Passive Immunization Prevents Induction of Lyme Arthritis in LSH Hamsters

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We determined that sera obtained from hamsters infected with *Borrelia burgdorferi* could prevent the induction of Lyme arthritis. When irradiated hamsters were administered immune serum and subsequently challenged with *B. burgdorferi*, no evidence of infection was detected. Recipients failed to develop swelling of the hind paws, and no histopathologic changes were detected. In addition, *B. burgdorferi* was not recovered from tissues of hamsters that were passively immunized. By contrast, irradiated hamsters that were administered normal hamster serum or saline and infected with the Lyme spirochete developed arthritis. Extensive histopathologic changes occurred in the hind paws and knee joints, and spirochetes were recovered from most of the tissues examined. These results show that immune serum can confer complete protection on recipient hamsters to challenge with *B. burgdorferi*.

Lyme disease is a multisystem infection that is often accompanied by an expanding skin lesion; erythema migrans; and concomitant or subsequent development of arthritic, cardiac, or neurologic complications (17, 23, 26, 27). The disease is caused by a spirochete, *Borrelia burgdorferi*, and is transmitted primarily to human hosts by ixodid ticks (3, 5, 25). Lyme disease has become the most frequently reported tick-borne illness in the United States since it was first reported in 1977, as described recently (6).

Despite considerable interest in the clinical manifestations of Lyme disease, the mechanisms of pathogenesis are poorly understood. A major factor responsible for the slow progress in deciphering these mechanisms has been the absence of an animal model. Rabbits and guinea pigs develop skin lesions resembling human erythema migrans after infection with *B. burgdorferi* (4, 15, 16). These lesions, however, do not develop consistently and are the only manifestation that has been noted. Adult hamsters (14), mice (18), and rats (2) can also be infected with the Lyme spirochete but do not develop clinical manifestations of Lyme disease. Only mild histologic changes are detected, despite the persistence of spirochetes in tissues for several months (7).

Recently, Barthold et al. (2) demonstrated that weanling and 3-week-old inbred Lew/N rats developed arthritis after infection with *B. burgdorferi*. These studies were the first to demonstrate a sustained clinical manifestation of Lyme disease in animals.

We induced Lyme arthritis in nonirradiated inbred LSH/Ss Lak hamsters (9, 19). Hamsters developed a histologically demonstrable arthritis after injection of *B. burgdorferi* in the hind paws (9, 19). When hamsters were exposed to radiation and infected with *B. burgdorferi*, the hind paws became severely inflamed (19). The prolonged inflammation (7 weeks) resulted in destructive and erosive bone changes in the joints of the hind paws (9). Most importantly, the arthritic lesions found in hamsters (9, 19) and rats (2) resembled those found in humans with Lyme arthritis.

The pathogenesis of Lyme arthritis and the immune response to *B. burgdorferi* can be studied in these animals (2, 9, 19). We therefore determined whether sera obtained from hamsters infected with *B. burgdorferi* could prevent the induction of Lyme arthritis.

MATERIALS AND METHODS

Animals. Inbred LSH/Ss Lak hamsters (age, 6 to 8 weeks) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Hamsters (weight, 60 to 100 g) were housed at three or four per cage at an ambient temperature of 21°C.

Organism. *B. burgdorferi* 297 was obtained from Russell C. Johnson (University of Minnesota, Minneapolis). The strain was originally isolated from human spinal fluid (24) and has been maintained by passage in Barbour-Stoenner-Kelly medium (BSK) and hamsters (10–14, 19).

The hamster-passed spirochetes were grown in BSK at 35°C for 5 days. The suspension of *B. burgdorferi* was adjusted with fresh BSK to contain approximately 10^7 organisms per ml. One-milliliter samples were then dispensed in vials, which were sealed and stored in liquid nitrogen until use.

