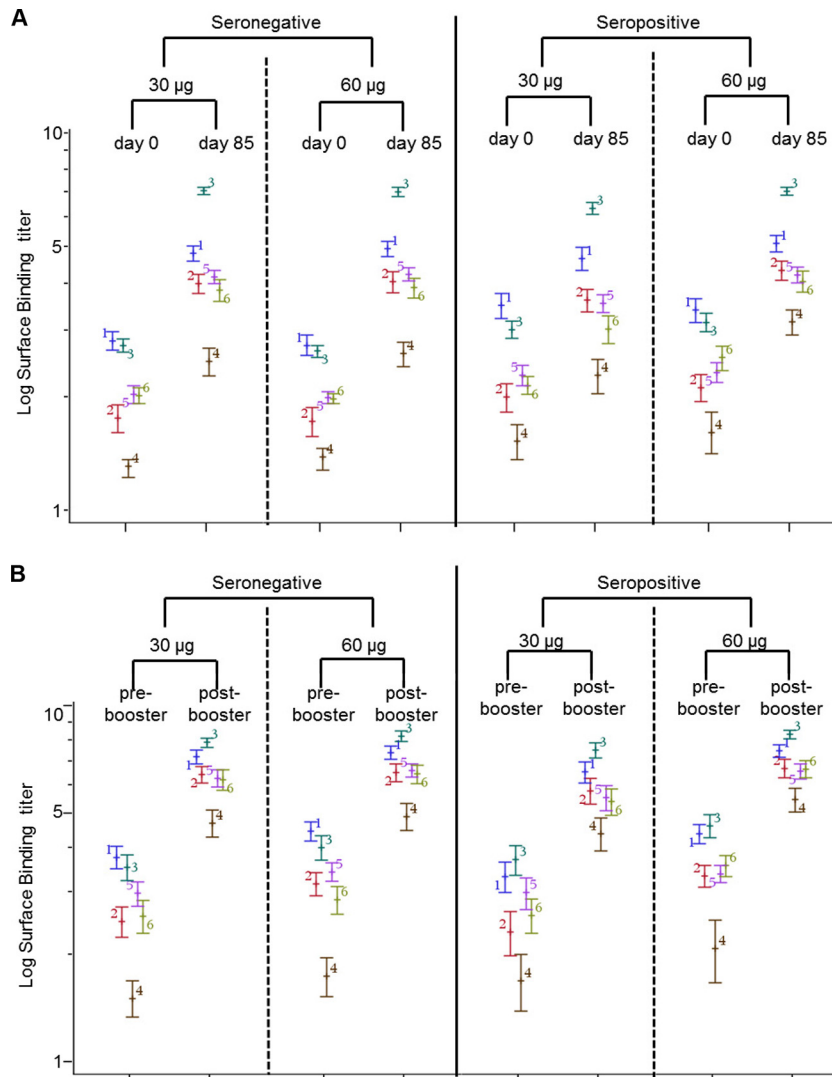
The primary ELISA-based immunogenicity data are supported by the demonstration that vaccine-induced antibodies are functionally capable of binding to *B. burgdorferi*, *B. afzelii*, *B. bavariensis*, and *B. garinii* species expressing OspA serotypes 1 to 6, which are representative of all major human-pathogenic species. In pre-clinical studies in mice, OspA and surface-binding antibody titers have been shown to correlate with protection from *Borrelia* challenge (39). Importantly, the antibody titers measured by the surface-binding assay were highly correlated with those measured by ELISA, and they were also in agreement with the ELISA data with respect to the differences in dose response in the seronegative and seropositive subjects, as well as the higher titers induced by the 9- to 12-month booster than those of the 6-month booster.

Taken together, the study data suggest that the novel multiva-

lent OspA vaccine is equally safe and well tolerated in seronegative and seropositive subjects, and it induces substantial antibody responses against all six OspA serotypes included in the vaccine. A substantial booster response was induced in both populations by a booster vaccination either 6 months or 9 to 12 months after the first immunization. The study data also demonstrate that a 60- $\mu$ g dose is the preferred dosage for entry into phase III trials, as this dose induces significantly higher titers than does a 30- $\mu$ g dose in the seropositive population, with no significant difference in tolerability. This is the first study reporting the direct comparison of an OspA vaccine in *B. burgdorferi sensu lato*-seronegative and -seropositive populations. The potential limitations of this study include the fact that the screening C6-ELISA may not discriminate 100% between subjects previously exposed to LB and seronegative subjects. Additionally, our study did not include patients who had preexisting antibody titers to OspA, as is sometimes found in patients with late-stage Lyme disease. We also did not systematically

**FIG 3** ELISA titers induced against OspA serotypes 1 to 6 (marked by different color bars and labeled by number) in seronegative and seropositive participants receiving the 30- $\mu$ g and 60- $\mu$ g doses at baseline and 28 days after the third priming immunization (day 85) (A), before the 6-month booster (prebooster) and 28 days after the 6-month booster (postbooster) (B), and before the 9- to 12-month booster (prebooster) and 28 days after the 9- to 12-month booster (postbooster) (C). The data are the GMTs and 95% CIs.





**FIG 5** Surface-binding antibody titers induced against OspA serotypes 1 to 6 in seronegative and seropositive participants receiving three priming immunizations with the 60-µg dose and a booster at 9 to 12 months after the first immunization at baseline and 28 days after the third priming immunization (day 85) (A) and before the 9- to 12-month booster (prebooster) and 28 days after the 9 to 12 month booster (postbooster) (B). The data are the GMTs and 95% CIs.

capture a detailed medical history on previous LB manifestations in the seropositive individuals. However, as seropositive individuals remain at high risk for subsequent infection with *B. burgdorferi sensu lato*, it is reassuring that the study data suggest that the novel multivalent OspA vaccine should be effective in both populations.

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