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DNA Characterization of Lyme Disease Spirochetes

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Lyme disease spirochetes (LDS) have phenotypic characteristics of both treponemes and borreliae. To ascertain whether one or more species of LDS exist, as well as their taxonomic status, we determined the DNA base (G + C) content for three strains of LDS, the DNA relatedness of ten strains isolated in the United States or Europe, and the DNA relatedness of LDS to other spirochetes. The G + C content of the three LDS strains was 28.1-29.0 mol %, most similar to those of *Borellia hermsii* (30.6 mol %) and *Treponema hyodysenteriae* (25.6 mol %) among the other spirochetes tested. DNA hybridization studies of nine LDS strains to a reference strain isolated from human blood revealed divergence (unpaired bases) within related nucleotide sequences of only 0.0-1.0 percent, indicating the strains were one species. Similarly, relatedness values of seven strains to the reference strain were high: 58-98 percent (mean, 71 percent) in 50°C reactions and 50-93 percent (mean, 69 percent) in 65°C reactions. Labeled DNA from *B. hermsii* was 30-40 percent related to three Lyme disease spirochete strains in 50°C reactions and 8-10 percent related in 65°C reactions. In contrast, DNA from the reference LDS strain showed relatedness of only 1 percent to DNAs of two leptospire and only 16 percent to DNA from *T. hyodysenteriae*. We conclude that LDS are a single species, genetically unlike treponemes or leptospire, which belong in the genus *Borrelia*.

In 1982, a newly recognized spirochete was isolated from *Ixodes dammini* ticks [1], and then from ECM skin lesions [2,3], cerebrospinal fluid [2], and blood [2,4] of patients with Lyme disease. Subsequently, this spirochete was recovered from the white-footed mouse (a preferred host of immature *I. dammini*) [5], and *I. ricinus* ticks (the recognized vector of ECM in Europe) [6]. The taxonomic status of these organisms is unclear [1,2]. Of the three genera of *Spirochaetaceae* pathogenic for man—*Leptospira*, *Borrelia*, and *Treponema*—the morphology and growth characteristics of Lyme disease spirochetes are most like those of the treponemes and borreliae [1,2]. To clarify the taxonomy of these isolates, we determined the guanine-plus-cytosine (G + C) content of the DNA of three strains, the DNA relatedness of ten strains from Europe or the United States, and the relatedness of Lyme disease spirochetes to selected other spirochetes. These data are reported in greater detail elsewhere [7].

METHODS

All Lyme disease spirochetes and *B. hermsii* were grown at 33°C in BSK medium [6], leptospire were cultured at 27°C in Ellinghausen's medium [8], and treponemes

were grown at 37°C in Trypticase soy broth containing 0.25 percent dextrose and 10 percent bovine fetal serum [9] in an atmosphere of 90 percent N₂ and 10 percent CO₂. Cells were harvested when growth appeared maximal, after three to ten days' incubation. The G + C content of DNA from Lyme disease spirochetes was determined by denaturing DNA in a spectrophotometer (Gilford Instruments model 2600). Native DNA (in 1X SSC) was adjusted to an optical density of 1.0 and placed in microcuvettes at 258 nm. Using a thermal programmer (Gilford Instruments model 2527), the sample was allowed to equilibrate at 50°C for 15 minutes and the temperature was raised one degree per minute to 100°C. The thermal midpoint of denaturation was determined and the mol % G + C was calculated by the equation of De Ley [10]. The preparation of unlabeled and labeled DNA from Lyme disease spirochetes and selected other spirochetes, and the procedure used for DNA hybridization, were done as previously described [11,12]. After phenol and chloroform extractions, Lyme disease spirochete strain TLO-005, a human blood isolate, was labeled *in vitro* with ³²P by nick translation. Both labeled and unlabeled DNAs were sheared by sonication and denatured by boiling. Approximately 2,000 cpm of labeled DNA (approximately 0.01 µg) was added to 15 µg of unlabeled DNA in 1.0 ml of 0.28 M phosphate buffer and incubated at 50°C or 65°C for 16 hours to insure almost complete reassociation of labeled with unlabeled DNA. After incubation, the DNA mixtures were diluted to a 0.14 M phosphate buffer concentration and passed through hydroxyapatite. Double-stranded DNA was eluted from hydroxyapatite either by the addition of 0.4 M phosphate buffer or, to determine divergence in related DNA sequences, by a series of elutions with 0.14 M phosphate buffer at increasing temperature.

