JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1987, p. 2296–2301
0095-1137/87/122296-06$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 25, No. 12

Characterization of *Borrelia burgdorferi* Strains Isolated from *Ixodes pacificus* Ticks in California

MARJORIE L. BISSETT* AND WARREN HILL

Microbial Diseases Laboratory, California Department of Health Services, Berkeley, California 94704

Received 8 June 1987/Accepted 18 August 1987

In a survey of 1,714 adult *Ixodes pacificus* ticks collected in northern California, 24 (1.4%) were found to be infected with spirochetes that reacted with an anti-*Borrelia burgdorferi* polyvalent conjugate in direct immunofluorescence tests. Eleven isolates of *B. burgdorferi* from these ticks were characterized by monoclonal antibody, polyacrylamide gel electrophoresis, and Western blot (immunoblot) analyses. Ten of the isolates had molecular and antigenic characteristics similar to those of other U.S. isolates. One strain, cloned by limiting-dilution techniques, was different from any previously reported U.S. strain, but similar to reported European strains. The cloned strain, DN127-Cl9-2, did not react with monoclonal antibodies to Osp A and Osp B major proteins found in most of the U.S. strains. It exhibited an abundant protein with an apparent molecular weight of 25,000.

Lyme disease is a vector-borne disease caused by a spirochete, *Borrelia burgdorferi*, which is transmitted by the bite of an infected ixodid tick (11, 13, 20, 21). The disease is characterized most frequently by an initial distinctive skin lesion known as erythema migrans or erythema chronicum migrans depending upon the length of time that the lesion persists. The lesion(s) may be followed weeks to months later by neurologic or cardiac manifestations. Arthritis may occur months to years later. In the United States, the disease occurs in the Northeast, the Midwest, and the West, but most frequently in the northeastern coastal states. In some areas of the Northeast, 80 to 100% of the *Ixodes dammini* ticks are infected (10, 14). The disease also occurs in other parts of the United States, Europe, and Australia (18). The primary vector in Europe is *Ixodes ricinus*.

The clinical manifestations of Lyme disease in North America, although similar in general to those seen in Europe, differ in some respects, particularly in a more frequent association of arthritis with the disease (1, 16, 18, 19). The first case of erythema chronicum migrans acquired in California was described in 1978 by Naversen and Gardner (17). The tick vector was an adult western black-legged tick (*Ixodes pacificus*). Burgdorfer et al. (12) performed a tick-spirochete survey of adult *I. pacificus* collected in southwestern Oregon and northern California, and they found that 1.1% of the ticks collected in California were infected with the spirochete. Spirochetes isolated from one of the infected ticks were indistinguishable from the type strain of *B. burgdorferi*.

Barbour et al. (5) have characterized human, animal, and tick strains of *B. burgdorferi* from the United States and Europe. Three major proteins were described and studied by polyacrylamide gel electrophoresis (PAGE), monoclonal antibody, and Western blot (immunoblot) analyses. A major protein of the outer surface of the spirochete with an apparent molecular weight (M_r) of 31,000 (31K) reacted with a monoclonal antibody, H5332, and was designated Osp A (5). Another major protein of the outer surface, Osp B, varied with different strains in its M_r and in its reaction to two other monoclonal antibodies. A protein with an M_r of 41,000 was found to be a major constituent of the periplasmic flagella of the spirochete (4). A monoclonal antibody (H9724) to this protein was specific for the genus *Borrelia*. Barbour and his colleagues found that most of the isolates from Europe differed from U.S. isolates in their Osp A proteins (5).

Our laboratory, in cooperation with the California State Vector Surveillance and Control Branch, has been involved in a continuing survey of spirochetal infection of adult *I. pacificus* ticks collected primarily from areas surrounding reported California cases of Lyme disease. During this study, we isolated 11 strains of *B. burgdorferi*; the characterization of these strains, including a strain with properties different from those of other U.S. isolates, is the subject of this report.

