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Identification of the Tick-Borne Relapsing Fever Spirochete *Borrelia hermsii* by Using a Species-Specific Monoclonal Antibody

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Borrelia hermsii **causes a relapsing fever in humans and is one of several species of tick-borne spirochetes known to occur in the western United States. Spirochetes observed in the peripheral blood of patients acutely ill have been presumptively identified in the past by the geographic location of exposure and the probable species of tick vector. We describe a monoclonal antibody (H9826) that bound to the flagellar protein of *B. hermsii* but not to those of any of the other species tested, which included *B. parkeri*, *B. turicatae*, *B. coriaceae*, *B. anserina*, *B. burgdorferi*, and *Leptospira interrogans* serovar ballum. This antibody bound efficiently to *B. hermsii* in an indirect immunofluorescence assay and was used to rapidly detect and identify this spirochete in the peripheral blood of experimentally infected mice and in the central ganglia of *Ornithodoros hermsi* ticks. H9826 can rapidly confirm the identification of *B. hermsii* to increase our understanding concerning the geographic distribution, vector specificity, and epidemiological significance of this zoonotic human pathogen.**

Tick-borne relapsing fever in the United States was first reported in Colorado in 1915 (30), and since then numerous endemic foci have been identified throughout the western states, with a small number of human cases being recognized each year (11, 15). In California, which is the only state that currently requires that cases of this disease be reported, tick-borne relapsing fever was first recognized in 1921 (12), and in recent years an average of 10 cases per year have been reported in this state (15). The disease is thought to be caused by two or three species of closely related spirochetes, including *Borrelia hermsii*, *B. turicatae*, and possibly *B. parkeri* (4, 15). Historically, the identification of these bacteria has been based on the species of soft tick in the genus *Ornithodoros* that transmits and possibly serves as a reservoir for the spirochetes in nature (18). Therefore, *B. hermsii*, *B. turicatae*, and *B. parkeri* are found in and are transmitted by the ticks *Ornithodoros hermsi*, *O. turicata*, and *O. parkeri*, respectively, and these vector-pathogen associations are thought to be specific (18). Since the development of an artificial medium for cultivating *Borrelia* species in vitro (1, 24) and newer techniques for characterizing the bacteria at the molecular and genetic levels, little effort has been made to clarify and confirm the distribution of the various species of relapsing fever spirochetes in nature. While some workers have recently questioned the specific status of *B. hermsii*, *B. turicatae*, and *B. parkeri* on the basis of DNA-DNA hybridization studies (22), these spirochetes are still considered to be valid and separate species.

In the past, the identification of spirochetes detected in blood smears from human cases of relapsing fever was based on the probable geographic location of exposure and the suspected tick vector (9, 11). Such a presumptive identification may not accurately identify the infecting organisms and is complicated by the presence in the wild of a diversity of other species of ticks and spirochetes. In California, for example, the relapsing fever ticks *O. hermsi*, *O. turicata*, and *O. parkeri* all occur, as does *O. coriaceus* (21), from which another spirochete, *B. coriaceae*, has been isolated (23, 28). Complicating the epidemiological picture further is the wide distribution of the hard tick *Ixodes pacificus* throughout much of California (21); this tick harbors the Lyme disease spirochete, *B. burgdorferi* (14). Although human cases of Lyme disease have been reported in most of the western United States (16), such reports are based on varied clinical manifestations without isolation or detection of spirochetes and serological tests lacking specificity (16, 36). Monoclonal antibodies that differentiate the genus *Borrelia* from other genera of pathogenic spirochetes (5) and that also specifically differentiate *B. burgdorferi* (7) and *B. coriaceae* (10) from other members of the genus have been developed. Monoclonal antibodies to serotype-specific variable outer surface proteins of *B. hermsii* have also been developed (2), but these antibodies do not identify other serotypes or isolates of this species. Given the uncertainty regarding which *Borrelia* species cause relapsing fever and the intriguing speculation that a chronic form of relapsing fever that mimics chronic forms of Lyme disease may exist (29), diagnostic techniques are required to help delineate the epidemiological significance of the various *Borrelia* species.

