JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1996, p. 524–529 Vol. 34, No. 3
0095-1137/96/$04.00+0

Novel *Borrelia burgdorferi* Isolates from *Ixodes scapularis* and *Ixodes dentatus* Ticks Feeding on Humans

JOHN F. ANDERSON,[1]* RICHARD A. FLAVELL,[2] LOUIS A. MAGNARELLI,[1] STEPHEN W. BARTHOLD,[3] FRED S. KANTOR,[4] REINHARD WALLICH,[5] DAVID H. PERSING,[6] DANE MATHIESEN,[6] AND EROL FIKRIG[7]

Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504[1]; Section of Immunobiology and Howard Hughes Medical Institute,[2] Section of Comparative Medicine,[3] Section of Immunobiology,[4] and Section of Rheumatology,[7] Yale University School of Medicine, New Haven, Connecticut 06520; Department of Applied Immunology, German Cancer Research Center, INF 280, Germany[5]; and Mayo Clinic, Rochester, Minnesota 55905[6]

Received 10 October 1995/Returned for modification 13 November 1995/Accepted 28 November 1995

Seven cultures of *Borrelia burgdorferi* differing from strains B31 and ZS7 were identified from among 99 isolates from *Ixodes scapularis* ticks and from white-footed mice (*Peromyscus leucopus*) and 1 isolate from an *Ixodes dentatus* tick. Five of the six novel isolates from *I. scapularis* and the isolate from *I. dentatus* were from ticks feeding on humans. The six isolates from *I. scapularis* lacked OspA and OspB, four possessed an OspD band, and two reacted with an anti-OspC monoclonal antibody. Restriction fragment length polymorphisms of *Hin*dIII-digested DNAs from six OspA-negative isolates did not hybridize with radiolabeled *ospA* or LA88 DNA, and only isolate 46047 hybridized with the *pG* gene. Fragments similar to those recorded for the standard *B. burgdorferi* sensu stricto strains B31 and ZS7 were obtained with the *fla* and the HSP70 genes. Pulsed-field gel electrophoresis patterns of DNA digested with *Mlu*I included the specific *B. burgdorferi* sensu stricto band at 135 kbp for the five OspA-negative isolates from *I. scapularis* ticks. The six novel isolates apparently lack the 55-kbp plasmid encoding OspA. The pG-containing plasmid may be missing from all but isolate 46047. The isolate from the *I. dentatus* tick was similar to previous isolates from *I. dentatus* ticks feeding on rabbits. None of the isolates could be recovered from inoculated C3H/HeNCrlBR or white-footed mice. All isolates reacted with sera from humans with early or late Lyme disease. Our studies demonstrate that these borreliae occur in ticks feeding on humans, and therefore, at least some humans in the northeastern United States are likely being exposed to borreliae other than the classic B31-type strains that have thus far been isolated from humans.

Lyme disease was responsible for more than 90% of the arthropod-associated illnesses reported in the United States during 1992 (18). The principal vector of the etiologic agent, *Borrelia burgdorferi* (21), in the eastern United States is *Ixodes scapularis* (16), formerly known as *Ixodes dammini*, a distinct mitochondrial haplotype (31).

Isolates of *B. burgdorferi* from humans in Europe exhibit antigenic heterogeneity (9, 40, 42) and have now been identified as three distinct species: *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* (6, 17, 21). In contrast, spirochetes cultured from persons diagnosed with Lyme disease in the United States have all been remarkably similar to the *B. burgdorferi* sensu stricto strain B31 (9, 12, 13, 29, 33, 36). Furthermore, isolates from U.S. *I. scapularis* ticks likewise have been generally indistinguishable from the B31 strain (9) except for four isolates from upstate New York (5). Cultured spirochetes from *Ixodes pacificus*, *Ixodes neotomae*, and *Ixodes dentatus* ticks, however, have shown extensive antigenic heterogeneity, as have strains recovered from dusky-footed wood rats (*Neotoma fuscipes*) and kangaroo rats (*Dipodomys californicus*) in California and from eastern cottontail rabbits (*Sylvilagus floridanus*) in New York State (4, 14, 15, 23). We report here on isolates from *I. scapularis* ticks collected while feeding on humans or while questing that apparently lack the 55-kbp OspA-encoding plasmid. Additionally, we report on a strain differing from B31 isolated from an *I. dentatus* tick that had fed on a human.

* Corresponding author. Mailing address: The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, CT 06504-1106. Phone: (203) 789-7214. Fax: (203) 789-7232.

MATERIALS AND METHODS

Isolation and growth of borreliae. One hundred isolates of borreliae cultured from unfed *I. scapularis* ticks ($n = 24$), from *I. scapularis* ticks feeding on humans ($n = 39$) or animals ($n = 25$), from white-footed mice (*Peromyscus leucopus*) ($n = 11$), and from an *I. dentatus* tick feeding on a human ($n = 1$) collected in Connecticut, New York, New Jersey, and Maryland from June 1990 through December 1992 were tested for their similarity to *B. burgdorferi* B31 (Table 1). All borreliae were isolated in semisolid Barbour-Stoenner-Kelly medium (BSK) containing agarose and rifampin (7, 20, 22). The specific methods for making isolations of spirochetes from ticks and mice were described previously (2). Primary cultures were subpassed in liquid BSK without rifampin for analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with a variety of monoclonal antibodies (MAbs) directed against different epitopes. Aliquots of each original culture were frozen in liquid nitrogen.

