THE AGENTS OF LYME BORRELIOSIS

G. Baranton and I. Saint Girons

SUMMARY

Several species within the *Borrelia burgdorferi* complex responsible for Lyme borreliosis have been delineated, namely *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. This genetic diversity is also revealed at the phenotypic level. Correlation of each species with different clinical traits has been made. The genome of *Borrelia burgdorferi* is unusual. In contrast to most bacteria, its chromosomes as well as several plasmids are linear.

KEY WORDS:

Lyme borreliosis, Borrelia burgdorferi, new species, taxonomy, linear genome

1. INTRODUCTION

Cutaneous symptoms and epidemiological traits of what is now named Lyme borreliosis have been described since the beginning of the century in Europe. However, some symptoms such as erythema migrans (EM) (1), acrodermatitis chronica atrophicans (ACA) (2), or lymphadenosis benigna cutis (LBC) (3), had been separately described. These entities were connected only in the fifties (4). The bacterial nature of the infectious agent and its transmission by ticks were obvious at this time. Interest was aroused in the New World by clustered cases of arthritis in a wooded area around the small town of Lyme (5). This turned out to represent a new aspect for this "old disease". In 1982, W. Burgdorfer isolated a spirochete from Ixodes ticks from the USA (6). Spirochetes were also isolated from European ticks (7).

This spirochete was classified as belonging to the *Borrelia* genus. A new species (*burgdorferi*) was created on both genomic and phenotypic criteria (8). A multiplicity of *Borrelia* species and hematophagous vectors had been described and each bacterial species

was usually associated with a vector species. However, surprisingly, Hyde and Johnson demonstrated that *B. parkeri, B. turicatae* and *B. hermsii,* responsible for North American recurrent fever, constituted a unique genomic species (9) although transmitted by three *Ornithodoros* species. In the same report, they showed that *Borrelia burgdorferi* consists of a single species although transmission was achieved by different *Ixodes* vector species. Indeed, vectors differ from one continent to another but also on the same continent (i.e. *I. ricinus* in Europe; *I. dammini* (identical to *I. scapularis*) and *I. pacificus* in North America).

The following paragraphs are dealing with the agents of Lyme disease within the *Borrelia burgdorferi* complex. The question of the correlation of each species with different clinical traits in various continents is addressed (10). The genome organization of this human pathogen is compared to that of other spirochetes and procaryotes in general.

2. PHENOTYPIC AND GENETIC DIVERSITY OF B. BURGDORFERI

Many epidemiological and clinical features suggested that *B. burgdorferi* strains are extremely heterogeneous. Different vectors harbored *B. burgdorferi* isolates, mainly *Ixodes* within the "*ricinus* complex". However, *B. burgdorferi* isolates were extracted from other *Ixodes* spp. non included in the latter group such as *I. ovatus* in Japan, *I. neotomae* in California. Differences in pathogenicity for mammals have been observed depending on strains: some *I. damini* isolates were found of low pathogenicity (11), as well as some *I. ovatus* isolates (12). Geographical distribution of the bacterial agent spanned at least three continents. The pleiomorphous symptomatology is associated with a non homogeneous geographical distribution: predominance of arthritis in the U.S.A., of neurological forms in western Europe, of late cutaneous symptoms in Scandinavia (not found in U.S.A.).

Protein profiles and monoclonal antibodies (Mabs) reactivity led A. Barbour (13) and B. Wilske (14) to define serogroups or serotypes among *B. burgdorferi.* European strains were shown to be more heterogeneous than U.S. strains and a certain homogeneity could be recognized among European strains when classified by their organotropism (15).

Genotypic methods revealed that *B. burgdorferi* was highly polymorphic at the genetic level. Among the methods used, some had a taxonomical significance. Those either explored the whole genome (DNA/DNA relatedness (8, 9, 16), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) (17), Pulsed Field Gel Electrophoresis (PFGE) (18), or several chromosomal polyallelic genes (Multilocus Enzyme Electrophoresis) (19), or conserved genes such as rDNA genes (ribotyping) (16) and fla gene sequencing (20, 21) or rRNA sequencing (22, 23). The results of these different approaches lead to reconsider the somewhat surprising results of species uniqueness for the agent of Lyme borreliosis.

