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Development of Destructive Arthritis in Vaccinated Hamsters Challenged with *Borrelia burgdorferi*

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We present the first direct evidence that adverse effects, particularly severe destructive arthritis, can develop in vaccinated hamsters after challenge with *Borrelia burgdorferi* sensu lato isolates. Hamsters were vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant. A severe destructive arthritis was readily evoked in vaccinated hamsters challenged with the homologous *B. burgdorferi* sensu stricto isolate C-1-11 before high levels of protective borreliacidal antibody developed. Once high levels of C-1-11 borreliacidal antibody developed, hamsters were protected from homologous challenge and development of arthritis. Vaccinated hamsters, however, still developed severe destructive arthritis when challenged with other isolates of the three genomic groups of *B. burgdorferi* sensu lato (*B. burgdorferi* sensu stricto isolate 297, *Borrelia garinii* isolate LV4, and *Borrelia afzelii* isolate BV1) despite high levels of C-1-11 specific borreliacidal antibody. Vaccines that contained whole spirochetes in adjuvant induced destructive arthritis, but this effect was not dependent on the isolate of *B. burgdorferi* sensu lato or the type of adjuvant. These studies demonstrate that caution is necessary when employing whole spirochetes in adjuvant for vaccination to prevent Lyme borreliosis. Additional studies are needed to identify the antigen(s) responsible for the induction and activation of arthritis and to define the immune mechanisms involved.

Lyme borreliosis is caused by the spirochete *Borrelia burgdorferi* sensu lato (3, 5, 19) and is transmitted primarily to human hosts by *Ixodes* ticks (6, 49, 50). Its most characteristic clinical feature is an expanding skin lesion, erythema migrans, yet patients with the illness may present with arthritic, cardiac, or neurological symptoms without a skin lesion (46, 47, 53). The signs and symptoms of the disease change frequently and are often intermittent. If the infection is left untreated, chronic arthritis may develop in weeks or months. Today, Lyme borreliosis has become the most frequently reported tick-associated illness in the United States (8) since it was first consistently reported in the 1970s (48, 51, 52).

The high level of interest in Lyme borreliosis has facilitated a worldwide effort to develop an effective vaccine against *B. burgdorferi* sensu lato. The feasibility of vaccination has been demonstrated in dogs (9, 26), gerbils (33), hamsters (17, 18, 20), and mice (12, 13, 43, 44, 54, 55). Viable (21, 23, 30, 32, 36, 39, 40) and nonviable (9, 17, 18, 20, 22, 26) Lyme borreliosis spirochetes and several of their outer surface proteins, including OspA (12, 13, 34, 35, 37, 38, 43, 44, 54, 55), OspB (14, 34, 35, 55), OspC (33), and the 39-kDa protein (41), have been shown to induce protective antibodies capable of killing isolates of *B. burgdorferi* sensu lato in vitro or preventing infection in animals. Other spirochetal components may also be involved. Recently, a whole-cell vaccine for dogs has become available commercially (9, 18, 26), and field trials involving humans with OspA, as a vaccine are being conducted. Although vaccination studies are progressing, there are important concerns about the heterogenicity (4, 15, 24, 31, 56–59) and immunogenicity (10, 54) of nonviable whole spirochetes and subunit components. In addition, the possibility of adverse effects developing from vaccination with or without adjuvants must be considered.

In this study, we present the first direct evidence that an adverse effect, especially severe destructive arthritis, can develop after vaccination against the Lyme borreliosis spirochete. We describe the development of severe destructive arthritis in mature immunocompetent hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu lato in adjuvant after challenge with isolates of the three genomic groups of *B. burgdorferi* sensu lato.

MATERIALS AND METHODS

Hamsters. Six- to 8-week-old inbred LSH/Ss WSLH hamsters were obtained from our breeding colony located at the Wisconsin State Laboratory of Hygiene. Hamsters weighing 60 to 120 g were housed three per cage at an ambient temperature of 21°C. Food and water were available ad libitum.

Organisms. Low-passage (< 10) virulent isolates of the three genomic groups (28, 29) of *B. burgdorferi* sensu lato (*B. burgdorferi* sensu stricto isolates C-1-11 and 297, *Borrelia garinii* isolate LV4, and *Borrelia afzelii* isolate BV1) and *Borrelia hermsii* were cultured once in modified Barbour-Stoenner-Kelly (BSK) medium (7) at 32°C to a concentration of 5×10^7 spirochetes per ml. Five-hundred-microliter samples were then dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, N.C.) containing 500 μl of BSK supplemented with 30% glycerol (Sigma, St. Louis, Mo.), sealed, and stored in liquid nitrogen. When needed, a frozen suspension of spirochetes was thawed, and an aliquot was used to inoculate fresh BSK. The culture was incubated at 32°C for 72 h and diluted with fresh BSK to yield 5×10^6 spirochetes per ml. Spirochetes were enumerated by dark-field microscopy and with a Petroff-Hauser counting chamber. Isolates were obtained from S. M. Callister, R. C. Johnson, and G. Stanek.

