Reservoir Targeted Vaccine for Lyme Borreliosis Induces a Yearlong, Neutralizing Antibody Response to OspA in White-Footed Mice

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Lyme disease caused by Borrelia burgdorferi, is the most common vector-borne disease in North America and Europe (2), and the area of where the disease is endemic is expanding (28, 39, 57). B. burgdorferi infection produces a progressive disease with a wide array of clinical manifestations involving the skin, heart, joints, and central and peripheral nervous system (46, 49–51). Disseminated infection can cause permanent damage to some of these systems (50). Lyme disease can be successfully treated with antibiotics, but recovery can involve a substantial convalescence period (31, 40, 41, 58). No human vaccine is commercially available.

In eastern North America, B. burgdorferi is transmitted among wildlife hosts and humans by Ixodes scapularis (1, 4, 5, 34). I. scapularis has a 2-year life cycle with four life-stages: egg, larva, nymph and adult (1). The nymph is the tick life stage that infects humans (3, 26, 55). Control measures aimed at disrupting the life cycle of B. burgdorferi have been difficult because there are no effective measures to control B. burgdorferi; there is no human vaccine available, and existing vector control measures are generally not acceptable to the public. However, if B. burgdorferi could be eliminated from its reservoir hosts or from the ticks that feed on them, the enzootic cycle would be broken, and the incidence of Lyme disease would decrease. We developed OspA-RTV, a reservoir targeted bait vaccine (RTV) based on the immunogenic outer surface protein A (OspA) of B. burgdorferi aimed at breaking the natural cycle of this spirochete. White-footed mice, the major reservoir species for this spirochete in nature developed a systemic OspA-specific IgG response as a result of ingestion of the bait formulation. This immune response protected white-footed mice against B. burgdorferi infection upon tick challenge and cleared B. burgdorferi from the tick vector. In performing extensive studies to optimize the OspA-RTV for field deployment, we determined that mice that consumed the vaccine over periods of 1 or 4 months developed a yearlong, neutralizing anti-OspA systemic IgG response. Furthermore, we defined the minimum number of OspA-RTV units needed to induce a protective immune response.

MATERIALS AND METHODS

Lyophilization and viability of E. coli expressing B. burgdorferi OspA. A culture of Escherichia coli BL21(DE3)(pLysS) harboring a plasmid containing the full-length sequence of OspA from B. burgdorferi (strain B31) was induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and the cells were harvested, resuspended in a solution of TBY containing 12% sucrose (27), and quickly frozen. The antigen was placed in a lyophilizer (Labconco) overnight and stored at −70°C for future use. The viability of the lyophilized bacteria expressing the lipidated form of OspA was determined by adding 200 mg of lyophilized vaccine to 1 ml of phosphate-buffered saline (PBS) and plating 1/10 of the suspension onto TBY plates supplemented with proper antibiotic. Two hundred milligrams of E. coli cells expressing OspA contains approximately 2 mg/ml of lipidated OspA. The parental Escherichia coli strain transformed with the empty plasmid was used as a control.

Bait vaccine production. One vaccine dose was made using 200 mg of lyophilized, previously induced bacteria, resuspended in 200 μl of water, and mixed with rolled oats (approximately 2 mg/ml of lipidated OspA). The mixture was made daily before immunization and offered ad libitum for ingestion. To test exposure to high temperature and humidity, the edible bait was treated for 24 h at 34°C with 60% humidity.

Immunization schedule. Outbred white-footed mice (Peromyscus leucopus; Peromyscus Stock Center, University of South Carolina) were used. Initially, we

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TABLE 1. Transmission of *B. burgdorferi* in ticks that fed on bait-vaccinated white-footed mice (*P. leucopus*) as determined by OspC-PCR