3. A TAXONOMIC BASIS FOR A NEW NOMENCLATURE

Since 1987, the delineation of a bacterial species is based upon DNA/DNA hybridization studies and ΔTm determination (24). Two isolates belong to the same species if DNA relatedness is higher than 70% and the ΔTm is lower than 5%. The rationale for the first whole DNA/DNA relatedness study (9), mentioned in the introduction, was mainly to demonstrate that the *I. dammini* spirochete was responsible for human disease and also to measure the extent of divergence between a *I. ricinus* European isolate (IRS) and a *I. dammini* US isolate. Another study (25), including a larger number of US isolates and a single European isolate (the same IRS strain), confirmed *B. burgdorferi* homogeneity. Since more isolates were described as phenotypically heterogeneous, it was necessary to study numerous isolates mainly from Europe. A study of a large number of isolates, relying on the bacterial species concept (26) led to the delineation of at least three species: *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* (27) within the nomenspecies *B. burgdorferi* sensu lato. Another genomic species was reported in Asia (28) (Table). Evidence for new species in Europe or in the U.S.A is quite likely.

This new nomenclature for the agents of Lyme borreliosis allowed to unify the various terminologies already used for a given group (i.e. serogroup II (29) serotype 4 (15), group 3 (20), class 2 (30) correspond to *B. garinii*). Furthermore a reference strain was designated for each member of the *B. burgdorferi* complex (Table). B31 remains the type strain for *B. burgdorferi* sensu stricto, 20047 represents *B. garinii* while VS461 is the type strain for *B. afzelii* (27). F63B is the type strain for the non pathogenic genospecies (28).

4. A LINEAR GENOME FOR THE LYME DISEASE AGENTS

A living organism is ultimately characterized through determination of the whole DNA sequence which constitutes its genomic inheritage. Development of new techniques have very recently allowed the sequencing of whole genomes. The first step towards sequencing the thousands of genes of a medium-sized procaryotic genome is the establishment of a genetic map. Identified genes are localized on these maps relative to one another on graphic representations of chromosomes or plasmids which constitute the genome. These representations allow the architecture of a genome to be judged. However, for organisms for which genetics knowledge is limited, physical maps of genomes may be constructed. They are based on organization of large DNA fragments generated by restriction endonucleases.

The presence of linear plasmids in *B. burgdorferi* was demonstrated in 1987 (31). In 1989, the linear nature of the chromosome of *B. burgdorferi* was discovered (32, 33). The telomeres of the linear plasmids are covalently closed (31), while the architecture of the ends of the chromosome is not known. The physical and genetical map of *B. burgdorferi* sensu stricto has been constructed and is consistent with the linearity of the chromosome (34, 35). The physical maps of *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* are quite different although the genetic maps are quite similar (Davidson, Ojaimi, Old and Saint Girons, unpublished results).

Genome linearity is not a characteristic of spirochetes. The chromosome of *T. pallidum* although of the same small size as that from *Borrelia burgdorferi* (1 Mbp) is circular (36). The genome of *Leptospira interrogans* consists of two circular chromosomes (4.5 and 0.35 Mbp) (37, 38, 39). The chromosome of *Borrelia spp.* was the first to be shown as linear. However, recently other linear chromosomes have been reported among eubacteria such as *Rhodococcus fascians* (40), *Streptomyces lividans* (41) *Agrobacteriurn tumefaciens* (Allardet-Servant et al, unpublished results).

