Lyme Borreliosis: From Etiological Agent to Human Pathogenesis

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Abstract

Lyme borreliosis, a multisystem disorder, is caused by spirochaetes of the *Borrelia* (*B.*) *burgdorferi* sensu lato complex, which are transmitted by ticks. In the last years the disease has become a global public health problem and a prototype of an emerging infection. This review presents a large body of information on cellular and molecular biology of *B. burgdorferi*, interaction with the human host, immune response and disease pathogenesis.

Keywords: *Borrelia burgdorferi* sensu lato, Lyme borreliosis, pathogenesis.

1. INTRODUCTION

Lyme borreliosis (LB), a tick borne disease with the highest incidence in Europe and North America, is a zoonosis; *Borrelia burgdorferi* sensu lato, the etiological agent, circulates between the *Ixodes ricinus* complex ticks and a variety of vertebrate hosts like rodents, squirrels and many species of birds. Natural hosts do not appear to develop clinical disease, while in humans LB presents with a variety of clinical signs and symptoms, affecting the skin, the heart, the nervous and musculoskeletal systems, and several variations in the course of the disease. LB has been first described in 1977 in the United States, though documentations of the disease exist from the end of the XIXth century in Europe. Recent molecular studies have identified sequence of *B. burgdorferi* DNA in “the Iceman” from Tyrol, a 5300 years old human body discovered in the Alps (Keller *et al.*, 2011). As in the last years the disease has become a global public health problem and the interest of medical personnel and researchers is constatly increasing, the review presents information on cellular and molecular biology of *B. burgdorferi*, interaction with the human host, immune response and disease pathogenesis.

2. THE ETIOLOGY OF LYME BORRELIOSIS

The first description of LB was made in Germany in 1883 as a chronic dermatological disease, named acrodermatitis chronica atrophicans (ACA). In 1909, the Swedish dermatologist Afzelius described a slowly expanding cutaneous lesion, named erythema migrans (EM) and in 1930 Hellerstrom suggested a relationship between tick bite, EM and disorders of the nervous system. In 1975-1976 in the town of Lyme, USA, cases of arthritis in children were described, characterized by debut in summer or autumn, short and recurrent attacks of tumefaction and pain in the large joints, preceded by a skin rash. This new entity was named Lyme arthritis, but in the following years a multisystemic disorder was described, named Lyme disease and later, after the discovery of the etiological agent, Lyme borreliosis (Burgdorfer *et al.*, 1982).

In 1982 Burgdorfer described a spirochete of the genus *Borrelia* in the body of the *Ixodes* ticks, identified as the etiological agent of LB and named *Borrelia burgdorferi*. Due to the development of new molecular diagnostic tools, new species of *B. burgdorferi* were described in the following years;

nowadays 19 different species are identified with different geographical distribution (Franke *et al.*, 2013).

The genus *Borrelia*, a member of the spirochaete phylum, is divided into three major pathogenic groups of organisms, all transmitted by arthropods: *B. burgdorferi* sensu lato (the agent of LB), the relapsing fever borreliae, and the etiological agent of avian spirochaetosis, *Borrelia anserina*. Members of the genus *Borrelia* have a very complex lifestyle as they are host associated spirochaetes that shuttle between haematophagous arthropods and vertebrates.

2.1. Morphology, Shape and Membrane Structure

B. burgdorferi is a thin, elongated, motile, wave-like bacteria with the diameter of 0.2 µm and length of 15 µm. The bacterium has a fragile outer membrane surrounding the protoplasmic cylinder. This cylinder consists of a peptidoglycan layer, a cytoplasmic membrane (inner membrane) and the enclosed cytoplasmic contents (Johnson *et al.*, 1984). 7–11 bipolar flagella are located in the periplasmic space attached to the poles and wrapped around the cell cylinder, giving the bacterium its characteristic flat wave shape (Motaleb *et al.*, 2000).