Monoclonal antibody H9826 was originally shown to bind to the pII (invariable) protein of serotypes C, 7, and 21 of *B. hermsii* HS1 (2). In the present report, we demonstrate that this monoclonal antibody binds to the flagellar proteins of various strains of *B. hermsii* but not to those of the other members of the genus *Borrelia* tested. H9826 allows for the rapid detection and identification of *B. hermsii* in blood smears and ticks to clarify the distribution and epidemiological significance of this zoonotic tick-borne pathogen.

MATERIALS AND METHODS

Borrelia **strains and cultivation.** *B. hermsii* HS1 (ATCC 35209) serotype C originated from *O. hermsi* collected near

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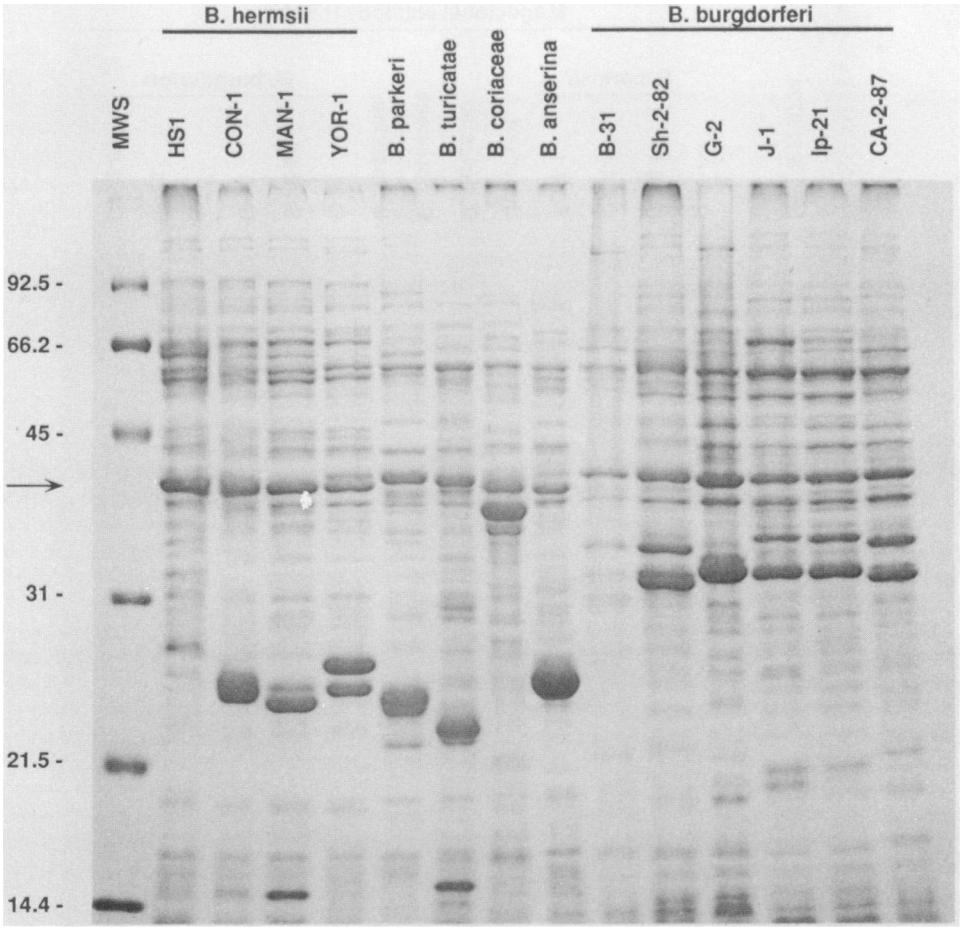

FIG. 1. SDS-PAGE of *Borrelia* whole-cell lysates used for Western blot analysis with monoclonal antibody H9826. The 12.5% gel was stained with Coomassie brilliant blue, and molecular weight standards (MWS) from Bio-Rad are given on the left in kilodaltons. The arrow on the left indicates the predominant flagellar protein that varies in apparent molecular mass from 38 to 41 kDa among the spirochetes examined and with which the antibody reacts.

