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Infection of Syrian Hamsters with Lyme Disease Spirochetes

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Syrian hamsters were shown to be susceptible to infection by the Lyme disease spirochete *Borrelia burgdorferi*. Although these spirochetes did not cause any outward signs of illness in these animals, they did cause a generalized infection. Spirochetemia was present during the first 6 days of infection. At 14 days postinfection, spirochetes could be isolated from one or more of the following organs: spleen, eyes, kidneys, liver, testes, and brain. Spirochetes were isolated from the eyes and kidneys of one animal 52 days postinfection, suggesting that these organisms may cause a persistent infection. Virulence of *B. burgdorferi* is maintained by animal passage but is lost upon prolonged in vitro cultivation.

Lyme disease is a newly described tick-borne spirochetosis (3, 5, 10). Genetic (6, 8) and phenotypic (R. C. Johnson, F. W. Hyde, and C. M. Rumpel, Yale J. Biol., Med., in press) studies have shown that this spirochete represents a new species of *Borrelia* and that the various isolates from different geographical areas belong to the same species (6, 8). This new species of *Borrelia* has been named *Borrelia burgdorferi* (7).

The spirochete is transmitted by several species of the ixodid ticks. Mice and deer appear to be important reservoir hosts for the spirochete (1, 4). A characteristic and diagnostic feature of the early phase of the disease in humans is the development of an expanding erythematous skin lesion, erythema chronicum migrans (9). The major sequela of untreated Lyme disease patients in the United States is various degrees of arthritis (11). Understanding of the host-parasite relationship in Lyme disease has been hindered because of the lack of a suitable experimental animal host.

In this report, we describe the susceptibility of Syrian hamsters to infection by *B. burgdorferi*.

MATERIALS AND METHODS

Origin and cultivation of Lyme disease spirochetes. *Ixodes dammini* New York (ATCC 35210) and New Jersey (WB) isolates and the *Ixodes ricinus* Switzerland (ATCC 35211) isolates were provided by Willy Burgdorfer and Allen Barbour, Rocky Mountain Laboratories, Hamilton, Mont. Human spinal fluid (TLO-030), blood (TLO-005), and skin (TLO-031) isolates were supplied by Allen Steere, Yale University, New Haven, Conn. The spirochetes were routinely cultivated in Barbour-Stoenner-Kelly (BSK) medium prepared as described by Barbour (A. G. Barbour, Yale J. Biol. Med., in press) at 30°C in air. Isolation medium consisted of BSK medium plus 0.1 to 0.2% agarose (Seakem LE; FMC Corp., Rockland, Maine).

Inoculation and isolation of *B. burgdorferi* from hamsters. Male or female Syrian hamsters (Engle Laboratories, Farmington, Ind.; 5 to 10 weeks old) were injected intraperitoneally with ca. 10^8 cells of *B. burgdorferi*. Routinely at 14 days postinfection, the hamsters were sacrificed, and the appropriate specimens were cultured. Hamsters in groups of five were used to establish the infectious nature of the test organisms.

Samples of one to two drops of blood, obtained by cardiac puncture, were added to 10 ml of media. Freshly collected urine was diluted 1:10 and 1:100 in culture media. At necropsy a 10% (wt/vol) suspension of the kidneys, brain, eyes, testes, liver, or spleen in culture media was prepared with a tissue homogenizer, sand, or mortar and pestle or by extrusion through a 3-ml syringe (without a needle). The extrusion method was used routinely because of its simplicity. After allowing the larger tissue debris to settle, duplicate 1:10 and 1:100 dilutions of the supernatant fluid were cultured. Cultures were examined by dark-field microscopy at weekly intervals for the presence of spirochetes and considered negative if spirochetes were not seen after 6 weeks of incubation.

The identify of isolated spirochetes as *B. burgdorferi* was established with rabbit antisera to *B. burgdorferi* and by the indirect immunofluorescence test (2). Antiserum was obtained from White New Zealand rabbits 1 week after five weekly intravenous injections of 10^8 viable *B. burgdorferi* (ATCC 35210). The endpoint titer of this antiserum was 1:2,048 (±one dilution) with *B. burgdorferi* and the hamster isolates.

RESULTS

The human spinal fluid (HSF) and *I. dammini* New Jersey isolates were infectious for the hamsters, whereas the *I. dammini* New York, *I. ricinus* Switzerland, and the human skin and blood isolates lacked this property. The first two spirochetes had been subcultured a few times since isolation, whereas the later spirochetes were transferred many times in culture media. This suggested that prolonged in vitro cultivation of the spirochetes could be responsible for the difference in infectiousness observed. This possibility was investigated by injecting 5 hamsters with the HSF spirochete isolated from hamsters and transferred once in culture media, 5 hamsters with the same isolate transferred 3 times at weekly intervals in media, and 10 hamsters with the same original (hamster-infectious) HSF isolate that had been transferred 30 times at weekly intervals in media. Spirochetes were isolated from all hamsters inoculated with the spirochetes passaged one or three times through the media. In contrast, none of the 10 hamsters injected with cells from subculture 30 were culture positive for spirochetes. Because of the loss of the ability to infect hamsters by spirochetes transferred many times in culture media, only cells that had been transferred no more than three times in vitro were used in subsequent studies.

The susceptibility to infection of male and female hamsters ranging in age from 4 to 52 weeks was examined. The

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number of culture-positive organs from 10 female and 10 male hamsters were 20 and 21, respectively. Similarily, no significant difference in the number of culture-positive organs was observed from groups of five hamsters of 4, 10, and 52 weeks of age.