Preparation of vaccines. *B. burgdorferi* sensu stricto isolates C-1-11 and 297 were grown in 4 liters of BSK to 5×10^6 spirochetes per ml. Spirochetes were harvested by centrifugation (10,000 × *g*, 4°C, 30 min) after three washes with phosphate-buffered saline (PBS, pH 7.4). The pellet was suspended in 1% Formalin and incubated at 32°C for 30 min. The Formalin-inactivated spirochetes were then washed three times by centrifugation and suspended in PBS. Five-hundred-microliter samples containing 5×10^9 spirochetes were dispensed into 1.5-ml screw-cap tubes (Sarstedt) and stored at −70°C. Subsequently, frozen samples of Formalin-inactivated *B. burgdorferi* C-1-11 and 297 were thawed and suspended in 10 ml of aluminum hydroxide gel (HPA-3; Reheis, Inc., Berkeley Heights, N.J.), aluminum hydroxide (Imject alum; Pierce, Rockford, Ill.), Freund's incomplete adjuvant (Sigma), or PBS.

Vaccination of hamsters. Hamsters were mildly anesthetized with ether contained in a nose-and-mouth cup and vaccinated intramuscularly in each hind leg with a single dose containing 0.2 ml of 10^8 Formalin-inactivated organisms of *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in various adjuvants. The protein concentration was 50 to 100 μg per inoculum (Bio-Rad Laboratories, Hercules, Calif.). Controls consisted of nonvaccinated hamsters and hamsters inoculated with 0.2 ml of the adjuvants alone or Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in PBS. Similar or higher concentrations of spirochetes have been used to vaccinate hamsters (20, 22).

Hamster sera. Sera were obtained from hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 with and without adjuvant at various intervals after vaccination. Concomitantly, sera were obtained from noninfected normal hamsters. Hamsters were anesthetized with ether and bled by intracardiac puncture. The blood was allowed to clot, and serum was separated by centrifugation at 500 × *g*, pooled, divided into 1-ml aliquots, dispensed into 1.5-ml screw-cap tubes (Sarstedt), and frozen at −20°C until used.

Borreliacidal assay. A previously described procedure (30) was modified and used to determine borreliacidal activity. Sera from vaccinated and nonvaccinated hamsters were heat inactivated at 56°C for 30 min, diluted 1:10 with fresh BSK, and filter sterilized through a 0.22-μm-pore-size filter apparatus (Acrodisc; Gelman Sciences, Ann Arbor, Mich.). Frozen suspensions of *B. burgdorferi* sensu stricto isolates C-1-11 and 297, *B. garinii* isolate LV4, *B. afzelii* isolate BV1, and *B. hermsii* in BSK were thawed, inoculated into fresh BSK, and incubated at 32°C for 72 h. Spirochetes were enumerated by dark-field microscopy and with a Petroff-Hauser counting chamber, and the suspensions were adjusted to contain 10^5 spirochetes per ml with BSK. One-hundred-microliter samples of the spirochetal suspensions were added to round-bottomed wells of a 96-well microtiter plate (GIBCO Laboratories, Grand Island, N.Y.). Subsequently, 100 μl of sera or twofold dilutions of sera from vaccinated and nonvaccinated hamsters and 20 μl of sterile guinea pig complement (hemolytic titer, 200 CH50 units per ml; Sigma) were added to each well of the microtiter plate. The plate was shaken gently and incubated at 32°C for 16 h. All assays were performed in duplicate.

Flow cytometry data acquisition and analysis. After incubation of assay samples, 100 μl was removed and diluted 1:5 with PBS (pH 7.4), and 50 μl of acridine orange (5.4 nM; Sigma) was added (27). Controls included samples containing normal serum with viable or heat-killed (56°C for 30 min) spirochetes in BSK and complement. The samples were then analyzed with a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) with FACScan LYSIS II software for data acquisition. Initially, viable and heat-killed spirochetes were detected and differentiated from BSK, serum, and complement particles by using forward scatter, side scatter, and acridine orange fluorescence. Live gating was performed only on profiles of spirochetes during data acquisition to exclude all BSK, serum, and complement particles. Data were acquired for 1 min. Assay samples were then analyzed by histogram profiles of acridine orange fluorescence with FACScan LYSIS II software. Gates were established for viable and heat-killed spirochetes based on their incorporation of acridine orange. Three parameters were evaluated: events per minute (number of labeled spirochetes), percent shift in fluorescence (number of dead spirochetes), and mean channel fluorescence (intensity of fluorescently labeled spirochetes). Borreliacidal activity was determined by a decrease in events per minute and increases in percent shift in fluorescence and mean channel fluorescence compared with values obtained with normal serum. Spirochetes were sorted based on the flow cytometric parameters. Sorted spirochetes were incubated in fresh BSK medium and monitored for growth for 5 weeks. No growth of spirochetes was detected (27). The borreliacidal titer was the highest dilution of immune serum that killed spirochetes compared with normal serum.

