Isolation of *Borrelia* Spirochetes from Patients in Texas

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The Texas Department of Health Laboratory began culturing the Lyme disease spirochete *Borrelia burgdorferi* in 1985. This organism was subsequently isolated from blood, cerebrospinal fluid, joint fluid, skin, bone, and autopsy tissues from humans. Fluorescent-antibody tests with murine monoclonal antibodies confirmed that seven of these isolates were *B. burgdorferi* and that two others belonged to the genus *Borrelia*.

Lyme disease, first recognized in 1975, is a tick-borne infection that can result in long-term rheumatologic or neurologic disorders. A spirochete isolated from *Ixodes dammini* in 1982 was later found to be the etiologic agent of the illness (6, 16). Since then, this bacterium, called *Borrelia burgdorferi*, has been detected in tissue specimens from patients who were diagnosed clinically as having Lyme disease (4, 5, 8, 9, 16). These specimens include blood, cerebrospinal fluid (CSF), joint fluids, and skin tissues.

At the Texas Department of Health, we began culturing for *B. burgdorferi* during the spring of 1985. Blood, CSF, joint fluids, bone scrapings, autopsy tissues, and skin tissues from patients whose physicians suspected Lyme disease were submitted to our laboratory. These specimens were placed in modified BSK II medium in an attempt to isolate *B. burgdorferi* (1). Our findings are reported here.

**MATERIALS AND METHODS**

Specimens were collected by physicians and shipped overnight to the Texas Department of Health Laboratory in Austin. Blood, CSF, and joint fluids were sent either on wet or dry ice; skin and autopsy tissues were shipped in approximately 1 ml of either brain heart infusion or thioglycolate medium; and bone scrapings (from the right wrist of the patient) were placed directly into BSK II medium by the physician.

On receipt, unprocessed specimens were placed in 3 ml of modified BSK II medium containing glutamine and 6% normal rabbit serum. Unlike the BSK II previously described, CMRL with glutamine was used in our medium (1a). An additional 1 ml of fresh 3% L-glutamine per 100 ml of BSK II was added every 2 weeks after the medium had been stored for 1 month. All specimens were incubated at 34°C, and samples of each culture were examined for spirochetes by dark-field microscopy after 14 to 21 days.

A 20-week-old meadow vole (*Microtus pennsylvanicus*) was inoculated intraperitoneally with 0.5 ml of concentrated *B. burgdorferi* (pellet and washed) that had been cultured from the skin lesions of one patient. A second vole was inoculated intraperitoneally with 0.5 ml of BSK II medium and served as a negative control. The vole that was injected with spirochetes was found dead 7 days postinoculation. Samples of its heart, spleen, liver, lung, and kidney were examined directly by dark-field microscopy and Giemsa staining. In addition, a portion of each tissue was placed in 3 ml of BSK II medium and incubated at 34°C for 14 days.

When spirochetes were observed, a confirmatory indirect fluorescent-antibody test was performed. For this test undiluted murine monoclonal antibodies H9724 (which recognizes an antigenic determinant common to the genus *Borrelia*) and H5332 (which recognizes an antigenic site specific for *B. burgdorferi* species), kindly donated by Alan Barbour of the Rocky Mountain Laboratories, Hamilton, Mont., and fluorescein-conjugated goat anti-mouse immunoglobulins (1:100 dilution; lot no. 17543; Cappel Laboratories, Cochranville, Pa.) were used. A subculture of *B. burgdorferi* B31, supplied by the Centers for Disease Control, Atlanta, Ga., served as our positive control. Methods for the indirect fluorescent-monoclonal antibody procedure have been described elsewhere (3).

An indirect fluorescent-antibody test for detecting antibody in human serum was performed as described previously (13). *B. burgdorferi* antigens were either prepared at the Texas Department of Health Laboratory or provided by K. E. Hechemy of the New York State Health Department, Albany. Rabbit anti-human globulin was produced by Miles Laboratories, Inc., Naperville, Ill.

**RESULTS**

During this study, blood specimens from 100 patients were cultured for *B. burgdorferi*. We cultivated spirochetes from two (2%) specimens: one chilled whole blood and one frozen clot. A total of 31 skin specimens were placed in culture, resulting in 8 (26%) isolates, and spirochetes were observed in 1 of 7 (14%) cultured CSF specimens. Tissues from two autopsies (kidney, liver, spleen, lung, and lymph node) were submitted, and spirochetes were detected in the liver and spleen of one patient. Spirochetes were isolated from one of three (33%) joint fluids (knee) and from the one bone specimen that was submitted (wrist).

Altogether, spirochetes were cultured from 14 patients. Positive reactions with monoclonal antibody H5332 confirmed that seven of these isolates were *B. burgdorferi*. Two additional isolates (from CSF and skin) reacted with H9724. This suggests that they were *Borrelia* spirochetes, but because they did not bind with antibody H5332, the species was not determined. The 14 specimens from which isolates were obtained were submitted by physicians from six counties in eastern Texas.

