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Recombinant OspA Protects Dogs against Infection and Disease Caused by *Borrelia burgdorferi*

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Twenty-two specific-pathogen-free beagles were vaccinated with recombinant OspA (*ospA* gene derived from *Borrelia burgdorferi* B31) alone or with adjuvant (QuilA, Montanide ISA25, or aluminum hydroxide) at 6 weeks of age. Thirteen dogs were used as nonvaccinated controls with or without adjuvant. Three dogs were kept as contact controls and received neither vaccine nor challenge. Six weeks or 6 months after the first vaccination, the vaccinated (20 of 22) and nonvaccinated dogs (13) were challenged by exposure to adult ticks (*Ixodes scapularis*) naturally that were infected with *B. burgdorferi* (tick infection rate, ≥60%) and that were collected from Westchester County, N.Y. Protection from infection was evaluated by culture for *B. burgdorferi* from skin biopsies taken near the sites of tick bites. Skin biopsies were taken at monthly intervals for 3 months. *B. burgdorferi* was not isolated from any of the vaccinated dogs. In contrast, 12 of 13 control dogs challenged by exposure to the ticks were culture positive. The histopathology of the joint capsules 3 months after the challenge was used to evaluate protection from arthritis. Eight of 13 control dogs showed synovitis in single or multiple joints, while only 1 of the 22 vaccinated dogs had a single focus of mild inflammation in a single joint. At the time of the challenge, the vaccinated dogs had antibody to *B. burgdorferi*, which was demonstrable by kinetic enzyme-linked immunosorbent assay, Western blotting (immunoblotting), and a serum growth inhibition assay. The vaccinal antibody declined gradually after the challenge, especially in dogs vaccinated with OspA without adjuvants. Antibodies in the challenge control dogs were only detectable by 4 to 6 weeks after the challenge and remained at high levels until the termination of the study. Contact control dogs showed no antibody responses or histopathologic lesions and were culture negative. By Western blot analysis, antibodies to OspA first appeared in the sera of vaccinated dogs 3 weeks after the first vaccination. The absence of additional bands after the challenge suggests that infection in vaccinated dogs was blocked. Results from this study show that vaccination with recombinant OspA protected dogs against infection and disease after an experimental challenge with *B. burgdorferi* by exposure to ticks.

Lyme disease caused by *Borrelia burgdorferi* and related borrelias is one of the most common tick-transmitted diseases in the world (4, 39, 44). The spirochetes are transmitted by *Ixodes* ticks (1, 4, 9). In the United States, human Lyme disease was first discovered in Lyme, Conn. (45), and was subsequently found in dogs, horses, cattle, and cats (5, 10, 24, 29, 32, 34). The main clinical feature in humans is erythema migrans followed by joint, cardiac, or neurologic disease (44, 45). In dogs, the dominant clinical sign of Lyme borreliosis is acute lameness (24, 26, 29).

Dogs may develop arthritis after natural infection, and renal disease (21) and cardiac and neurologic disorders have been claimed (for a review, see reference 26). In a previous report, we demonstrated that experimental infections in dogs exposed to *B. burgdorferi*-infected adult ticks (*Ixodes scapularis*) resulted in an acute, recurrent lameness with fibrinopurulent arthritis (2). The dogs recovered but developed a persistent mild polyarthritis (2).

Two preparations of *B. burgdorferi* (from Fort Dodge Laboratories [8, 22, 27] and Solvay Animal Health, Inc. [unpublished data]) have been commercialized for vaccination of dogs against Lyme disease in the United States. However, Lim et al. recently reported that the vaccination of hamsters with a formalin-killed bacterin induced a destructive arthritis (28). This suggested that one or several antigens within the spirochete might play a role in the induction and activation of arthritis. Thus, it is essential to develop a vaccine which provides protection in dogs without the potential side effect of a bacterin.

Several outer surface lipoproteins from *B. burgdorferi* have been evaluated for their capacities to induce protective immunity (14, 33, 37, 38, 40, 43, 46, 48). One outer surface protein (OspA) has been reported to induce an active and protective immunity in mice (14–20, 40, 42, 43) and rhesus monkeys (36). OspA is also immunogenic in humans (23). Two brief reports described the protection of dogs of OspA (3, 11). In this paper, we report that a recombinant OspA (rOspA) is capable of inducing active immunity in specific-pathogen-free dogs which prevents infection and disease following challenge with *B. burgdorferi* delivered by infected ticks (2).

MATERIALS AND METHODS

Dogs. Thirty-eight 6-week-old specific-pathogen-free beagles from the Baker Institute colony were used. The dogs were kept in P2 isolation units. They were fed a commercial ration and water ad libitum. All dogs were observed daily for clinical signs, and their daily body temperatures were recorded. Body weights were measured weekly.