SDS-PAGE and immunoblotting. Isolates that had been passed two or three times were compared with strain B31 by SDS-PAGE (9). Each isolate was tested by SDS-PAGE in 15% polyacrylamide gels in a Hoefer SE250 mini gel apparatus (Hoefer Scientific Instruments, San Francisco, Calif.). Protein bands were stained with Coomassie brilliant blue R-250. Low-range SDS-PAGE standards (Bio-Rad Laboratories, Richmond, Calif.) were used to estimate the molecular weights of major proteins.

Proteins from each isolate were transferred from SDS-polyacrylamide gels to nitrocellulose membranes (Nitroplus nitrocellulose Transfer membrane; Micron Separations, Inc., Westboro, Mass.) with a Hoefer TE22 transfer apparatus (4, 37). Membranes were exposed for 2 h to murine MAbs diluted 1:15 to 1:500 in Tris-buffered saline. Horseradish peroxidase color developer (4-chloro-1-naphthol; Bio-Rad) was used to visualize the reactions. The molecular weights of the

TABLE 1. Sources of 100 primary *B. burgdorferi* isolates used in the study

State	Tick or animal source	No. of isolates
Connecticut	*I. scapularis* female off of a human	21
	I. scapularis nymph off of human	14
	White-footed mouse	11
	I. scapularis female, host seeking	9
	I. scapularis female off of a deer	17
	I. scapularis male off of a human	1
	I. scapularis male, host seeking	15
	I. scapularis male off of a deer	5
	I. dentatus nymph off of a human	1
	I. scapularis larva off of a white-footed mouse	1
Maryland	*I. scapularis* female off of a pet	1
New Jersey	*I. scapularis* female off of a dog	1
	I. scapularis male off of a human	1
	I. scapularis nymph off of a human	1
New York	*I. scapularis* female off of a human	1

protein bands that reacted positively in immunoblots were estimated with low-range biotinylated SDS-PAGE molecular weight protein standards.

Nine immunoglobulin G (IgG) MAbs were used in the study. Three were against epitopes of OspA (H5332, H3TS, and 8C4BC) (9, 10, 35), two were against OspB (H6831 and 7E6C) (8, 19), two were against flagellin (H9724 and L41 1C11) (8, 41), and one each was against OspC (L22 1F8) (41) and OspD (1C8-3B11) (30).

Restriction fragment length polymorphism (RFLP) analyses. Plasmid-enriched genomic DNA was removed from 200 ml of each isolate grown to the stationary phase in BSK (7). Approximately 7.5 μg of DNA was digested with 5 μl of *Hin*dIII (20 U/μl). DNA was separated by electrophoresis on a 0.7% agarose gel and was transferred to a Nytran Plus nylon membrane (Schleicher & Schuell, Keene, N.H.). Following UV cross-linking of the DNA, the membrane was dried and hybridized with ^{32}P-labeled probes. Gene probes were derived from *B. burgdorferi* ZS7 and included the chromosomal probes *fla* and HSP70; *ospA* and LA88, which are on the same 53-kbp linear plasmid (37a, 39); and *pG*, which is on a 48-kbp linear plasmid (38). The membrane was left in the ^{32}P solution at 65°C overnight and was autoradiographed on Kodak XAR safety film with intensifying screens at −70°C for 20 min to 12 h.

Pulsed-field gel electrophoresis (PFGE). Selected *Borrelia* isolates were grown to confluency in 20 ml of BSK-H (Sigma Chemical Co., St. Louis, Mo.) plus 6% rabbit serum. The cells were harvested by centrifugation at 8,000 × *g* for 15 min. The cell pellet was resuspended in 300 to 500 μl of EET (100 mM EDTA, 10 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], 10 mM Tris [pH 8.0]) and warmed to 42°C. Agarose plugs were made by diluting 1.6% SeaPlaque agarose (FMC BioProducts, Rockland, Maine) in EET heated to 60°C with an equal volume of resuspended cells for a final concentration of 0.8%. After the plugs (50 μl) were soaked in *Mlu*I restriction buffer, *Mlu*I (3 μl at 10 to 12 U/μl) was added, and the plug was digested overnight at 37°C.

A 1.2% Fastlane (FMC) agarose gel was used to separate the restriction fragments in a Bio-Rad CHEF-DRII electrophoresis chamber, and the gel was prerun (no voltage) for 1 h. Conditions for running the gel were an initial switch time of 1 s, a linear ramp with a final switch time of 10 to 15 s, and 200 V (6 V/cm) for 16 to 17 h. The pulsed-field gel was stained with ethidium bromide; DNA was visualized with UV light.

DNA fragments were transferred from the agarose gel to a Nytran membrane (Schleicher & Schuell) and were probed for *ospA* by using the ECL Direct Nucleic Acid Labeling and Detection System (Amersham, Arlington Heights, Ill.). The chemiluminescent 742-bp probe specific for the *B. burgdorferi* gene encoding OspA (32) was hybridized and detected as described previously (34).