Preparation of *B. burgdorferi* for infection of hamsters. A frozen vial containing a suspension of *B. burgdorferi* was thawed and used to inoculate fresh BSK. The culture was grown for 5 days at 35°C and diluted with BSK to contain 5 $\times 10^6$ organisms per ml. Hamsters were infected subcutaneously in each hind paw with 0.2 ml of this suspension.

Irradiation of hamsters. Groups of hamsters were exposed to 600 rads of gamma radiation with a cobalt-60 irradiator (Picker Corp., Cleveland, Ohio). Hamsters survived this level of radiation without reconstitution with normal bone marrow cells.

Preparation of hamster serum. Ten LSH hamsters were infected subcutaneously in each hind paw with 0.2 ml of BSK containing 10^6 viable *B. burgdorferi* organisms. Three weeks after infection hamsters were mildly anesthetized by ether inhalation and were bled by intracardiac puncture. The blood was allowed to clot; and the serum was separated by centrifugation, pooled, divided into 1-ml portions, and frozen at −20°C until use. Concomitantly, pooled normal serum was obtained from noninfected hamsters.

Passive transfer of resistance. Groups of four irradiated hamsters each were injected intravenously in the sublingual

vein with 0.4 ml of saline or normal or immune serum at 3-day intervals (−3, 0, +3, and +6 days) for 9 days. In some experiments dilutions of immune serum were administered to hamsters. Three days after the first injection (day 0), hamsters were irradiated and injected in each hind paw with 10^6 B. burgdorferi organisms. A fourth group of four irradiated hamsters was injected with 0.2 ml of BSK in each hind paw.

Assessment of arthritis. Swelling of the hind paws of irradiated hamsters was used to evaluate the inflammatory response to infection. The volume of each hind paw was measured with a plethysmograph (Buxco Electronics, Sharon, Conn.) on days 1, 5, and 7 to 11 after challenge with B. burgdorferi. Measurements were obtained by lightly anesthetizing the hamsters and carefully dipping a hind paw into a column of mercury up to the ankle and recording the amount (in milliliters) of mercury displaced. The mean plethysmograph values were obtained from four hamsters (eight paws) and were used as an index of the severity of arthritic swelling. Mercury displacement was standardized with a volume calibrator.

Isolation of B. burgdorferi from tissues. Thirteen days after infection, hamsters were sacrificed by ether inhalation. The left kidney, spleen, and urinary bladder were removed aseptically and homogenized separately with 1 ml of BSK in a sterile petri dish. The tissue suspensions were then inoculated into 4 ml of BSK and incubated at 35°C. Cultures were monitored weekly for spirochetes by dark-field microscopy. If cultures failed to demonstrate growth of B. burgdorferi, portions of the cultures (2 ml) were subcultured to 4 ml of fresh BSK and examined weekly for 3 weeks.

Preparation of tissue for histology. The pelvis with attached hindquarters was removed, skinned distally to the hind paws, fixed in 10% neutral Formalin, and transferred to 10% formic acid for decalcification for 5 days. The rear limbs were amputated at the midfemur and bisected in its longitudinal axis, thus exposing the hind paw and knee joints. The tissue specimens were routinely processed, embedded in paraffin, cut into 6-μm sections, and stained with hematoxylin and eosin. Sections were also stained for spirochetes by a modified microwave technique of the Steiner silver impregnation method (8).

Statistical analysis. The plethysmograph values obtained from irradiated hamsters were tested by analysis of variance. The Fisher least-significant-difference test (22) 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

Early course of arthritis in hamsters. Two groups of 21 irradiated hamsters each were inoculated in each hind paw with 0.2 ml of BSK or BSK containing 10^6 B. burgdorferi organisms. A third group of 21 irradiated, noninfected hamsters was used to obtain base-line volume measurements of the hind paws. Plethysmograph measurements of the hind paws were obtained 7, 9, 14, and 21 days after infection.

There was an increase ($P \leq 0.001$) by day 7 in the mean paw volume of irradiated hamsters infected with B. burgdorferi compared with that of the controls (Fig. 1). The swelling peaked at day 10 and was still detected on days 14 and 21. No statistically significant differences ($P \geq 0.05$) were detected between the irradiated, noninfected hamsters and the irradiated hamsters inoculated with BSK.

