JOURNAL OF CLINICAL MICROBIOLOGY, May 1991, p. 894–896
0095-1137/91/050894-03$02.00/0
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Vol. 29, No. 5

Persistent Cardiac and Urinary Tract Infections with *Borrelia burgdorferi* in Experimentally Infected Syrian Hamsters

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Received 19 November 1990/Accepted 5 February 1991

The heart can be severely affected in humans with Lyme disease, causing conduction defects and, rarely, heart failure. Although immunodeficient and young mice may develop cardiac lesions, cultivation of *Borrelia burgdorferi* from cardiac tissues of experimentally infected animals has not been reported previously. We infected Syrian hamsters with *B. burgdorferi* 297 and found a marked tropism of the spirochete for myocardial and urinary tract tissues. Fifty-six of 57 hearts (98%) and 52 of 58 bladders (90%) were culture positive. The cardiac infection was persistent and could be documented in 21 of 22 hearts (96%) cultured from days 28 to 84 postinfection. The urinary tract was also a site of persistent infection in most animals, with 18 of 23 bladders (78%) being culture positive from days 28 to 84. The persistence of spirochetes was specific for the heart and bladder, as indicated by negative cultures of specimens from the liver and spleen, in which only 1 of 23 cultures was positive from days 28 to 84. Because of the high isolation rates, tropism, and persistence that we found for *B. burgdorferi* in the hamster heart and bladder, these sites will be useful and important for the cultivation of spirochetes in experimental studies that evaluate the efficacies both of candidate vaccines in preventing infection and of antibiotics in eradicating organisms from privileged sites. In addition, the clear demonstration of persistent cardiac infection with *B. burgdorferi* may provide a useful model for studying the pathogenesis of cardiac Lyme disease.

The experimental infection of laboratory animals with *Borrelia burgdorferi* is of potential importance in studying the pathogenesis, diagnosis, treatment, and prevention of Lyme disease. We have previously demonstrated that the Syrian hamster is susceptible to *B. burgdorferi* infection, that an early spirochetemia occurs, and that the spirochete can be recovered from the spleens of a majority of animals (10). It has also been demonstrated that hamsters develop synovitis after inoculation of the organism directly into the foot (6). These observations have provided a useful in vivo model for the assessment of the efficacy of antimicrobial agents and candidate vaccines (8, 9). Other rodents, including rats (3) and laboratory mice (2), can also be infected. The tissue obtained with ear punch biopsy has also been shown to be culture positive in the majority of infected rodents (16). In *Peromyscus leucopus* (the white-footed mouse), the bladder has been shown to be a consistent site of infection (15), a useful finding for an experimental model. The severe combined immunodeficiency (*scid*) mouse is highly susceptible to infection and also develops pathological changes in various organs, including the heart (14), but cultivation and recovery of the organism from the cardiac tissues of these or other infected animals have not been reported.

The heart is an important target of infection in humans with Lyme disease (18); this is most frequently manifested by conduction system disturbances but, occasionally, by congestive heart failure. The spirochete has been seen in or cultivated from cardiac tissue in rare instances (12, 13, 17).

Because of the importance of cardiac involvement in humans with Lyme disease, we sought to cultivate spirochetes from the hearts of experimentally infected hamsters. We report that *B. burgdorferi* can be recovered from the hearts of nearly all infected hamsters and that the cardiac infection persists for at least 84 days. This finding is consistent with the myocardial tropism observed in humans with infection and provides a useful source for the cultivation of spirochetes from animals with experimental infections. In addition, we confirmed and extended to hamsters the finding of persistent urinary bladder infection previously described in the white-footed mouse (15).

MATERIALS AND METHODS

Infecting organism. *B. burgdorferi* 297, which was originally isolated from human spinal fluid, was obtained from Allen Steere (19). The spirochetes were isolated from the spleens of experimentally infected hamsters. They were then cultivated twice in BSK medium (1) at 30°C, prior to their enumeration in a Petroff-Hausser chamber and use in the hamster experiments.

Hamster inoculation. Male Syrian hamsters (age, 4 weeks) were inoculated intraperitoneally with 10^8 cells of *B. burgdorferi* 297 in 1 ml of BSK medium or with 1 ml of BSK medium alone. At the time points indicated in Table 1, the hamsters were sacrificed by CO_2 inhalation.

Cultivation of *B. burgdorferi* from infected hamsters. For blood samples, 1 to 2 drops of whole blood were obtained by cardiac puncture and inoculated into 6 ml of BSK medium plus 0.1% agarose (Seakem LE; FMC Corp., Rockland, Maine). Urine was aseptically removed from intact bladders with a 25-gauge needle, and 1 drop was cultured in 6 ml of BSK medium. Hamster organs were homogenized with 6 ml of BSK medium in individual bags by using a Stomacher Lab-Blender (Tekmar Co., Cincinnati, Ohio). The supernatants from each organ were cultured both undiluted and in duplicate 1:10 dilutions and examined for spirochetes by dark-field microscopy after 3 and 6 weeks of incubation at 30°C. Spot checks were performed on selected positive cultures by using monoclonal antibodies specific for

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TABLE 1. Isolation of *B. burgdorferi* from blood and organs of infected hamsters

Day	No. positive/total no. for the following culture sources:						
	Heart	Bladder	Kidney	Urine	Blood	Liver	Spleen
5	3/3	3/3	3/3	0/2	2/3	ND[a]	3/3
7	5/5	5/5	ND	1/5	1/5	3/5	ND
14	23/23	23/23	13/18	0/9	0/13	2/5	16/18
28	9/9	9/9	4/4	0/9	0/9	0/5	0/4
60	8/8	6/9	1/4	0/9	0/4	0/5	1/4
84	4/5	3/5	ND	0/3	0/5	0/5	ND
Total (% positive)	56/57 (98)	52/58 (90)	21/29 (72)	1/41 (2)	3/39 (7)	5/25 (20)	20/29 (69)

[a] ND, Not done.