The location of the HSF isolate in infected hamsters was assayed. Daily blood samples were obtained from five hamsters (anesthetized with diethyl ether) during the first 9 days of infection. Spirochetemia was detected by culture during the first 6 days of infection. The number of hamsters that were culture-positive on days 1, 2, 3, 4, 5, and 6 were 2, 3, 2, 1, 2, and 2, respectively. Blood cultures were negative for days 7, 8, and 9. The spleens, kidneys, urine, eyes, brains, and blood of 21 hamsters were cultured 14 days postinfection. Of the 21 animals, 19 were culture positive for at least one specimen. Blood and urine were uniformly culture negative, and only a single isolate was obtained from the brains. The number of animals with the following culture-positive organs were: spleens, 12 (62%); eye, 9 (47%); and kidneys, 8 (42%). Of the culture-positive hamsters, 10 (53%) had one organ positive, 5 (26%) had two organs positive, and 4 (21%) had three organs positive. Because spirochetes were not isolated from livers in preliminary studies, this organ was not included in this study. However, since this part of the study was completed, the livers of four infected hamsters were cultured, and two were found to be positive. Additionally, the testes of these four animals were cultured, and one animal had culture-positive testes. Of the five hamsters infected with the *I. dammini* New Jersey isolate, two animals had culture-positive spleens, kidneys, and livers. One of these hamsters also had culture-positive eyes and testes. The remaining three animals had culture-positive spleens and kidneys.

The HSF and the *I. dammini* New Jersey isolates, although infectious for hamsters, did not elicit any obvious signs of disease in these animals. However, isolation of the HSF isolate from the eyes and kidneys of a hamster (only one animal available) 52 days postinfection indicated that this spirochete has the potential to persist in these animals for extended periods of time. Additionally, seven serial animal passages of the HSF isolates appeared to have neither increased nor decreased its virulence for hamsters.

DISCUSSION

Syrian hamsters, regardless of sex or age, appear to be susceptible to infection by recent human and tick isolates of *B. burgdorferi*. At least 91% of the animals had one or more culture-positive organs when infected with 10^8 spirochetes. The percentage of culture-positive animals is probably greater, as the isolation medium apparently varied in its growth-supporting properties. Of 53 duplicate 1:10 dilutions of hamster organ suspensions, only 48% were culture positive in duplicate tubes. This is in contrast to stock cultures which consistently grow in duplicate tubes from an inoculum of 10 cells. Because of the inconsistency encountered with the isolation medium, we did not determine a 50% infectious dose for the HSF isolate. An early attempt to do this was unsatisfactory due to erratic results. However, we were able to infect one of five animals with 10^3 spirochetes. Steere et al. (10) have also encountered difficulties isolating *B. burgdorferi* from humans, whereas these spirochetes could be readily isolated from infected ticks. In spite of this limitation, useful information can be generated by using hamsters as an experimental animal host.

Cultural studies have shown that *B. burgdorferi* invades the blood, skin, and spinal fluid of humans (3, 10) and the blood of raccoons, deer, and mice (1). Burgdorfer (W. Burgdorfer, Yale J. Biol. Med., in press), using infected *I. dammini* ticks, produced an erythematous skin response and spirochetemia in New Zealand white rabbits. Whether other human or animal tissues are invaded by *B. burgdorferi* is presently not known. Our studies with Syrian hamsters indicate that *B. burgdorferi* of either human or tick origin causes a generalized infection without any obvious adverse effects. A spirochetemia is present for the first 6 days of infection. At 14 days postinfection, a time at which spirochetemia cannot be detected, spirochetes were isolated from the spleens, kidneys, eyes, livers, testes, and brains. The spleen was the organ most frequently culture positive, and these cultures usually became positive sooner than those of other organs. Some spleen cultures became positive within 2 days, whereas 1 week or longer was usually required for the other organ cultures. This observation suggests that the spleen may contain more spirochetes than other organs or that it may serve as a nutritional supplement for the isolation medium or both. Although culture became positive 2 to 3 days earlier when incubated at 34°C, as compared with 30°C, the latter incubation temperature was used because the spirochetes remained viable longer.

B. burgdorferi apparently produces a persistent infection in hamsters. The presence of this spirochete in as many as six different organs at 14 days postinfection suggests that the hamster mounts a slow or ineffective immune response or the spirochete is resistant to or has taken up residence in a location(s) where it is protected from the immune mechanism of the host. Although long-term persistence of the spirochete was not part of this study, we had one infected animal that was not sacrificed until 52 days postinfection that had culture-positive eyes and kidneys. It appears that this spirochete can persist in human infections since it was isolated from the spinal fluid of one patient 10 weeks after the onset of illness (10). Cultural studies of organs of wild animal reservoirs of this spirochete, such as the mice and deer, have not been reported. The results of our studies with hamsters suggests that cultivation of organs of host animals could be useful for identifying the host range of this spirochete and identifying endemic areas during seasonal periods when ticks are not available.

Only recent isolates that have been transferred a few times in culture media should be used for infecting hamsters. We found that the virulence of the human spinal fluid isolate was lost upon prolonged subculture in media. Virulence was maintained by passage through hamsters and at least three subcultures in media. The tick isolates appear to respond in a similar manner to in vitro cultivation since only the recently isolated *I. dammini* New Jersey spirochete was infectious for hamsters.

The hamster is a useful experimental animal for increasing our knowledge of the interaction of *B. burgdorferi* with animal hosts. It is a well-studied, relatively inexpensive animal that can be used to study such areas as the immune response to this spirochete, the feasibility of a Lyme disease vaccine, and in vivo antibiotic susceptibility.

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