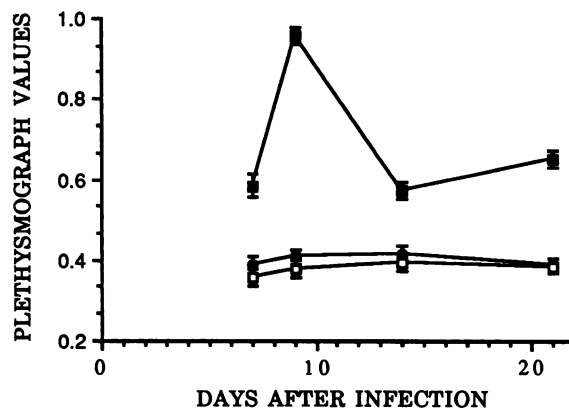**Effect of immune serum on induction of arthritis.** The purpose of this experiment was to determine whether treat-

FIG. 1. Plethysmograph values (mean ± standard error) for noninfected, irradiated hamsters (●) and irradiated hamsters injected with B. burgdorferi (■) or BSK (□).

ment of irradiated hamsters with sera from nonirradiated hamsters infected with B. burgdorferi for 3 weeks could prevent the induction of arthritis.

Three groups of four irradiated hamsters each were injected with saline, normal serum, or immune serum and subsequently challenged with B. burgdorferi. A fourth group of four irradiated hamsters was injected with BSK.

Inflammation of the hind paws was detected in hamsters that were infused with normal serum or saline 7 days after challenge with B. burgdorferi (Fig. 2). The response peaked on day 8 of infection and then declined. By contrast, hamsters infused with immune serum showed no evidence of swelling of the hind paws (Fig. 2 and 3A). Hind paw swelling in hamsters infused with immune serum did not differ significantly from that in noninfected irradiated hamsters injected with BSK (Fig. 2 and 3A and D). Similar results were obtained when this experiment was repeated. Furthermore, hamsters infused with dilutions of immune serum (up to 20-fold) failed to develop swelling of the hind paws after challenge with B. burgdorferi (data not shown).

Isolation of B. burgdorferi from tissues. Thirteen days after infection, hamsters were sacrificed; and the spleen, left kidney, and urinary bladder were cultured in BSK for B. burgdorferi.

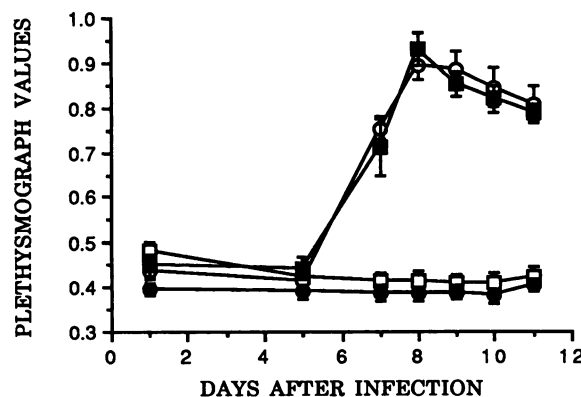The Lyme spirochete was detected in all of the cultures of urinary bladders from hamsters that were infused with