Inoculation and attempted isolation procedures. The infectivities of isolates with protein bands distinctly different from those of strain B31 were determined by syringe inoculation of second- through fifth-passage isolates in 3-week-old C3H/HeNCrlBR (C3H) mice, 7-week-old Syrian hamsters, or 6- to 20-week-old white-footed mice. All animals except the white-footed mice, which were reared in the laboratory, were obtained from Charles River Laboratories, Raleigh, N.C. White-footed and C3H mice were inoculated with 10^4 spirochetes intradermally into the upper right or left portion of the back or with 10^7 cells intraperitoneally into the lower left abdominal quadrant. Hamsters were inoculated only with strain 48081 by intraperitoneal procedures as described above. In addition, 1-day-old C3H mice were inoculated intracerebrally or intradermally with 10^4 or 10^6 spirochetes of strain 49736, and 1-week-old C3H mice were inoculated intradermally with 10^6 spirochetes of strain 49736 and were assessed for infection (culture) at 2 weeks after inoculation. The numbers of spirochetes were counted in a chamber (Hausser Scientific Partnership, Horsham, Pa.) by dark-field microscopy.

Attempts to isolate borreliae from blood and tissues of skin, bladder, spleen, kidney, joint, and brain from C3H and white-footed mice were made at variable times ranging from 14 to 101 days after inoculation. Attempts at isolation from hamsters were made 49 days after inoculation.

ELISAs. Class-specific enzyme-linked immunosorbent assays (ELISAs) were used to detect IgM and IgG antibodies to *B. burgdorferi* in 31 serum samples. Samples obtained from 25 persons who had early or late Lyme disease were chosen for analyses. The first study group (19 serum samples from 15 persons) consisted of patients who had histories of tick bites and physician-diagnosed erythema migrans. Blood samples were collected between 3 and 6 weeks after the onset of illness. The second group included 12 serum samples from 10 persons who also had erythema migrans and who subsequently developed arthritis. Blood samples were obtained from these subjects 6 weeks or later following the diagnosis of characteristic expanding skin lesions. Although information on the sources of serum specimens has been reported previously (24–27), records of antibiotic treatment were unavailable. Details on the materials (including control sera) and methods used in these tests and on the sensitivities and specificities of the antibody assays have been described previously (24–27).

TABLE 2. *B. burgdorferi* strains which lack OspA from *I. scapularis* ticks and from an *I. dentatus* tick

Isolate no.	Date of collection (mo/day/yr)	State	Tick species and stage
46047	3/09/1992	Connecticut	Unengorged *I. scapularis* female tick off of a human
46794	5/14/1992	Connecticut	Slightly engorged *I. scapularis* female tick off of a human
48080	6/22/1992	Connecticut	Slightly engorged *I. dentatus* nymph off of a human
48081	6/22/1992	Connecticut	Unengorged *I. scapularis* nymph off of a human
48510	7/02/1992	New Jersey	Slightly engorged *I. scapularis* nymph off of a human
49736	10/14/1992	Connecticut	Unengorged *I. scapularis* female tick off of a human
50772	12/02/1992	Connecticut	Host-seeking *I. scapularis* male tick

RESULTS

Ninety-three of the 100 isolates described in Table 1 had protein banding patterns similar to those of *B. burgdorferi* B31 by SDS-PAGE. That is, they had similar patterns with prominent bands at 31, 34, 41, and 60 kDa. All 93 isolates reacted with MAbs to OspA (H5332 and H3TS) and to flagellin (H9724). Forty-five of the 93 isolates reacted with the anti-OspB MAb H6831. In addition, 62 of these 93 isolates were tested with additional MAbs to OspA (8C4BC), OspB (7E6C), OspC (L22 1F8), OspD (1C8-3B11), and flagellin (L41 1C11). These 62 isolates all reacted with MAbs to OspA (8C4BC), OspB (7E6C), and flagellin (L41 1C11). Thirty isolates reacted with the anti-OspD 1C8-3B11, and six isolates reacted with the anti-OspC L22 1F8, although only three of the latter isolates reacted strongly.

The other 7 of the 100 isolates had proteins distinctly different from those of strain B31 (Table 2). These isolates can be placed into two groups on the basis of their protein banding profiles in SDS-polyacrylamide gels (Fig. 1 and 2) or reactions to MAbs (Table 3). Six of the isolates were from nymphal or female ticks feeding on humans; one was from a host-seeking male *I. scapularis* tick.

Isolate 48080 from an *I. dentatus* nymph had prominent protein bands at 31 and 34 kDa, like strain B31 (Fig. 2), but unlike strain B31, this isolate reacted only with MAbs H9724

TABLE 3. Immunoblot reactions of *B. burgdorferi* B31 and seven antigenically different isolates from *I. scapularis* and *I. dentatus* ticks

Isolate no.	Tick species	Reactions with MAbs[a]								
		OspA			OspB		OspC, L22 1F8	OspD, 1C8-3B11	Flagellin	
		H5332	H3T5	8C4BC	H6831	7E6C			H9724	L41 1C11
B31	*I. scapularis*	P	P	P	P	P	N	N	P	P
46047	*I. scapularis*	N	N	N	N	N	N	P	P	P
46794	*I. scapularis*	N	N	N	N	N	N	N	P	P
48080	*I. dentatus*	N	N	P	N	N	N	N	P	P
48081	*I. scapularis*	N	W	N	N	N	W	N	P	P
48510	*I. scapularis*	N	N	N	N	N	N	P	P	P
49736	*I. scapularis*	N	W	N	N	N	P	P	P	P
50772	*I. scapularis*	N	N	N	N	N	N	P	P	P

[a] P, positive; N, negative; W, weak positive reaction.

and L41 1C11 directed against flagellin and MAb 8C4BC directed against OspA (Table 3).

