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Borrelia burgdorferi: Cell Biology and Clinical Manifestations in Latent Chronic Lyme

Aaron J. Smith, John Oertle, Dino Prato

Envita, Scottsdale, AZ, USA
Email: Aaron@Envita.com, JohnO@envita.com, DinoPrato@envita.com

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Abstract

Chronic Lyme disease is predicated by an infection with *Borrelia burgdorferi* via tick vector. *B. burgdorferi* has been extensively researched with regard to its genome and cell biology. There are many unique characteristics to the bacteria itself; however, serological diagnostics and diagnosis based on symptoms can be complicated and potentially misleading. Other promising diagnostics were also evaluated in this review. Treatment of the chronic Lyme disease can be complicated and at times ineffective. The purpose of this review is to examine *B. burgdorferi* from a biological and clinical perspective.

Keywords

Lyme Disease, *Borrelia Burgdorferi*, Genome, Cellular Process, Epidemiology, CD57, C4a, Diagnostics, Treatment

1. Introduction

The most common vector borne disease in America is Lyme disease. The disease was first categorized by European physicians as Garin-Bujadoux-Bannwarth syndrome [1] and in the US as Lyme arthritis [2]. The bacteria *Borrelia burgdorferi* (Bb) has the capacity to live in both Ixodes ticks in addition to mammalian hosts. This spirochete is unusual with respect to its genetic makeup in addition to its ability to scavenge for nutrients that are commonly synthesized by most microorganisms. Bb has the capacity to evade and attenuate the immune system which under certain circumstances, like acute early infections, can be difficult to detect Bb for diagnostic purposes. The current diagnostic criteria is problematic and often leads to type I and type II errors in addition to doctor ambiguity with regard to recognizing the symptoms associated with Lyme disease since the symptoms of

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Lyme disease mimic other common diseases. Chronic Lyme disease symptoms overlap with diseases like chronic fatigue, fibromyalgia rheumatoid arthritis and Parkinson's disease. The migration of the disease by Ixode ticks also plays a role in diagnostic criteria since locations more commonly associated with Lyme disease are more likely to be diagnosed by doctors. In other words, areas not normally associated with Lyme disease are less likely to undergo diagnostic measures to detect Bb. Difficulty in syndromic definitions can lead to failure to respond with proper antimicrobial therapy where disease progression is often associated with misdiagnosis or treatment failure.

2. Genome

There are currently 12,543 genes identified for numerous strains of Bb with various virulences. Wei-Gang Qiu and Che L. Martin [3] reviewed and analyzed 28 known strains of Bb even though there are many strains that have yet to be discovered. Bb's genome as a whole is one of the most complicated genome of any bacteria [4]-[7]. The genome consists of an approximately 950 kb in addition to several circular and linear plasmids that consists of 9 to 62 kb in length. The genome is not GC rich with about 28% of the genome consisting of G + C base pairing. Linear plasmids consist of covalently sealed telomere region [8] that requires resolvase ResT for replication [6] [9]. The vast majority of the house-keeping genes are found in the genome but there are other house-keeping genes found in various plasmids. Lipoproteins are mostly encoded in the plasmids and represent 7.8% of all open reading frames. These lipoproteins, in the enzootic cycle, are differentially expressed in plasmids [10]-[12].

Coevolution of Bb with mammalian and tick host is likely the reason why Bb lacks the ability to synthesize nucleotides, amino acids, and fatty acids [7] [13]. Bb has the capacity to import these essential precursors to functional macromolecules by importing them with possibly more than 52 transporter and/or binding proteins to sequester carbohydrates, peptides, and amino acids [14].

vls (variable major protein-like sequence) is used as a mechanism to alter the epitopes of lipoproteins in a manner that evades the mammalian host immune system [15]. There are approximately 15 cassettes upstream to the vlsE gene loci where the cassettes and the vlsE region are flanked with 17 bp of highly repetitive nucleotides. The DNA at the vlsE locus is shuffled by nonreciprocal homologous recombination [16] creating altered amino acid sequence which protects Bb from being detected by the host's adaptive immunity respectively.