Infection of hamsters. Vaccinated hamsters were mildly anesthetized with ether and challenged subcutaneously in each hind paw with 0.2 ml of BSK containing 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11 or 297, *B. garinii* isolate LV4, *B. afzelii* isolate BV1, or *B. hermsii*. Controls included nonvaccinated hamsters and hamsters vaccinated with various adjuvants alone or Formalin-inactivated spirochetes in PBS and challenged with 10^6 viable spirochetes or BSK. We have infected hamsters with 10^2 to 10^8 *B. burgdorferi* organisms and recovered spirochetes from bladders, spleens, kidneys, and hearts cultured in BSK medium. Recovery rates varied (≥66%) for challenge inocula of between 10^2 and 10^4 spirochetes. When hamsters are challenged with 10^6 spirochetes, the measurement of arthritis by plethysmograph is less variable and spirochetes are recovered from all tissues.

Assessment of arthritis. Swelling of the hind paws of hamsters challenged with isolates of *B. burgdorferi* sensu lato and *B. hermsii* was used to evaluate the inflammatory response. The hind paws were measured every other day for 21 days with a plethysmograph (Buxco Electronics, Sharon, Conn.). Measurements were obtained by mildly anesthetizing hamsters with ether, carefully dipping a hind paw into a column of mercury up to the ankle, and measuring the amount of mercury displaced (in milliliters). The mean plethysmograph value for three hamsters (six hind paws) per group was used as an index of severity of swelling from arthritis. Mercury displacement was standardized with a volume calibrator.

Recovery of spirochetes from tissues. Twenty-one days after challenge, hamsters were killed by CO_2 inhalation. The urinary bladder, spleen, left kidney, and heart were removed aseptically, homogenized through a 5-ml syringe, and inoculated into 5 ml of BSK supplemented with rifampin (100 μg/ml; Sigma) and phosphomycin (100 μg/ml; Sigma). Cultures were incubated at 32°C and examined weekly for 2 weeks by dark-field microscopy for motile spirochetes. If spirochetes were not detected, 0.5 ml of the culture was inoculated into 4 ml of BSK supplemented with antibiotics and examined weekly for another 3 weeks. If no spirochetes were detected, the culture was

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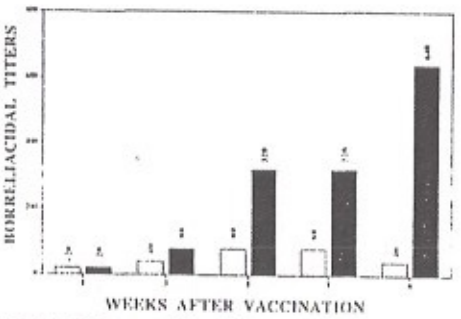

FIG. 1. Development of borreliacidal antibody titers in hamsters after vaccination with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in PBS (stippled bars) or aluminum hydroxide gel (solid bars).

considered negative. For recovery of *B. hermsii*, blood was inoculated into 5 ml of BSK supplemented with antibiotics.

Preparation of tissues for histology. The hind legs of all vaccinated and nonvaccinated hamsters were amputated 21 days after challenge at the midfemur, fixed in 10% neutral buffered Formalin, placed in decalcifying solution (Lerner Laboratories, Pittsburgh, Pa.) for 18 h, and stored in 10% Formalin prior to processing. The knees and hind paws were bisected longitudinally, embedded in paraffin, cut into 6-μm sections, placed on glass slides, and stained with hematoxylin and eosin. Hind legs were randomly selected from each group of hamsters for histopathological examination.

Statistics. The plethysmograph values obtained from hamster hind paw measurements were tested by analysis of variance. The Fisher least-significant-difference test (45) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before the experiments were started.

RESULTS

Development of borreliacidal antibody in vaccinated hamsters. Two groups of 15 hamsters each were vaccinated with a single dose of a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in PBS or an aluminum hydroxide gel. Borreliacidal antibody titers were determined at 1, 3, 5, 7, and 9 weeks after vaccination by flow cytometry. One week after vaccination, low levels of borreliacidal antibody were detected in pooled sera from three hamsters vaccinated with the preparations of Formalin-inactivated spirochetes (Fig. 1). However, hamsters vaccinated with Formalin-inactivated spirochetes in adjuvant had a 2- to 16-fold increase in borreliacidal antibody titer 3 weeks or later after vaccination.