2.1.1. Flagella, motility and chemotaxis

The periplasmic flagella, with an important role in morphology and motility, presents a filament, a hook and basal body, but it is composed by two classes of proteins, instead of one as in other bacteria. The external flagellar protein Fla A is characteristic to the spirochetes, while Fla B presents homology to the flagellar proteins of other bacteria. A feature of *B. burgdorferi* s.l. is its capacity to swim efficiently in a viscous medium such as the connective tissue where other bacteria are slowed down or immobilized.

2.1.2. Structure of the outer membrane

Since *B. burgdorferi* s.l. possesses both an outer membrane and a cytoplasmic membrane, the surface of this bacterium is analogous to enteric Gram-negative bacteria, but it has also several distinct features. For example, *B. burgdorferi* s.l. has a high abundance of membrane proteins covalently modified with lipids, has no lipopolysaccharide (LPS) but presents glycolipid antigens other than LPS. The *B. burgdorferi* outer membrane contains a low density of transmembrane-spanning proteins, being more

susceptible to disruption by routine physical manipulations and to detergents compared with Gram-negative bacteria (Bergström *et al.*, 2002).

A. The lipoproteins

While the spirochete passes from the arthropod to the vertebrate, there are changes from an environment with no immune system based on antibodies to an environment with high immunological pressure. To persist in its host, *B. burgdorferi* changes its surface antigens. More than 100 polypeptides have been identified in the outer membrane, but the sequencing of *B. burgdorferi* genome has defined many other hypothetical lipoproteins. The most studied are the major **outer surface proteins** (Osps). Until now 6 Osps have been characterised, OspA-F, exposed to the surface and fixed through a lipidic fraction to the external membrane.

The first *B. burgdorferi* gene sequenced, *ospA*, encodes the most studied protein of the bacteria. OspA is expressed by the spirochete in the midgut of unfed tick and mediates binding to the intestinal epithelium. OspA and OspB are implied also in the adherence and penetration of the mammalian host cell, having a role in the pathogenesis of LB. OspC is very heterogenic, 19 major OspC groups being identified. The expression of OspA and OspB decreases while the tick is feeding. OspC expression is induced 36–48 hours after the blood meal starts and mediates the escape of the spirochaete from the tick midgut via the haemolymph to the salivary glands from where it enters the new vertebrate host. OspD is another component of the outer membrane, while OspE and OspF are the founding members of a protein family known as the **OspEF-related proteins** (Erps), important for the virulence of *B. burgdorferi* (they bind complement factor H and inhibit complement activation). Recombination of the genes encoding the Erp antigens might contribute to the evasion of the mammalian immune response.

The decorin-binding proteins. Microbial adhesion and colonization of host tissues are important events in the bacterial infection process. Spirochaetes injected in the skin are found associated with collagen fibres in the extracellular matrix, but they do not attach directly to collagen but to decorin, a small proteoglycan. There are two borrelial-encoded adhesins that can bind to decorin, DbpA and DbpB (**decorin-binding protein**), and a protein that can bind fibronectin-

BBK32. The interaction between BBK32 and fibronectin seems to be an important pathogenic mechanism for dissemination and persistence of *B. burgdorferi* into the mammalian host.

The vmp-like sequence, Vls, of *B. burgdorferi* s.l. Persistence of *B. burgdorferi* in the host can be achieved by a mechanism called antigenic variation; this mechanism has been studied for several years in relapsing fever borreliosis where new serotypes appear during relapses due to a change in surface proteins called variable major proteins (Vmps). In *B. burgdorferi* s.s a linear plasmid was found to have a sequence that resembles the Vmp system in *B. hermsii* - *vls* locus. Recombination occurs *in vivo* as early as 4 days after experimental infection of mice but not *in vitro*, suggesting that the mammalian host provides the signal for *vls* recombination (Zhang and Norris, 1998).

RevA protein. Another surface expressed protein, revA is expressed in the mammalian host due to increase in temperature (36°C compared to 23°C in ticks). The expression of RevA is also influenced by the pH (Caroll *et al.*, 2001).