FIG. 2. Plethysmograph values (mean ± standard error) for irradiated, noninfected hamsters (●) and irradiated hamsters administered saline (○) or immune (□) or normal (■) serum and infected with B. burgdorferi.
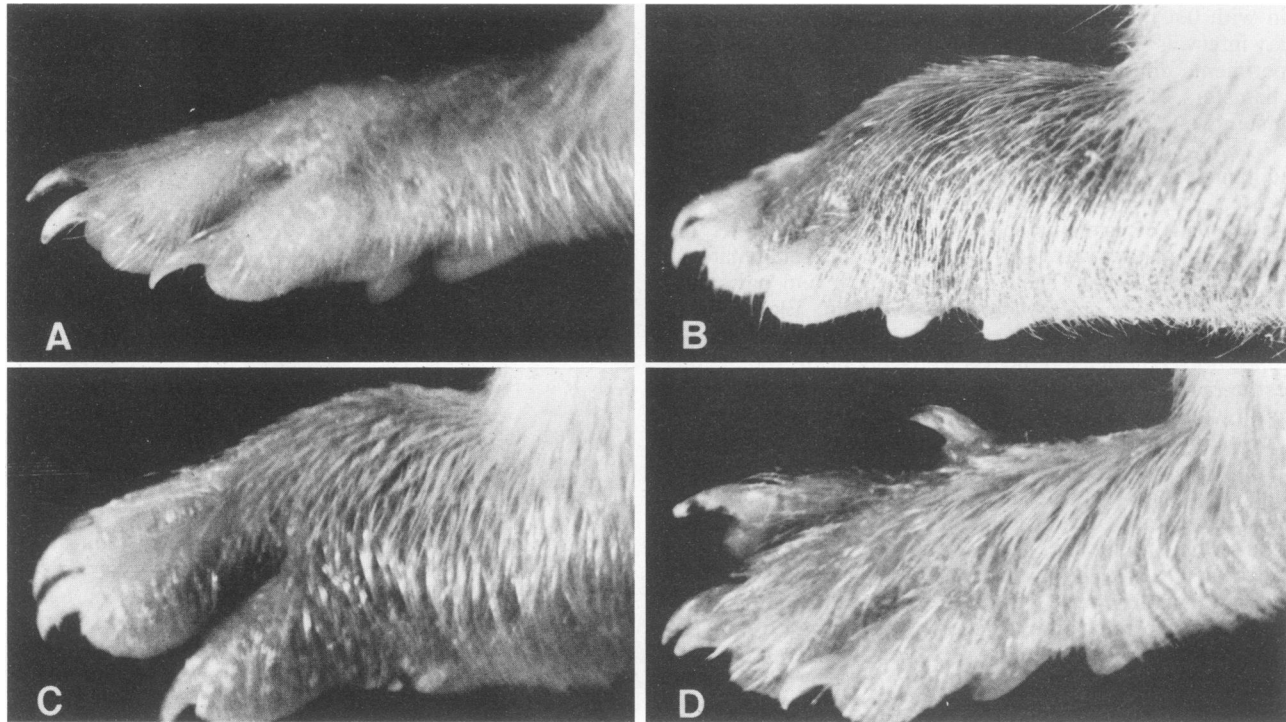
FIG. 3. Appearance of hind paws 8 days after infection with *B. burgdorferi* of irradiated hamsters administered immune (A) or normal (B) serum or saline (C) and appearance of hind paws of noninfected, irradiated hamsters (D).

normal serum or saline (Table 1). Spirochetes were also detected in most of the cultures of kidneys or spleens from these hamsters. By contrast, no spirochetes were detected in cultures of tissues from *B. burgdorferi*-infected hamsters infused with immune serum. Spirochetes were also not detected when the cultures were subcultured to fresh BSK.

Histopathology of the hind paws and knee joints. Extensive pathologic changes were detected in the hind paws and knee joints of *B. burgdorferi*-infected hamsters administered saline (Fig. 4C) or normal serum (Fig. 4B). The tibiotarsal, intertarsal, and interphalangeal joints showed evidence of inflammation. The synovial linings of the hind paws and knee joints were hypertrophic and hyperplastic with focal areas of ulceration. Adherent fresh fibrin protruded into the joint spaces and was associated with many neutrophils. The neutrophils also permeated the subsynovial connective tissue and periarticular structures, including the tendons, tendon sheaths, ligaments, fibrous capsule, and periosteum. The acute inflammatory reaction of the hind paws and knee joints was also associated with many spirochetes in the synovial and periarticular tissues (data not shown).