Development of severe destructive arthritis in vaccinated hamsters. Five groups of three hamsters each were vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel. At 1, 3, 5, 7, and 9 weeks after vaccination, hamsters were challenged subcutaneously in the hind paws with 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11. Hamsters challenged 5 weeks or less after vaccination developed severe swelling of the hind paws (Fig. 2 and 3C). Swelling was detected 5 days after challenge and increased rapidly, with peak swelling occurring on days 14 to 16 after challenge before gradually decreasing. Spirochetes were also recovered from the urinary bladder, spleen, kidney, and heart of these hamsters after cultivation in BSK for 10 days

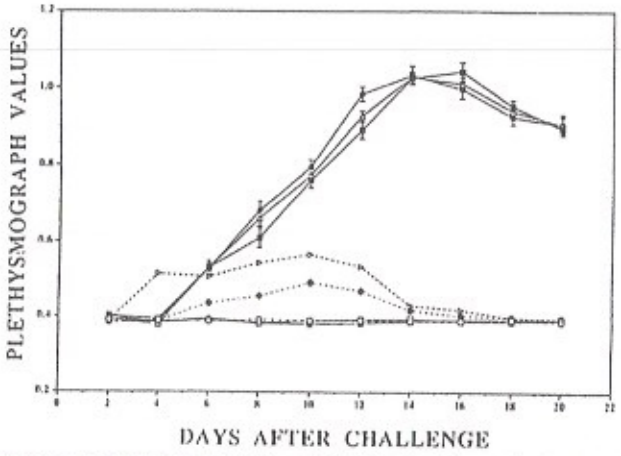

FIG. 2. Development of hind paw swelling in hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 and vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel for 1 (-■-), 3 (-△-), 5 (-●-), 7 (-□-), and 9 (-▲-) weeks. Controls included nonvaccinated hamsters challenged with BSK (- - ○ - -) or *B. burgdorferi* sensu stricto isolate C-1-11 (- - ◆ - -) and hamsters vaccinated with aluminum hydroxide gel and challenged with *B. burgdorferi* sensu stricto isolate C-1-11 (- - ▷ - -). Nonchallenged vaccinated hamsters showed no adverse clinical manifestations.