Spokane, Wash. (38). *B. hermsii* CON-1, MAN-1, and YOR-1 were isolated from the blood of humans with relapsing fever in California (26). *B. coriaceae* CO53 (ATCC 43381) was isolated from *O. coriaceus* collected in California (23, 28). *B. parkeri*, *B. turicatae*, and *B. anserina* were isolated from *O. parkeri*, *O. turicata*, and a domestic chicken, respectively, and were part of the Rocky Mountain Laboratories bacterial pathogen collection. Strains of *B. burgdorferi* included B-31 (ATCC 35210) and Sh-2-82, both isolated from *I. dammini* collected on Shelter Island, N.Y. (13, 34); J-1 and Ip-21, which originated from *I. persulcatus* collected in Japan and the Soviet Union, respectively (25, 32); CA-2-87, which was isolated from *I. pacificus* collected in California (34); and G-2, which was isolated from human cerebrospinal fluid in Germany (37). *Leptospira interrogans* serovar ballum was obtained as a pellet of frozen cells from D. Denee Thomas, The Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N.C.

Live borrelial cultures were maintained in BSK-II medium (1) at 34°C and passaged twice a week.

Monoclonal antibody and isotype. Antibody H9826 was the product of a fusion of a BALB/c mouse spleen with NS1 myeloma cells as described previously (8). In brief, on day 1 the mouse was infected with live cells of *B. hermsii* serotype 7. Beginning on day 4 the mouse was given tetracycline in water for 14 days. On day 21 the mouse was boosted intravenously with live serotype 7 cells. The spleen was harvested on day 24 for fusion and subsequent cloning on semisolid medium as described previously (20).

The isotype of monoclonal antibody H9826 was determined by Western blot (immunoblot) analysis with whole-cell lysates of *B. hermsii* serotype C and rabbit anti-mouse immunoglobulin M (IgM), IgG1, IgG2A, and IgG2B (Cappel-Organon Teknika Corp., West Chester, Pa.) and by ^{125}I-protein A radiography.

SDS-PAGE and Western blot analysis. Whole-cell lysates of borrelia cultures were prepared as described previously (35). The Laemmli buffer system (27) was used with a vertical gel electrophoresis system (Bethesda Research Laboratories-GIBCO, Gaithersburg, Md.) or a Mini-Protean II dual slab cell (Bio-Rad Laboratories, Richmond, Calif.) in accordance with the instructions of the manufacturers. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), lysates were transferred to nitrocellulose in either a Trans-Blot or a Mini Trans-Blot electrophoresis transfer cell (Bio-Rad) in accordance with the instructions of the manufacturer. The larger gel and Trans-Blot system were used to test borrelial cell lysates with undiluted supernatant