Ticks. Adult ticks (*I. scapularis*) infected with *B. burgdorferi* were collected by flagging in a forested area of Westchester County, N.Y. The ticks were maintained at the Cornell Entomology Laboratory at 94% relative humidity at 10°C

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TABLE 1. Isolation of *B. burgdorferi* from skin biopsies and tissues and histopathology of joints from dogs immunized with 100 μg of rOspA plus adjuvant (vaccine trial I)

Dog	Vaccine[a]	No. of days from first vaccination to challenge	Isolation of *B. burgdorferi* from:				Histopathology of joint[b]
			Skin biopsies by mo postchallenge			Tissues[c]	
			1	2	3		
1	OspA + A	173	−	−	−		NL
2	OspA + A	173	−	−	−		NL
3	OspA + A	42	−	−	−		NL
4	OspA + A	42	−	−	−		NL
5	OspA + B	173	−	−	−		NL
6	OspA + B	173	−	−	−		NL
7	OspA + A + B	173	−	−	−		NL
8	OspA + A + B	173	−	−	−		NL
9	A + B	173	−	+	+	J, S	NL
10	B	173	+	+	+	S	NSM
11	A	42	−	−	−	J, M	NL
12	A	42	+	+	+	J, S	NSP
13		42	−	+	+	J, M, Me, S	NL
14		42	+	+	+	J, M, S	NSM
15		42	+	+	+	Me, S	NL
16		42	+	+	+	M, Me, S	NSM
17[d]	OspA + A		−	−	−		NL
18[d]	OspA + A		−	−	−		NL
19[e]			−	−	−		NL
20[e]			−	−	−		NL

[a] Vaccinated dogs were inoculated with 100 μg of rOpsA twice with a 3-week interval with adjuvants (A, 100 μg of QuilA; B, 100 μg of ISA25).
[b] NL, not significant lesion; NSM, nonsuppurative monoarthritis; NSP, nonsuppurative polyarthritis.
[c] Tissues positive for *B. burgdorferi*. J, joint capsules; M, muscle; Me, meninges; S, skin.
[d] Dogs 17 and 18 were vaccinated but unchallenged.
[e] Dogs 19 and 20 were neither vaccinated nor challenged (contact control).

for 2 months. To determine the percentage of ticks infected with *B. burgdorferi*, 20 male or female ticks were ground and cultured individually in BSK-II medium with 8 μg of kanamycin per ml and 50 μg of rifampin per ml as previously described (2, 7, 41). The cultures were then examined over a 6-week period for *B. burgdorferi* by dark-field microscopy and by indirect immunofluorescence. They showed a 60 to 80% infectivity rate.

Overexpression of rOspA. rOspA derived from *B. burgdorferi* B31 was overexpressed by a T7 promoter and purified by immobilized metal ion affinity chromatography as previously described (7). Purified rOspA was kept at −20°C until used. rOspA supplied by Connaught Laboratories Inc., Swiftwater, Pa., for vaccine trial II was prepared as described previously (13).

Vaccination of dogs. We evaluated the immunogenicity and efficiency of the recombinant vaccine in three trials.

(i) Vaccine trial I. Twenty 6-week-old male and female beagles were used in this study. The dogs were randomly allotted to a vaccination group (10 dogs) and a nonvaccinated control group (10 dogs). Each dog in the vaccinated group was injected intramuscularly twice with a 3-week interval with 100 μg of rOspA in adjuvant. Six dogs received 100 μg of QuilA in a 1-ml emulsion as an adjuvant (Accurate Chemistry and Scientific Company, Westbury, N.Y.), two dogs received 25 μg of Montanide ISA25 (Seppic, Paris, France), and two dogs received both 100 μg of QuilA and 25 μg of Montanide ISA25. Control dogs were injected intramuscularly twice with the adjuvants only, two dogs were injected with 100 μg of QuilA, one dog received 25 μg of Montanide ISA25, and one dog received a combination of both adjuvants (100 μg of QuilA plus 25 μg of Montanide ISA25). Four additional nonvaccinated dogs (Table 1) were challenged by exposure to ticks; two dogs without any injection or challenge were maintained as contact control dogs but were kept in the same unit with the infected dogs. Six vaccinated dogs and two control dogs were challenged 6 months after the first vaccination. Two vaccinated dogs and the contact control dogs were not challenged. All other dogs were challenged 6 to 7 weeks after the first vaccination (Table 1).