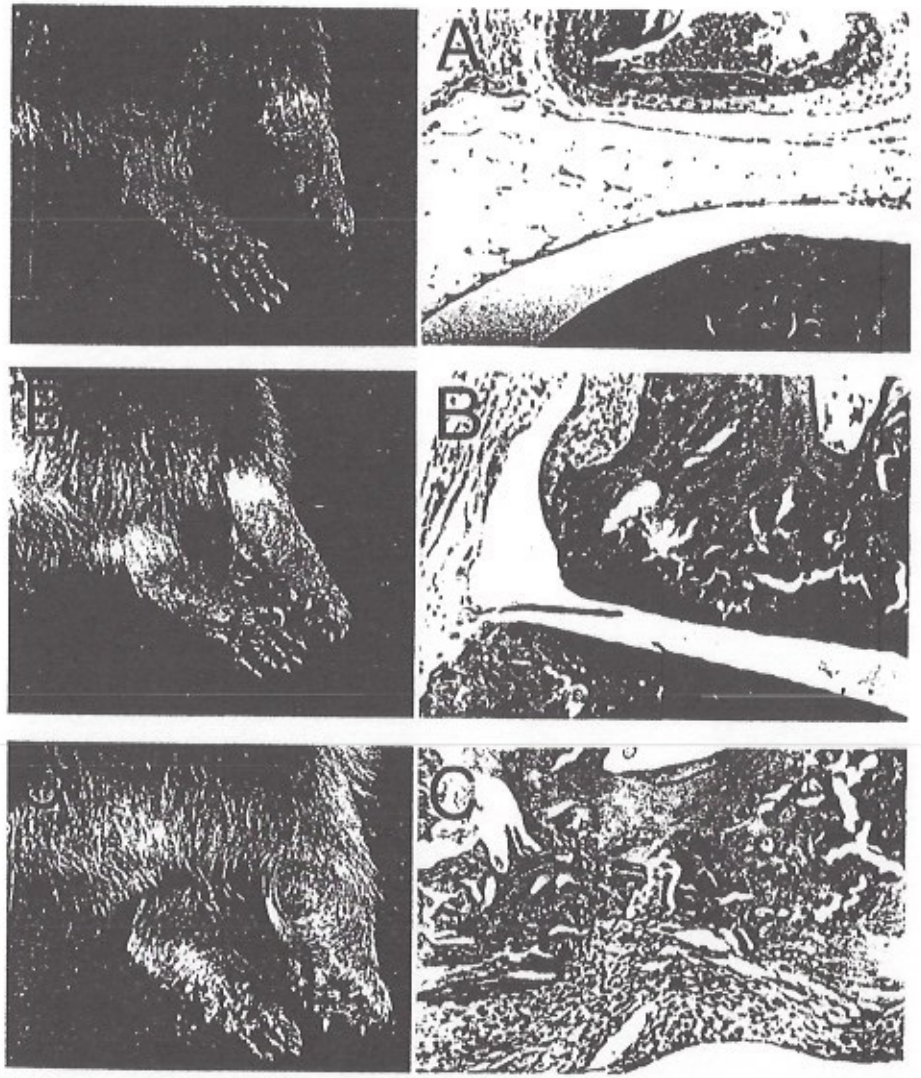

FIG. 3. Appearance (left) and histopathology (right) of hind paws. (A) Nonvaccinated hamster challenged with BSK. (B) Nonvaccinated hamster challenged with *B. burgdorferi* sensu stricto isolate C-1-11. (C) Hamster vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel and challenged with the same isolate. Results were determined 21 days after challenge. Arrows point to areas of infiltration of inflammatory cells into the synovium. Destructive arthritis was also observed in hamsters vaccinated with *B. burgdorferi* sensu stricto isolate 297 in adjuvant and challenged with *B. burgdorferi* sensu stricto isolate 297. In addition, destructive arthritis was evoked when hamsters vaccinated with *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in adjuvant were challenged with *B. garinii* isolate LV4 and *B. afzelii* isolate BV1. The presence of high-titer isolate-specific borreliacidal antibody prevented the induction of arthritis when vaccinated hamsters were challenged with the homologous isolate. However, arthritis still developed in vaccinated hamsters challenged with nonhomologous isolates of *B. burgdorferi* sensu lato.

TABLE 1. Recovery of *B. burgdorferi* from tissues[a]

Group and time of challenge (wk postvaccination)	No. of animals positive for spirochetes in:			
	Urinary bladder	Spleen	Kidney	Heart
Vaccinates				
1	3	2	3	3
3	3	2	2	2
5	2	3	2	3
7	0	0	0	0
9	0	0	0	0
Controls				
Nonvaccinated	3	3	3	3
Adjuvant alone	3	2	3	2

[a] Hamsters were vaccinated with Formalin-inactivated *B. burgdorferi* isolate C-1-11 in aluminum hydroxide gel and challenged 1, 3, 5, 7, and 9 weeks after vaccination with *B. burgdorferi* isolate C-1-11. Controls included nonvaccinated hamsters and hamsters inoculated with adjuvant alone. There were three hamsters in each group.

(Table 1). By contrast, hamsters challenged 7 and 9 weeks after vaccination failed to develop swelling of the hind paws (Fig. 2), and their tissues did not grow spirochetes when cultured in BSK (Table 1). Slight swelling of the hind paws was detected when nonvaccinated hamsters and hamsters inoculated with adjuvant alone were challenged with *B. burgdorferi* sensu stricto isolate C-1-11 (Fig. 2 and 3B). The swelling, however, was significantly less ($P < 0.01$) and of shorter duration than the swelling in hamsters challenged 1, 3, and 5 weeks after vaccination with Formalin-inactivated spirochetes in adjuvant. The tissues of these hamsters also grew spirochetes when cultured in BSK for 10 days (Table 1). In addition, nonvaccinated hamsters and hamsters vaccinated with BSK failed to develop any swelling of the hind paws (Fig. 2 and 3A). Vaccinated hamsters that were not infected showed no adverse clinical manifestations. Likewise, vaccinated and nonvaccinated hamsters challenged with nonviable spirochetes failed to develop arthritis. In other experiments, arthritis was evoked in vaccinated hamsters challenged intradermally and intraperitoneally. The onset of arthritis was delayed by approximately 20 days and the swelling of the hind paws was less severe after challenge by these routes.

Changes in movement and behavior of hamsters. Hamsters that had been vaccinated 5 weeks or less previously with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel were challenged subcutaneously in the hind paws with 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11. These hamsters displayed unusual behavior patterns and had difficulty moving about their cages. They became unusually vicious about 10 days after challenge and refused to eat and drink despite placement of food and water directly on the floors of the cages. Simply touching the cages caused the hamsters to squeal. Hamster movement was greatly restricted because they could not move their legs. Hamsters maneuvered about the cages extremely slowly on their abdomens, dragging their hind legs. These responses gradually waned 20 days after challenge and correlated with the onset and decrease in the swelling of the hind paws. These clinical findings may be the result of severe pain rather than inflammation of the central nervous system. No unusual changes were observed in nonvaccinated hamsters or hamsters inoculated with adjuvant alone and then challenged with *B. burgdorferi* sensu stricto isolate C-1-11 or with BSK.