The exported plasmid protein A (*eppA*) genes are only expressed during infection in the mammalian host. The widespread occurrence of *eppA* suggests that they contribute to the survival of *B. burgdorferi* s.l. in nature, and possibly to the pathogenesis of LB (Miller *et al.*, 2000).

BmpA, BmpB, BmpC and BmpD constitute a family of homologous lipoproteins that are immunogenic during infection in vertebrate hosts and BmpA (p39) is widely used in the diagnostic assays. BmpA activates the proinflammatory response in the human synovial cells (Yang *et al.*, 2008).

B. The porins

The permeability of the bacterial cell envelopes is the result of channel forming proteins in their outer membranes, called porins. Their function is to uptake the substances from the environment. *Borrelia* are limited in their metabolic and biosynthetic capacities and therefore highly dependent on nutrients provided by their hosts. Tree porins have been described in *B. burgdorferi*: P13, P66 (Oms66) and Oms 38/ Dip A. DipA protein, which exhibits high homology to the Oms38 porin of relapsing fever *Borrelia*, presents a binding site specific for dicarboxylates which play important roles in the deficient

metabolic and biosynthetic pathways of *Borrelia* species (Bergstrom and Zuckert, 2010).

C. The envelope-spanning protein complexes

Efflux systems and transporters. A system called BesC was identified in the outer membrane of *B. burgdorferi* with properties of efflux system and export through membrane, that was shown to be necessary for infection in mice and involved in resistance to antibiotics. This efflux system has a role in maintenance of cellular homeostasis, export of exogenous toxic agents and survival in different host environments (Bunikis *et al.*, 2008). Glucose is the major energy source for *B. burgdorferi*, involving several glucose transporters. Mannose host proteins are also a carbohydrate source for *B. burgdorferi*, with BB0408 and BB0629 proteins suggested to be mannose transporters (von Lackum and Stevenson, 2005).

The oligopeptide permease (Opp A-1, 2 and 3) are used for transportation of peptides into bacteria, as carbon and nitrogen sources.

2.1.3. Blebs and cysts formation

B. burgdorferi s.l. when grown on stress culture media (antibiotics, low pH) is capable of producing extracellular vesicles (blebs), that contain an outer membrane, while some also contain cytoplasmic membrane (Radolf *et al.*, 1994). The cyst formation is a response to starvation in serum-free medium and it implies changes in the cell integrity and form (Alban *et al.*, 2000). It is a slow but active process that needs protein synthesis, reversible in 50% of the cases. It is not known if cyst formation is implied in the persistence of *B. burgdorferi* in the host, with a role in pathogenesis, immune evasion and/or resistance to antibiotics (Brorson and Brorson, 2006; MacDonald, 2006).

2.2. The Genome and Genome Organization

The genetic organization of *B. burgdorferi* is unusual in the sense that it contains a small linear chromosome and 21 linear and circular plasmids (most bacterial species present a circular chromosome and circular plasmids). Most of the genome of the *B. burgdorferi* sensu stricto (s.s) strain B31 was published in 1997 (Fraser *et al.*, 1997) and it was completed in 2000 (Casjens *et al.*, 2000), with a genome size of 1.52 Mbp: 910 725 bp on the linear chromosome, and 611 kbp divided on 12 linear (lp) and 9 circular plasmids (cp), and only 1283 genes (compared with the 4405 genes present in *E. coli*). The small number of genes in

B. burgdorferi s.s is similar to the number of genes in the related spirochaete *Treponema pallidum*; a number of genes encoding cellular biosynthetic enzymes are missing. The genome of 14 strains of *B. burgdorferi* s.s. was completed till now (Casjens *et al.*, 2000; Schutzer *et al.*, 2011), *B. afzelii*, *B. garinii*, *B. bissettii*, *B. valaisiana* and *B. spielmanii* (Schutzer *et al.*, 2012).