3. Cellular Processes

Analysis of the genome of Bb revealed limitations of its ability to undergo de novo biosynthetic synthesis which suggests Bb dependence to the host for metabolic processes [17]. The mechanisms for acquiring nutrients from the host must be dynamic since the Bb is a parasite for two vastly different hosts; ticks and mammals. For example, host defense often involves the sequestration of key nutrients for cellular processes; an example being iron. However, Bb has evolved in a way that allows the bacteria to survive without Iron and instead uses manganese and zinc.

Bb does not have the capacity for the synthesis of purines which suggest a process that involves the uptake of guanine for the synthesis of DNA [18]-[20]. Hypoxanthine is also imported into Bb before it is modified to be incorporated into DNA as dGMP or RNA as GMP. Bb is also thought to import nucleotide monophosphates (NMP) as well as deoxynucleated monophosphate to be used for the synthesis of RNA and DNA. This notion is supported by an identified nucleotidase as a means to dephosphorylate NMP and dNMP in addition to a nucleoside transport system [7] [21]. Bb also scavenges amino acids via oligopeptide permease A (oppA) [7]. The expression of oppA-II, oppA-III, and oppA-V in murine infections suggest the possibility that these genes are induced in humans [22].

Bb has limited ability to synthesize various organic molecules and therefore is found in niche areas particularly in the enzootic lifecycle [7]. Glucose, mannose, GlcNAc, maltose, glycerol, and chitbiose are utilized by Bb *in vitro* [23]. Chitobiose utilization suggests that Bb can use ticks as a carbon source [23] [24]. Glycerol is another carbon source that is used by *B. burgdorferi* as studied in vitro by attenuating glpD and by deletion of the glp operon [25] [26].

There are no genes that encode the synthesis of fatty acids in Bb and it is hypothesized that it acquires fatty acids through scavenging the host. The cellular membrane of Bb is composed of phosphatidylglycerol (PG), phosphatidylcholine (PC), sterol galactoside, and monofalatosyl diacylglycerol (MGalDAG). There is a signifi-

cantly high proportion of polyunsaturated fatty acids in Bb than in other bacteria and it is hypothesized that this is the case since it is highly likely that Bb scavenges for fatty acids [20] [27] [28]. There is also functional evidence in vitro using *E. coli* as a model to study the synthesis of MGalDAG by monogalctosyl-1,2-diacylglycerol synthase [29], PC by phosphatidylcholine synthase, and PG by phosphatidylglycerolphosphate synthase [30].

DNA repair mechanisms for Bb are significantly limited compared to other bacterial species [31] even though UvrA was identified as intra-chain and inter-chain DNA damage repair. UvrB, UVrC and UvrD are other components associated with DNA excision repair pathways [32] and are involved in the protection of DNA from both DNA damage from UV radiation and nitrosative damage.

Motility and chemotactic function is distributed by 6% of their genes found in the linear chromosome [33]. Motility for Bb involves two motor-hook-filament structures [34] with a ribbon within the periplasmic space [35]. These structures allow Bb to travel through liquid environments like blood, cerebral spinal fluid, and lymph in addition to being able to tunnel within extracellular matrix as wells as connective tissue. Bb twin flagella are found in the front and back of the cell where the bacteria uses a clockwise and counter clockwise motion in order to control its motility.

4. Evading Immune System

Bb must evade the immune system in both early and late stages of infection. Early immune evasion involves avoiding the innate response which includes defending itself against oxidative defense, Toll-like receptors, NOD-like receptors, and complement system [36]. Avoiding adaptive immune responses involves antigenic recombination in addition to down-regulating immunodominant antigens to deceive the later stages of adaptive immunity [37].