MATERIALS AND METHODS

Tick examinations. Vector Surveillance and Control Branch staff of the California Department of Health Services collected and submitted 1,714 *I. pacificus* ticks from 12 northern California counties from December 1984 to May 1985 and from October 1985 to May 1986. Collections were usually made in areas surrounding locales where human cases of Lyme disease had occurred. In the laboratory, the ticks were dipped in alcohol and dissected in BSK II medium (2) by the method of Burgdorfer et al. (12). Portions of the midgut were examined by dark-field microscopy and direct immunofluorescent-antibody (DFA) tests. The DFA test was performed by using a fluorescein isothiocyanate-conjugated polyclonal rabbit antiserum prepared against *B. burgdorferi* B31 (3, 7).

Culture procedures. The remaining portions of the midguts of ticks with spirochetes, as demonstrated by dark-field microscopy and DFA tests, were triturated in a small tissue grinder with 0.5 ml of BSK II medium. The resulting suspension was transferred to a tube containing 11 ml of BSK II. A 1-ml portion of BSK II was used to rinse the tissue grinder, and this rinse was also added to the undiluted tube. Tenfold dilutions with and without 50 μl of rifampin per ml were made from the undiluted suspension in tubes containing 9 ml of BSK II. Inoculated tubes were incubated

* Corresponding author.

at 35°C for a minimum of 4 weeks. The tubes were examined for the presence of spirochetes by dark-field microscopy three to four times during the first 10 days of incubation and weekly thereafter. When spirochetes were observed in the culture at a density of approximately one spirochete per 40× high dry field, 1 ml of the culture was transferred to 9 ml of BSK II and incubated at 35°C. When this transfer culture exhibited 1 to 10 actively motile spirochetes per high dry field, the culture was frozen (8) and stored at −70°C.

B. burgdorferi B31 (ATCC 35210) and *Borrelia hermsii* HS1 (ATCC 35209) (kindly provided by Alan Barbour) were used as control strains in the study.

Monoclonal antibodies. The following monoclonal antibodies were supplied to us as hybridoma supernatants through the generosity of Alan Barbour: H5332 and H3TS (monoclonal antibodies to different epitopes of the Osp A protein) (5, 8), H6831 and H5TS (monoclonal antibodies to Osp B proteins) (6), H9724 (genus-specific monoclonal antibody to periplasmic *Borrelia* flagella) (4), and H4825 (monoclonal antibody specific for *B. hermsii* outer membrane protein) (6).

IFA test. Borrelia suspensions for indirect immunofluorescent-antibody (IFA) tests were prepared by centrifuging 3- to 4-day-old cultures of the spirochetes at 2,000 × *g* for 15 min in a GLC-2B centrifuge (Beckman Instruments, Inc., Fullerton, Calif.), discarding the supernatant, and washing the sediment one time in M/15 phosphate-buffered saline (PBS) with 5 mM magnesium chloride (8). The sediment was again suspended in PBS-MgCl. After adjustment to approximately 100 organisms per high dry field, 20 μl of the suspension was added to each well of an eight-well microscope slide (Cel-line Associates, Inc., Newfield, N.J.). The excess fluid was removed from each well with a Pasteur pipette, and the slides were allowed to air dry. The slides were fixed for 10 min in 10% methanol, air dried, and stored frozen at −70°C until use.

IFA tests were performed by adding 15 μl of antibody (undiluted hybridoma supernatant) to each well and incubating the slides for 30 min at 35°C. After the slides were washed for 10 min in PBS, 20 μl of fluorescein-conjugated goat anti-mouse immunoglobulin (Antibodies, Inc., Davis, Calif.) was added to each well at a dilution previously determined to be optimum by titration (usually 1:200). Slides were incubated for 30 min at 35°C and then washed in PBS as described above. Slides with cover slips mounted were then examined for fluorescence under a Zeiss epifluorescence microscope using a 40× high-dry-objective lens. Fluorescence of spirochetes was read on a scale of 1+ to 4+, with 2 to 4+ fluorescence considered positive.