2.3. *B. burgdorferi* Sensu Lato Complex

The 19 different genomic species of *B. burgdorferi* are classified based on their pathogenicity to humans.

2.3.1 *Borrelia* Species Pathogenic for Humans

B. burgdorferi sensu stricto (s.s). In USA it is the only human pathogenic species, associated mainly to the musculoskeletal manifestations of LB, but also to the cutaneous and neurological manifestations. In Western Europe it is the species mainly isolated from arthritis (Jaulhac *et al.*, 2000).

B. garinii. 6 serotypes have been described using monoclonal antibodies, out of which serotype 4 has been further classified as a different genospecies, *B. bavariensis* (Margos *et al.*, 2009). Concerning the organotropism of *B. garinii*, the neural apparatus is the main target (Wilske *et al.*, 1996), but rarely *B. garinii* has been isolated from the synovial fluid (Fingerle *et al.*, 2008), or even from the cutaneous lesions of ACA.

B. afzelii has an organotropism for skin, being the etiological agent mainly isolated from ACA (Lünemann *et al.*, 2001), but it was also isolated from the cerebrospinal fluid (CSF) or joints.

B. spielmanii. Until now it was isolated only from the EM lesion (Maraspin *et al.*, 2006); it is not clear if it has an invasive potential.

B. bissettii. In Europe it was rarely isolated from the EM lesions (Strle *et al.*, 1997), from the cardiac valve tissue (Rudenko *et al.* 2008), from the serum of some patients with the suspicion of LB (Rudenko *et al.* 2009), or from the CSF (Fingerle *et al.*, 2008).

B. valaisiana. The pathogenic potential has rarely been described, being isolated by polymerase chain reaction (PCR) from skin (Rijpkema *et al.*, 1997), CSF (Diza *et al.*, 2004), but it was not isolated by cultivation from humans.

B. lusitaniae has been isolated from the skin of a portuguese patient (de Franca *et al.*, 2005).

2.3.2. *B. burgdorferi* species with no proven human pathogenic potential

Eleven species have not been isolated from human so far: *B. americana*, *B. andersonii*, *B. californiensis*, *B. carolinensis*, *B. japonica*, *B. tanukii*, *B. turdi*, *B. sinica*, *B. yangtze*, *B. kurtenbachii* and *B. finlandensis*.

Several strains of unclear phylogenetic identity still need to be characterized and, if applicable, defined as new species (Franke *et al.*, 2013).

2.3.3. *B. burgdorferi* species in Romania

B. afzelii, *B. garinii*, *B. burgdorferi* s.s., *B. valaisiana* and *B. lusitaniae*, species with human pathogenic potential, have been identified in Romania in questing ticks (Kalmár *et al.*, 2013) and in ticks collected from humans or animals (Briciu *et al.*, 2014; Ionita *et al.*, 2013).

3. EPIDEMIOLOGY OF LB

3.1. Terminology

A vector tick is able to acquire the pathogen during the blood meal, maintains it through one or more life stages and passes it on to other hosts when feeding again. In rare cases uninfected ticks can also acquire an infection when feeding together - "co-feeding"- with infected vector ticks on a host, when mating, or the female infects transovarially the eggs.

Tick hosts include all vertebrates that ticks feed on in nature. A reservoir host must take up a critical number of infectious agents during an infectious tick bite, must allow the pathogen to multiply and to survive in its body and must allow the pathogen to find its way into other feeding ticks. Non-reservoir hosts may have contact with infected ticks and may or may not develop a long-lasting infection but are incapable of transmitting the infection to ticks. Barrier hosts eliminate the infection in feeding ticks (zooprophylactic host), or destroy a large numbers of vector ticks because of efficient grooming or an acquired anti-tick immunity (Kahl *et al.*, 2002).