Histopathology of hind paws. Twenty-one days after challenge with *B. burgdorferi* sensu stricto isolate C-1-11, an erosive and destructive arthritis was detected in the hind paws of hamsters vaccinated 5 weeks earlier with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant (Fig. 3C). The synovia of the tibiotarsal and intertarsal joints displayed chronic hypertrophy and hyperplasia characterized by bridging of villi mixed with fibrin (pannuslike), erosion of the articular cartilage, and focal destruction of underlying bone (Fig. 3C). A

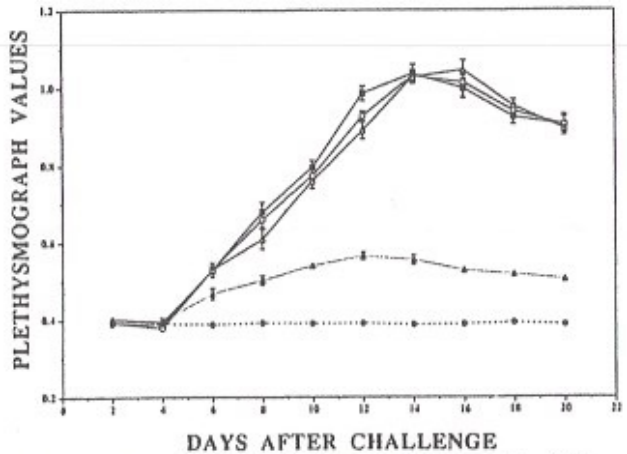

FIG. 4. Development of hind paw swelling in hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel (-■-), aluminum hydroxide (-○-), and Freund's incomplete adjuvant (-△-) after challenge with *B. burgdorferi* sensu stricto isolate C-1-11. Controls included nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 (- - ▲ - -) or BSK (- - ● - -).

TABLE 2. Recovery of *B. burgdorferi* from tissues of hamsters vaccinated with different preparations[a]

Group and prepn	No. of animals positive for spirochetes in:			
	Urinary bladder	Spleen	Kidney	Heart
Nonvaccinated	3	2	3	3
Vaccinated				
Aluminum hydroxide gel	3	3	3	3
Aluminum hydroxide	2	2	3	3
Freund's incomplete adjuvant	2	3	2	3

[a] Hamsters were vaccinated with the indicated preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 and then challenged 5 weeks later with the same isolate. Nonvaccinated hamsters were used as controls and were also challenged. There were three hamsters in each group.

cellular infiltrate of neutrophils, macrophages, mast cells, lymphocytes, and plasma cells was also present in the subsynovial and periarticular tissues. Chronic arthritis was pronounced and characterized by fibromyxoid changes with residual granulation tissue and occasional cartilaginous metaplasia. In contrast, the hind paws of nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 displayed an acute synovitis (Fig. 3B). A cellular inflammatory infiltrate was present, but the joint spaces were free of pannus tissue formation and the development of erosive and destructive arthritis. (Fig. 3B). Similar histopathological findings were also seen in the hind paws of hamsters infected with *B. burgdorferi* sensu stricto isolate C-1-11 after vaccination with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in PBS or with adjuvant alone (data not shown). Nonvaccinated hamsters inoculated with BSK also failed to develop any significant histological changes (Fig. 3A).

Effects of various adjuvants on development of severe destructive arthritis. Three groups of three hamsters each were challenged subcutaneously in the hind paws with 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11 5 weeks after vaccination with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel, aluminum hydroxide (Imject alum), or Freund's incomplete adjuvant. All vaccinated hamsters developed severe swelling of the hind paws 5 days after infection (Fig. 4). The swelling peaked on days 14 to 16 after challenge and gradually decreased. Spirochetes were also recovered from their tissues after cultivation in BSK for 10 days (Table 2). Although nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 developed slight swelling, the severity of swelling was significantly less ($P < 0.01$) than that measured in vaccinated hamsters and was of shorter duration (Fig. 4). Nonvaccinated hamsters inoculated with BSK failed to develop any swelling of the hind paws. When these experiments were repeated with Formalin-inactivated or other preparations of whole *B. burgdorferi* sensu lato isolates in various adjuvants, similar results were obtained.