The remaining six isolates lacked OspA and OspB bands by SDS-PAGE (Fig. 1). Four isolates (isolates 46047, 48510, 49736, and 50772) had distinct strong or weak bands at 28 kDa. These four isolates reacted with the anti-flagellin MAbs H9724 and L41 1C11 and the anti-OspD MAb 1C8-3B11 (Table 3). None reacted with the two OspB MAbs, and with the exception of isolate 49736, none reacted with MAbs directed against OspA or OspC. Isolate 49736 reacted weakly with anti-OspA MAb H3TS and strongly with anti-OspC MAb L22 1F8.

Isolates 46794 and 48081 lacked OspA, OspB, and OspD protein bands (Fig. 1). Both isolates reacted to the two anti-flagellin MAbs H9724 and L41 1C11, and strain 48081 reacted weakly to the anti-OspA MAb H3TS and to OspC MAb L22 1F8 (Table 3). These two isolates did not react with the remaining MAbs tested.

RFLP analyses with five radiolabeled DNA probes were performed with the six isolates lacking OspA by SDS-PAGE and standard isolates representing three different European species of *Borrelia*. The patterns of *Hin*dIII-digested DNA hybridized with radiolabeled *fla*, *ospA*, *pG*, HSP70, and LA88 genes are summarized in Table 4. In contrast to the three standard species tested, no fragments were apparent in the novel isolates with the 53-kb linear plasmid-encoded *ospA* and LA88 gene probes, and only isolate 46047 exhibited fragments at 1.8 and 3.8 kbp with the *pG* gene probe. A fragment of 1.5 kbp was present in all isolates and strain ZS7 with the *fla* gene probe. All six isolates from *I. scapularis* ticks possessed a 0.8-kbp fragment with the HSP70 gene, as did strains ZS7 and ACA-1; additionally, isolate 48510 had a second weak fragment at 0.9 kbp.

The PFGE patterns of DNAs digested with *Mlu*I exhibited diversity among five OspA-lacking isolates (Fig. 3, lanes 4 to 6, 10, and 11). While all DNAs had digestion products at 135, 110, and 50 kbp, differences in the locations of other bands were apparent. The standard N40 *B. burgdorferi* sensu stricto strain (Fig. 3, lane 7) also had digestion products at 135, 110, and 50 kbp, but unlike the five new novel isolates, isolates B31 and N40 possessed a band at about 55 kbp which apparently corresponded to the plasmid encoding *ospA* and *ospB.*

The *ospA* probe hybridized only with PFGE blots containing the 55-kbp plasmid. There was no hybridization with the five isolates that apparently lacked this plasmid species.

In contrast to strain N40, which infected the three C3H mice tested, none of the novel isolates could be isolated from inoculated laboratory animals (Table 5).

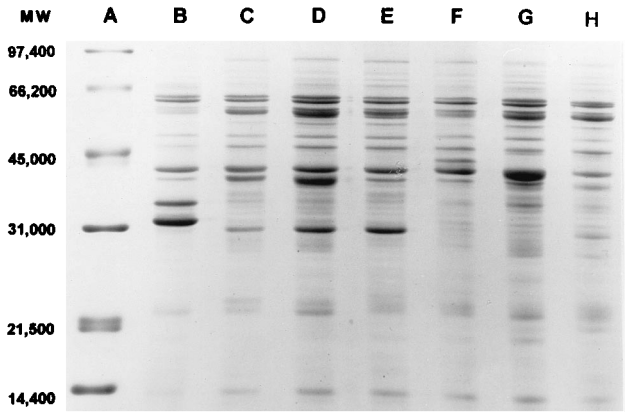

FIG. 1. Coomassie blue-stained proteins of whole-cell lysates of *B. burgdorferi* isolates from *I. scapularis* ticks that were feeding on humans or questing tested by SDS-PAGE. The molecular weights (MW) of the protein standards are given to the left of the first lane. Lanes: A, low-molecular-weight standard proteins; B, B31; C, 46047; D, 48510; E, 50772; F, 46794; G, 48081; H, 49736.

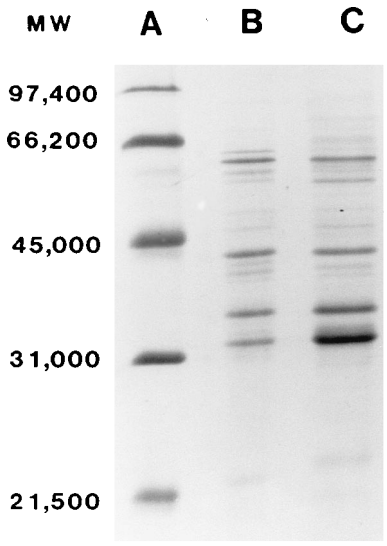

FIG. 2. Coomassie blue-stained proteins of whole-cell lysates of *B. burgdorferi* sensu stricto isolate B31 and isolate 48080 from an *I. dentatus* tick feeding on a human tested by SDS-PAGE. The molecular weights (MW) of the protein standards are given to the left of the first lane. Lanes: A, low-molecular-weight standard proteins; B, B31; C, 48080.