3.2. Vectors

Ticks are classified in the class Arachnida, subclass Acari, order Parasitiformes, and suborder Ixodida. There are approximately 878 species, divided into four families, specifically the Argasidae (soft-bodied ticks), Ixodidae (hard-bodied ticks), Nuttalliellidae, and Laelaptidae (Anderson JF, Magnarelli, 2008). The identification of ticks uses a holistic system (Estrada-Peña *et al.*, 2004).

I. ricinus, the most important vector of *B. burgdorferi* in Europe, has a life cycle between 2-6

years (it may vary from one habitat to another, influenced by climatic factors). The stages of development are represented by eggs, larva, nymphs and adults. *I. persulcatus*, the taiga tick is the vector of LB in the southern forest zone of Eurasia and some countries from Asia. *I. ricinus* and *I. persulcatus* overlap in far-eastern Europe. In the East and Center of North America the main vectors are *I. scapularis* ticks, while *I. pacificus* is the main vector in the Western regions, along the Pacific coast.

B. burgdorferi s.l., ingested with the blood meal, colonize the mid-gut of the tick and persist there along the different stages of development (trans-stadial transmission).

3.3. Reservoir hosts

At least 237 animal species are implied in the maintenance of *I. ricinus* population and are susceptible to be reservoir hosts for *B. burgdorferi* (Gern, 2008). The xenodiagnosis, the only way to prove the infectivity of a vertebrate species, implies capturing and keeping in captivity the vertebrate. The reservoir host does not develop the disease, though minor symptoms may be present. It was proved that rodents, squirrels and many species of birds are reservoir hosts. Out of the large mammals, the red foxes are reservoir hosts while large ungulates are not, but they contribute indirectly to spirochetes transmission, being hosts for ticks. Migratory birds play a key role for LB distribution, since they are not only a reservoir for important *Borrelia* spp., but they also act as vehicles of infected ticks over long distances (Rudenko *et al.*, 2011). The description of new species of *Borrelia* in the last years has opened a new domain in LB ecology that suggests an association between certain genospecies and vertebrate hosts.

4. B. BURGDORFERI INTERACTION WITH THE HOST

4.1. The Bite Site

The tick creates a feeding pit with its mouthparts, using its hypostome (a barbed protuberance) as an anchor to the skin of the host, while bioactive agents from tick saliva are inoculated into the skin that increase *B. burgdorferi* chances of survival (Radolf *et al.*, 2012). Proteins BbCRASPs (*B. burgdorferi* complement regulator-acquiring surface proteins) bind complement factor H and complement factor Hlike protein

1. The first recognition of *B. burgdorferi* by the mammalian host is performed by the dendritic cells and macrophages in the dermis. Production of cytokines and chemokines by neutrophils results in the recruitment of additional inflammatory cells to the bite site. *B. burgdorferi* is a very motile organism that may escape from these phagocytes (Moriarty *et al.*, 2008); the spirochetes that do not escape are ingested and degraded into the phagosomes. Dendritic cells that have taken up spirochaetes migrate to the lymph nodes, where they present processed borrelial antigens to T cells and B cells. Sensitized T cells enter the circulation and are recruited to the site of infection. Plasma cells secrete specific antibodies that can kill *B. burgdorferi* via complement-dependent and -independent pathways.

4.2. Dissemination

After a delay of up to 2 days, *B. burgdorferi* begins to spread to distant tissues. To disseminate, *B. burgdorferi* penetrates the matrix between cells and enters capillary beds. The spirochaete circumvents its inability to produce enzymes capable of digesting extracellular matrix components by appropriating host proteases, such as plasminogen and urokinase. Egress from the circulation into tissues involves adhesion to vascular endothelium, followed by extravasation and expression of several adhesins that mediate attachment to host tissue.

4.3. Immune Response

Both the innate and adaptive immune systems are important for controlling infection during the disseminated phase, as mice that are deficient in either system have greater bacterial burdens than wild-type mice (Weis and Bockenstedt, 2010). The early development of IgM antibodies independent of T cells is crucial for clearing the pathogen. T cell-dependent production of IgG by B cells is detectable later during infection.