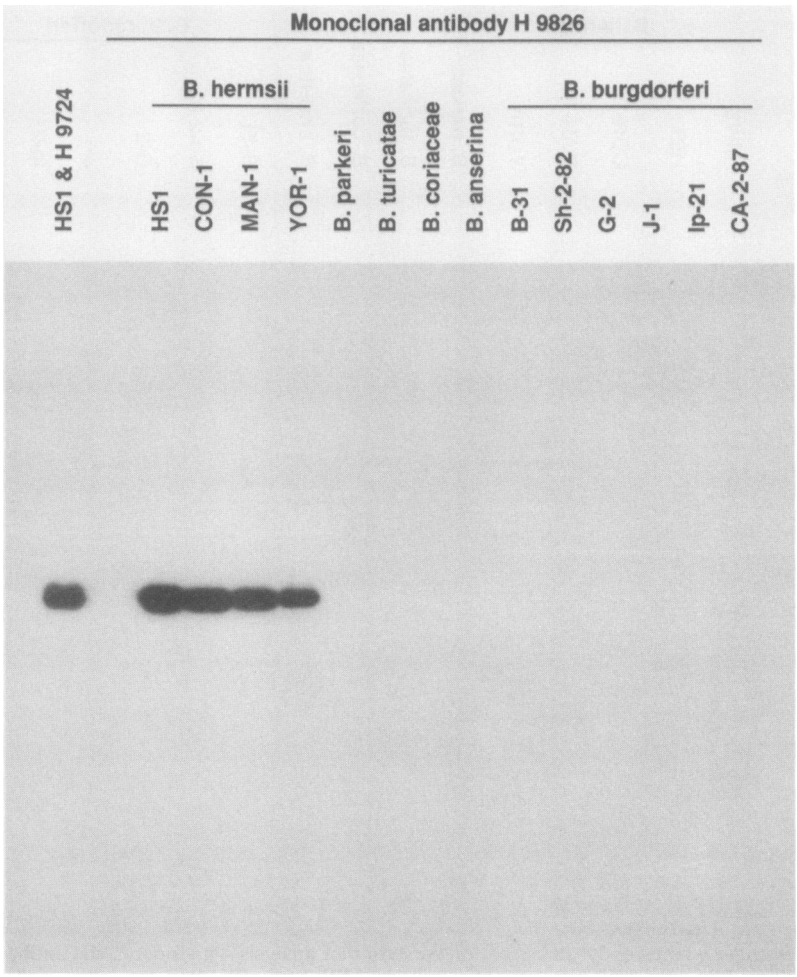

FIG. 2. Western blot analysis of monoclonal antibody H9826 for reactivity with the flagellar proteins of the various *Borrelia* species and strains shown in Fig. 1. At the left is the genus-specific monoclonal antibody (H9724) reacted with the flagellar protein of *B. hermsii* HS1. Bound antibody was detected by ^{125}I-protein A radiography.

of H9826. The *Borrelia* genus-specific monoclonal antibody H9724 (5) was used at a dilution of 1:50. The smaller gel and Mini Trans-Blot system were used to determine the isotype of H9826 with different specific rabbit anti-mouse immunoglobulin sera diluted 1:50. Membranes were prepared and examined by ^{125}I-protein A radiography as described previously (35).

Immune electron microscopy. Whole cells of *B. hermsii* HS1, *B. hermsii* YOR-1, and *B. burgdorferi* B-31 were removed from BSK-II medium by centrifugation and resuspended in phosphate-buffered saline–5 mM $MgCl_2$. The cells were centrifuged, washed three times, disrupted by vortexing of the pellets, and adsorbed onto electron microscope grids. Monoclonal antibody H9826 was labeled with protein A-colloidal gold and used as described previously (5, 6).

IFA. Undiluted tissue culture supernatant containing monoclonal antibody H9826 was tested to determine its reactivity with spirochetes in blood smears from mice experimentally infected with *B. hermsii* YOR-1, central ganglia from *O. hermsi* ticks infected with *B. hermsii* YOR-1, slides of fixed cells of *B. hermsii* HS1 serotype C, slides of fixed cells of *B. burgdorferi* B-31, and midgut tissues from *I. dammini* ticks infected with *B. burgdorferi* JD-1 (37) by an indirect immunofluorescence assay (IFA) as described previously (13). *B. hermsii* cells fluorescing under UV irradiation were photographed with a Zeiss fluorescence microscope at ×400 with Kodak Tri-X Pan film (ASA 400) and a 75-s exposure.

RESULTS

Four strains of *B. hermsii*, six strains of *B. burgdorferi*, one strain of *B. coriaceae*, and one undesignated strain each of *B. parkeri*, *B. turicatae* and *B. anserina* were first examined by SDS-PAGE and Coomassie brilliant blue staining (Fig. 1) (*L. interrogans* was also examined but is not shown). All the *Borrelia* lysates had numerous polypeptides, including a dominant band representing the flagellar protein, ranging in apparent size from 38 kDa in *B. anserina* to 41 kDa in *B. burgdorferi* (arrow in Fig. 1). This result was confirmed by reactivity with monoclonal antibody H9724 (data not shown), which was shown previously to bind to the flagellar proteins of members of the genus *Borrelia* but not to those of other genera of pathogenic spirochetes (5). When identical amounts of each lysate were separated by SDS-PAGE and transferred to nitrocellulose by Western blotting, only the four strains of *B. hermsii* bound monoclonal antibody H9826 (Fig. 2) (*L. interrogans* is not shown). Mono-