B. burgdorferi. *P* values were calculated by the Fisher exact test.

RESULTS

The combined data from three individual experiments are presented in Table 1. We isolated *B. burgdorferi* from the cardiac tissues of 56 of 57 (98%) infected hamsters from which samples were obtained for culture. No organs from control uninfected animals were culture positive. The cardiac infection was present at all times at which samples were obtained from 5 to 84 days postinoculation (Table 1). Similarly, we also found the urinary tract to be a major target of infection. Of 29 kidneys, 21 (72%) removed for culture were positive, although at the latest time point when kidneys were cultured (60 days), only 1 of 4 kidneys was positive. We found that the bladders of 52 of 58 hamsters (90%) were culture positive. The bladders were always culture positive through day 28, but 5 of 14 were culture negative at days 60 and 84 postinfection. Despite the nearly universal finding of urinary tract infection with *B. burgdorferi*, we were able to isolate *B. burgdorferi* from only 1 of 41 urine samples tested.

Organ involvement was not nonspecific, as indicated by cultures of reticuloendothelial organs. Thus, although positive cultures of samples from the liver or spleen were common early in infection (24 of 31 positive from days 5 to 14), only 1 of 23 such cultures was positive at later time points (days 28 to 84), a period during which 21 of 22 hearts (96%) ($P < 10^{-8}$ versus liver or spleen) and 18 of 23 bladders (78%) ($P < 10^{-6}$ versus liver or spleen) were still positive. Finally, the positive cardiac and urinary tract cultures were not merely due to spirochetemia, since all cultures of blood obtained at 14 days and later were negative.

DISCUSSION

Our results demonstrate, in the hamster model, a marked tissue tropism of *B. burgdorferi* for the heart, an important site of involvement in humans with Lyme disease. We also believe that this finding is representative of natural infections of rodents. Ongoing studies in our laboratories have demonstrated cardiac infection in 80% of specimens of *Peromyscus leucopus* captured near the St. Croix River, as well as in *Tamias striatus* (Eastern chipmunks) and *Microtus pennsylvanous* (meadow voles) (unpublished data). Although the isolation of *B. burgdorferi* from the hearts of experimental animals has not, to our knowledge, been reported previously, histopathologic evidence of carditis has been noted in various species of young or genetically immunodeficient nude (2) and severe combined immunodeficiency (14) mice. Our studies suggest that such changes may, in fact, be due to direct infection with the spirochete. We documented cardiac infection in 98% of infected hamsters, yet no pathologic changes were noted (data not shown). This discrepancy may be explained in several ways. First, rodents are primarily reservoir hosts and would not necessarily be expected to develop pathologic signs or disease. Previously, for example, although local synovial pathology has been noted following direct inoculation of the organism into the feet of hamsters (6), few pathologic changes have been noted in systemically infected hamsters (4). Second, although it is highly infectious, strain 297 is not as virulent as fresh isolates obtained from ticks (data not shown). Third, only a small percentage (<10%) of humans with untreated Lyme disease develop clinical cardiac disease (18), suggesting that subclinical cardiac infection might not be uncommon, while only rare hosts develop pathologic changes. Finally, a time period longer than 84 days may be required for pathologic alterations to develop, or such changes may be highly restricted within the heart and may not have been noted by our pathologic studies.

We also demonstrated tropism for the urinary tract, most strikingly, the bladder. This finding is consistent with the report of Schwan et al. (15), who demonstrated bladder involvement in the mouse and who suggested that the bladder wall was infected. Although there is no documented syndrome of urinary pathology in humans with Lyme disease, *B. burgdorferi* has been implicated in some cases of canine nephritis (11). In studies in our laboratories, we have demonstrated that *B. burgdorferi* antigens (7) and, more recently, spirochetal DNA (5) can be found in the urine of some patients with active Lyme disease. It is of interest that although the bladder is commonly involved, it is difficult to recover spirochetes from urine. In this and other studies (data not shown), we found that less than 5% of urine samples were culture positive, even though nearly all of the bladders were infected. Possible factors contributing to this discrepancy could include the rapid inactivation of *B. burgdorferi* by human urine, the small amounts of urine cultured, and the presence of spirochetes in a nonviable or nonreplicating state. We are studying hamster urine samples for the presence of both *B. burgdorferi* antigens and, by the polymerase chain reaction, spirochetal DNA in order to determine whether there is evidence for nonculturable spirocheturia.

The high isolation rates, tropism, and persistence that we reported for *B. burgdorferi* in the hamster heart and urinary bladder are of interest both for the potential pathogenic implications discussed above and because of the utility of these tissues for use in the cultivation of spirochetes from animals with experimental infections. Cultivation of these

tissues could be conveniently incorporated into studies of candidate antibiotics and vaccines to provide a reproducibly high rate of isolation of *B. burgdorferi* from infected animals.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AR 40448 (to J.L.G.) and AR 34744 (to R.C.J.).

We thank Susan Bowers for assistance in examining pathologic materials and Marilyn Gropper and Jodi Aasmundrud for assistance in preparing the manuscript.

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