The need for serological tests to diagnose LB has led to increased research on the immune response during *B. burgdorferi* infection. Flagellin B induces an early and intense response in antibodies, but has the disadvantage of cross-reactivity with antigens of other bacteria. OspC induces an intense immune response early during infection but presents a heterogeneity of epitopes in the different strains. In spite of its role in antigenic variation, VlsE presents a highly immunogenic epitope both in early and late infection, being used

in diagnostic assays. The IgM and IgG antibodies develop slowly, directed towards a large number of proteins, as long as infection progresses. The first appear the antibodies against flagellin (FlaB) and p66, followed, as *B. burgdorferi* disseminates in the host, by antibodies anti OspC, VlsE, BBK32, Fla A, BmpA (Wilske *et al.*, 2007).

As almost half of the patients with EM do not present detectable antibodies, the lack of serological response does not exclude the diagnostic of EM (Dandache and Nadelman, 2008). The existing data show an unpredictable response in antibodies after therapy, variable and not correlated with clinical evolution (Glatz *et al.*, 2006). The persistence of IgM and IgG antibodies has been described 10-20 years after infection in asymptomatic patients (Kalish *et al.*, 2001). It is assumed that the sustained positive titers indicate a long-term serologic memory that results from an antigen-independent polyclonal activation and differentiation of memory B cells. IgG antibodies are found in high titers in patients with Lyme arthritis.

4.4. Immune Evasion

Antigenic variation (VlsE) represents the main mechanism for infection in mammalian hosts (Bankhead and Chaconas, 2007).

Inhibition of complement cascade. Through BbCRASP *Borrelia* binds the factor H proteins and inhibits the binding of active C3b on its surface, protecting the bacterium against complement-mediated killing (Kraiczy *et al.*, 2001).

Protective niches. *B. burgdorferi* may survive for at least one year in the tissue of experimentally infected mice, and due to its motility and invasiveness, it may penetrate in the conjunctive tissue (Cabello *et al.*, 2007). Though in vitro data have shown that *B. burgdorferi* may survive in intracellular niches (Girschick *et al.*, 1996), in vivo spirochetes have been visualized only extracellular. New studies are needed to sustain the role of niches in persistent infection.

4.5. Persistence of Infection

B. burgdorferi has the particularity of an extracellular pathogen that can cause persistent infection. Although there are many studies that suggest the role of immune response in controlling infection, in some tissues, like joints, the evasion of the immune response may be explained by the affinity of the spirochetes to the relatively avascular conjunctive tissue, that could impede

the penetration of phagocytes, antibodies or even antibiotics. The persistence of spirochetes in the vascular cutaneous tissue of immunocompetent mouse, visualizing the spirochetes without an associated immune infiltrate, is rather difficult to explain (Hodzic *et al.*, 2003). An explanation could be that in this "dormant" phase the spirochetes are "seen" as harmless by the immune system, possibly hiding under the host's proteins or diminishing the antigen expression or proinflammatory molecules. Another explanation could be that spirochetes in the persistent phase of infection induce changes in the environment, that suppress the recruitment of inflammatory cells. Embers *et al.* (2012) evaluated the primates *Rhesus macaque* laboratory infected with *B. burgdorferi*, proving by xenodiagnosis post-therapeutical persistence of *B. burgdorferi*. The persistence of *B. burgdorferi* was proved in mice and dogs (Straubinger *et al.*, 1997; Hodzic *et al.*, 2008), but no *Borrelia* transmission to naive mice was achieved from ticks that fed on the infected mice. It was suggested that the persistent spirochetes are attenuated and noninfectious, representing a subpopulation of persisters tolerant to antibiotics. The persistence of *B. burgdorferi* in the host and the resistance to antibiotics was suggested by some authors to be due to a phenotypic resistance and cyst formation (Hunfeld *et al.*, 2006; Sapi *et al.*, 2011) or to a biofilm (Sapi *et al.*, 2012). New studies are needed to support these theories. The main questions regarding LB are that if the spirochetes remain pathogen after antibiotic therapy, if they will persist for a long time or if they will be cleared by the immune response of the host.