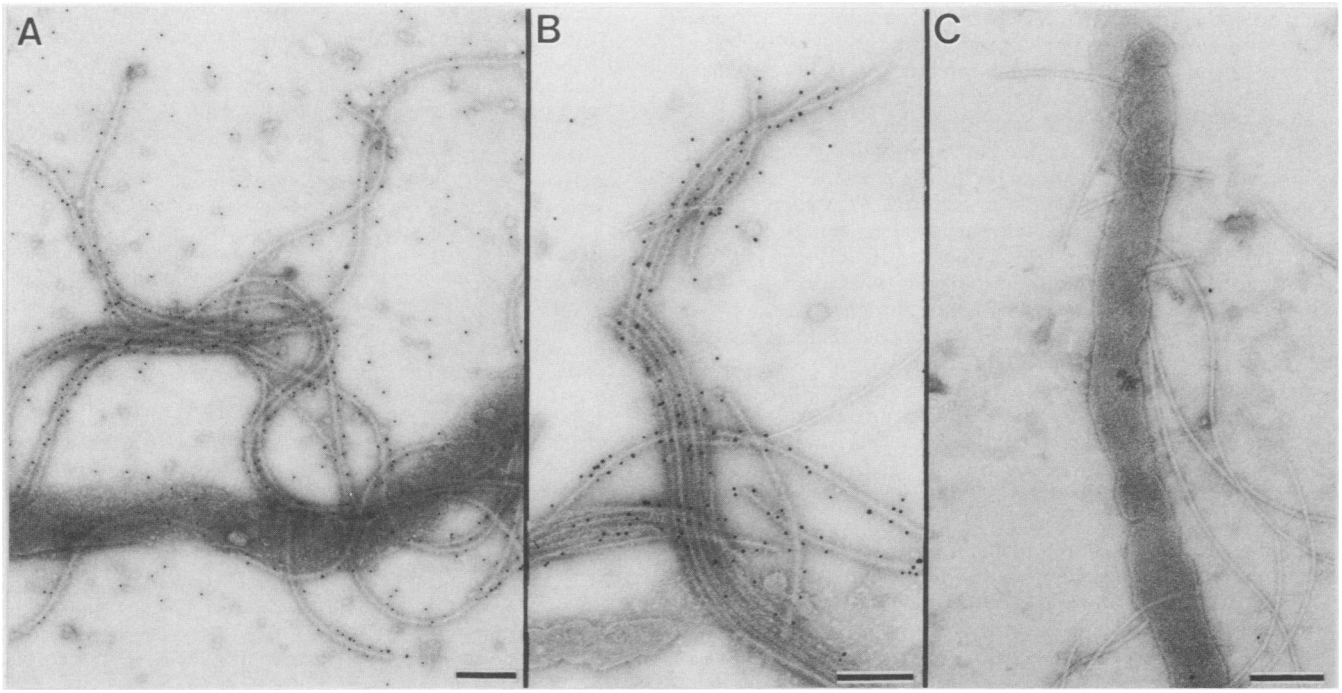

FIG. 3. Binding of monoclonal antibody H9826 and protein A-colloidal gold to the flagellar protein (axial filaments) of *B. hermsii*. (A) Disrupted cells and axial filaments of *B. hermsii*. (B) Free axial filaments of *B. hermsii*. (C) Disrupted cell and axial filaments of *B. burgdorferi*. The stain was 0.5% ammonium molybdate. Bars, 100 nm.

clonal antibodies H9826 and H9724 bound to antigens in *B. hermsii* HS1 with the same apparent migration (two left lanes in Fig. 2), suggesting that H9826 also binds to the flagellar protein. This result was confirmed by immune electron microscopy, when the antibody-colloidal gold complex bound to the axial filaments emerging from lysed cells of *B. hermsii* but not *B. burgdorferi* (Fig. 3).

IFA staining demonstrated that H9826 bound efficiently to whole cells of *B. hermsii* HS1 serotype C (Fig. 4) but not *B. burgdorferi* B-31 when cultured spirochetes were fixed on glass slides. The antibody also bound to *B. hermsii* YOR-1 in blood smears from two experimentally infected BALB/c mice and central ganglia from two infected *O. hermsi* ticks. *B. burgdorferi* JD-1 in midgut tissues from five *I. dammini* nymphs bound monoclonal antibody H9724 but not H9826 (data not shown).