<table>
<thead>
<tr>
<th>Vaccinea</th>
<th>No. of mice</th>
<th>No. of ticks infected/total (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>OspA-RTV (EcA)</td>
<td>12</td>
<td>0/63 (0)*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0/15 (0)†</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5/54 (15)%</td>
</tr>
<tr>
<td>Control (Ec)</td>
<td>3</td>
<td>7/17 (41)*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>6/9 (67)%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5/6 (83)%</td>
</tr>
</tbody>
</table>

a OspA-RTV (EcA), reservoir targeted vaccine, which is a bait vaccine composed of *E. coli* cells expressing *B. burgdorferi* OspA (EcA) mixed with oatmeal; Control (Ec), *E. coli* cells transformed with empty vector mixed with oatmeal.

b P values were determined by two-tailed Fisher’s exact test: *, P < 0.0001; †, P = 0.0006; §, P = 0.002; ¶, P = 0.05.

ND, not determined.

used an immunization schedule comprised of offering one vaccine unit per day during 5 days a week for 4 weeks, followed by a 2-week boost, for a total of 30 vaccine units per a period of 8 weeks (1 unit = 200 mg *E. coli* cells expressing *B. burgdorferi* OspA ~ 2 mg/ml of lipitated OspA). For the studies aimed at optimizing immunization schedules for field application, five protocols were tested. In the first, each white-footed mouse received 1 vaccine unit daily on 5 days a week during 4 weeks, for a total of 20 vaccine units per month. In the second, each mouse received 1 vaccine unit daily on 5 days a week during 16 weeks, for a total of 80 vaccine units per 4 months. In the third, each mouse received 1 vaccine unit daily on 3 days a week during 16 weeks, for a total of 48 vaccine units per 4 months. In the fourth, each mouse received 1 vaccine unit daily on 2 days a week during 16 weeks, for a total of 32 vaccine units per 4 months. In the fifth, each mouse received 1 vaccine unit daily on 1 day a week during 16 weeks, for a total of 16 vaccine units per 4 months.

**Challenge with *B. burgdorferi*-infected ticks.** Challenge was performed 2 weeks after the last vaccine intake by placing 6 to 8 *B. burgdorferi*-infected nymphal ticks for 2 h on the back of the head of restrained mice. Three days later, engorged ticks were collected after naturally falling off, counted, and a daily record was kept for each mouse. Three weeks later, mice were euthanized and blood, heart, and bladder tissues were obtained to assess for protection or *spirochete dissemination*. All experiments involving animals were performed after obtaining proper University of Tennessee Health Science Center (UTHSC) IACUC approval.

**Determination of vaccine efficacy.** (i) Antibody assays. Total IgG was determined by enzyme-linked immunosorbent assay (ELISA) using 0.5 μg/ml of purified recombinant OspA and serum from immunized white-footed mice (1:100) and horse-radish peroxidase-conjugated anti-*Peromyscus leucopus* secondary antibody (1:16,000) (KPL). The immunoblot test (Virablot; Viralab) was used to screen for anti-*B. burgdorferi* IgG antibodies in serum from vaccinated mice after challenge. A pattern of 5 out of 10 bands positive (93, 66, 58, 45, 41, 39, 30, 28, 23, and 18 kDa) was considered evidence of infection.

(ii) LA-2 equivalent assay. The LA-2 equivalent assay was performed as described in reference 30.

**Neutralization assay.** *Borrelia burgdorferi* strain BL206 (courtesy of G. Wormser, New York Medical College, Valhalla, NY) was grown in BSK to 5×10^7 cells per ml at room temperature. Eight microliters of the culture were mixed with 8 μl of BSK medium (Sigma, Saint Louis, MO) and 4 μl of fresh *Peromyscus leucopus* serum. Controls included heat-inactivated preimmune sera without guinea pig complement (Rockland Laboratories, Gilbertsville, PA), complement only, and pooled anti-OspA antisera with complement. The samples were incubated at 37°C for 18 h. Live and dead *B. burgdorferi* spirochetes were recorded in five high-power fields using a 400× dark-field microscope (AxioImager, Zeiss, Germany). Spirochetes showing signs of mobility were counted as live; nonmotile spirochetes were counted as dead.

