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Antigenic and genetic structure of *Borrelia burgdorferi*

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Abstract

Lyme borreliosis is a disease caused by the spirochaetes *Borrelia burgdorferi*, *Borrelia afzelii* and *Borrelia garinii* and it is transmitted by ticks. Most of the proteins (outer surface proteins, flagellar proteins and other uncertain location proteins) have a strong antigenic variability. Osp A protein genetic and serological studies facilitated the differentiation of seven serotypes strongly correlated with the known genospecies. The genetic structure of these spirochaetes included a large linear chromosome, several linear microchromosomes as well as a number of circular plasmids.

Introduction

Lyme borreliosis is a worldwide disease caused by the spirochaete *Borrelia burgdorferi*, transmitted by *Ixodes* species (Huppertz, 1990). The earliest clinical descriptions were recorded in Europe at the beginning of this century. The disease was recognized by Steere *et al.* (1977) in Lyme, Connecticut, U.S.A., in an oligoarticular arthritis epidemic in children residing in this locality. Burgdorfer and Kieraus (1982) isolated the aetiological agent from the mid-intestine of *Ixodes scapularis* var. *dammini* from an endemic zone in New York State. This spirochaete, designated *B. burgdorferi*, was later isolated from cerebrospinal fluid (CSF) and blood of some patients (Steere *et al.*, 1983; Benach *et al.*, 1983). The number of reported cases of Lyme borreliosis has increased significantly in recent years due the current availability of serological diagnostic methods and the awareness of clinicians.

There are geographical differences with regard to the type of causative spirochaete and the clinical symptoms of the disease. A genetic variability between several types of causative disease of Lyme borreliosis has been reported. Three genospecies have been designated *B. burgdorferi*, *B. afzelii*, and *B. garinii*, and seven serotypes have been defined by their reactivity with antibodies against several protein epitopes from the external surface (Osp A): serotype 1 corresponds to *B. burgdorferi sensu stricto*, serotype 2 corresponds to *B. afzelii* and serotypes 3 to 7 to *B. garinii* (Wilske *et al.*, 1990).

Recently, *B. japonica* has been accepted (group F63B). It was isolated from *Ixodes ovatus* but its pathogenic effects were not proved (Baranton *et al.*, 1992; Canica *et al.*, 1993; Kawabata *et al.*, 1987; Postic *et al.*, 1993).

Various pathogenic and organotropic potentials have been suggested for the different Osp A serotypes (Wilske *et al.*, 1993a; Adam *et al.*, 1991; Peter and Bretz, 1992). The wide spectrum of a clinical outbreak can be correlated by the awareness of clinicians, at the beginning of the disease, to the genetic tendency in the population and to strain diversity. According to Holt *et al.* (1994), spirochaetes of the genus *Borrelia* belong to the family Spirochaetaceae in the *Spirochaetales*. Morphologically, they are spiral micro-organisms with three to ten spirals, 3–20 μm long and 0.2–0.5 μm in diameter. An external membrane encloses the protoplasmic cylinder formed of a cap of glycopeptides and a cytoplasmic membrane which encloses the protoplasmic cellular content (Figure 1).

Antigenic structure

Outer surface proteins (Osp)

A number of lipoproteins from the external membrane have been identified and designated Osp (outer surface protein) A, B, C, D, and F. They are encoded by linear and circular plasmids (Figure 1). The Osp A protein has a molecular weight of 30.5–33.0 kD. The European and American strains belonging to *B. burgdorferi sensu stricto* express a 31 kD Osp A. The Osp A proteins in strains from *B. garinii* and *B. afzelii* have an apparent molecular weight of 32 to 33 kD (Baranton *et al.*, 1992; Adam *et al.*, 1991). The strains from *B. japonica* have an Osp A protein of 31 kD, which is the same molecular weight as *B. burgdorferi sensu stricto* (Postic *et al.*, 1993).

Variability in the size of Osp B is notably greater in American strains of *B. burgdorferi sensu stricto* (Lane and Pascocello, 1989). Strains of *B. burgdorferi sensu stricto* and *B. afzelii* have an Osp B protein of 34 and 35 kD, respectively. The 34 kD protein might correspond to Osp B in strains from group F63B. This lipoprotein is apparently not present in strains from *B. garinii,* but such lipoproteins can be induced after numerous passages *in vitro*.

The monoclonal antibody I-17,3 (Schwan and Burgdorfer, 1987) specifically reacts with the 35 kD protein corresponding to Osp B in strains from *B. afzelii*. European strains (40%) show a protein of ~22 kD, designated Osp C (Canica *et al.*, 1993). Likewise ~50% of American strains express an analogous protein of 20 to 25 kD (Lane and Pascocello, 1989; Bisett and Hill, 1987).