(ii) Vaccine trial II. In order to perform a titration of the protective antigen dose and to compare the capacities of the adjuvants to elicit maximum protection for humans, six 6-week-old beagles were allotted to a vaccination group (four dogs) and a nonvaccination control group (two dogs). Each dog in the vaccine group was given two subcutaneous injections of 10 μg of rOspA in 10 μg of 1% aluminum hydroxide (rOspA and the adjuvant were supplied by Connaught

TABLE 2. Isolation of *B. burgdorferi* from skin biopsies and tissues and histopathology of joints from dogs immunized with 10 μg of rOspA plus adjuvant (vaccine trial II)

Dog	Vaccine[a]	No. of days from first vaccination to challenge	Isolation of *B. burgdorferi* from:				Histopathology of joint[b]
			Skin biopsies by mo postchallenge			Tissues[c]	
			1	2	3		
21	OpsA + H	42	−	−	−		NL
22	OpsA + H	42	−	−	−		NL
23	OpsA + H	42	−	−	−		NL
24	OpsA + H	42	−	−	−		NSM
25		42	+	+	+	J, M, P, S	NSM
26		42	+	+	+	J, M, P, S	NSP

[a] Vaccinated dogs were inoculated with 10 μg of rOspA plus aluminum hydroxide (H) twice with a 3-week interval.
[b] NL, not significant lesion; NSM, nonsuppurative monoarthritis; NSP, nonsuppurative polyarthritis.
[c] Tissues positive for *B. burgdorferi*. J, joint; M, muscle; P, pericardium; S, skin.

TABLE 3. Isolation of *B. burgdorferi* from skin biopsies and tissues and histopathology of joints from dogs immunized with 100 μg of rOspA without adjuvant (vaccine trial III)

Dog	Vaccine[a]	No. of days from first vaccination to challenge	Isolation of *B. burgdorferi* from:				Histopathology of joint[b]
			Skin biopsies by mo postchallenge			Tissues[c]	
			1	2	3		
27	OspA	49	−	−	−		NL
28	OspA	49	−	−	−		NL
29	OspA	49	−	−	−		NL
30	OspA	49	−	−	−		NL
31	OspA	49	−	−	−		NL
32	OspA	49	−	−	−		NL
33	OspA	49	−	−	−		NL
34	OspA	49	−	−	−		NL
35		49	+	+	+	M, S	NL
36		49	+	+	+	J, M, S	NSM
37		49	+	+	+	J, M, S	NSM
38[d]			−	−	−		NL

[a] Vaccinated dogs were inoculated twice with 100 μg of rOspA with a 3-week interval.
[b] NL, not significant lesion; NSM, nonsuppurative monoarthritis.
[c] Tissues positive for *B. burgdorferi*. J, joints; M, muscle; S, skin.
[d] Dog 38 was neither vaccinated nor challenged (contact control).

Laboratories) with a 3-week interval. The two control dogs were not inoculated. All dogs were challenged by exposure to ticks (Table 2).

(iii) Vaccine trial III. Twelve 6-week-old beagles were used. Each of eight dogs in the vaccine group was injected intramuscularly twice with a 3-week interval with 1 ml of 100 μg of rOspA without adjuvant. Four dogs were kept as nonvaccinated controls. All eight dogs in the vaccinated group and three of four dogs in the nonvaccinated group were challenged by exposure to ticks. One nonvaccination dog was kept as a contact control (Table 3).

Challenge infection of dogs after vaccination. The challenge infection of the dogs was made by placing 15 female and 7 male adult ticks (*I. scapularis*) onto the clipped side of a dog as previously reported (2). The infectivity rate of the ticks was ≥60%. The ticks were allowed to feed and engorge for 6 days, when at least 50% of the female ticks were fully engorged.

Collection of serum and tissue samples. Serum samples were obtained from each dog at the time of the first and second vaccinations, at the challenge, and at 2-week intervals for 3 months thereafter. The sera were tested by Western blotting (immunoblotting), kinetic enzyme-linked immunosorbent assays (KELA), and *B. burgdorferi* growth inhibition assays. After the dogs were exposed to the ticks, they had skin biopsies taken at monthly intervals for the isolation of spirochetes. Three months after the challenge, all dogs were euthanized and tissues were removed for the culture of *B. burgdorferi* and for histopathology.

Isolation of *B. burgdorferi*. To test for protection from infection, attempts to isolate *B. burgdorferi* from skin biopsies and from various tissues were made. Samples from skin punch biopsies (diameter, 4 mm) collected at monthly intervals after exposure to the ticks and pieces of tissue (approximately 0.2 to 1 gm) from necropsy were homogenized in 3 ml of BSK-II medium in a tissue homogenizer (stomacher; Tekmar, Cincinnati, Ohio) and then transferred to 27 ml of prewarmed BSK-II medium. At necropsy, the following tissues were removed aseptically for culture: four limb muscles, six joint capsules (right and left stifle, elbow, and shoulder joints), the pericardium, the peritoneum, lymph nodes, and meninges (dura mater). The cultures were checked weekly for up to 6 weeks for the presence of *B. burgdorferi* by dark-field examination and by the indirect fluorescence assay.