(iii) *B. burgdorferi* culture. The heart and bladder were individually cultured in Barbour-Stoenner-Kelly (BSK-H) medium (New York Medical College) with an antibiotic mixture for *Borrelia* (Sigma) for up to 6 weeks at 34°C. Cultures were checked every week by dark-field microscopy.

**RESULTS**

Host reservoir competence and vector transmission probability. To determine the efficacy of OspA-RTV bait vaccination in clearing *B. burgdorferi* from the vector (nymph), we tested the infection prevalence of the ticks after challenge of immunized *P. leucopus* mice (day 75). To evaluate transmission of *B. burgdorferi* to the next tick cohort (larvae), we tested the ticks placed on the same mice 3 weeks after challenge (xenodiagnosis, day 98). Results of three independent experiments are summarized in Table 1. Total DNA extracted from ticks was tested by PCR using *B. burgdorferi* OspC primers. *B. burgdorferi* was cleared from most nymphal ticks (day 75) that fed on the OspA-RTV group but not from ticks that fed on the control group of white-footed mice. In two experiments, nymphal infection prevalence (NIP) was reduced from 41% to 0% (P < 0.0001) and from 67% to 0% (P = 0.0006) as a result of OspA-RTV transmission. In a third experiment, NIP was reduced from 83% to 15% as a result of OspA-RTV consumption (P = 0.002). Overall, reductions in NIP ranged from very to extremely statistically significant. When we performed xenodiagnosis, we observed that larval infection prevalence (LIP) was reduced from 57% to 13% (P = 0.05) as a result of OspA-RTV consumption. Reduction in LIP was borderline statistically significant.

To determine the efficacy of OspA-RTV bait vaccination in preventing dissemination of *B. burgdorferi* in the rodent host (white-footed mouse, *P. leucopus*), we analyzed the tissues collected at termination (day 105) following a protocol of bait vaccination, challenge, and xenodiagnosis (Table 2). Groups of *P. leucopus* mice (n = 16) received 200 mg of *E. coli* cells expressing OspA (EcA; 2 mg/ml of OspA) mixed with oatmeal, during 5 days per week for 4 consecutive weeks, rested 2 weeks and received a boost over 2 weeks (30 vaccine units over 8

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**TABLE 2. Dissemination of *B. burgdorferi* in bait-vaccinated white-footed mice (*P. leucopus*) at termination (day 105)**

<table>
<thead>
<tr>
<th>Vaccinea</th>
<th>No. of mice</th>
<th>Anti-<em>B. burgdorferi</em> IgG (Western blot)</th>
<th>Dark-field microscopy of <em>B. burgdorferi</em> culture</th>
<th>Tissue PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>OspA-RTV (EcA)</td>
<td>16</td>
<td>1/16 (6.25)</td>
<td>1/16 (6.25)</td>
<td>2/16 (12.5)</td>
</tr>
<tr>
<td>Control (Ec)</td>
<td>14</td>
<td>14/14 (100)</td>
<td>10/10 (100)</td>
<td>11/12 (91.6)</td>
</tr>
</tbody>
</table>

a OspA-RTV (EcA), reservoir targeted vaccine, which is a bait vaccine composed of *E. coli* cells expressing *B. burgdorferi* OspA (EcA) mixed with oatmeal; Control (Ec), *E. coli* cells transformed with empty vector mixed with oatmeal.

b P < 0.001 for all intercolumnar comparisons by two-tailed Fisher’s exact test.
Immunization with OspA-RTV formulation and E. coli cells expressing B. burgdorferi OspA (EcA) mixed with oatmeal; NeutrA, neutralization assay; cPCR, PCR done with cultures of bladder tissue. Data are representative of one of two independent experiments.