Resistance to oxidative stress is associated with not uptaking iron where the limited intracellular iron provides an environment that intrinsically avoids oxidative damage from the host immune system [38]. Bb also does not have enzymes associated with the tricarboxylic acid cycle and respiration, a common target for ROS [7] [39]. dps, /napA, sodA, and cdr are associated with BosR gene regulation and contribute to the ROS resistance [40]-[44]. dps proteins have the capacity to sequester iron which is associated with the prevention of ROS-resistant nucleoids [45]. sodA codes for superoxide dismutase containing a manganese cofactor which detoxifies ROS species [7] [46]-[49]. Since Bb does not code for catalase or peroxidase enzymes, it is likely that the accumulated ROS are detoxified by CoA disulfide reductase or cdr to regenerate the oxidized state of CoA [7] [40].

Variation in antigenic surface proteins is a process used by many pathogenic bacteria to evade the immune system by changing epitopes so they are not targeted by the adaptive immune system [50]-[54]. The structure of these surface proteins involves the variable region to be presented on the outside of the cellular membrane while the highly antigenic constant region is buried in the cellular membrane [55]-[57]. The variation of the surface epitopes occurred in various tissues by the vlsE recombination events. The function of vlsE recombination is unknown but thought to be a decoy antigen specifically made for the evasion of the host immune system.

Bb's survival in serum is critical in a given host and must evade the host immune system by host complement inhibitors especially since C3 has the capacity to bind to most strains of Bb [58] [59]. This is done in part by complement regulator acquiring surface proteins (CRASPs) to complimentarily bind to factor H, factor H-like proteins 1 (FHL-1) or related proteins to factor H which is associated with the cleavage and inactivation of C3b [59]-[64]. There are several recognized CRASP genes which bind to factor H with differing affinities. Such redundancy is thought to allow *B. burgdorferi*'s ability to evade the host immune system in various vertebrates [65] [66].

Chemoreceptor arrays at the poles of Bb allow Bb to follow chemoattractant trails that lead to host cell compartments [67] [68]. Bb will then use adhesins to bind to glycosaminoglycans, fibronectin, and decorin [69]-[71]. Such niche seeking behavior allows Bb to evade the immune system by essentially seeking an environment that contains nutrients for Bb to survive while simultaneously hiding from the immune system. The host immune system is able to clear Bb, even in immune compromised individuals, if Bb loses its ability to recognize chemoattractants [72].

While chronic bacterial infections are virtually always associated with biofilms, acute infections are typically free floating and disseminate throughout the body accordingly. Those that have chronic infections of Bb are likely to have Bb within a biofilm community. Biofilms are a community of a complex of polymicrobia within

an exopolymeric gel. These microenvironments are also regulated by quorum sensing which detects and then responds to cell density by releasing diffusible signals that induce changes in gene expression among other nearby species of cells [73]. When Bb recognizes cell density described in a quarum, it will begin to release anti-inducing molecules including pheromone known as autoinductor (AI)-2 via LuxS enzyme synthesis [74]. In addition to Bb ability to protect itself from the immune system within biofilm, the biofilm also protects the bacteria from antibiotics.

5. Epidemiology

Geographic range change is one process in which diseases can emerge or re-emerge [75]. The emergence and change in range of *Ixodes scapularis* is documented by location in order to understand the spread of tick borne infectious disease like Lyme disease. Current northward expansion in the United States in recent decades and increase of incidence in the north eastern region of the United States of tick borne infections like Lyme disease has captured the attention of epidemiologist [76]-[79]. Monitoring the spread of and genetic diversity of ticks and tick borne pathogens is significant from an ecological, pathological, and diagnostic detectability of the ticks and their association with Lyme disease [80].

The two major features of the spread of Lyme disease involves an epidemiological and ecological understanding of the life cycle of tick borne infections in new regions and the mechanism of tick borne dispersion in the endemic regions between hosts and new ecological suitable locations. Both are associated with the density of ticks and the density of tick hosts where tick density is a major factor for the threshold of the tick borne pathogen transmission cycle and higher tick density is associated with spread of Lyme disease [81] [82].

Patterns of diversity among ticks are associated with the population expansion. The mortality rate of ticks in an environment associated with extreme temperature, drowning, desiccation and potentially predation are significant factors in the geographic spreading of Lyme disease [83]-[85]. Strains that are more adaptable might be favored in northeastern states in the US as compared to the Midwest US [82] since *I. scapularis* in larval and nymphal has a higher seasonal coincidence in the Midwest [86].