Induction of severe destructive arthritis in vaccinated hamsters challenged with other isolates of the three genomic groups. Five groups of three hamsters each were vaccinated with formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in aluminum hydroxide gel and challenged 7 and 3 weeks later, respectively, with 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11 or 297, *B. garinii* isolate LV4, *B. afzelii* isolate BV1, or *B. hermsii*. Hamsters vaccinated with *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in adjuvant had borreliacidal antibody titers of 1:320 and 1:1,280, respectively, at the time of challenge (Table 3). No swelling of the hind paws was detected in vaccinated hamsters challenged with the homologous isolate, nor were spirochetes isolated from the tissues of these animals. When vaccinated hamsters were challenged with the nonhomologous isolate of *B. burgdorferi* sensu stricto, *B. garinii*, or *B. afzelii*, severe swelling of the hind paws developed and spirochetes were isolated from the tissues of these hamsters even though high levels of isolate-specific borreliacidal antibody were present (Table 3). Hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in adjuvant failed to develop any swelling of the hind paws when challenged with *B. hermsii*, although spirochetes were isolated from the blood.

DISCUSSION

This is the first documentation that severe destructive arthritis can develop after vaccination against infection with *B. burgdorferi* sensu lato. Arthritis was readily evoked in vaccinated hamsters challenged with isolates of *B. burgdorferi* sensu lato before high levels of protective borreliacidal antibody had developed. Once high levels of isolate (vaccine)-specific borreliacidal antibody developed, hamsters were protected from homologous challenge and development of arthritis. However, vaccinated hamsters still developed severe destructive arthritis when challenged with other isolates of the three genomic groups of *B. burgdorferi* sensu lato. Our results demonstrate

TABLE 3. Induction of severe destructive arthritis in vaccinated hamsters[a]

Challenge isolate	No vaccine			Isolate C-1-11 vaccine			Isolate 297 vaccine		
	Borreliacidal antibody titer	No. with arthritis	No. positive by culture	Borreliacidal antibody titer	No. with arthritis	No. positive by culture	Borreliacidal antibody titer	No. with arthritis	No. positive by culture
B. burgdorferi sensu stricto isolates									
C-1-11	<20	0	3	320	0	0	<20	3	3
297	<20	0	3	20	3	3	1,280	0	0
B. garinii LV4	<20	0	3	<20	3	3	<20	3	3
B. afzelii BV1	<20	0	3	20	3	3	<20	3	3
B. hermsii	<20	0	3	<20	0	3	<20	0	3

[a] Groups of three hamsters were vaccinated with whole-cell preparations of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in aluminum hydroxide gel and then challenged with the same or a different isolate. Nonvaccinated hamsters were used as controls. The borreliacidal antibody titer of nonvaccinated and vaccinated hamsters was determined prior to challenge. The number of hamsters with severe destructive arthritis and the number of hamsters whose urinary bladder, spleen, kidney, or heart tissues were positive for growth of spirochetes in culture were also determined. Blood samples were cultured in BSK for recovery of *B. hermsii*.

that vaccination induces protection against homologous challenge, but vaccination also primes hamsters for development of arthritis. If vaccinated hamsters are challenged with isolates of *B. burgdorferi* sensu lato that are resistant to the vaccine-induced specific antibody, they will develop severe destructive arthritis. This is an important finding and suggests that vaccines must be composed of several isolates of *B. burgdorferi* sensu lato to induce a comprehensive borreliacidal antibody response to prevent development of arthritis.

Adjuvants are frequently used in vaccines to augment immune responses without major side effects (2). We showed that vaccination with Formalin-inactivated spirochetes in the absence of adjuvant induced only a weak borreliacidal antibody response. Borreliacidal antibody accurately reflects the level of protective antibody (40). When Formalin-inactivated spirochetes were incorporated in adjuvant, high levels of borreliacidal antibody developed 7 weeks or more after vaccination. Vaccinated hamsters were protected from homologous challenge. Surprisingly, hamsters vaccinated with spirochetes in adjuvant developed severe destructive arthritis when challenged with the homologous isolate before high levels of borreliacidal antibody were detected. Furthermore, vaccinated hamsters developed severe destructive arthritis when challenged with other isolates of the three genomic groups of *B. burgdorferi* sensu lato, even when high levels of isolate-specific borreliacidal antibody were present. Our results also showed that development of arthritis was caused by the spirochetes and not by the adjuvants. These findings suggest that vaccines prepared from whole spirochetes should be tested for their ability to induce arthritis or other clinical manifestations of Lyme borreliosis by challenging vaccinated animals with multiple isolates of *B. burgdorferi* sensu lato before and after the establishment of high levels of protective borreliacidal antibody.