Weeks). Groups of P. leucopus (n = 14) received 200 mg of E. coli cells mixed with oatmeal and were used as controls. Mice were allowed to rest for 2 weeks, and then tick challenge was performed (day 70) using naturally infected field-caught nymphal ticks. Three weeks later (day 98), mice were subjected to xenodiagnosis with clean larval ticks, and 1 week later (day 105), they were terminated and blood and tissues were collected to evaluate spirochetal dissemination. Blood was collected throughout the protocol to monitor the systemic IgG response to OspA. Results of three independent experiments are summarized in Table 2. All 14 mice (100%) that received oatmeal control developed evidence of B. burgdorferi dissemination: 14 had low OspA-specific IgG (optical density at 450 nm [OD450] of 0.065 with a standard deviation [SD] of 0.033), 14 had a positive Western blot, 10/10 had a positive B. burgdorferi culture, and 11/12 (92%) showed a positive tissue PCR. In contrast, only 6 to 13% of the 16 P. leucopus mice that consumed the OspA-RTV (EcA) showed evidence of B. burgdorferi dissemination: 16 mice developed high OspA-specific IgG (OD450 1.08; SD, 0.280), 1/16 (6.25%) had a positive Western blot, the same mouse had a positive tissue PCR, and an additional mouse had positive PCR tissue (12.5%). Differences between these groups are extremely statistically significant by the two-tailed Fisher’s exact test. Thus, the triad vector-B. burgdorferi-hor host was disrupted as a result of immunization with OspA-RTV, and dissemination of B. burgdorferi only occurred in mice that did not develop sufficient levels of anti-OspA antibodies.

Correlates of protection. Through our extensive experimentation with the OspA-RTV formulation and P. leucopus over the past 6 years, we observed that an effective immune response could be measured by a titer of antibody specific to OspA equivalent to an OD450 of ≥1. The LA-2 equivalent antibody assay is generally accepted as a good correlate of vaccine protection (30). To test our hypothesis, we analyzed the LA-2 equivalent antibody present in the serum from white-footed mice after consumption of the bait RTV and evaluated its correlation to protection as measured by a neutralization assay and by assessment of B. burgdorferi dissemination in the mouse after challenge by PCR. P. leucopus mice (OspA-RTV, n = 10; control, n = 6) received 30 units of oral RTV (200 mg bait, 2 mg/ml of lipidated OspA dose per unit) over a period of 8 weeks. Blood was collected on days 14, 28, 42, 56, and 70 to evaluate the levels of OspA-specific IgG (OD450) and the amount of LA-2 equivalent antibody (ng/ml). Blood collected on day 70 was used in the neutralization assay. Mice were challenged with naturally infected field nymphal ticks, and 4 weeks later (day 105), they were terminated and bladder tissue was tested for the presence of B. burgdorferi by culture and PCR. OspA-RTV (EcA), reservoir targeted vaccine, which is a bait vaccine composed of E. coli cells expressing B. burgdorferi OspA (EcA) mixed with oatmeal; Control (Ec), E. coli cells transformed with empty vector mixed with oatmeal; NeutrA, neutralization assay; cPCR, PCR (Fig. 1; data are representative of one of two experiments). In the group vaccinated with OspA-RTV, two mice developed low titers of anti-OspA antibody (0.5 < OD450 < 0.8) but no LA-2 equivalent antibodies, and serum from these mice failed to completely neutralize B. burgdorferi in culture (about 8 or 9 spirochetes were counted per mouse). As expected, these mice became infected as a result of nymphal challenge. The remaining eight mice developed high titers of antibody to OspA (2 < OD450 > 1); ¾ of these presented with high concentration of anti-LA-2 antibodies; serum from all eight mice neutralized B. burgdorferi in culture (0 live B. burgdorferi spirochetes counted per mouse), and none showed evidence of B. burgdorferi dissemination after nymphal challenge, by OspC PCR of bladder culture. Thus, LA-2 equivalency was only a correlate of protection for 75% of the mice that produced anti-OspA IgG titers superior to 1. None of the six controls developed antibodies to OspA or LA-2 equivalent antibodies, and serum from all six failed to neutralize B. burgdorferi in culture (a range of 20 to 45 live spirochetes were counted per mouse). Furthermore, all six mice provided evidence of B. burgdorferi dissemination by OspC PCR of bladder culture. Considering that 25% of white-footed mice in our study that were protected from infection after tick challenge week...
developed a high titer of total IgG but did not develop measurable amounts of LA-2 equivalent antibody, in this study, we defined a titer of antibody to OspA superior to 1 (OspA OD$_{450}$ of $>1$) as a correlate of protection.