TABLE 4. RFLP analysis of five radiolabeled genes probed with *Hin*dIII-digested DNA from three *B. burgdorferi* species and six *B. burgdorferi* isolates lacking OspA from *I. scapularis*

Isolate identification	*Hin*dIII fragment size (kbp)				
	Genes on linear 53-kbp plasmid		Gene on linear 48-kbp plasmid *pG*	Genes on chromosome	
	ospA	*LA88*		*fla*	HSP70
B. burgdorferi ZS7	1.2, 0.3	2.8	1.8, 3.8	1.5	0.8
B. afzelii ACA-1	1.7, 2	1.7	2.4, >7	1.2, 0.5	0.8
B. garinii ZQ1	0.9, 0.4	1.3	1.7	1.2, 0.5	0.7
B. burgdorferi					
46047	N[a]	N	1.8, 3.8	1.5	0.8
46794	N	N	N	1.5	0.8
48081	N	N	N	1.5	0.8
48510	N	N	N	1.5	0.8, 0.9
49736	N	N	N	1.5	0.8
50772	N	N	N	1.5	0.8

[a] N, negative.

Human serum samples reacted with all nine strains of *B. burgdorferi* tested by ELISAs (Table 6). In analyses for IgM antibodies, seropositivity was the highest (73.7 to 84.2%) when strains 48510, 48081, and 2591 were coated onto polystyrene plates. With the exception of strains 46047 and 49736, reactivities for the remaining strains exceeded 52%. In analyses for IgG antibodies in a separate group of sera, reactivities were likewise relatively high (41.7% or greater). When results for both groups are compared, ELISAs with strain 46047 appeared to have low levels of sensitivity.

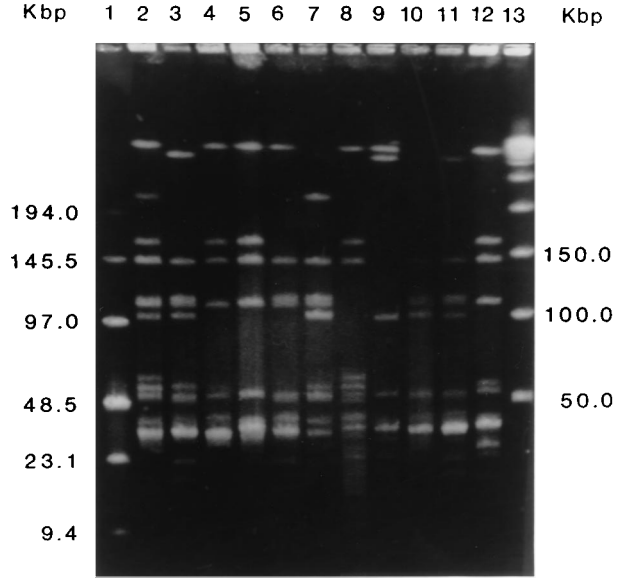

FIG. 3. PFGE banding patterns of *Mlu*I DNA digests of 11 *B. burgdorferi* isolates, 5 of which from *I. scapularis* ticks lack OspA (lanes 4 to 6 and 10 and 11). Lane 1, 0.1- to 200-kbp ladder; lane 2, 50830 from a questing *I. scapularis* tick in Lyme, Conn.; lane 3, 48234 from an *I. scapularis* nymph off of a human in Rocky Neck, Conn.; lane 4, 50772 (see Table 2); lane 5, 46794 (see Table 2); lane 6, 46047 (see Table 2); lane 7, N40 from a questing *I. scapularis* tick in Westchester County, N.Y.; lane 8, B31 seminal *B. burgdorferi* sensu stricto; lane 9, 25015 from *I. scapularis* larva off of a white-footed mouse in Millbrook, N.Y.; lane 10, 48510 (see Table 2); lane 11, 49736 (see Table 2); lane 12, 26861 from an *I. dentatus* tick off of an American robin in East Haddam, Conn.; lane 13, 50- to 1,000-kbp ladder.

Geometric means and ranges of reciprocal antibody titers were determined to compare the reactivities of sera in ELISAs with those of different strains of *B. burgdorferi*. Similar to previous results, geometric mean values for assays with strains 49736 ($\bar{x} = 54$) and 46047 ($\bar{x} = 60$) were markedly lower than those calculated for assays that included other strains (Table 7). Differences ranged from about 3- to 10-fold in analyses for IgM antibodies and from about 2- to 10-fold in tests for IgG antibodies. Maximal IgG antibody titers (1:40,960) were usually four- to eightfold higher than those recorded in IgM antibody analyses of a separate group of sera.

DISCUSSION

Ticks feeding on humans in the northeastern United States can carry borreliae distinctly different from the *B. burgdorferi* sensu stricto strain B31. Unlike the situation in Europe, where *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* have been isolated from humans, only *B. burgdorferi* strains similar to B31 have been isolated from humans diagnosed with Lyme disease in the United States. Until strains differing from B31 have been isolated from humans in the United States, the ability of these types of borreliae to cause disease will remain unknown. Nonetheless, our study documents that borreliae lacking OspA occur in *I. scapularis* ticks that have fed on humans. This tick species is the most important vector of borreliae that cause Lyme disease in the United States. It is therefore likely that, at least on occasion, humans are being exposed to these other forms of borreliae.