FIG. 4. IFA staining of *B. hermsii* HS1 with monoclonal antibody H9826. Magnification, ×1,500.

Isotyping with the specific rabbit anti-mouse immunoglobulin sera and Western blot analysis showed monoclonal antibody H9826 to be IgG2A.

DISCUSSION

Spirochetes in humans acutely ill with relapsing fever are often easily detected by light microscopy by examination of thick drops and thin smears of peripheral blood stained with Giemsa stain (9). Fresh wet drops of blood can also be examined by dark-field microscopy and, when live spirochetes are abundant in such a preparation, the field of view is quite spectacular. For many years, animal inoculation has also been useful to demonstrate spirochetes in human blood when the level of spirochetemia is below that detectable by microscopy (9). More recently, a liquid medium was developed and improved to successfully isolate and culture many *Borrelia* species in vitro (1, 24), although attempts to culture spirochetes from relapsing fever patients have not yet been made in most clinical laboratories. None of these methods, however, identifies the spirochetes, and this drawback has posed a difficulty in advancing our understanding of the epidemiological significance of the various *Borrelia* species. Certainly, the inability to culture *Borrelia* species until the 1970s was a major factor. Also, the primary means of identification, namely, determining the specificity of the different spirochetes for certain species of *Ornithodoros* ticks, is not possible for clinical laboratories. Even though it can be done in research laboratories, xenodiagnosis to identify relapsing fever spirochetes (19) is not practical. For

such an endeavor, uninfected laboratory colonies of various species of *Ornithodoros* ticks are required to allow them to first feed on mice experimentally infected with the spirochetemic blood from a relapsing fever patient. These ticks are then kept for several weeks until they molt to the next stage, when they are allowed to feed on uninfected mice. Daily examinations of the peripheral blood of these mice are made to determine which ticks have transmitted spirochetes. The identification of the spirochetes originally seen in the patient's blood is based on which species of tick transmitted them to mice (19). Host susceptibility has also been used to determine whether the relapsing fever spirochetes are of the louse-borne or tick-borne type (17), but this method is also impractical and is of uncertain value for distinguishing species of tick-borne spirochetes. Given such problems, it is understandable that most clinical laboratories have based the identification of relapsing fever spirochetes in a patient's blood on the probable tick vector.

The ability now to cultivate large numbers of spirochetes in vitro, free of other bacterial contaminants, has provided the opportunity to purify and analyze molecular and genetic determinants that may be useful in their identification. One DNA hybridization probe that specifically identified *B. hermsii* has been described; however, a minimum of approximately 2,800 spirochetes were required for detection and identification in a dot blot assay (37). More recently, the polymerase chain reaction has been used (31, 32). In one study, *B. hermsii*, *B. parkeri*, and *B. turicatae* were differentiated from other *Borrelia* species but not from each other (32). When sequences unique to the internal variable region of the flagellin gene (*fla*) were used for polymerase chain reaction primers, *B. hermsii* was distinguished from the other species of North American tick-borne relapsing fever spirochetes (31). As mentioned earlier, DNA-DNA hybridization studies have also been used to examine the genetic relatedness of some *Borrelia* species (22). However, the complex nature of the *Borrelia* genome, with numerous sizes of both linear and supercoiled plasmids (3, 33), and their potential loss with in vitro cultivation, as has been described for *B. burgdorferi* (3, 33), require that plasmid-free chromosomal preparations be available for such analyses. Once relapsing fever spirochetes are detected, staining with a species-specific monoclonal antibody in an IFA is by far the most practical method for differentiating *B. hermsii* from other tick-borne spirochetes. We have described a monoclonal antibody that accomplishes this differentiation and binds to an epitope unique to the flagellin of *B. hermsii*. Whether this epitope is a primary structure of the protein encoded by a recently reported (31) unique DNA sequence of the *B. hermsii fla* gene or a conformational epitope is not known.

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