RESULTS

Of three Lyme disease spirochete strains isolated from *I. dammini* or human blood in the United States, or *I. ricinus* in Europe, the G + C content was 28.1–29.0 mol %. Compared to our reference strain, TLO-005, the DNA hybridization studies revealed divergence (unpaired bases) within related nucleotide sequences of 0.0–1.0 percent for all nine Lyme disease spirochete strains (Table 1). Relatedness values of seven strains to TLO-005 were 58–98 percent (mean, 71 percent) in 50°C reactions and 50–93 percent (mean, 69 percent) in 65°C reactions. Two other strains, from which very low yields of DNA were obtained, showed less relatedness (36 percent and 50 percent at 50°C, 38 percent and 47 percent at 65°C).

When the G + C content of Lyme disease spirochetes was compared to selected other spirochetes (*Borrelia hermsii* ATCC 35209, *Treponema hyodysenteriae* ATCC 27164, *Leptospira interrogans* serotype *mankarso*, *L. biflexa* serotypes *patoc* and *illini*), the G + C content of Lyme disease spirochete strains was most similar to those of *B. hermsii* (30.6 mol %) and *T. hyodysenteriae* (25.6 mol %). It was dissimilar from *L. interrogans* serotype *mankarso* (36.7 mol %), and *L. biflexa* serotypes *illini* (47.6 mol %) and *patoc* (39.0 mol %). In DNA relatedness studies, the Lyme disease spirochete strain TLO-005 had only 1 percent relatedness with the leptospires *L. interrogans* serotype *mankarso* and *L. biflexa* serotype *illini* and only 16 percent relatedness with *T. hyodysenteriae*.

Conversely, we determined relatedness between labeled DNA from *B. hermsii* and unlabeled DNA from three Lyme disease spirochetes (strains TLO-004, TLO-005, TLO-033). Relatedness at 50°C was 30–40 percent and at 65°C was 8–10 percent; divergence was 16.5–18.5 percent.

TABLE 1
DNA Relatedness of Lyme Disease Spirochete Strains

Source of Unlabeled DNA	DNA Relatedness to Labeled DNA from Lyme Disease Spirochete Strain TLO-005		
	RBR, 50°C ^a	D ^b	RBR, 65°C
TLO-005 (Human blood, CT)	100	0.0	100
TLO-008 (<i>Ixodes ricinus</i> , Switzerland)	98	1.0	93
TLO-032 (Mouse, NY)	80	1.0	71
B31 (<i>Ixodes dammini</i> , NY)	67	0.5	71
TLO-024 (<i>Ixodes dammini</i> , NY)	67	0.0	50
TLO-004 (<i>Ixodes dammini</i> , MA)	65	0.0	70
TLO-029 (Human skin, NY)	64	1.0	68
TLO-033 (Human blood, NY)	58	1.0	61
TLO-031 (Human skin, CT)	50	0.5	47
TLO-030 (Human spinal fluid, CT)	36	0.0	38

^aRBR = relative binding ratio = % (heterologous DNA bound to hydroxyapatite)/(homologous DNA bound to hydroxyapatite) × 100

^bD = divergence, calculated to the nearest 0.5 percent was calculated on the assumption that a 1°C decrease in thermal stability of a heterologous DNA duplex compared to that of the homologous DNA duplex is caused by each 1 percent of the bases that are unpaired.

Each reaction was done two to four times. In control reactions in which labeled DNA was incubated in the absence of unlabeled DNA, 1.0 percent to 2.5 percent of labeled DNA bound to hydroxyapatite. These control values were subtracted before RBR was calculated. Binding to hydroxyapatite in homologous TLO-005 reactions was 51 percent in 50°C reactions and 55 percent in 65°C reactions.

DISCUSSION

Based upon our characterization of the DNA of Lyme disease spirochetes, we believe that they are a single species. The three strains tested had similar G + C contents. In addition, DNA hybridization studies revealed divergence within related nucleotide sequences of only 0.0–1.0 percent compared to our reference strain TLO-005 and, for seven isolates, high relatedness at either 50°C or 65°C reactions. Although two of the nine strains showed less relatedness to the reference strain, we believe that they belong to the same species because of the low amount of divergence and because of the lack of relatedness in reactions done at 65°C compared to 50°C. Furthermore, one of these two strains (TLO-030) has been shown by Johnson and co-workers to have a high degree of homology with two of the LDS strains tested by us [13]. Thus, we believe that the apparent lesser relatedness of these two strains was because of low yields of DNA.

Lyme disease spirochetes are genetically unlike treponemes or leptospirae. Although we studied only one treponeme, *T. hyodysenteriae* was selected because it is the only one that has a G + C content close to Lyme disease spirochetes. In contrast, the DNA from *B. hermsii* was related enough to Lyme spirochetes to place them in the genus *Borrelia*. In addition, the morphologic characterization of these spirochetes by others is most consistent with that of other borreliae [1,2,6]. Thus, we conclude that Lyme disease spirochetes are a single species which belong in the genus *Borrelia*.

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