SDS-PAGE. Frozen suspensions of in vitro-cultivated spirochetes were rapidly thawed, centrifuged, and washed twice in PBS-MgCl (7, 8). The organisms were suspended in glass-distilled water, and the protein concentration was determined by the Bradford method (9). The suspension was diluted to a concentration of 1.28 mg of protein per ml with glass-distilled water so that a mixture of two parts of the suspension with one part of the sample buffer would provide a final protein concentration of 0.85 mg/ml. The sodium dodecyl sulfate (SDS)-PAGE was performed essentially by the method of Barbour et al. (7). In preparing the sample buffer, DL-dithiothreitol (50 mM final concentration) was used as the reducing agent instead of 2-mercaptoethanol. The pH of the separating gel buffer was 8.6. The concentration of acrylamide in the separating gel was 12.5%. Gels were stained with Coomassie brilliant blue R-250. Molecular weight standards were run with each gel (low-molecular-weight protein standard; Bio-Rad Laboratories, Richmond, Calif.; or prestained protein low-molecular-weight standards; Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

Western blots. Proteins were transferred from the gels to nitrocellulose paper in a Transphor cell (Hoefer Scientific Instruments, San Francisco, Calif.) containing buffer prepared by the method of Towbin et al. (22). Electrophoresis was carried out at 20°C at 100 V for 3 h. After transfer, the protein antigen-monoclonal antibody tests were performed, according to the manufacturer's instructions, using the Vectastain immunodetection ABC Kit (Vector Laboratories, Burlingame, Calif.). Briefly, the blot was first immersed in Tween-PBS (TPBS) and incubated for 30 min at room temperature. The blot was then transferred to a 1:50 or 1:100 dilution of hybridoma supernatant prepared in TPBS and incubated at room temperature for 60 min. The dilution of hybridoma antibody supernatants used depended on the results of antigen-antibody titration on blots (data not shown). After a suitable washing with TPBS, the blots were immersed in a 1:200 dilution of biotinylated anti-mouse immunoglobulin G (heavy and light chain specific; Vector Laboratories, Burlingame, Calif.) and incubated at room temperature for 30 min. The blots were washed with TPBS, then transferred to Vectastain ABC reagent for a 30-min incubation at room temperature. After being washed with PBS, the blot was transferred to a peroxidase substrate solution, and color was allowed to develop for 15 min. Color development was stopped with two washes of distilled water, and the blots were dried. All incubation periods were accompanied by gentle agitation.

RESULTS

Tick examinations. A total of 1,714 adult ticks were examined during the 1984 to 1985 and 1985 to 1986 tick seasons. Spirochetes were found by dark-field microscopy and DFA tests in 24 (1.4%) of these ticks. The number of ticks examined and the number found positive for spirochetes (arranged by county of collection) are given in Table 1.

Isolation of spirochetes. Attempts to isolate and grow the spirochetes from the tick midguts were successful with 11 of the 24 positive ticks. Of the 11 cultures, 9 showed typical spirochetes within 2 to 5 days (average, 3.4 days); spirochetes were observed in the other two cultures after 7 to 9 days of incubation. Of the 13 positive ticks from which we were unable to culture the spirochetes, 2 showed 4+ fluorescence of the spirochetes in the DFA test, but there were

TABLE 1. Origin (county), number, and percent of ticks with spirochetal infection

County	No. of ticks examined	No. of ticks with spirochetes	% Positive[a]
Alameda	70	0	0
Del Norte	42	2	4.8
Humboldt	630	6	1.0
Lake	25	1	4.0
Mendocino	210	4	1.9
Monterey	34	0	0
Napa	167	2	1.2
Placer	15	0	0
San Benito	56	0	0
Santa Clara	54	1	1.9
Shasta	16	0	0
Sonoma	395	8	2.0

[a] Mean percent positive, 1.4%.

very few spirochetes present. The inability to successfully grow these spirochetes may have been due to their limited distribution in the tick tissue. In the other 11 specimens in which spirochetes were demonstrated but cultures were negative, the DFA tests showed reduced fluorescence of the spirochetes (2+). This reduced fluorescence was demonstrated by repeated DFA tests with specimens from these ticks. One of these eleven midgut specimens was also tested against an Osp A monoclonal antibody (H5332) in an IFA test. The spirochetes in the specimen showed a 4+ fluorescence with this antibody. We were unable to culture spirochetes from midgut material in every instance for which DFA test results showed the reduced fluorescence. The number of spirochetes present in wet mounts and DFA tests of these 11 specimens ranged from few (less than 10 per entire smear) to many (10 to 40 per high dry field).