Aiming for the lightest vaccine schedule in preparation for our intensive field study, we compared our efficacy-proven 30-unit vaccination schedule delivered in 6 weeks ($n=5$ mice) with a 20-unit vaccination schedule delivered over a period of 4 weeks ($n=5$ mice). We found that the 30-unit schedule induced titers of antibody specific to OspA and LA-2 equivalent antibodies in all mice comparable to the 20-unit schedule. Furthermore, we determined that mice receiving the 20-unit vaccination schedule were equally protected from *B. burgdorferi* dissemination after tick challenge as mice receiving the 30-unit schedule.

Optimization of OspA-RTV for field distribution. We developed a vaccination protocol for the white-footed mouse (*P. leucopus*) to be applicable in the field. To identify alternative immunization schedules, we tested the following: one group of mice consumed a unique dose of 200 mg of OspA-RTV ($\approx 2$ mg/ml of OspA) per day, 5 times per week for a period of 4 weeks (20 units); a second group consumed a dose of 200 mg of OspA-RTV per day, 5 times per week, for a period of 16 weeks (80 units); a third group consumed a dose of 200 mg of OspA-RTV per day once per week, for a period of 16 weeks (16 units); a forth group consumed a dose of 200 mg of OspA-RTV per day twice per week for a period of 16 weeks (32 units). Groups consisted of four mice. The respective control was added to each group. The immune response to OspA was monitored for 1 year (Fig. 2). Blood samples were collected on day 0 and subsequently at 2- to 3-week intervals for 365 days. OspA-specific total IgG antibody was determined against purified recombinant OspA by ELISA. Mice that consumed OspA-RTV 5 times per week, either 20 or 80 units (Fig. 2A and B), developed a quicker immune response to OspA showing protection correlate results (OD$_{450}$ of $>1$) by day 14. Mice that consumed fewer units of OspA-RTV per week developed an equivalent amount of antibody to OspA later: by day 21 (32 units twice a week; Fig. 2C) and by day 28 (16 units once a week; Fig. 2D). Mice that consumed the oatmeal control did not develop an immune response to OspA (data not shown). Thus, the intake in number of vaccine units per week correlates with how fast the mouse mounts an immune response to OspA in the first 4 weeks of immunization. Over the entire immunization schedule (1 month in Fig. 2A and 4 months in Fig. 2B, C, and D), there was no significant statistical difference between the distributions of 16, 32, and 80 units over a period of 4 months and that of 20 units over a period of 1 month. In conclusion, all four immunization schedules tested are applicable in the field, although the least cumbersome schedule—20 units distributed 5 times per week over a period of 4 weeks—has the additional advantage of inducing a quicker immune response to OspA.

When we compared the three immunization schedules over the period of exponential increase of antibody to OspA after vaccination (first 6 weeks), we determined the smallest number...
of vaccine units needed to induce a protective immune response (Fig. 3). On days 28 (4 weeks) and 42 (6 weeks), there were no statistically significant differences between the antibody responses to OspA in the group of mice that received 1 (4 to 6 units) or 5 (20 to 30 units) vaccine units per week. Thus, we conclude the minimum number of vaccine units (200 mg RTV with 2 mg/ml of OspA/unit) to induce a potentially protective immune response to OspA to be around 5.

To finalize optimization of the reservoir targeted vaccine, we tested the OspA-RTV resistance to natural field conditions. White-footed mice were fed a single dose of OspA-RTV (200 mg, ~2 mg/ml of OspA) pretreated at 34°C with 60% humidity following three schedules (Fig. 4). On the first schedule, mice consumed the OspA-RTV three times per week for a period of 16 weeks (48 units; Fig. 4A). On the second, mice consumed RTV once per week for a period of 16 weeks (16 units; Fig. 4B). Groups consisted of four mice. The respective control was added to each group. As described above, the immune response to OspA was monitored for 1 year and the OspA-specific total IgG antibody level was determined against purified recombinant OspA by ELISA. Controls did not develop antibody responses to OspA (data not shown). We determined that treatment of the vaccine using conditions of temperature and humidity that mimic natural field conditions did not have adverse effects on the RTV competence to induce an immune response to OspA.