Although not yet completely immunogenetically studied, it has been suggested that the Osp D protein is a virulence factor in *B. burgdorferi*. (Norris *et al.*, 1992). The Osp D molecular weight is ~30 kD and it is encoded by a linear plasmid of 38 kb (Norris *et al.*, 1992).

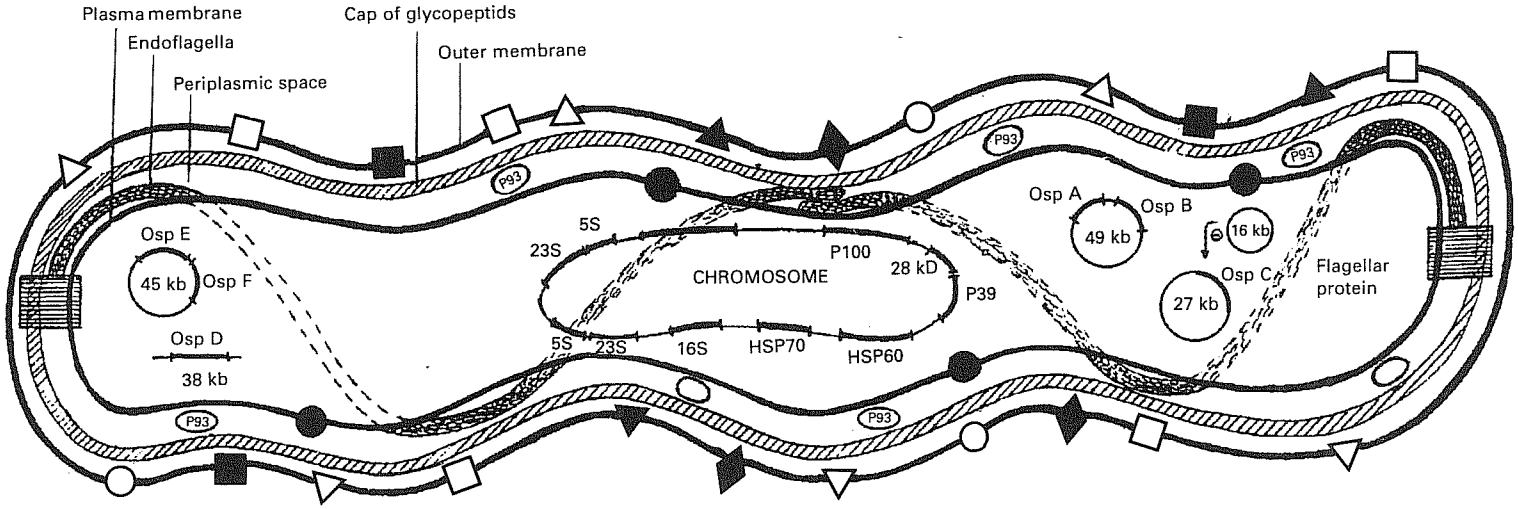

Figure 1 Antigenic and genetic structure of *B. burgdorferi sensu stricto*. Unknown location proteins, HSP60, HSP70, 12 kD proteins. Osp A, △; Osp B, □; Osp C, ○; Osp D, ▲; Osp E, ■; Osp F, ◆; P39, ●.

Recently genes for another two lipoproteins from the outer surface have been cloned, and designated Osp E and Osp F, with molecular weights of ~19 and 26 kD, respectively, and encoded by the 45 kb plasmid (Lam *et al.*, 1994). Spontaneous variations in number and size of Osp proteins have been seen in subcultures *in vitro* and this may explain the lack of effectiveness of the host immunity mechanisms.

Flagella

The flagella are located under the external membrane, linked to the opposite extremes of the protoplasmic cylinder, and consist of seven to thirty axial filaments, periplasmic flagella or endoflagella. Their free extremes extend to the middle of the cell, and are superimposed here (Figure 1). The Western blot band of 41 kD corresponds to this flagellar antigen. Other oral *Borrelia* and *Treponema* species, causal agents of periodontal disease, and occasionally *Leptospira*, have a similar protein (Barbour and Schrumpf, 1986). This antigenic component and the one corresponding to the 93 kD band are not chemically lipoproteins (Wilske *et al.*, 1993a). Antibodies against the 41 kD protein have been more frequently found in the series studied, and they are the earliest antibodies appearing during infection, at the same time as antibodies against the Osp C protein. The flagellar antigen is one of the most immunogenic and unspecific antigens. The presence of antibodies against the 41 kD flagellar protein may be the cause of many clinical outbreaks in the course of neuroborreliosis. Moreover, it can be the consequence of the release of antigens from the nervous system. The immune system contact with this antigen shares reactivity with that protein (Gutierrez *et al.*, 1994).