Serology: KELA, immunoblots, and growth inhibition tests. The KELA for measuring the levels of serum antibody to *B. burgdorferi* was described previously (2, 41). Each unit of slope was designated as a KELA unit. Western blot confirmation of sera indicated that the cutoff separating negative from positive sera was 100 KELA units (2, 41). Briefly, diluted serum was added to duplicate wells in microtiter plates containing antigens of French-pressed *B. burgdorferi* lysate. Bound antibody was detected by using second antibodies of a goat anti-canine antibody of heavy- and light-chain specificity conjugated to horseradish peroxidase (Cappel Research Products, Durham, N.C.). Color development with the chromogen tetramethylbenzidine with H_2O_2 as a substrate was measured kinetically and expressed as the slope of the reaction rate between the enzyme and the substrate solution.

The procedure for the Western blot analysis was previously described (2, 7). French-pressed *B. burgdorferi* lysate was used as an antigen and was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7). Western blot analysis was performed in a miniblotter (2). Test sera from experimental animals were used as a first antibody, and goat anti-dog immunoglobulin G conjugated to horseradish peroxidase was used as a second antibody.

The growth inhibition assay was done as described elsewhere (6, 39, 47). Briefly, serial dilutions of serum in microtitration plates were incubated with 10^6 live *B. burgdorferi* per ml in BSK-II medium for 30 min, and guinea pig complement was then added. The microtitration plates were sealed and incubated at 34°C for 4 to 6 days. Bacterial growth was measured as a function of the pH, which was indicated by a color change from red to yellow and which was determined with a microplate reader set at 570 nm.

Gross pathology and histopathology. All vaccinated and nonvaccinated dogs were euthanized approximately 3 months after exposure to the ticks and were examined for gross and histopathologic lesions to document protection from disease. The following tissues were fixed in 10% neutral formalin: joint capsules (elbow, shoulder, stifle, and tarsus) and lymph nodes. Tissues were embedded in paraffin wax, sectioned, and stained by conventional methods for histopathologic evaluation.

Statistical analysis. The protective effect of the vaccine against the development of infection and disease while controlling for different times of challenge was evaluated by logistic regression analysis with BMDP statistical software (BMDP Statistical Software Inc., Los Angeles, Calif.). The analysis was performed for each adjuvant separately. To evaluate the effect of using the aluminum hydroxide adjuvant, Fisher's exact test was used.

RESULTS

Clinical signs. None of the vaccinated dogs showed any clinical signs (lameness, anorexia, or depression) or elevated body temperature following vaccination or challenge by exposure to the ticks. Local reactions to adjuvant inoculation were mild swelling and reddening, which disappeared by 3 days after inoculation. Among the nonvaccinated control dogs, only one dog (dog 36) became lame for a 4-day period 3 months after the challenge. Thirteen nonvaccinated control dogs had sporadic elevated body temperatures for 1 or 2 days. In contrast to the vaccinated dogs, nonvaccinated control dogs were frequently reluctant to get up and appeared depressed. Anorexia and loss in body weight were not seen in any of the dogs. Clinical signs and temperature elevation were not found in the three contact control dogs and in the two vaccinated dogs that were not challenged.

Isolation of *B. burgdorferi*. *B. burgdorferi* was not isolated from any of the skin biopsies taken from the vaccinated dogs at monthly intervals after the challenge or from the skeletal muscles, joint capsules, pericardium and peritonium samples, lymph nodes, and meninges collected at necropsy. Twelve of 13 dogs in the nonvaccinated, challenged control group were culture positive (Tables 1 to 3). The skin and tissue samples from the two dogs vaccinated with 100 μg of rOspA plus 100 μg of

QuilA that were not challenged and from nonvaccinated contact control dogs were all culture negative (Table 1 and 3). There was a significant difference between the vaccinated and nonvaccinated groups with regard to the development of challenge infections ($P < 0.05$). There was no significant difference among dogs of the vaccinated group with respect to the intervals between vaccination and challenge or to the different adjuvants ($P > 0.05$).