By contrast, the hind paws and knee joints of *B. burgdorferi*-infected hamsters infused with immune serum were

TABLE 1. Isolation of *B. burgdorferi* from tissues of hamsters administered immune or normal serum[a]

Serum treatment	Spleen	Bladder	Kidney
Immune[b]	0/7	0/7	0/7
Normal	7/8	8/8	5/8
Control (saline)	8/8	8/8	7/8

[a] Results are from two experiments. Results are given as total number of tissues containing *B. burgdorferi*/total number tested.
[b] One hamster died.

uniformly free of histopathologic changes (Fig. 4A). Pathologic changes were also not detected in the hind paws or knee joints of noninfected hamsters inoculated with BSK (Fig. 4D).

DISCUSSION

The mechanism(s) by which experimental animals and human hosts acquire resistance to infection with *B. burgdorferi* is poorly understood. We reported that sera obtained from hamsters infected with *B. burgdorferi* can prevent the induction of arthritis. In addition, immune serum conferred complete protection against infection of hamsters with the Lyme spirochete.

When irradiated hamsters were administered immune serum and challenged with *B. burgdorferi*, recipients failed to develop swelling of the hind paws. Histopathologic changes also were not detected in the hind paws or knee joints. In addition, *B. burgdorferi* was not recovered from tissues after the tissues were incubated in BSK. By contrast, irradiated hamsters administered normal hamster serum or saline developed arthritis. Extensive histopathologic changes were detected in the hind paws and knee joints, and *B. burgdorferi* was recovered from most of the tissues. Our results confirm and extend the findings of Johnson et al. (10), who demonstrated the efficacy of immune serum.

These findings and those of Johnson et al. (10–13) are important. They demonstrate unequivocally that antibody-mediated immunity plays an important role against infection with *B. burgdorferi*. Johnson et al. (10) also showed that exposure of hamsters to small amounts (0.0125 ml) of immune serum confers protection. We showed that sera from *B. burgdorferi*-infected hamsters has considerable anti-*B. burgdorferi* activity. Induction of arthritis was prevented, even though immune serum was diluted 20-fold.
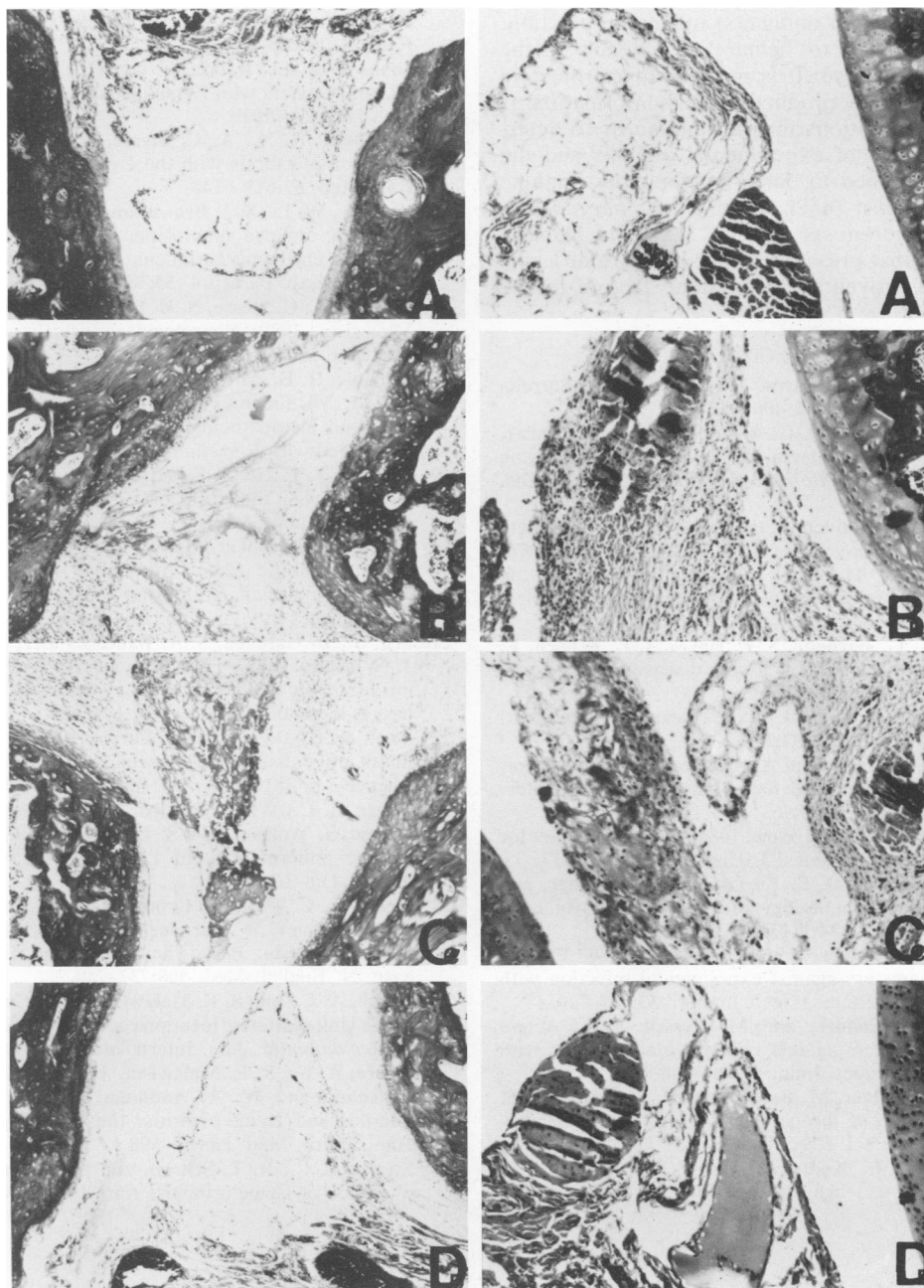
FIG. 4. Pathology of the tibiotarsal (left panels) and knee joints (right panels) of irradiated hamsters infected with *B. burgdorferi* and administered immune (A) or normal (B) serum or saline (C) and appearance of hind paws of noninfected, irradiated hamsters (D).