Unknown or undefined locations

The 93 kD protein seems to be located in the periplasmic space (Volkman *et al.*, 1991). It is encoded by the chromosome, is species-specific, and may be a marker in Lyme disease.

The antigen corresponding to the Western blot 39 kD band is the most specific one in *B. burgdorferi*. Therefore it should be considered as one of the major antigenic components used for serological diagnosis by ELISA (Sullivan *et al.*, 1994). It is likely to be located in the cytoplasmic membrane, and sometimes inside the cytoplasm (Sullivan *et al.*, 1994). Simpson *et al.* (1990) have shown that the 6.3 kb fragment encodes the P39 lipoprotein and the 28 kD antigen of *B. burgdorferi*. The 12 kD protein is species-specific but its function and location are currently unknown.

Heat shock proteins (HSP) have been examined, especially HSP60 and HSP70, due to their immunogenicity in Lyme borreliosis patients (Anzola *et al.*, 1992; Girouard *et al.*, 1993). These proteins are

encoded by chromosomal genes and their polymorphism is lower than Osp genes. It has been shown by the comparison of HSP60 sequences from two strains belonging to two different genetic groups. Their homology is >95% at the DNA sequences level and >99% at the protein level (Wallich *et al.*, 1992).

Genetic structure

The structural analysis of the *B. burgdorferi* genome is composed of a large linear chromosome, with ~10^6 bases, additional linear micro-chromosomes and several circular, coiled plasmids (Figure 1). These organisms have vesicles containing nucleic acids. The vesicles may mediate the transfer of DNA among borrelias (Bergstrom *et al.*, 1992). The plasmid size is 1.5–50 kb. In the genetic map, Osp A and Osp B genes have been located in the 49 kb linear plasmid (Bergstrom *et al.*, 1991).

The Osp A gene in B31 (*B. burgdorferi sensu stricto*), PKo (*B. afzelii*) and PBi (*B. garinii*) strains has been studied. It is constituted by an 819 to 822 b region. The sequence of the open reading frame in *B. garinii* and *B. afzelii* strains contains six bases more than B31 strain (Johnson *et al.*, 1992a; Marconi *et al.*, 1993b; Zumstein *et al.*, 1992). The Osp A sequence analysis shows that the leader sequence is included in the first third of the protein, and it is more conserved than the terminal C-domain. The greater variability is observed in the middle zone of the protein. The homology among Osp A from B31 and ZS7 strains in *B. burgdorferi* is 99%. Among strains belonging to *B. garinii* and *B. afzelii*, the homology is 73 and 83%, respectively (Zumstein *et al.*, 1992).

A typing method based on the genetic DNA hybridization has been proposed. The oligonucleotides used are derived from the Osp A gene variable region (Zumstein *et al.*, 1992). The variability of the Osp A phenotype was used to establish a serotyping system perfectly correlated with the genetic classification and correlated with the different clinical outbreaks of the disease. At least 10% of strains did not express Osp A (Wilske *et al.*, 1993a).

The Osp B gene in the B31 strain contains an open reading frame with 888 nucleotides which encode a 296 amino acid protein. Genes in the strains from *B. afzelii* (ACA1 strain) and *B. garinii* (Ip90 strain) show an homology of 79% with Osp B from B31 and 81% among them. The Osp B locus shows great heterogeneity in its nucleic acid sequence. Thus, Osp B polymorphism was seen in a clonal strain from *B. burgdoferi*, related to the apparent molecular weight and to reactivity with monoclonal antibodies. This suggests that the Osp B locus is not a good candidate for diagnosis (Shoberg *et al.*, 1994).

The Osp C gene was cloned and sequenced by Fuchs *et al.* (1992). It is located in a 27 kb circular plasmid and is down-regulated by a repressor encoded by a 16 kb linear plasmid (Marconi *et al.*, 1993b; Sadziene *et al.*, 1993). The gene length varies according to genetic species. It can be 630, 636 and 621 nucleotides, corresponding to a 210, 212, 207 amino acid protein in *B. burgdorferi*, *B. afzelii* and *B. garinii*, respectively (Fuchs *et al.*, 1992; Jauris-Heipke *et al.*, 1993). Furthermore, there is variability in the Osp C gene in different isolates. Although this gene is found in all of them, not all the isolates transcribe it actively. The B31 strain, used mainly in serological methods in Lyme disease, does not express Osp C (Padula *et al.*, 1993). Marconi *et al.* (1993b) have found the Osp C gene in every 21 isolates studied, expressing or not expressing protein. The gene can be controlled by several promoters which are very similar in their nucleotide sequence. The gene sequence analysis shows much polymorphism among species and even in the same species, particularly in *B. garinii*. The observed homology was 70 to 74% in the Osp C protein from the three species (Wilske *et al.*, 1993b; Jauris-Heipke *et al.*, 1993). The comparison among protein sequences does not show similarity among Osp C and Osp A (Fuchs *et al.*, 1992), although an antigenic relationship has been revealed among both proteins using monoclonal antibodies (Wilske *et al.*, 1988). When oligonucleotide probes were used for the Osp C gene from several spirochaetes *B. burgdorferi*, *B. garinii* and *B. afzelii*, an homologous Osp C was detected in other species of Borrelias, including *B. coriaceae*, *B. hermsii*, *B. anserina*, *B. turicatae* and *B. parkeri*.