Serology. At the time of the challenge, all vaccinated dogs had antibody to *B. burgdorferi* as determined by KELA, with antibody levels of between 300 to 600 KELA units which gradually declined after the challenge (Fig. 1). Antibody levels in dogs vaccinated with rOspA without adjuvant declined more rapidly compared with those in dogs vaccinated with rOspA with adjuvants (Fig. 1). Antibodies (as determined by KELA) in the nonvaccinated, challenged control dogs were detectable by 4 to 6 weeks after challenge (Fig. 1). Western blot analysis showed OspA antibody in the 32-kDa region 3 weeks after the first vaccination. Bands became denser after the second vaccination. Bands also appeared in the 20-kDa and 62-kDa regions. Additional bands were not seen after the challenge with the ticks. In contrast, multiple bands were seen for the nonvaccinated control dogs by 4 to 6 weeks after exposure to the infected ticks (Fig. 2). The *B. burgdorferi* growth inhibition assay with sera from the vaccinated dogs showed titers of 1:40 to 1:2,560 at the time of the challenge, with no statistically significant difference among the formulations of the vaccine with different adjuvants ($P > 0.05$). However, like the levels of antibodies determined by KELA, those determined by the growth inhibition assay declined more rapidly in dogs that were vaccinated with rOspA without adjuvant (data not shown). Sixty days after the second vaccination, antibody titers by the growth inhibition assay had declined to levels of $\leq$1:40 in dogs that had received 100 μg of rOspA without adjuvant. In contrast, dogs that received 100 μg of rOspA together with QuilA or ISA25 had antibody titers of $\geq$1:160 by 6 months after the first vaccination. Three of 4 dogs vaccinated with 10 μg of rOspA plus aluminum hydroxide had antibody titers of $\geq$1:160 by 4 months after the second vaccination. Sera from the control dogs did not show growth inhibition before the challenge (Fig. 3). The contact control dogs did not develop titers by either KELA or the growth inhibition assay (data not shown).

Histopathology. Histopathologic examinations were performed on joint tissues from the elbow, shoulder, stifle, and tarsal joints (eight specimens per dog). Of 13 nonvaccinated control dogs challenged by exposure to ticks, 8 showed a nonsuppurative synovitis characterized by a perivascular-to-diffuse infiltrate with plasma cells and lymphocytes (Fig. 4). This infiltrate was commonly seen close to the synovial surface but sometimes was deeper in the joint capsule. In affected joints, multiple foci of these mononuclear cells were seen; a few dogs showed inflammation in more than one joint (polyarthritis). With the exception of one focal joint lesion in one dog (dog 24) (Table 2), no histopathologic joint lesions were found in the vaccinated dogs. The two dogs that were given only rOspA (dogs 17 and 18) (Table 1) but that were not challenged showed no joint lesions. Lymph nodes, especially the axillary or prescapular, were moderately to markedly increased in size on the side of the dog where the challenge with the ticks had occurred. There was mild follicular and parafollicular hyperplasia in the vaccinated dogs. The two vaccinated but unchallenged dogs (dogs 17 and 18) also showed a mild follicular and parafollicular hyperplasia. The follicular and parafollicular hyperplasia was more pronounced in nonvaccinated dogs. Large follicles were often formed within both the cortex and medulla of the lymph node. In this study, all combinations of rOspA (10