5. CLINICAL AND EPIDEMIOLOGICAL IMPLICATIONS OF SEVERAL SPECIES RESPONSIBLE FOR LYME DISEASE

The main criticism addressed to taxonomic changes is the following: is there a correlation between a species and epidemiology or clinics? Sera from

Table. Schematic epidemiological and clinical distinctions between the members of the *B. burgdorferi* sensu lato complex

	Type strain	Ixodes vector	Geographical location	Preferred evolutive symptom[a]
B. burgdorferi sensu stricto	B 31	*I. dammini* *I. ricinus*	U.S.A. Europe	Arthritis
B. garinii	20047	*I. ricinus* *I. persulcatus*	Eurasia	Neurological manifestations
B. afzelii	VS461	*I. ricinus* *I. persulcatus*	Eurasia	Late cutaneous symptoms A.C.A.
F63B group	F63 B	*I. ovatus*	Japan	No pathogenicity[b]

a) as inferred from preferential reactivity of sera from patients against representatives of the Lyme borreliosis agents

b) no pathogenicity of *I. ovatus* for human has been reported

patients suffering from different clinical presentations were analyzed by western-blot against representatives of each Lyme borreliosis agents (10) (42). A relative preferential reactivity associated arthritis with *B. burgdorferi* sensu stricto, neurological forms with *B. garinii* and mainly ACA with *B. afzelii*. Furthermore, it could be observed that the geographical distribution of isolates belonging to a given species was correlated with the locally predominant symptoms associated with the latter. Another indirect evidence for association of a clinical symptom with a given species was the identification of strains isolated from a given organ or cutaneous lesion. For example, using Mabs reactivities (27) and nucleotide sequencing (43), it could be observed that most of the ACA isolates belonged to *B. afzelii*.

Several questions remain unsolved: how to explain why a closer similarity is sometimes found between two isolates, one from Europe, the other from U.S.A., than among US strains or European strains belonging to *B. burgdorferi* sensu stricto? Geographic isolation, as well as transmission by different vectors, should have led to bacterial specification on each side of the Atlantic. In addition, the relatively recent discovery of Lyme borreliosis in the U.S.A., as well as the finding of *B. burgdorferi* sensu stricto but not the other species suggested the anteriority of presence of *Borrelia* in Eurasia. However since *B. burgdorferi* sensu stricto seems absent from Asia, how did this species settle in the U.S.A.?

4. CONCLUSION

The genetic diversity of *B. burgdorferi* sensu lato lead to reconsider the taxonomic status of this human pathogen. It also allowed to define peculiar organotropisms and geographical predominance of each of the new species thus delineated.

However, there is probably another aspect which could be of interest in this strain diversity, namely the problem of cross protection. The major outer membrane protein OspA is considered as a vaccine candidate (44) in spite of low cross-protection offered by some strains. Since it was observed that OspA gene sequence variations are as high as 20 % (45), an extensive study of bacterial diversity seems to be a prerequisite to analyze the levels of cross protection.

REFERENCES

1. Afzelius, A. Verhandlungen der dermatologischen Gesellschaft zu Stockholm on October 28, 1909. Arch Dermatol Syph 1910; 101: 404.

2. Buchwald, A. Ein Fall von diffuser idiopathischer Haut-Atrophie. Arch Dermatol Syph. 1883;10: 553-556.

3. Burckhardt, J. L. Zur Frage der Follikel- und Keimzentrenbildung in der Haut. Frankf Z Pathol 1911; 6: 352-359.

4. Hauser, W. Zur Kenntnis der Akrodermatis chronica atrophicans. Arch Dermatol Syph 1955; 199: 350-393.

5. Steere, A. C., S. E. Malawista, D. R. Snydman, R. E. Shope, W. A. Andiman, M. R. Ross and F. M. Steele. Lyme arthritis: An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 1977; 20: 7-17.

6. Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grundwald and J. P. Davis. Lyme disease: A tick-borne spirochetosis? Science 1982; 216: 1317-1319.

7. Barbour, A. G., W. Burgdorfer, S. F. Hayes, O. Peter and Aeschlimann. Isolation of a cultivable spirochete from Ixodes ricinus ticks of Switzerland. Current Microbiol 1983; 8: 123-126.

8. Johnson, R. C., G. P. Schmid, F. W. Hyde, A. G. Steigerwalt and D. J. Brenner. Borrelia burgdorferi sp nov. etiological agent of Lyme disease. Int J Syst Bacteriol 1984; 34: 496-497.

9. Hyde, F. W. and R. C. Johnson. Genetic relationship of Lyme disease spirochetes to Borrelia, Treponema and Leptospira spp. J Clin Microbiol 1984; 20: 151-154.