IFA tests. The results of reactions of isolated strains subjected to IFA tests with monoclonal antibodies are shown in Table 2. All of the California strains with the exception of SON2110 and DN127 gave positive reactions with the Osp A (H5332 and H3TS), Osp B (H6831 and H5TS), and flagellar (H9724) monoclonal antibodies. All strains of *B. burgdorferi* were negative when tested against the monoclonal antibody H4825, which is specific for a protein of *B. hermsii*. The SON2110 strain did not react with the Osp B H6831 monoclonal antibody. The DN127 strain did not react with either of the Osp B monoclonal antibodies. The reaction of this strain with Osp A monoclonal antibodies H5332 and H3TS was atypical; only about 10% of the organisms bound these monoclonal antibodies. Limiting-dilution cloning of this strain was performed. The clone that was produced (DN127-Cl9-2) was negative with all of the monoclonal antibodies except the genus-specific H9724.

SDS-PAGE. The 11 isolates, the cloned strain (DN127-Cl9-2), and the control strains B31 and HS1 were subjected to SDS-PAGE (Fig. 1). The isolates, with the exception of DN127 and DN127-Cl9-2, had Osp A protein bands with relative mobilities (M_r) of 31,000. DN127 showed a major protein band with an M_r of 32,000; the derived clone, DN127-Cl9-2, did not have an apparent protein band in this region. Osp B proteins (with bands at 33,000) were present in most of the California isolates. SON188 and HUM115 each had an Osp B protein at 33,500. There was no apparent Osp B protein band present in DN127-Cl9-2. All of the isolates had another major protein band with an M_r of 40,000. DN127 and DN127-Cl9-2 were unique in having an abundant major protein with an M_r of 25,000. Other strains had less-abundant but still-significant protein bands in the 20,000- to 25,000-M_r region of the gel, particularly SON188, SON335, HUM7814, and MEN2523 (Fig. 1).

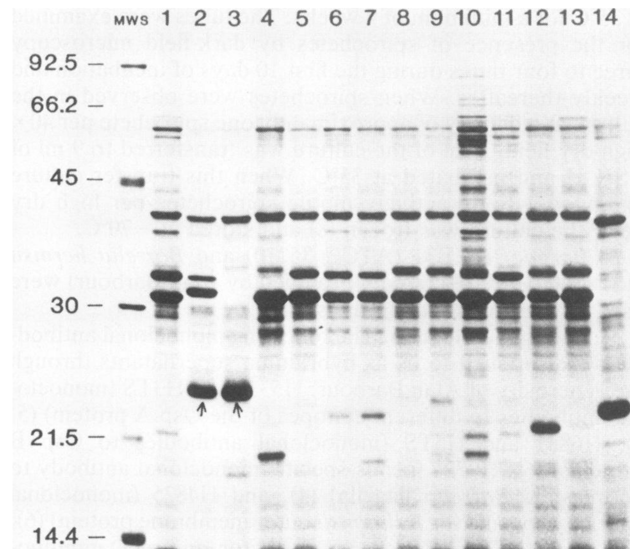

FIG. 1. Coomassie blue-stained SDS-PAGE of whole-cell lysates of *B. burgdorferi* isolates from ticks in California. The isolates and control strains are as follows. Lanes: 1, B-31; 2, DN127; 3, DN127-Cl9-2; 4, SON188; 5, SON2110; 6, SON328; 7, SON335; 8, HUM115; 9, HUM3336; 10, HUM7814; 11, MEN115; 12, MEN2523; 13, LAKE339; 14, HS-1. The M_r values (10^3) of the molecular weight standards (low-molecular-weight protein standard; Bio-Rad) are shown on the left. The arrows indicate the 25K major protein. Note that other strains, particularly SON188 (lane 4), SON335 (lane 7), HUM7814 (lane 10), and MEN2523 (lane 12), have significant protein bands in the 20K to 25K region.