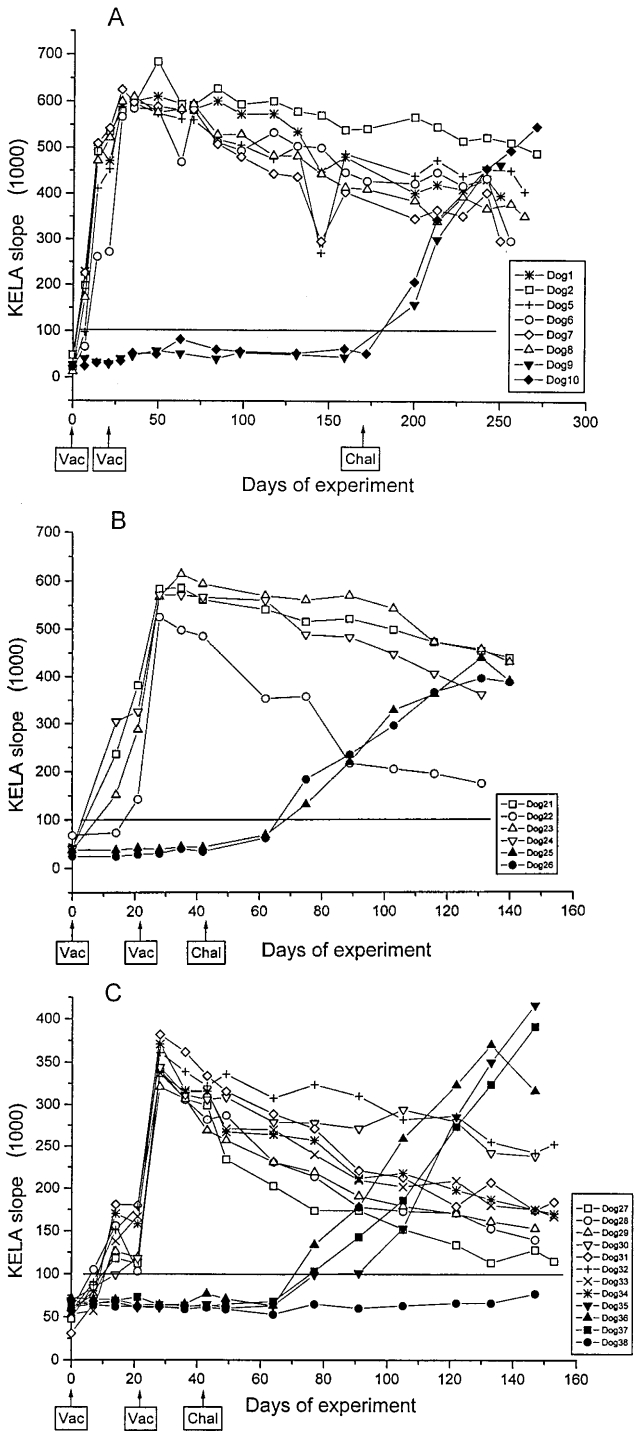

FIG. 1. Antibody levels in vaccinated and unvaccinated dogs as determined by KELA. The line at 100 KELA units represents the cutoff between positive and negative sera. Open symbols indicate vaccinated dogs, and solid symbols indicate nonvaccinated dogs. (A) Vaccinated dogs were inoculated twice with 100 μg of rOspA plus adjuvants intramuscularly with a 3-week interval and were challenged with *B. burgdorferi*-infected ticks 6 months later. (B) Vaccinated dogs were inoculated twice with 10 μg of rOspA plus adjuvant subcutaneously with a 3-week interval and were challenged with *B. burgdorferi*-infected ticks at 42 days after the first vaccination. (C) Vaccinated dogs were inoculated twice with 100 μg of rOspA without adjuvants intramuscularly with a 3-week interval and were challenged with *B. burgdorferi*-infected ticks at 49 days after the first vaccination. All unvaccinated dogs in each group were kept with vaccinated dogs and were challenged at the same time. Vaccination (Vac) and challenge (Chal) times are indicated by arrows.

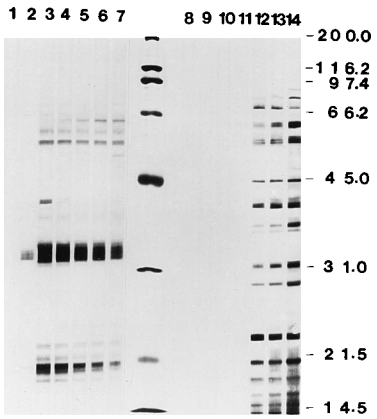

FIG. 2. Western blot analysis of the antibody response of a dog (dog 4) vaccinated with 100 μg of rOspA and QuilA (lanes 1 to 7; see also Table 1) and of an unvaccinated dog (dog 14) (lanes 8 to 14; see also Table 1). Lane 1, preimmune serum; lane 2, 3 weeks after the first vaccination; lane 3, 6 weeks after the first vaccination (at the time of the challenge with the ticks); lane 4, 10 weeks after the first vaccination; lane 5, 13 weeks after the first vaccination; lane 6, 16 weeks after the first vaccination; lane 7, 20 weeks after the first vaccination. Lanes 8 to 14 correspond to lanes 1 to 7 but are for unvaccinated dog 14. The molecular weight marker lane is at the center of the figure, between lanes 7 and 8. The biotinylated SDS-PAGE standard broad-range molecular markers (Bio-Rad Laboratories, Richmond, Calif.) were used. The numbers at the right indicate molecular weights.

or 100 μg) plus various adjuvants (QuilA, ISA25, and aluminum hydroxide) provided protection from infection and disease.

DISCUSSION

Our criterion for the protection of dogs from infection was the failure to isolate live *B. burgdorferi* from monthly skin biopsies after the challenge with ticks and from a variety of tissues taken by necropsy at 3 months after the exposure. We did not use PCR to detect *B. burgdorferi* DNA, because positive results would not differentiate between viable and nonviable organisms. Except for one dog in trial I (dog 11) (Table 1), *B. burgdorferi* was isolated from skin biopsies or other tissues from all nonvaccinated control dogs challenged by the exposure to ticks. In contrast, attempts to isolate the organism were negative for all vaccinated dogs (Tables 1 to 3).

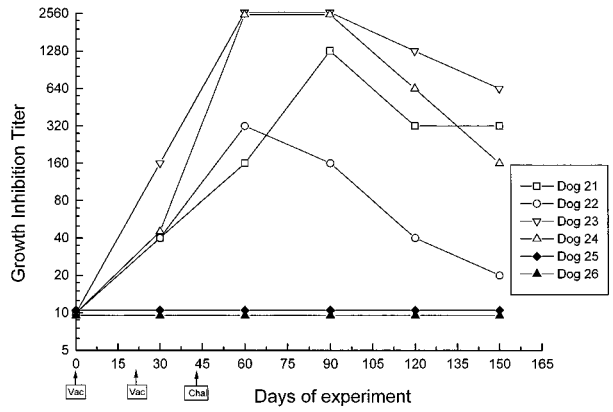

FIG. 3. Antibody titers from the vaccinated and unvaccinated dogs of vaccine trial II as determined by the growth inhibition assay. Open symbols indicate vaccinated dogs, and solid symbols indicate nonvaccinated dogs. Sera were from the same group of dogs as that reported on in Fig. 1B. Vaccination (Vac) and challenge (Chal) times are indicated by arrows.