10. Assous, M., D. Postic, G. Paul, P. Nevot and G. Baranton. Western blot analysis of sera from Lyme borreliosis patients according to the genomic species

of the Borrelia strains used as antigens. Eur J Clin Microbiol Infect Dis 1993; 12: 261-268.

11. Anderson, J. F., S. W. Barthold and L. A. Magnarelli. Infectious but nonpathogenic isolate of Borrelia burgdorferi. J Clin Microbiol 1990; 28: 2693-2699.

12. Nakao, M., K. Miyamoto, K. Uchikawa and H. Fujita. Characterization of Borrelia burgdorferi isolated from Ixodes persulcatus and Ixodes ovatus ticks in Japan. Am J Trop Med Hyg 1992; 47: 505-511.

13. Barbour, A. G. A proposal for a serotyping system based upon major outer surface proteins of Borrelia burgdorferi. Lyme borreliosis Newletters 1986; 2: 7-9.

14. Wilske, B., A. G. Barbour, S. Bergström, N. Burman, B. I. Restrepo, P. Rosa, T. Schwann, Soutschek and R. W. Wallich. Antigenic variation and strain heterogeneity in Borrelia spp. Res Microbiol 1992; 143: 583-596.

15. Wilske, B., V. Preac-Mursic, G. Schierz, R. Kühbeck, A. G. Barbour and M. Kramer. Antigenic variability of Borrelia burgdorferi. Ann NY Acad Sci 1988; 539: 126-143.

16. Postic, D., C. Edlinger, C. Richaud, F. Grimont, Y. Dufresne, P. Perolat, G. Baranton and P. A. D. Grimont. Two genomic species in Borrelia burgdorferi. Res Microbiol 1990;140: 507-516.

17. Welsh, J., C. Pretzman, D. Postic, I. Saintgirons, G. Baranton and M. Mcclelland. Genomic fingerprinting by Arbitrarily Primed Polymerase Chain Reaction resolves Borrelia burgdorferi into 3 distinct phyletic groups. Int J Syst Bact 1992; 42: 370-377.

18. Belfaiza, J., D. Postic, E. Bellenger, G. Baranton and I. Saint Girons. Genomic fingerprinting of Borrelia burgdorferi sensu lato by pulsed field electrophoresis. J Clin Microbiol 1993; 31: in press

19. Boerlin, P., O. Peter, A. G. Bretz, D. Postic, G. Baranton and J. C. Piffaretti. Population genetic analysis of Borrelia burgdorferi isolates by multilocus enzyme electrophoresis. Infect Immun 1992; 60: 1677-1683.

20. Adam, T., G. S. Gassmann, C. Rasiah and U. B. Gobel. Phenotypic and genotypic analysis of Borrelia burgdorferi isolates from various sources. Infect Immun 1991; 59: 2579-2585.

21. Picken, R. N. 1992. Polymerase chain reaction primers and probes derived from flagellin gene sequences for specific detection of the agents of Lyme disease and North American relapsing fever. J Clin Microbiol 30: 99-114.

22. Marconi, R. T. and C. F. Garon. 1992. Phylogenetic analysis of the genus Borrelia a comparison of North American and European isolates of Borrelia burgdorferi. J Bacteriol 174: 241-244.

23. Marconi, R. T. and C. F. Garon. 1992. Identification of a 3rd genomic group of Borrelia burgdorferi through signature nucleotide analysis and 16S rRNA sequence determination. J Gen Microbiol 138: 533-536.

24. Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr and H. G. Trüper. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37: 463-464.

25. Schmid, G. P., A. G. Steigerwalt, S. E. Johnson, A. G. Barbour, A. C. Steere, I. M. Robinson and D. J. Brenner. 1984. DNA characterization of the spirochete that causes Lyme disease. J Clin Microbiol 20: 155-158.

26. 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.

27. 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 4: in press.

28. Postic, D., J. Belfaiza, E. Isogai, I. Saint Girons, P. A. D. Grimont and G. Baranton. A n e w genomic species in Borrelia burgdorferi sensu lato isolated from japanese ticks. Res Microbiol 1993; 144: in press.