While markedly different from strain B31, the six OspA-negative isolates from *I. scapularis* ticks described in this report may still belong to *B. burgdorferi* sensu stricto. PFGE revealed diversity among the five isolates tested, but all possessed a 135-kbp fragment which presumably is specific for *B. burgdorferi* sensu stricto (11). Furthermore, 23S rDNA sequencing showed these isolates to be indistinguishable from B31 (28a), and RFLP analysis of *fla* and HSP70 genes revealed fragment patterns similar to those of *B. burgdorferi* sensu stricto (39).

Although possessing some key characters of *B. burgdorferi* sensu stricto, the OspA-negative isolates from *I. scapularis* ticks differ both phenotypically and genetically as well as in their ability to infect laboratory rodents. The absence of OspA and OspB with or without OspD as determined by SDS-PAGE and immunoblotting distinguishes these isolates from all pre-

TABLE 5. Infectiousness of *B. burgdorferi* N40 and seven antigenically different *B. burgdorferi* isolates

Isolate identification	No. of animals infected/no. of animals inoculated		
	White-footed mice	C3H mice	Hamsters
N40	ND[a]	3/3	ND
46047	0/3	0/5	ND
46794	0/3	0/6	ND
48080	ND	0/3	ND
48081	0/3	0/6	0/3
48510	0/3	0/6	ND
49736	0/2	0/3[b]	ND
50772	0/3	0/5	ND

[a] ND, not determined.
[b] In addition zero of five 1-week-old C3H mice and zero of six 1-day-old C3H mice inoculated with 10^4 or 10^6 spirochetes became infected.

vious borreliae recovered from *I. scapularis* ticks. The weak immunoblot reactions of isolates 49736 and 48081 with anti-OspA MAb H3TS may be cross-reactions with a related epitope on another protein, or these isolates may contain more than one strain of spirochete. Furthermore, our inability to document infection in three laboratory rodent species inoculated with these isolates suggests to us that, unlike B31-type strains, which are infectious in rodents, these specific isolates may have different reservoir hosts. *I. scapularis* ticks feed on at least 120 different species of mammals, birds, and reptiles in the eastern United States (3). While isolates from 82 *I. scapularis* ticks and from 11 white-footed mice in the present study were similar to *B. burgdorferi* sensu stricto strain B31, the 6 *I. scapularis* ticks harboring the novel isolates may previously have fed on infected nonrodent host animals.

The fragment patterns showing an absence of RFLP fragments determined with *ospA* and *pG* gene probes were unlike the fragment patterns of the three standard species used as controls. An exception was strain 46047, which possessed two fragments that were similar to those of strain ZS7 when the probe was *pG*. (We previously incorrectly reported a single fragment at 1.7 kbp in isolate 48510 when *ospA* was used as a probe [1].) The absence of DNA fragments which hybridize with *ospA* and LA88 genes in the six isolates from *I. scapularis* ticks without OspA (both genes are located on the same linear plasmid [37a]) indicates that this specific plasmid is likely missing altogether from these isolates. Additionally, the five isolates without OspA tested by PFGE uniformly lacked the *ospA*-encoding fragment at about 55 kbp which was evident in the other isolates. Furthermore, the plasmid encoding *pG*, which is distinct from the plasmid harboring the *ospA* gene, may likewise be missing from five of the six isolates, or alternatively, the *pG* gene exhibits only little nucleotide sequence identity with the ZS7 gene; strain 46047 is an exception.

TABLE 6. Reactivities of human sera to various strains of whole-cell *B. burgdorferi* in an ELISA for IgM or IgG antibodies

B. burgdorferi strain	No. (%) serum samples testing positive for[a]:	
	IgM antibodies	IgG antibodies
2591	14 (73.7)	8 (66.7)
N40	10 (52.6)	7 (58.3)
46047	4 (21.1)	5 (41.7)
46794	11 (57.9)	7 (58.3)
48080	12 (63.2)	8 (66.7)
48081	14 (73.7)	10 (83.3)
48510	16 (84.2)	10 (83.3)
49736	3 (15.8)	9 (75.0)
50772	11 (57.9)	9 (75.0)

[a] There were two study groups with different serum specimens for analyses of IgM or IgG antibodies. A total of 19 serum samples were tested for IgM antibodies to each strain, and 12 serum samples were tested for IgG antibodies to each strain.

TABLE 7. Geometric means and ranges of antibody titers for human sera tested with whole cells of *B. burgdorferi* strains in an ELISA for IgM and IgG antibodies

B. burgdorferi strain	IgM antibody titer		IgG antibody titer	
	Geometric mean[a]	Range	Geometric mean	Range
2591	533	N[b]–5,120	1,280	N–20,480
N40	166	N–2,560	905	N–40,960
46047	60	N–640	302	N–20,480
46794	199	N–2,560	854	N–40,960
48080	248	N–5,120	2,032	N–40,960
48081	214	N–2,560	3,225	N–40,960
48510	429	N–2,560	1,810	N–40,960
49736	54	N–160	480	N–5,120
50772	214	N–2,560	1,522	N–40,960

[a] A value of 40 (average titer for negative sera) was used to compute geometric means.
[b] N, negative.

Most strains of *B. burgdorferi* performed comparably when they were used in an ELISA to detect IgM or IgG antibodies. These results are consistent with those published earlier (26) when a different set of antigens was tested. It is clear that there are marked differences in protein profiles among strains regarding the presence or absence of some key immunodominant antigens, but the ability to detect antibodies to *B. burgdorferi* by an ELISA is not usually affected. Numerous common antigens are shared among the multitude of *B. burgdorferi* sensu lato strains described. However, as in previous work (26), ELISAs with some strains appear to be less sensitive. Aside from normal test variability, there may be substantial differences in antigenic components that have yet to be identified. Changes in antigenic composition can occur naturally or can be a result of subculturing. Moreover, host immune responses vary, and it is unclear how common certain strains of *B. burgdorferi* are in populations of ticks.