In our earlier studies of canine Lyme disease (2), we found that clinical signs of lameness with joint swelling and stiffness were seen when puppies at 6 weeks of age were exposed to infected ticks. We have seen lameness or other clinical signs in only about 5% of control dogs challenged when 12 weeks of age or older (2). That percentage corresponds with the incidence of Lyme disease in dogs in the field (25). Accordingly, one of our criteria for protection from the disease was the presence or absence of histopathologic changes in joint tissues at 3 months after the challenge. Mono- or polyarthritis was found in 8 of 13 nonvaccinated control dogs exposed to infected ticks. In contrast, except for one lesion in one joint of one dog (dog 24) (Table 2), vaccinated dogs were free of joint lesions. Whether the single lesion was caused by the vaccination, the challenge, or other undefined causes remains unknown. Nevertheless, the possibility of OspA-induced joint lesions cannot be ruled out. It seems unlikely that this single focal lesion was caused by *B. burgdorferi*, as all tissues cultured from vaccinated dogs were negative. The lymph nodes from vaccinated, unchallenged dogs (dogs 17 and 18) (Table 1) also showed a mild follicular and parafollicular hyperplasia. Whether the mild-to-moderate hyperplasia observed in vaccinated dogs reflected a response to the rOspA, the adjuvant, or both elements is unknown. We speculated that the adjuvant is the more likely inciting factor.

High antibody levels in vaccinated dogs prior to the challenge, as determined by KELA, apparently correlated with protection against the challenge (Fig. 1). However, the antibodies in vaccinated dogs reflected antibody to the 32-kDa OspA, which is not present in the dogs after the exposure to the ticks (2). Western blots had reliable markers associated with protection. In all vaccinated dogs, Western blots expressed a wide band in the 32-kDa OspA region. Additionally, we saw weaker bands in the 20-kDa region that might indicate breakdown products of OspA. We also saw bands in the 60-kDa region which may reflect antibody responses to a dimer or polymer of OspA. The Western blot pattern for vaccinated dogs did not change after the challenge, suggesting that challenge infections did not become established in these vaccinated dogs. The 32-kDa bands waned with time (Fig. 2, lanes 4 to 7), suggesting that a drop in vaccinal antibody levels occurred as a function of time.

A correlation between serum growth inhibition of *B. burgdorferi* and protection from infection was found. By the growth inhibition assay, all the vaccinated dogs had high antibody titers after vaccination (Fig. 3) that gradually declined after the challenge with the ticks. It is possible that antibodies neutralized *B. burgdorferi* in the tick gut and prevented migration to the salivary gland and into the host as reported previously (17). We have not attempted to isolate *B. burgdorferi* from ticks after their engorgement on vaccinated dogs. Following the challenge, the nonvaccinated dogs lacked antibodies to *B. burgdorferi* as determined by the growth inhibition assay, even though they showed high antibody titers by KELA (Fig. 1). Western blot analysis also showed a strong antibody response to the *B. burgdorferi* antigens (Fig. 2, lanes 12 and 13). However, no significant anti-OspA antibodies can be detected with these sera (47). This indicated that the anti-OspA antibodies are a critical factor in the inhibition of *B. burgdorferi* growth.

The use of adjuvant is important in combination with rOspA. Although dogs vaccinated with rOspA without adjuvant were protected when challenged 4 weeks after the last vaccination (dogs 27 to 34) (Table 3), antibodies (by both assays) declined rapidly within 2 months of the challenge. In contrast, dogs