29. Barbour, A. G. and M. Schrumpf. Polymorphisms of major surface proteins of Borrelia burgdorferi. Zbl Bakt Hyg A 1986; 263: 83-91.

30. Rosa, P. A., D. Hogan and T. G. Schwan. Polymerase chain reaction analyses identify two distinct classes of Borrelia burgdorferi. J Clin Microbiol 1991; 29: 524532.

31. Barbour, A. G. and C. F. Garon. Linear plasmids of the bacterium Borrelia burgdorferi have covalently closed ends. Science 1987; 237: 409-411.

32. Baril, C., C. Richaud, G. Baranton and I. Saint

Girons. Linear chromosome of Borrelia burgdorferi. Res Microbiol 1989; 140 :507-516.

33. Ferdows, M. S. and A. G. Barbour. Megabase-sized linear DNA in the bacterium Borrelia burgdorferi, the Lyme disease agent. Proc Natl Acad Sci USA 1989; 86: 59695973.

34. Davidson, B. E., J. MacDougall and I. Saint Girons. Physical map of the linear chromosome of the bacterium Borrelia burgdorferi 212, a causative agent of Lyme Disease, and localization of rRNA genes. J Bacteriol 1992; 174: 3766- 3774.

35. Old, I. G., J. Mac Dougall, I. Saint Girons and B. E. Davidson. Mapping of genes on the linear chromosome of the bacterium Borrelia burgdorferi: possible locations for its origin of replication. FEMS Microbiol Lett 1992; 99: 245- 250.

36. Walker, E. M., J. K. Arnett, J. D. Heath and S. J. Norris. Treponema pallidum Subsp pallidum has a single, circular chromosome with a size of approximately 900 kilobase pairs. Infect Immun 1991; 59: 2476- 2479.

37. Zuerner, R. L. Physical map of chromosomal and plasmid DNA comprising the genome of Leptospira interrogans. Nucleic Acids Res 1991; 19: 4857-4860.

38. Baril, C., J. L. Herrmann, C. Richaud, D. Margarita and I. Saint Girons. Scattering of the rRNA genes on the physical map of the circular chromosome of Leptospira interrogans serovar icterohaemorrhagiae. J Bacteriol 1992; 174: 7566-7571.

39. Zuerner, R., J. Hermann and I. Saint Girons. Comparison of genetic maps for two Leptospira interrogans serovars provides evidence for two chromosomes and intraspecies heterogeneity. J Bacteriol 1993; 175: 5445-5451.

40. Crespi, M., E. Messens, A. B. Caplan, M. Van Montagu and J. Desomer. Fascination induction by the phytopathogen Rhodococcus fascians depends upon a linear plasmid encoding a cytokinin synthase gene. The EMBO J 1992; 11: 795- 804.

41. Leblond, P., M. Redenbach and J. Cullum. Physical map of the Streptomyces lividans 66 genome and comparison with that of the related strain Streptomyces coelicolor A3(2). J Bacteriol 1993; 175: 3422- 3429.

42. Anthonissen, F. M., M. De Kesel, P. P. Hoet and G. H. Bigaignon. Evidence for the involvement of different genospecies of Borrelia in the clinical outcome of Lyme disease in Belgium. Res Microbiol submitted

43. Wilske, B., V. Preac-Mursic, U. B. Gobel, B. Graf, S. Jauris, E. Soutschek, E. Schwab and G. Zumstein. An OspA serotyping system for Borrelia burgdorferi based on reactivity with monoclonal antibodies and OspA sequence analysis. J Clin Microbiol 1993;31:340-350.

44. Fikrig, E., S. W. Barthold, F. S. Kantor and R. A. Flavell. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. Science 1990; 250: 553-556.

45. Zumstein, G., R. Fuchs, A. Hofmann, V. Preac-Mursic, E. Soutschek and B. Wilske. Genetic polymorphism of the gene encoding the outer surface protein-A (OspA) of Borrelia burgdorferi. Med Microbiol Immunol 1992; 181: 57-70.

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