TABLE 2. IFA reactivities of monoclonal antibodies against 12 strains of *B. burgdorferi* isolated from ticks in California

Strain	IFA reactivity[a] of:					
	Osp A		Osp B		Genus-specific	*B. hermsii*
	H5332	H3TS	H6831	H5TS	H9724	H4825
B31[b]	+	+	+	−	+	−
HS1[b]	−	−	−	−	+	+
DN127	+[c]	+[c]	−	−	+	−
DN127-Cl9-2	−	−	−	−	+	−
SON188	+	+	+	+	+	−
SON2110	+	+	−	+	+	−
SON328	+	+	+	+	+	−
SON335	+	+	+	+	+	−
HUM115	+	+	+	+	+	−
HUM3336	+	+	+	+	+	−
HUM7814	+	+	+	+	+	−
MEN115	+	+	+	+	+	−
MEN2523	+	+	+	+	+	−
LAKE339	+	+	+	+	+	−

[a] +, Positive (4+, 3+, and 2+ fluorescence); −, negative (±, 1+, and negative fluorescence).

[b] Control strains: B-31, type strain *B. burgdorferi*; HS-1, strain of *B. hermsii*.

[c] Only 10% of the organisms bound the antibody.

Western blots. Western blots of PAGE-separated proteins were performed with monoclonal antibodies (Table 3). The major protein with an apparent molecular weight of 40,000, which was present in all of our isolates, reacted only with the H9724 monoclonal antibody. It is therefore comparable to the major 41K protein described by Barbour et al. (4) and represents a protein of the periplasmic flagella of the spirochete.

The Osp A monoclonal antibodies (H5332 and H3TS) bound the 32K band of DN127 and the 31K bands of all other strains (Fig. 2). The DN127-Cl9-2 strain showed only a very faint band in the 32K region when tested with the Osp A antibody H5332 and showed no reaction with H3TS.

Tests with the Osp B monoclonal antibodies (H6831 and H5TS) showed that they reacted with the bands appearing in the 33K and 33.5K regions with all strains except SON2110, DN127, and DN127-Cl9-2 (Fig. 3). SON2110 reacted with Osp B monoclonal H5TS but showed no reaction with H6831. DN127 and DN127-Cl9-2 were negative with both Osp B monoclonal antibodies in the Western blot test, even though DN127 had an obvious 33K protein band in PAGE tests. None of the monoclonal antibodies reacted with the 25K major protein of DN127 or DN127-Cl9-2.

TABLE 3. Relative mobilities of protein bands of California isolates reacting with monoclonal antibodies in Western blot tests

Strain	Relative mobility (M_r, 10^3) of protein reacting to[a]:					
	Osp A		Osp B		Genus-specific	*B. hermsii*
	H5332	H3TS	H6831	H5TS	H9724	H4825
B31	31	31	33	33	40	–
HS1	–	–	–	–	38.5	24
DN127	32	32	–	–	40	–
DN127-C19-2	32*	–	–	–	40	–
SON188	31	31	33.5	33.5	40	–
SON2110	31	31	–	33	40	–
SON328	31	31	33	33	40	–
SON325	31	31	33	33	40	–
HUM115	31	31	33.5	33.5	40	–
HUM3336	31	31	33	33	40	–
HUM7814	31	31	33	33	40	–
MEN115	31	31	33	33	40	–
MEN2523	31	31	33	33	40	–
LAKE339	31	31	33	33	40	–

[a] –, No binding of antibody detected; *, only a very faint band was observed (see Fig. 2).