The Osp D gene was located in the linear plasmid from *B. burgdorferi* strains with a low number of passages but it was absent in non-virulent strains with a high number of passages. The Osp D gene was not found in all the spirochaetes isolated in Lyme disease, but it appeared in 90 and 24% of *B. garinii* and *B. burgdorferi*, respectively. The gene analysis showed that it was the target of some recombinations and was recently acquired by *B. burgdorferi* and transferred laterally among species (Marconi *et al.*, 1994).

Recently, Osp E and Osp F genes have been cloned. They were structurally classified in a transcriptional unit under the control of a common promoter. The Osp E gene was located in the 5′ end and was composed of 513 nucleotides encoding a 171 amino acid protein. The Osp F gene was located 27 bp downstream the stop codon of the previous gene, and consisted of 690 nucleotides, generating a 230 amino acid protein (Lam *et al.*, 1994).

Most spirochaetes show complex flagellar filaments constituted by a set of polypeptides. *B. burgdorferi* and *Spirochaete zuelzerae* are

an exception. Their filaments are composed of a single protein formed by 336 amino acids in *B. burgdorferi* (Wallich *et al.*, 1990). The flagellin gene is located in the chromosome (Gassmann *et al.*, 1991). This gene shows a high degree of conservation among different strains of *B. burgdorferi*, and constitutes an adequate sequence for its own amplification (Kramer *et al.*, 1990; Johnson *et al.*, 1992b). Greater variability is evident in the central region protein (Gassmann *et al.*, 1991; Jiang *et al.*, 1992; Luft *et al.*, 1991). The epitope recognized by monoclonal antibodies H9724 (Barbour *et al.*, 1986), specific to the *Borrelia* genus, is located between amino acids 90 and 266 (Collins and Peltz, 1991).

Luft *et al.* (1991) showed that the immune response in patients was targeted against antigenic domains located in the variable and preserved regions of the protein. The nucleotide differences in the variable parts of the flagellin were used by Picken (1992) to build probes in order to differentiate the three *B. burgdorferi*, *B. garinii* and *B. afzelii* species. The variability in the region between amino acids 180 and 230 was confirmed by Jauris-Heipke *et al.* (1993).

The 16S rRNA sequence analysis shows that spirochaetes as a rule constitute a coherent taxon composed of six greater classes or groups. The third group of spirochaetes includes *B. burgdorferi*, *B. anserina*, *B. hermsii*, and a tick of rabbit *borrelia* strain. Borrelias constitute a close filogenetic class, with a mean similarity of 97% (Paster *et al.*, 1991).

There is a single gene *rrs* in *B. burgdorferi* rRNA encoding the 16S rRNA unit and there were two copies of both genes *rrl* and *rrf* encoding 23S rRNA and 5S rRNA units, respectively. These genes are located within a chromosomic fragment which consists of ~9.5 to 10 kb. The 23S and 5S rRNA genes are arranged in the sequence 23S-5S-23S-5S and they are not apparently joined to the 16S rRNA gene, which is located 2 kb upstream of the 23S-5S duplication. The individual copies from the 23S-5S duplication are separated by a 182 bp space. Within each 23S-5S unit, an identical 22 bp space separates the 23S and 5S rRNA sequences. This fact has not been observed in other bacteria (Davidson *et al.*, 1992; Fukunaga *et al.*, 1992). These rRNA genes are independently expressed in *B. burgdorferi* (Fukunaga *et al.*, 1992).

Various geographical isolates from *B. burgdorferi* can be differentiated on the basis of restriction fragment length polymorphism (RFLP). This polymorphism can be a useful instrument in determining genetic relationships among different isolates from *B. burgdorferi* (Schwartz *et al.*, 1992). On the other hand, this important genetic variability produces different results in the

diagnostic methods and constitutes the basis for the use of various antigens for indirect diagnosis in Lyme disease.

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