Our results suggest that alternative approaches, besides whole spirochetes, need to be considered for the development of Lyme borreliosis vaccines. One approach would be to use selected proteins or other antigens of *B. burgdorferi* sensu lato that are known to induce protective borreliacidal antibody. Several *B. burgdorferi* sensu lato proteins, including OspA (12, 13, 43, 44, 54, 55), OspB (14, 34, 35, 55), OspC (33), and the 39-kDa protein (40) have been shown to induce killing antibody. Although OspA is currently the leading vaccine candidate, it has considerable immunologic (4, 15, 38) and molecular (24, 31, 39) heterogeneity. This may require several OspA proteins for development of a comprehensive vaccine. We have shown that isolates of *B. burgdorferi* sensu lato can be separated into at least five distinct seroprotective groups (28, 29). This suggests that combinations of *B. burgdorferi* sensu lato protective proteins may be required to provide a comprehensive vaccine for humans and other animals. The ability of these proteins alone or in combination to induce or elicit adverse clinical manifestations is unknown and must be evaluated before their inclusion in a vaccine. Likewise, the immunogenicity of these purified proteins must be determined. An adjuvant may be required for the induction of an adequate protective antibody response and may enhance the potential for adverse effects. Another approach for vaccination would be to use whole spirochetes and eliminate or remove the antigen(s) responsible for the induction of arthritis or other possible autoreactive pathologic responses (1, 16, 25, 42). Although this approach is not popular, it should be evaluated because of the immunogenic and molecular heterogeneity of vaccine protein candidates and the number of antigens that can induce borreliacidal activity.

Why has severe destructive arthritis not been documented in other vaccinated animals? Vaccination of dogs (9, 26) and hamsters (17, 18, 20) has been done previously with whole spirochetes in adjuvant. One explanation is that vaccinated animals are commonly challenged with the homologous infectious *B. burgdorferi* sensu lato isolate during periods when levels of borreliacidal antibody are high. Generally, several weeks elapse between vaccination and challenge, allowing sufficient time for development of specific borreliacidal antibody. In this study, the protective antibody response to vaccination with *B. burgdorferi* sensu stricto isolate C-1-11 developed slowly. It took 7 weeks for the development of sufficiently high levels of borreliacidal antibody to prevent infection and the development of arthritis. By contrast, hamsters vaccinated with *B. burgdorferi* sensu stricto isolate 297 developed high levels of borreliacidal antibody as early as 1 week after vaccination and were protected from homologous challenge and development of arthritis. Another explanation is that investigators failed to challenge vaccinated animals with isolates of *B. burgdorferi* sensu lato belonging to distinct seroprotective groups. Lovrich et al. (28, 29) identified five seroprotective groups among North American and European isolates of *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* by using the borreliacidal assay. We showed that hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolates C-1-11 and 297 developed severe destructive arthritis when challenged with distinct seroprotective group isolates in the presence or absence of homologous borreliacidal antibody. A third explanation may be that the route of challenge influenced the development of arthritis. We have also evoked arthritis in vaccinated hamsters challenged intradermally and intraperitoneally. However, the onset of arthritis was delayed by approximately 20 days and the swelling of the hind paws was less severe in these animals. The late development of arthritis may not have been observed by other investigators. A fourth explanation is that the hamster is unique. Hamsters may process whole spirochetes in adjuvant differently than other animals. It is important to note that serious adverse reactions in dogs to a commercially available whole-cell vaccine containing an adjuvant have not been reported to date (9, 26). Likewise, other animals have not developed arthritis despite vaccination with whole spirochetes in adjuvant (17, 18, 20). Even if other animal models do not develop arthritis after vaccination, the hamster's propensity for development of arthritis may lead to a better understanding of the immune mechanisms responsible for the induction and development of arthritis.

Our results also suggest that humorally mediated responses are not responsible for the development of severe destructive arthritis in hamsters. Arthritis was evoked in the presence and absence of borreliacidal antibody and of antibody used for the serodiagnosis of Lyme disease. Passive transfer of serum from hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 did not induce arthritis when naive syngenic recipient hamsters were challenged with the homologous isolate or other isolates of *B. burgdorferi* sensu lato, even after daily administration of serum for 7 days (data not shown). Likewise, Fikrig et al. (11) showed that active immunization with OspA did not enhance arthritis but hastened its resolution. These results and those obtained by histopathology suggest that cell-mediated responses are involved. Additional studies are needed to determine whether severe destructive arthritis can be passively transferred with cells or abrogated in vaccinated hamsters by treatment with specific T-lymphocyte reagents.

In summary, severe destructive arthritis developed in vaccinated hamsters. Although vaccinated hamsters failed to develop arthritis when challenged with the homologous isolate during periods when the levels of isolate-specific borreliacidal antibody were high, they developed severe destructive arthritis when challenged before borreliacidal antibody developed or when challenged with different isolates of *B. burgdorferi* sensu lato. The induction and development of arthritis was not dependent on the isolate of *B. burgdorferi* sensu lato or the type of adjuvant used. Additional studies are needed to define the antigen(s) responsible for the induction of arthritis and the mechanism(s) involved. These investigations are needed to understand the immune mechanisms responsible for arthritis and for the development of a safe vaccine for prevention of Lyme borreliosis.

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