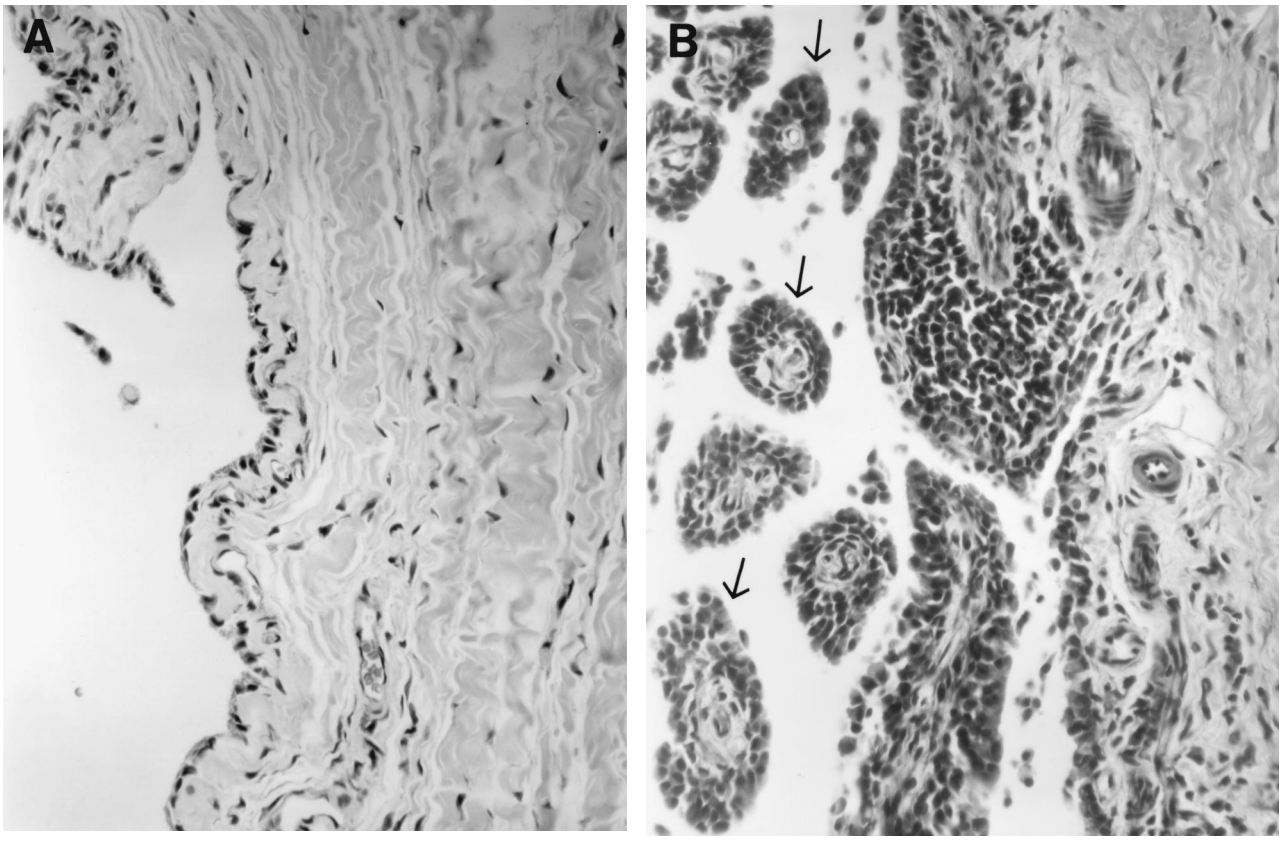

FIG. 4. Histologic appearance of synovial membranes from vaccinated and nonvaccinated dogs exposed to *B. burgdorferi*-infected ticks. (A) Dog 21. In this vaccinated dog, the synovial membrane is normal. The surface is slightly undulating with occasional folds. Hematoxylin-eosin stain was used. Magnification, ×250. (B) Dog 16. In this nonvaccinated control dog, there is synovial inflammation. The synoviocytes have proliferated into papillae (arrows), and a mononuclear cell infiltration is seen below the synovial surface. Hematoxylin-eosin stain was used. Magnification, ×250.

vaccinated with rOspA plus QuilA and/or ISA25 were protected from infection when they were challenged 6 months after the vaccination. Antibodies in these dogs remained high by both assays. Our data confirm the results reported by Ma et al. (31).

The heterogeneity of the OspA proteins in different *Borrelia* species has been reported mainly in Europe and Asia (4, 12, 35, 49). This aspect has to be considered with regard to immunization against Lyme disease. With few exceptions, one serotype seems to prevail in the United States (4, 12, 35). We, therefore, have not addressed the question of heterogeneity in our vaccine trial. However, as more data on *B. burgdorferi* serotype diversity in the United States are accumulating, this problem may have to be addressed (30).

The OspA subunit vaccine is a good candidate vaccine for dogs and is currently being tested for safety and immunogenicity in humans (23). The dog has proved to be a good model for human Lyme arthritis (2). A vaccine study in dogs, therefore, would also be valuable in the evaluation of human Lyme vaccines. Active and passive protection of mice by OspA against infection with *B. burgdorferi* when challenged by needle inoculation or exposure to ticks has been reported (14–20, 38, 40, 43). Our data show that dogs can also be protected by rOspA vaccination.

In summary, a rOspA subunit vaccine protected dogs against *B. burgdorferi* infection and disease. Further studies of the duration of protection after vaccination, safety, and cross protection against the possible heterogeneous OspA structures that may be found among new *B. burgdorferi* strains isolated in the United States are needed (30).

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