A dark-field examination of organs from the inoculated vole resulted in the detection of spirochetes in the kidney. Cultured tissues from this vole were also positive for spirochetes when examined by dark-field microscopy. No spirochetes were detected in the negative control.

Serum specimens were collected from each of the 14 patients from whom *Borrelia* spirochetes were recovered.

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Half of the samples contained antibody to *B. burgdorferi*, with titers ranging from 1:64 to 1:256 (Table 1). Serum samples collected from seven patients had no detectable antibody.

### DISCUSSION

Although serologic tests for the detection of antibodies to *B. burgdorferi* are available, they may prove unreliable when trying to confirm a clinical diagnosis of Lyme disease (10, 12, 13, 15). Currently used procedures have a less than optimal sensitivity for detecting antibody in specimens that have been collected during acute stages of infection. It appears that immunologic responses may be curtailed if antibiotic therapy is administered early in the course of the illness. Also, false-positive reactions have been reported with the sera of patients who have treponemal infections. Therefore, an additional form of laboratory diagnosis may include the isolation of Lyme disease spirochetes from patient tissues.

Since they are relatively easy to obtain, the majority of the samples cultured by this laboratory were blood samples. Our low percentage (2%) of recovery was consistent with values obtained previously (4, 16). There are several possible explanations. Samples may not have been collected early during the bacteremic phase of the disease process; there may have been interference if antibiotic therapy had already been initiated; or conditions of transport may have been less than desirable.

In contrast, 26% of the skin specimens were culture positive. These included fluids from vesicular or ulcerating skin lesions and scrapings or punch biopsies from unexplained rashes. Each of these samples was transported in approximately 1 ml of either brain heart infusion or thioglycolate medium in an attempt to keep any organs viable during shipment. These media were chosen since each is commonly found in clinical laboratories.

When placed in our BSK II medium, *B. burgdorferi* appeared to multiply readily. Other researchers have also been successful in detecting spirochetes in the skin of patients with Lyme disease (5). We suggest that skin tissues are more desirable than blood for isolating *B. burgdorferi*.

The isolate from an ulcerating skin lesion was further examined by investigators in another laboratory (although it was mistakenly identified as an isolate from human blood in the subsequent report [2]). Results of their polyacrylamide gel electrophoresis studies showed that the major proteins of our isolate were homogenous with those of other North American isolates. Ulcerating skin lesions are not a common characteristic of Lyme disease. The isolation of *B. burgdorferi* from these lesions was important for a definitive diagnosis.

*B. burgdorferi* isolated from skin lesions of one patient were inoculated into a meadow vole for two reasons: first, to see if they would multiply in the vole, and second, to attempt to purify the original culture, which was heavily contaminated. It is not known whether the vole died as a result of the spirochetal infection or due to an overwhelming infection caused by one or more of the contaminants, since cultures of the animal tissues were also grossly contaminated.

Although there have been few deaths attributed solely to Lyme disease, there has been a report of an infant death due to transplacental transmission of *B. burgdorferi* and a report of a patient who expired when concurrently infected with *B. burgdorferi* and a *Babesia* species (11, 14). Although the Lyme disease spirochete was isolated from autopsy tissues obtained from one patient in Texas, it was speculated (but not confirmed) that this patient was infected by additional pathogenic organisms. If so, these could have contributed to the death of the patient.

Results of serologic studies with monoclonal antibodies showed that the organism isolated from CSF was a member of the genus *Borrelia*. The species could not be identified because the organism never adapted to the medium and died off. It is possible this organism was, indeed, *B. burgdorferi* but that species-specific antigens were lost during long-term passage. On the other hand, this isolate could have been *Borrelia turicata*, the chief cause of tick-borne relapsing fever in Texas (7, 17). Monoclonal antibody H9724 is not able to distinguish between *Borrelia* species (3). Spirochetes from the joint of patients also did not adapt well to BSK II medium, even after 2 to 3 months of passage.

Laboratory culture of spirochetes is a slow and tedious process. Certainly, serologic procedures can be performed more quickly and efficiently. Because of sensitivity and cross-reactivity problems with the tests that are currently available, however, alternate methods may be necessary for a definitive diagnosis in some cases. Only 3 (21%) patients of the 14 studied had immunofluorescence antibody titers of ≥1:256, the cutoff designated as diagnostic by personnel at the Centers for Disease Control (13). Four patients had borderline titers (1:64 or 1:128), and seven (50%) had no detectable antibody to the Lyme disease spirochete. For these cases, isolation and identification of *B. burgdorferi* strengthened the clinical diagnosis of Lyme disease.

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### LITERATURE CITED


disease spirochetes and Ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. 41:795–804.