Lyme disease is also common in northern Europe in particular. Approximately 13.7% of all ticks in Europe are infected in Bb even though the prevalence of the disease is 18.6% in adult ticks as compared to 10.1% in nymphs [87]. The prevalence in Austria, Czech Republic, Germany, Switzerland, Slovenia, and Slovakia has is greater than 20% in adult ticks and greater than 11% in nymphs [87]. The spread of Bb is most common in spring and autumn in microenvironments where there is 85% humidity and deciduous or mixed woodland microenvironments [88] [89], suburban environments [90], and roadsides [91].

6. Chronic Lyme Disease

Often an infection of Bb will cause sub-acute symptoms, many times weeks to a few months after a tick bite before subsiding with or without antibiotic treatment [92]-[95]. The clinical manifestations may include neuroborreliosis, Lyme arthritis, Lyme carditis, skin manifestation, and flu like symptoms. The onset of neuroborreliosis can include mild meningism including headaches, lancinating radicular pain, and cranial neuritis. While chronic neuroborreliosis typically involves cognitive impairment, various kinds of paresis, extrapyramidal symptoms, bladder problems, psychosis, sensory disturbances, and spastic gate [96]. Stroke like symptoms are common and are caused by vasculitis [97] in addition to mononeuritis maultiplex or rediculitis [98]-[101]. In America, the most common symptoms of early onset include headache and meningitis while encephalopathy and neuropathy occur rarely [102].

It is important to note that early and chronic neuroborreliosis is typically indistinct. Lyme arthritis, often manifested in the knees, is characterized by inflammation of joints. Lyme arthritis can persist after the infection has been eliminated since the ailment is likely caused by an immunological mechanism. Lyme carditis ecompasses conduction defects, myocarditis and/or pericarditis [103]. Late forms of Lyme cardiac symptoms, in particular cardiomyopathy is rarely reported in Europe [104] [105] and not in America. Skin manifestations include erythema migrans and borrelia lymphocytoma and in later stages acrodermatitis chronica atrophicans (ACA), which may or may not be associated with focal neuropathy [106], is rarely seen in the United States [107].

Chronic infections of Bb have the capacity for creating an environment that leads to oncogenesis, particularly with non-Hodgkin lymphoma. The suspected mechanism of Bb leading to oncogenesis involves chronic antigen-dependent immunostimulation which is associated with constant and sustained lymphoid proliferation of

B-cells [108] [109]. Primary cutaneous B-cell lymphoma (PCBCL) has been found around skin lesions associated with area where the tick has bitten the skin in addition to patients that have serology of previous infection [110]-[112]. Horizontal gene transfer is likely the cause of Bb DNA to be found in nodal lymphoma like mantle cell lymphoma (MCL) in a patient with a history of being infected by Bb [113]. Having a history of being infected with Bb increases the likelihood of developing of acquiring MCL by 300% [114].

Transplacental transmission of Bb has been documented in various animal models with risk associated with adverse fetal outcomes [115]. In animal models, an infection at the beginning of the pregnancy might be correlated with an increase abortion rates in mice [116]. There are a few documented cases of women with Lyme disease having complications with their pregnancy. A premature birth and subsequently short life span of baby whose mother was diagnosed with Lyme disease during the pregnancy revealed severe cardiovascular defects and Bb like spirochetes found in the splean, kidney tubules, and bone marrow in the premature fetus [117]. Another case involving a mother with Lyme disease potentially leading to complications with child development involved a mother that gave birth to a child with spirochetes found in the brain [118]. Although there is significant evidence in animal models as well as some anecdotal cases in humans, studies have not confirmed causal relationships between Bb infections and adverse outcomes during pregnancy [119]. In addition, there is some evidence that acute infections of Lyme disease are more detrimental to the child than a mother who has chronic Lyme disease [119].