4.6. Reinfection

It was shown that human immune response is not protective against reinfection in patients with EM treated with antibiotics. Reinfection, clinically manifested as different EM lesions at different moments, is described in patients with repeated tick bites, but seems to be extremely rare in patients with late manifestations (i.e. arthritis). In case of late manifestations, sustained immune response seems to offer a better protection against reinfection. It is not known yet if immunosuppression increases the risk of reinfection. The variability of different strains may explain the escape from the existent immune response after a previous infection, followed by reinfection (Nadelman and Wormser, 2007).

5. PATHOGENESIS

B. burgdorferi genome does not encode any known toxins; tissue damage, and hence disease, is believed to be caused by multiplication, invasion, persistence of *B. burgdorferi* and to the inflammatory response elicited in the mammalian host. Data regarding the pathogenesis of LB has been obtained from studies on mice, seldom on dogs or monkeys, and a few studies on human cutaneous or synovial tissue, cerebrospinal or synovial liquid. The biopsies of EM lesion in humans showed T cells, macrophages, dendritic cells and neutrophils, cytokines and chemokines but no B cells. The CSF analysis in patients with early neuroborreliosis showed a lymphocytic infiltrate with moderately increased proteinorachia and antibodies against *B. burgdorferi*. The lymphocytic pleocytosis is caused by the lymphocytes B, as *B. garinii* may stimulate the monocytes in vitro to produce CXCL13, the lymphocytes B chemoattractant. The levels of C1q, C4, C3 and C3a are increased in the CSF but not in the plasma, suggesting that the inflammatory response is localized in the CSF (Rupprecht *et al.*, 2007).

Lyme arthritis presents in two clinical forms: an acute arthritis, reproduced by the murine model and a prolonged arthritis that resembles rheumatoid arthritis (Steere and Angelis, 2006). The synovial biopsies in nontreated patients showed lymphocytic infiltrates, rare macrophages, dendritic and plasmatic cells associated with spirochetes and antigen deposits in the perivascular space. The spontaneous remission of an acute arthritis episode is immune mediated, but the spirochetes may persist in the absence of antibiotic therapy and cause recurrent episodes of arthritis. 10% of the patients with Lyme arthritis develop chronic synovitis. In studies from the '80s, these patients were presenting arthritis >12 months and responded to antibiotic therapy when it was initiated (Steere *et al.*, 1987). In the following years, a second group of patients was identified, to whom arthritis was not responding to 3 months of antibiotic therapy (Steere and Angelis, 2006), and inflammation persisted in the absence of *B. burgdorferi*. These patients present high levels of TNF alpha, IL-1 beta and IFN gamma in the synovial liquid. Many theories have been proposed to explain the pathogenesis of refractory Lyme arthritis; the autoimmune theory is based on similarities between Lyme arthritis and

rheumatoid arthritis (the presence of HLA-DRB1 molecules) (Steere *et al.*, 2006).

Biopsies from the first cases of Lyme carditis showed myocardic infiltration with lymphocytes, plasmatic cells and macrophages. Murine carditis is characterized by bacterial infiltration of the conjunctive tissue around the aortic valve and the aorta at the heart base; the leucocytic infiltrate has a peak on the 15th day of infection, with remission of carditis and spirochetal clearance after 30 days (Barthold *et al.*, 1991).

Pathogenesis research in LB has passed into the genomic era and it is likely that functional genomics will shed more light on disease-inducing mechanisms of *B. burgdorferi* s.l. in the near future.

6. CONCLUSIONS

From the public health prospective, a better information on the etiology and epidemiology of *B. burgdorferi*, its interaction with the human host and disease pathogenesis, represents a key factor for a better approach to Lyme borreliosis of medical personnel.

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