**DISCUSSION**

Currently, there is no vaccine against Lyme disease for human use. The primary means of preventing this debilitating
disease is to avoid ticks by foregoing outdoor activities where Lyme disease risk is high. A promising alternative is to design control measures to reduce that risk. A highly specific, easily distributable, and inexpensive wildlife vaccine that targets the triad vector-\textit{B. burgdorferi}-host should reduce the prevalence of \textit{B. burgdorferi} in ticks and in the mouse reservoir around human communities. Disruption of the enzootic cycle of this pathogen could significantly reduce the incidence of Lyme disease cases within several years.

White-footed mice (\textit{P. leucopus}) are important reservoir hosts for \textit{B. burgdorferi}, infecting 80 to 90% of larval ticks that feed on them, thereby affecting dramatically the prevalence of \textit{B. burgdorferi}-infected ticks (7, 36, 42). The importance of mice as disease reservoirs and the ability to conduct controlled field experiments with this species make \textit{P. leucopus} the ideal target for a reservoir targeted vaccine (34, 45). We previously reported the development of an oral vaccine that disrupts the transmission cycle of \textit{B. burgdorferi} after testing the bait vaccine in a strain of inbred \textit{Mus musculus} (25). As a continuation, in this study we report the application of this bait vaccine to \textit{P. leucopus}, the natural vertebrate host of \textit{B. burgdorferi}, with the objective of working out protocols for field deployment.

\textit{B. burgdorferi} OspA has a unique mode of action that makes it an attractive candidate to develop an oral bait vaccine to control this spirochete. OspA is a mucosal immunogen and adjuvant (18) and oral delivery of OspA protected mice from systemic infection with \textit{B. burgdorferi} after needle challenge (17, 22, 38). Mice immunized with OspA produce antibodies that target spirochetes in the gut of the tick vector, thereby blocking transmission to the host. In addition, \textit{B. burgdorferi} is eliminated from ticks that feed on OspA-immunized mice (12–14, 17, 23). Furthermore, immunization decreases transmission of \textit{B. burgdorferi} from infected white-footed mice to \textit{I. scapularis} ticks both in the laboratory (11, 25) and in the field (53, 55). In addition, we and others have shown that oral gavage immunization of inbred \textit{Mus musculus} mice protects mice from challenge with infected ticks (25, 47). However, vaccinia virus-based vaccines have the drawback of being infectious to people who are immunocompromised or suffer from eczema (35, 56).

There is a precedent for the development of bait vaccines to be used as a strategy to reduce the risk of Lyme borreliosis (44). Baits and baiting systems for delivery of rabies vaccines (19, 32, 43), plague vaccines (9), and immuncontraceptive vaccines (6) have proven successful. Other systems of tick control have been explored. In one study, acaricide self-treatment of white-tailed deer resulted in reduction of tick density (10, 24, 29, 48). In another study, a rodent-targeted acaricide (fipronil) delivered to white-footed mice (\textit{P. leucopus}) in modified commercial bait boxes was also effective in reducing nymphal and larval tick infestations (tick density) (15). In an alternative approach, a doxycycline rodent bait formulation prevented tick transmission of \textit{B. burgdorferi} to vertebrate hosts as well as cured established infections in mice (16) (59). A decrease in tick density or tick infection, as well as the decrease of vertebrate host infection with \textit{B. burgdorferi}, is expected to result in the overall decrease in the human risk to Lyme disease.

