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Passive Immunization of Hamsters against Experimental Infection with the Lyme Disease Spirochete

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Hamsters passively immunized with as little as 0.0125 ml of immune rabbit serum (indirect fluorescent-antibody titer, 1:8,192) were protected from challenge with 1,000 50% infective doses of *Borrelia burgdorferi*. Cross-protection studies with Minnesota and Connecticut isolates of *B. burgdorferi* indicated no major differences in their capacity to elicit mutually protective antibodies in rabbits.

Lyme disease and related disorders, collectively known as Lyme borreliosis, are caused by the spirochete *Borrelia burgdorferi* (7, 9, 10, 13, 16). Rodents and other animals are the major reservoirs of the spirochete (1, 8); ixodid ticks are the primary vectors (1, 9, 10). After exposure of a human to an infected tick, *B. burgdorferi* gains access to the blood and lymphatic circulation, and a systemic illness ensues. The development of a characteristic expanding erythematous skin lesion, erythema chronicum migrans, at the site of the tick bite is an early and diagnostic feature of the disease (17). Some patients develop the chronic forms of the disease involving the joint synovia (arthritis [17]), the skin (acrodermatitis chronica atrophicans [11]), and the nervous system (Bannwarth's syndrome [3]). These forms of the disease are associated with the persistence of the spirochetes in the host (2, 14, 15, 18).

We examined serum from New Zealand White rabbits immunized with two isolates of *B. burgdorferi*, a human spinal fluid (HSF) isolate from Connecticut (16) and a Minnesota mouse (MM) isolate, for protective antibodies. Spirochetes were cultivated in Barbour-Stoenner-Kelly (BSK) medium (4) at 30°C. Rabbits received weekly intravenous injections of 10^8 viable cells washed twice with 0.01 M phosphate-buffered saline (pH 7.5). Serum was harvested 2 to 3 weeks after the final injection, and the antibody titer was determined by the indirect fluorescent-antibody assay (6). Rabbits immunized with the *B. burgdorferi* HSF isolate received four injections; the pooled serum had an indirect fluorescent-antibody titer of 1:8,192. Rabbits immunized with the *B. burgdorferi* MM isolate only received two injections to assay protective activity of a lower-titer serum. The pooled serum had an indirect fluorescent-antibody titer of 1:256. Syrian hamsters, which can be experimentally infected with *B. burgdorferi* (14), were used to assay for protective antibodies. Male and female hamsters, 5 to 10 weeks old, were injected with the test serum subcutaneously 18 h before challenge. Challenge consisted of the intraperitoneal injection of either 10^8 cells of the HSF isolate, which is equivalent to 1,000 50% infective doses (ID_{50}s), or 10^8 cells of the MM isolate (ID_{50} unknown). At 14 days postchallenge, hamsters were sacrificed, and the kid-

TABLE 1. Passive immunization of hamsters with rabbit serum

Test serum and vol administered (ml)	Antibody titer[a]	Challenge strain	No. of infected hamsters/total no. of hamsters	% Protection
Normal rabbit serum				
1.0	<1:8	HSF	10/10	0
0.5	<1:8	MM	5/5	0
Anti-*B. burgdorferi* HSF rabbit serum				
1.0	1:8,192	HSF	0/10	100
0.5	1:8,192	HSF	0/20	100
0.2	1:8,192	HSF	0/5	100
0.1	1:8,192	HSF	0/15	100
0.05	1:8,192	HSF	0/5	100
0.025	1:8,192	HSF	0/5	100
0.0125	1:8,192	HSF	0/5	100
0.1	1:8,192	MM	1/5	80
Anti-*B. burgdorferi* MM rabbit serum				
0.5	1:256	MM	0/5	100
0.5	1:256	HSF	0/5	100

[a] Indirect fluorescent-antibody titer.

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neys and spleens were cultured. Each hamster organ was placed in 6 ml of BSK medium and homogenized with a Stomacher Lab-Blender (Tekmar Co., Cincinnati, Ohio). After the larger tissue debris was allowed to settle, duplicate 1:10 dilutions of the supernatant were made in the isolation medium. Isolation medium was prepared by the addition of 0.15% agarose (SeaKem LE; FMC Corp., Marine Colloids Div., Rockland, Maine) to BSK medium. Cultures were examined for spirochetes by dark-field microscopy after 3 weeks of incubation at 30°C.

Control hamsters which received 1 ml of pooled normal rabbit serum were not protected from challenge with 1,000 ID_{50}s of the HSF isolate of *B. burgdorferi*. Of the 10 hamsters which received normal rabbit serum, 10 were culture positive (Table 1). In contrast, hamsters which received as little as 0.0125 ml of anti-*B. burgdorferi* (HSF isolate) serum were fully protected from challenge (Table 1). The MM isolate also elicited the formation of protective antibodies. Normal rabbit serum (0.5 ml) did not protect hamsters from challenge with 10^8 cells of the MM isolate. However, 0.5 ml of anti-*B. burgdorferi* (MM isolate) serum provided 100% protection against challenge with the MM isolate.

The immunogenic specificity of these two isolates from different geographical areas was investigated by cross-protection studies. Of hamsters passively immunized with 0.1 ml of anti-*B. burgdorferi* (HSF isolate) serum, 80% were resistant to challenge with the MM isolate (Table 1). Comparable results were observed with hamsters which received 0.5 ml of anti-*B. burgdorferi* (MM isolate) serum and were challenged with 1,000 ID_{50}s of the HSF isolate. Of these hamsters, 100% were resistant to challenge (Table 1).

The results of the passive immunization studies suggest that circulating antibodies can play a major role in resistance to *B. burgdorferi* infection. The cross-protection passive-immunization studies with rabbit antisera to isolates from two geographically different endemic areas of Lyme disease suggest that both isolates are similar in their protective antigen composition. Our cross-protection studies are in agreement with the report of Barbour et al. (5). They studied the antigens of a number of *B. burgdorferi* isolates from the United States and found their antigenic composition to be quite homogeneous.

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