The isolate from the *I. dentatus* nymph feeding on a human was not genetically analyzed, but its SDS-PAGE and immunoblotting reactions suggest that the isolate is similar to borreliae previously isolated from eastern cottontail rabbits and *I. dentatus* ticks feeding on cottontail rabbits (4). Although its infectiousness in humans is unknown, our isolation of a borrelia from an unengorged *I. dentatus* nymph feeding on a human suggests to us that, on at least rare occasions, humans are likely exposed to borreliae that occur naturally in cottontail rabbits. These borreliae have recently been named *Borrelia andersonii* (28).

ACKNOWLEDGMENTS

We thank Christiane Brenner, Bonnie Hamid, and Michael Vasil for technical assistance. Alan Barbour, University of Texas Health Science Center, San Antonio, and Bettina Wilske, Max von Pettenkofer Institut für Hygiene und Medizinische Mikrobiologie der Universitat München, provided MAbs.

This study was partially supported by NIH grants P01AI30548, 49387, AI30548, AR41497, and AI32403, by CDC grants U50/CCU 510343, U50/CCU106598, and U50/CCU106581, and by the Bundesministerium für Forschung und Technologie (01 KI 9104). Erol Fikrig is a

Pew Scholar in the Biomedical Sciences, and Richard A. Flavell is an investigator of the Harvard Hughes Medical Institute.

REFERENCES

1. **Anderson, J. F., R. A. Flavell, L. A. Magnarelli, S. W. Barthold, F. S. Kantor, R. Wallich, and E. Fikrig.** 1994. Variants of the prototype *Borrelia burgdorferi* B31 strain isolated from *Ixodes scapularis*, p. 23–26. *In* R. Cevenini, V. Sambri, and M. LaPlaca (ed.), Advances in Lyme borreliosis research. Proceedings of the VI International Conference on Lyme Borreliosis. University of Bologna, Bologna, Italy.
2. **Anderson, J. F., and L. A. Magnarelli.** 1992. Epizootiology of Lyme disease and methods of cultivating *Borrelia burgdorferi*. Ann. N. Y. Acad. Sci. **653:** 52–63.
3. **Anderson, J. F., and L. A. Magnarelli.** Enzootiology of Lyme borreliosis in the northeastern and north central United States. *In* Proceedings of the IX International Congress on Acrology. in press. Ohio Biological Survey, Columbus, Ohio.
4. **Anderson, J. F., L. A. Magnarelli, R. B. LeFebvre, T. G. Andreadis, B. McAninch, G.-C. Perng, and R. C. Johnson.** 1989. Antigenically variable *Borrelia burgdorferi* isolated from cottontail rabbits and *Ixodes dentatus* in rural and urban areas. J. Clin. Microbiol. **27:**13–20.
5. **Anderson, J. F., L. A. Magnarelli, and J. B. McAninch.** 1988. New *Borrelia burgdorferi* antigenic variant isolated from *Ixodes dammini* from upstate New York. J. Clin. Microbiol. **26:**2209–2212.
6. **Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont.** 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. Int. J. Syst. Bacteriol. **42:**378–383.
7. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.
8. **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.
9. **Barbour, A. G., Heiland, R. A., 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.
10. **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.
11. **Belfaiza, J., D. Postic, E. Bellenger, G. Baranton, and I. Saint Girons.** 1993. Genomic fingerprinting of *Borrelia burgdorferi* sensu lato by pulsed-field gel electrophoresis. J. Clin. Microbiol. **31:**2873–2877.
12. **Benach, J. L., E. M. Bosler, J. P. Hanrahan, J. L. Coleman, G. S. Habicht, T. F. Bast, D. J. Cameron, J. L. Ziegler, A. G. Barbour, W. Burgdorfer, R. Edelman, and R. A. Kaslow.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. **308:**740–742.
13. **Berger, B. W., R. C. Johnson, C. Kodner, and L. Coleman.** 1992. Cultivation of *Borrelia burgdorferi* from erythema migrans lesions and perilesional skin. J. Clin. Microbiol. **30:**359–361.
14. **Bissett, M. L., and W. Hill.** 1987. Characterization of *Borrelia burgdorferi* strains isolated from *Ixodes pacificus* ticks in California. J. Clin. Microbiol. **25:**2296–2301.
15. **Brown, R. N., and R. S. Lane.** 1992. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. Science **256:**1439–1442.
16. **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.
17. **Canica, M. M., F. Nato, L. du Merle, J. C. Mazie, G. Baranton, and D. Postic.** 1993. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. Scand. J. Infect. Dis. **25:**441–448.
18. **Centers for Disease Control and Prevention.** 1993. Lyme disease—United States, 1991–1992. Morbid. Mortal. Weekly Rep. **42:**345–348.
19. **Fikrig, E., H. Tao, F. S. Kantor, S. W. Barthold, and R. A. Flavell.** 1993. Evasion of protective immunity by *Borrelia burgdorferi* by truncation of outer surface protein B. Proc. Natl. Acad. Sci. USA **90:**4092–4096.
20. **Johnson, R. C., N. Marek, and C. Kodner.** 1984. Infection of Syrian hamsters with Lyme disease spirochetes. J. Clin. Microbiol. **20:**1099–1101.
21. **Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt, and D. J. Brenner.** 1984. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. Int. J. Syst. Bacteriol. **34:**496–497.
22. **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.
23. **Lane, R. S., and J. A. Pascocello.** 1989. Antigenic characteristics of *Borrelia burgdorferi* isolates from ixodid ticks in California. J. Clin. Microbiol. **27:** 2344–2349.
24. **Magnarelli, L. A., and J. F. Anderson.** 1988. Enzyme-linked immunosorbent assays for the detection of class-specific immunoglobulins to *Borrelia burgdorferi*. Am. J. Epidemiol. **127:**818–825.
25. **Magnarelli, L. A., J. F. Anderson, and R. C. Johnson.** 1987. Cross-reactivity in serological tests for Lyme disease and other spirochetal diseases. J. Infect. Dis. **156:**183–188.
26. **Magnarelli, L. A., J. F. Anderson, R. C. Johnson, R. B. Nadelman, and G. P. Wormser.** 1994. Comparison of different strains of *Borrelia burgdorferi* sensu lato used as antigens in enzyme-linked immunosorbent assays. J. Clin. Microbiol. **32:**1154–1158.
27. **Magnarelli, L. A., J. N. Miller, J. F. Anderson, and G. R. Riviere.** 1990. Cross-reactivity of non-specific treponemal antibody in serologic tests for Lyme disease. J. Clin. Microbiol. **28:**1276–1279.
28. **Marconi, R. T., D. Liveris, and I. Schwartz.** 1995. Identification of novel insertion elements, restriction fragment length polymorphism patterns, and discontinuous 23S rRNA in Lyme disease spirochetes: phylogenetic analyses of rRNA genes and their intergenic spacers in *Borrelia japonica* sp. nov. and genomic group 21038 (*Borrelia andersonii* sp. nov.) isolates. J. Clin. Microbiol. **33:**2427–2434.

