INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Oct. 1984, p. 496–497 Vol. 34, No. 4
0020-7713/84/040496-02$02.00/0

Borrelia burgdorferi sp. nov.: Etiologic Agent of Lyme Disease

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A review of reports on the genetic and phenotypic characteristics of strains of the spirochete which causes Lyme disease revealed that these organisms are representative of a new species of *Borrelia*. We propose the name *Borrelia burgdorferi* for this species. The type strain of *B. burgdorferi* is strain B31 (= ATCC 35210). In two separate studies the guanine-plus-cytosine content of the deoxyribonucleic acid of the type strain was determined to be 29.0 to 30.5 mol% (thermal denaturation method).

Erythema chronicum migrans, the skin lesion characteristic of Lyme disease, was first reported by Swedish physician Arvid Afzelius in 1909 (1). Subsequently, erythema chronicum migrans, occąsionally accompanied by neurological symptoms, was recognized throughout Europe (Schmid, Yale J. Biol. Med., in press). In 1970, the first case of erythema chronicum migrans acquired in the United States was reported (11), and in 1977 researchers investigating an unusual cluster of cases of childhood arthritis described the entire symptom complex now called Lyme disease (14).

In his original description, Afzelius recognized that the skin lesions occurred after the bite of a tick, and *Ixodes ricinus* was subsequently established as the vector of the disease in Europe. In the United States, *Ixodes dammini* and *Ixodes pacificus* were established as vectors (13). Despite these observations, the observation that erythema chronicum migrans could be transmitted between human volunteers (4), and the fact that the disease could be treated with antimicrobial agents (7, 15), a suspected infectious etiological agent eluded researchers until 1982.

In 1982, Burgdorfer et al. reported finding spirochetes in *I. dammini* ticks which were collected on Shelter Island, N.Y., a known endemic area for Lyme disease; these spirochetes reacted immunologically with the sera of patients with Lyme disease, and Burgdorfer et al. suggested that this organism was the etiological agent of Lyme disease (6). This suggestion was strongly supported by the subsequent isolation of seemingly identical spirochetes from the blood (3, 12), cerebrospinal fluid (12), and skin (12) of patients acutely ill with Lyme disease and the further demonstration of immunological reactivity of patient sera with the spirochetes (12). In 1983, similar spirochetes were isolated by the Burgdorfer group from *I. ricinus* ticks collected in Switzerland (2).

In the absence of taxonomic studies, the spirochetes which have been isolated have been termed Lyme disease spirochetes, *Ixodes dammini* spirochetes, or *Ixodes ricinus* spirochetes. We have reviewed reports of the immunological and phenotypic characteristics of these spirochetes, as well as the deoxyribonucleic acid (DNA) nucleotide relationships of these spirochetes to other spirochetes and among themselves. These reports indicate that all of the isolates thus far studied belong to one species and that this is a new species of *Borrelia*. For this species we propose the name *Borrelia burgdorferi* sp. nov. (burg.dorf er.i. N.L. gen. n. *burgdorferi* in honor of Willy Burgdorfer, who first discovered the organism in *I. dammini* ticks at the Rocky Mountain Laboratories, National Institutes of Health, Hamilton, Mont.).

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The DNA nucleotide composition and the DNA relatedness of Lyme disease spirochetes, borreliae, treponemes, and leptospires have been described recently independently by workers from our laboratories (8, 10). The guanine-plus-cytosine contents of Lyme disease spirochetes, as determined by thermal denaturation, were 27.3 to 30.5 mol%. These values were similar to values for *Borrelia* species (28.0 to 30.6 mol%), but differed from the values found for *Treponema* (35.0 to 53.0 mol%) and *Leptospira* (35.0 to 40.4 mol%) species. DNA hybridization studies done in both laboratories showed that all of the Lyme disease spirochete strains tested belong to a single species. In one laboratory workers found relatedness values of 76 to 100% (three strains, DNA filter method, 60°C, 2 × SSC [1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate]) (8). In the other laboratory workers found that 8 of 10 strains were highly related (61 to 98%) in studies done at both optimal and stringent criteria for DNA reassociation (hydroxyapatite method, 50 and 65°C). Two other strains were less related, but the related DNAs in all 10 strains showed little, if any (0.0 to 1.0%), sequence divergence (10). The levels of relatedness of Lyme disease spirochetes to *Borrelia* species were 31 to 59% in one study (8) and 30 to 40% in the other (10). Negligible relatedness was found between Lyme disease spirochetes or borreliae and species of *Treponema* or *Leptospira*. Workers in both laboratories concluded that all of the Lyme disease strains tested belong to a single group, which represents a previously undescribed species of *Borrelia*.

Description of the species. *Borrelia burgdorferi* possesses the basic phenotypic characteristics of the genus *Borrelia* (9; R. C. Johnson, F. W. Hyde, and C. M. Rumpel, Yale J. Biol. Med., in press). The spirochetes are flexible helical cells with dimensions of 0.18 to 0.25 by 4 to 30 μm. The organism is motile with both rotational and translational movements; the coiling of the cell is regular. On the average, seven periplasmic flagella are located at each cell end, and these flagella overlap at the central region of the cell. A multilayered outer envelope or membrane surrounds the protoplasmic cylinder, which consists of the peptidoglycan layer, cytoplasmic membrane, and the enclosed cytoplasmic contents. The diamino acid present in the peptidoglycan is ornithine. Cytoplasmic tubules are absent.

The cells are gram negative and stain well with Giemsa and Warthin-Starry stains. Unstained cells are not visible by bright-field microscopy but are visible by dark-field or phase-contrast microscopy. The optimal growth temperature is 34 to 37°C, and the organism has a generation time of 11 to 12 h at 35°C (A. G. Barbour, Yale J. Biol. Med., in press). The cells are catalase negative and microaerophilic. The

spirochetes are chemoorganotropic, when carbohydrates such as D-glucose are used as energy and carbon sources. D-Glucose is fermented to lactic acid (Johnson et al., in press). In contrast to the antigenic variability of the relapsing fever borreliae, this organism is antigenically stable. It is pathogenic for humans (3, 12) and animals (6). Infections are acquired from several tick species of the genus *Ixodes* which are parasitized by the spirochete. Transovarial transmission of the spirochete in ticks is infrequent and inefficient (W. Burgdorfer, Yale J. Biol. Med., in press). The type strain of *B. burgdorferi* is strain ATCC 35210. The guanine-plus-cytosine content of *B. burgdorferi* DNA is 27.3 to 30.5 mol%, as determined by thermal denaturation.

Description of the type strain. The type strain of *B. burgdorferi* is strain B31 (= ATCC 35210). This strain was the first isolate of *B. burgdorferi* and was isolated from a tick (*I. dammini*) collected on Shelter Island, N.Y. The guanine-plus-cytosine content of this strain is 29.0 to 30.5 mol%, as determined by the thermal denaturation method in two separate studies (8, 10).

We gratefully acknowledge support from Public Health Service grant AI 18153 from the National Institutes of Health (to R.C.J.).

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