DISCUSSION

California has a relatively low incidence of Lyme disease compared with endemic areas of the northeastern United States. A total of 98 cases of Lyme disease contracted in California have been reported from 1983 through 1985, and over 100 cases have been provisionally reported for 1986 (Robert Murray, Infectious Diseases Branch, California Department of Health Services, personal communication). Over 60% of the 98 cases reported in 1983 through 1985 contracted their infections in four northwestern coastal counties of the state (Humboldt, Mendocino, Sonoma, and Marin counties). In cooperation with the Vector Surveillance and Control Branch of the California Department of Health Services, we undertook a survey of ticks collected primarily from areas surrounding reported cases of human infection, although several collections not directly related to a human case were made from areas of concern such as recreational areas and parks. During two tick seasons (1984 to 1985 and 1985 to 1986), 1.4% of the adult *I. pacificus* ticks examined were infected. This is similar to the 1.1% infection rate reported by Burgdorfer et al. (12) in their earlier study of adult *I. pacificus* collected in northern California and parallels the 0.4% infection rate of *Ixodes scapularis* adults from North Carolina as reported by Magnarelli et al. (15). The low prevalence of infection of ticks is probably responsible for the relatively low numbers of cases of Lyme disease in California.

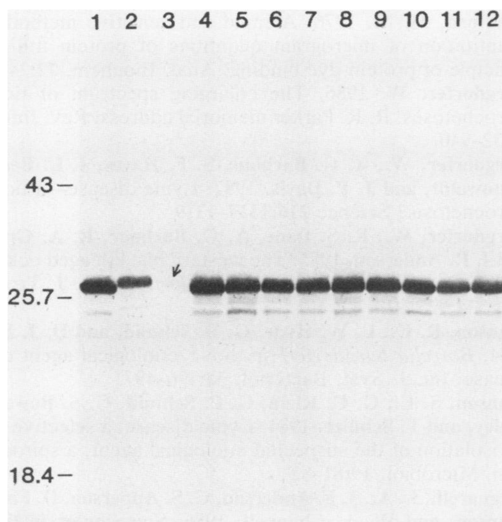

FIG. 2. Western blots of SDS-PAGE-separated proteins of whole-cell lysates of *B. burgdorferi* isolates tested with Osp A monoclonal antibody H5332. The isolates and control strain are as follows. Lanes: 1, B-31; 2, DN127; 3, DN127-C19-2; 4, SON188; 5, SON2110; 6, SON328; 7, SON335; 8, HUM115; 9, HUM3336; 10, MEN115; 11, MEN2523; 12, LAKE339. Strain HUM7814 was not included in this blot; reactions with this strain in separate Western blot tests were similar to those observed with strains in lanes 4 to 12. The M_r values (10^3) of the prestained protein low-molecular-weight standard (Bethesda Research Laboratories) are shown on the left. The arrow shows the area where a barely visible band indicated a slight binding of H5332 monoclonal antibody with a protein of strain DN127-C19-2.

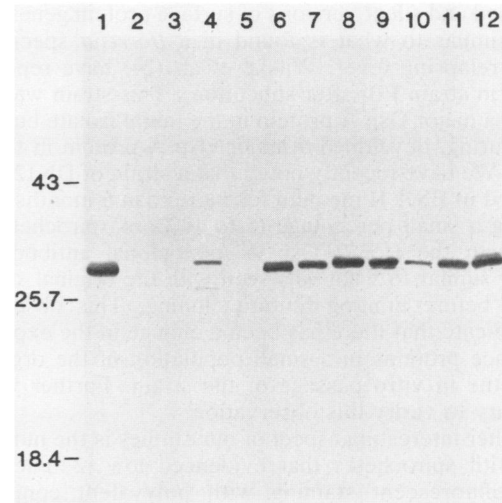

FIG. 3. Western blots of SDS-PAGE-separated proteins of whole-cell lysates of *B. burgdorferi* isolates tested with Osp B monoclonal antibody H6831. Lane designations and molecular weight standards are the same as in Fig. 2.