28a. **Mathiesen, D.** Unpublished data.

29. **Nadelman, R. B., C. S. Pavia, L. A. Magnarelli, and G. P. Wormser.** 1990. Isolation of *Borrelia burgdorferi* from the blood of seven patients with Lyme disease. Am. J. Med. **88:**21–26.
30. **Norris, S. J., C. J. Carter, J. K. Howell, and A. G. Barbour.** 1992. Low passage-associated proteins of *Borrelia burgdorferi* B31: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein. Infect. Immun. **60:**4662–4672.
31. **Oliver, J. H., Jr., M. R. Owsley, H. J. Hutcheson, A. M. James, C. Chen, W. S. Irby, E. M. Dotson, and D. K. McLain.** 1993. Conspecificity of the ticks *Ixodes scapularis* and *I. dammini* (Acari:Ixodidae). J. Med. Entomol. **30:**54–63.
32. **Persing, D. H., B. J. Rutledge, P. N. Rys, D. S. Podzorski, P. D. Mitchell, K. D. Reed, B. Liu, E. Fikrig, and S. E. Malawista.** 1994. Target imbalance: disparity of *Borrelia burgdorferi* genetic material in synovial fluid from Lyme arthritis patients. J. Infect. Dis. **169:**668–672.
33. **Rawlings, J. A., P. V. Fournier, and G. J. Teltow.** 1987. Isolation of *Borrelia* spirochetes from patients in Texas. J. Clin. Microbiol. **25:**1148–1150.
34. **Rys, P.** 1993. PCR Detection of *Borrelia burgdorferi*, p. 203–210. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.) Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
35. **Sears, J., E. Fikrig, T. Nakagawa, K. Deponte, N. Marcantonio, F. S. Kantor, and R. A. Flavell.** 1991. Molecular mapping of OspA mediated immunity against *Borrelia burgdorferi*, the agent of Lyme disease. J. Immunol. **147:** 1995–2000.
36. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, G. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308:**733–740.
37. **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.

37a. **Wallich, R.** Unpublished data.

38. **Wallich, R., C. Brenner, M. D. Kramer, and M. M. Simon.** 1995. Molecular cloning and immunological characterization of a novel linear-plasmid-encoded gene, *pG*, of *Borrelia burgdorferi* expressed only in vivo. Infect. Immun. **63:**3327–3335.
39. **Wallich, R., U. E. Schaible, Y. Lobet, S. E. Moter, M. D. Kramer, and M. M. Simon.** 1992. Evaluation of genetic divergence among *Borrelia burgdorferi* isolates by use of *ospA*, *fla*, HSP60, and HSP70 gene probes. Infect. Immun. **60:**4856–4866.
40. **Wilske, B., V. Preac-Mursic, U. B. Gobel, B. Graf, S. Jauris, E. Soutschek, E. Schwab, and G. Zumstein.** 1993. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. J. Clin. Microbiol. **31:**340–350.
41. **Wilske, B., V. Preac-Mursic, G. Schierz, and K. V. Busche.** 1986. Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **263:**92–102.
42. **Wilske, B., V. Preac-Mursic, G. Schierz, R. Kuhbecky, A. G. Barbour, and M. Kramer.** 1988. Antigenic variability of *Borrelia burgdorferi*. Ann. N. Y. Acad. Sci. **539:**126–143.