Our results suggest that OspA-RTV bait immunization of \textit{P. leucopus} drastically reduced vector transmission probability of \textit{B. burgdorferi}, given that in two independent experiments, we observed a reduction of NIP from 41% and 67% to 0%. The fact that ticks with the highest spirochete burden (83%) were still able to transmit \textit{B. burgdorferi} to the rodent host (15%) suggests that the amount of anti-OspA antibody circulating in the rodent was insufficient to block transmission in the nympha tick. Thus, the high spirochete burden in the tick overpowered RTV efficacy and is a factor to keep in mind when estimating the expected reduction of NIP as a result of RTV treatment over the years. In addition, in experiments in which we used xenodiagnosis to access host reservoir competence, we observed a solid disruption of transmission of \textit{B. burgdorferi} to the larvae even though clearance was not absolute. Although host reservoir competence was discretely affected after one round of RTV treatment, we expect the cumulative effect of this treatment over the years to interrupt transmission.

We investigated the LA-2 equivalency correlation to vaccine protection in serum from mice bait immunized with OspA-RTV. We observed that 20% of mice did not develop sufficient levels of anti-OspA antibody, and this lack of vaccine efficacy could be attributed either to the natural variability observed in an outbred population of mice or to \textit{B. burgdorferi} strain variability (37). However, all mice that were protected after challenge (80%) showed a high titer (OD$_{450}$ of $\geq$1) of total IgG specific to OspA: serum from all of these mice neutralized \textit{B. burgdorferi} in culture, but only 75% of these had a large amount of LA-2 equivalent antibody in the serum. In Johnson et al. (30), all 36% of hamsters that were protected after challenge had a high titer of total IgG specific to OspA, but only 60% of these had a large amount of LA-2 equivalent antibody. In another study (33) the natural reservoir host of \textit{B. burgdorferi} senso lato, the yellow-necked mouse (\textit{Apodemus flavicollis}), was immunized intraperitoneally with a suspension of lipidated OspA and subsequently tick challenged. In this study, 88% of mice were protected after challenge and developed high titers of antibody to OspA. Similarly to the present study, only 71% of these developed large amounts of LA-2 equivalent antibodies. These studies indicate that 25 to 40% of rodents vaccinated with lipidated or unlipidated OspA do not develop LA-2 equivalent antibodies, although these rodents provided additional evidence of protection in the three studies. In contrast, the neutralization assay shows that 100% of the mice that developed high IgG responses to OspA killed \textit{B. burgdorferi} in culture. Thus, our correlation of protection was set at a conservative OD$_{450}$ of $>1$ equivalent titer of OspA-specific IgG. Differences in vaccine efficacy are due to the fact that the bait vaccine used in this study expresses the lipidated form of OspA as well as in the study by Kurtenback et al. (33), while in the hamster (30), the unlipidated form of OspA was used.

Furthermore, we evaluated alternative immunization schedules and how natural field conditions can affect deployed RTV in order to develop immunization protocols applicable in the field. We determined that deployment of OspA-RTV once, twice, or three times a week for 4 months (16 weeks) will induce an equivalent immune response to deployment of vaccine consecutively 5 days per week for 1 month, consumption of more vaccine units early in the first weeks of immunization leads to a swifter response to OspA, and the natural high temperature and humidity conditions prevalent when nymphal and larval ticks are active in late spring and summer do not
affect OspA-RTV adversely, such that bait can be left in the field for at least 24 h without losing efficacy.

More importantly, our studies show that mice immunized with the OspA-RTV developed high titers of antibody to OspA that lasted throughout the full year in which we carried out the immunization (Fig. 2 and 4). Our data also shows that these titers of antibody to OspA correlate with protection. In addition, we determined that the minimum number of units required for white-footed mice to develop a protective immune response is 5. Considering that nymphal I. scapularis ticks that infect humans with B. burgdorferi are active in the spring and that the larval ticks that carry B. burgdorferi infection to the next tick cohort (which will become nymphs the following year) are active in the summer, our plan to deploy the OspA-RTV to wildlife includes distribution of bait two times per week for a period of 4 months, ranging from mid-April until mid-August. The rationale for a 4-month deployment rather than a less-labor-intensive 1-month potentially equally effective deployment is to vaccinate the young hosts that are born throughout both seasons.

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REFERENCES