Characterization by PAGE, monoclonal antibody, and Western blot analyses of the *B. burgdorferi* strains isolated from ticks indicates that 10 of the 11 isolates were similar to strains isolated in other parts of the United States. One strain, DN127-C19-2, was markedly different from any other reported U.S. isolate; it did not bind either of two Osp A monoclonal antibodies (H5332 and H3TS) in IFA tests; it reacted very weakly with Osp A monoclonal antibody H5332 in the Western blot; it was negative in tests with Osp B monoclonal antibodies; and it had an abundant major protein with an apparent molecular weight of 25,000. Most European strains do not react with H3TS, one of the 31K-specific monoclonal antibodies (5), and several European strains with major proteins in the 20K to 25K region have been reported (5, 23, 24). The characteristics of strain DN127-C19-2 are similar to an isolate from cerebral spinal fluid of a patient from the Federal Republic of Germany (strain PBi) (23, 24) and an isolate from *I. ricinus* from Sweden (strain F) (5). Barbour et al. (5) found that a DNA probe for an Osp A gene hybridized with DNA fragments of strain F and suggested that one explanation for this might be the existence of

expressed and silent versions of surface protein genes in this strain similar to what is found in a *Borrelia* species that causes relapsing fever. Wilske et al. (24) have reported a change in strain PBi after subculture. This strain was negative for a major Osp A protein in the initial isolate but, upon subculturing, developed a major Osp A protein in the 32K region. We have recently noted that a strain of DN127-Cl9-2 passaged in BSK II medium for more than 6 months is now showing a small percentage (5 to 10%) of spirochetes that react with the H5332 Osp A monoclonal antibody in a manner similar to what was seen with the original strain of DN127 before limiting-dilution cloning. This observation may indicate that there has been a change in the expression of surface proteins in a small population of the organisms during the in vitro passage of the strain. Further work is necessary to verify this observation.

Another interesting aspect of our studies is the number of ticks with spirochetes that evidenced low (2+) levels of immunofluorescent staining with polyvalent conjugates. Loss of immunofluorescence reactivity of spirochetes from the hemocele of ticks with generalized infections has been reported by Burgdorfer et al. (12). We did not examine tick tissues other than the midgut and therefore cannot state the number of ticks that might have had a generalized infection. In our survey, 11 ticks with spirochetes evidenced reduced fluorescence and none of these spirochetes was successfully cultured. There is a possibility that this reduced fluorescence represents a cross-reaction with an as-yet-undescribed species of *Borrelia*. However, one of the midgut specimens which showed spirochetes with reduced fluorescence to polyvalent conjugate was tested with monoclonal antibody H5332 in an IFA test. The positive reaction (4+) obtained with this antibody, which is specific for the Osp A protein of *B. burgdorferi*, is a strong indication that the spirochetes from the tick were, in fact, *B. burgdorferi*. Lane and Burgdorfer (R. S. Lane and W. Burgdorfer, Am. J. Trop. Med. Hyg., in press) have demonstrated in laboratory studies of infected *I. pacificus* ticks that transovarial and transstadial passage of *B. burgdorferi* occurs and that the progeny had generalized tissue infections, with spirochetes exhibiting reduced immunofluorescence staining with polyvalent conjugates. Spirochetes in tissue smears from the F_1 generation were nonreactive with H5332 monoclonal antibody, but spirochetes from tissue smears of F_2 progeny reacted with this monoclonal antibody. Lane and Burgdorfer were also unsuccessful in attempts to culture these spirochetes. These results suggest that the native ticks with spirochetes that showed reduced immunofluorescence in our study were transovarially infected and had generalized infections. Development of methods to isolate these spirochetes in order to characterize their antigens is in progress.

Investigators have raised the question of whether a particular phenotype of *B. burgdorferi* can influence the form of Lyme disease that is manifested during infection. The various differences in the clinical presentation of the disease in Europe and the United States may be related to the difference in characteristics of strains isolated in Europe and in the United States (5, 23). The isolation of a strain in this study that is more similar in its phenotype to European isolates than it is to U.S. strains has significance in attempts to answer this question. In addition, the suggestion that this strain may alter its expression of surface proteins during in vitro passage raises the possibility that the Lyme disease spirochete may exhibit a type of antigenic variation that is similar to that seen in the *Borrelia* spp. that cause relapsing fever (7).

ACKNOWLEDGMENTS

We thank James Clover, Bernard Nelson, and staff of the Vector Surveillance and Control Branch for collection of ticks; Robert Lane, Robert Murray, and Michael Janda for helpful discussion; Lu-Anne Dodge for preparation of the manuscript; and Sharon Kurashige and Hazel Anderholm for preparation of the photographs. We are indebted to Dr. Alan Barbour for his generous support, assistance, and review of the manuscript.