It is important that immune sera was obtained in this study from hamsters with active disease. Many spirochetes are found in the periarticular and synovial tissues of donor hamsters (9, 19). Donahue et al. (7) also showed that spirochetes could persist in hamsters for several months after infection. These observations suggest that hamsters can develop the humoral immune components necessary to confer protection on recipients but that these components are unable to destroy or eliminate *B. burgdorferi* in the donor hamsters. An explanation for the inability of hamsters to eliminate *B. burgdorferi*, despite the development of an effective antibody response, may be that the spirochetes are sequestered in host tissue that is not readily accessible to

protective antibody. Immunologically privileged sites have been proposed to be responsible for the chronic course of another spirochetal disease, syphilis (20, 21). This explanation may account for the failure of immune serum to protect hamsters from challenge with *B. burgdorferi* if immune serum is administered after infection (12). Another explanation is that *B. burgdorferi* may have an amorphous slime layer that protects it from an effective antibody response (1).

One of the goals of Lyme disease research is development of an effective vaccine. These studies and those of Johnson et al. (10–13) demonstrate that antibody-mediated immunity is involved. Additional studies are needed to isolate the protective factor in immune serum. This information would

be helpful in defining which antigen(s) induces protection. Other studies are needed to define the kinetics of the protective antibody response. It is possible that protective antibodies are transiently produced. This information is necessary to define conditions for vaccination or to determine the immune status of experimental animals and the human host. We also need to define whether our immune serum is effective against other strains of *B. burgdorferi*. There is evidence for strain specificity (12). The availability of animals (2, 9, 19) that present a clinical feature of Lyme disease that can be prevented by immune factors makes these studies feasible.

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