LITERATURE CITED

1. **Asbrink, E., and I. Olsson.** 1985. Clinical manifestations of erythema chronicum migrans Afzelius in 161 patients: a comparison with Lyme disease. Acta Dermato-Venereol. **65**:43–52.
2. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521–525.
3. **Barbour, A. G., W. Burgdorfer, S. F. Hayes, O. Peter, and A. Aeschlimann.** 1983. Isolation of a cultivable spirochete from *Ixodes ricinus* ticks in Switzerland. Curr. Microbiol. **8**:123–126.
4. **Barbour, A. G., S. F. Hayes, R. A. Heiland, M. E. Schrumpf, and S. L. Tessier.** 1986. A *Borrelia*-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. **52**:549–554.
5. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. **152**:478–484.
6. **Barbour, A. G., S. L. Tessier, and S. F. Hayes.** 1984. Variation in a major surface protein of Lyme disease spirochetes. Infect. Immun. **45**:94–100.
7. **Barbour, A. G., S. L. Tessier, and H. G. Stoenner.** 1982. Variable major proteins of *Borrelia hermsii*. J. Exp. Med. **156**:1312–1324.
8. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41**:795–804.
9. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
10. **Burgdorfer, W.** 1986. The enlarging spectrum of tick-borne spirochetoses: R. R. Parker memorial address. Rev. Infect. Dis. **8**:932–940.
11. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216**:1317–1319.
12. **Burgdorfer, W., R. S. Lane, A. G. Barbour, R. A. Gresbrink, and J. R. Anderson.** 1985. The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*. Am. J. Trop. Med. Hyg. **34**:925–930.
13. **Johnson, R. C., F. W. Hyde, G. P. Schmid, and D. J. Brenner.** 1984. *Borrelia burgdorferi* sp. nov.: etiological agent of Lyme disease. Int. J. Syst. Bacteriol. **34**:496–497.
14. **Johnson, S. E., G. C. Klein, G. P. Schmid, G. S. Bowen, J. C. Feeley, and T. Schulze.** 1984. Lyme disease: a selective medium for isolation of the suspected etiological agent, a spirochete. J. Clin. Microbiol. **19**:81–82.
15. **Magnarelli, L. A., J. F. Anderson, C. S. Apperson, D. Fish, R. C. Johnson, and W. A. Chappell.** 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York State, and North Carolina. J. Wild. Dis. **22**:178–188.
16. **Malawista, S. E.** 1984. Clinical features (Part Two). First International Symposium on Lyme Disease. Yale J. Biol. Med. **57**:505–506.
17. **Naversen, D. N., and L. W. Gardner.** 1978. Erythema chronicum migrans in America. Arch. Dermatol. **114**:253–254.
18. **Schmid, G. P.** 1985. The global distribution of Lyme disease. Rev. Infect. Dis. **7**:41–50.
19. **Stanek, G., G. Wewalka, V. Groh, R. Neumann, and W. Kristoferitsch.** 1985. Differences between Lyme disease and European arthropod-borne borrelia infections. Lancet **i**:401.

20. **Steere, A. C., S. E. Malawista, J. A. Hardin, S. Ruddy, P. W. Askenase, and W. A. Andiman.** 1977. Erythema chronicum migrans and Lyme arthritis: the enlarging clinical spectrum. Ann. Intern. Med. **86:**685–698.
21. **Steere, A. C., S. E. Malawista, D. R. Snydman, R. E. Shope, W. A. Andiman, M. R. Ross, and F. M. Steele.** 1977. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. **20:**7–17.
22. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
23. **Wilske, B., V. Preac-Mursic, and G. Schierz.** 1985. Antigenic heterogeneity of European *Borrelia burgdorferi* strains isolated from patients and ticks. Lancet **i:**1099.
24. **Wilske, B., V. Preac-Mursic, G. Schierz, and K. V. Busch.** 1986. Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. Zentralbl. Bakteriol. Hyg. A **263:**92–102.