7. Lyme Disease and Its Effect on CD57

The most common tickborne disease in the United States is Lyme disease [120] [121]. Lyme disease is considered acute within a period of a month after exposure while chronic Lyme disease can occur months or years after exposure [122]. Chronic exposure to Lyme disease takes a toll on the immunesystem; in particular CD57 [123]. CD57 is typically 60 - 360 cells/μl and although the CD57 count rebounds back to the normal range, Striker and Winger have shown that 51% of patients with Lyme disease have significantly lower levels of CD57 prior to antibiotic treatment [123]. CD57 levels might also be used to determine the effectiveness of the treating Lyme disease. Acutely infected patients with Lyme disease did not have a remarkably low level of CD57. Levels of CD4 and CD8 were also found to be normal in these patients. In comparison to CD4 and CD8, CD57 is poorly characterized [124] [125] but CD57 is known to have natural killer capability like CD56 but remains distinct form CD56 NK cells [126] [127]. Low levels of CD57 were predominantly shown in patients with neurological symptoms as opposed to patients with musculoskeletal disease. It is important to note that there are conflicting reports of CD57 levels in neurological and rheumatological diseases in general [128]-[132].

8. C4a Levels in Patients with Lyme Disease

Higher than normal levels of C4a in patients with Lyme disease are apparent in patients with acute [133] and chronic Lyme disease [134]. Patients that respond better to antibiotic treatment had significantly lower levels of C4a than patients who responded poorly. An increase in C4a in patients with musculoskeletal symptoms while neurologic symptoms associated with Lyme disease did not have an elevated level of C4a. Although C4a is an anaphylatoxin, C4a is not known to cause significant neurological inflammation in the CNS [135] [136] It is important to note that patients with chronic fatigue, a condition that could be mistakenly diagnosed instead of Lyme disease, C4a is also increased [137]. Although C4a levels have the potential to detect Lyme disease at an early stage, increased levels by itself is inadequate for a diagnosis of Lyme disease.

9. Diagnostics

The methods for diagnostics are often not sensitive enough or are administered too early to detect antibodies associated with an infection by Bb. Detection of serum IgG antibodies is the most prevalent in detecting Bb in 90% - 100% of patients with chronic Lyme disease. ELISA testing using recombinant antigens or single antigens for Bb to detect the presence of Bb is the most common form of diagnostic measures [138]-[141]. Western blot is used for further verification of Bb by ELISA however, detection using Western blot for and a negative results using ELISA are considered as antibody negative [142]-[144]. Serological test using IgM often have false positives and should not be used as a diagnostic criterion [145]. It is also possible that antibodies can exist even after Bb has been eliminated from the body, which can lead to false positives [146]. Methodology of diag-

nosis with PCR, by screening cerebral spinal fluid [143], chronic skin lesions [147] [148], synovial fluid [149] [150], blood [151], and urine [152], vary in sensitivity and specificity. PCR can also lead to false positives through improper laboratory techniques [153].

One of the challenges surrounding a proper diagnosis with Lyme disease is the symptoms of Lyme disease are very similar to other conditions. Often times the same symptoms are diagnosed differently depending on whether the patient is within an endemic region of Lyme disease. Two commonly confused diagnoses involve either fibromyalgia or chronic fatigue when a patient may actually be experiencing symptoms associated with chronic Lyme disease (**Table 1**). Symptoms related to neuroborreliosis can also resemble Parkinson's disease in some patients. It is important that the signs and symptoms vary from patient to patient, particularly in advanced stages of Lyme disease. Some of these symptoms can persist even after serological evidence suggests that Bb has been eliminated. It is also important to note again that even a positive serological diagnostics may be due to residual antibodies circulating after Bb has been eliminated.

10. Treatment of Lyme Disease

Treatments of Lyme disease depend on the progression of bacterial infection and its manifestation. The treatments also vary depending on the region where the patient resides. There are several treatment guidelines that can be used to treat chronic Lyme disease. ILADS recommends several months of IV prior to oral treatment of antibiotics [154] while IDSA [155] and EUCALB [156] recommend treatment for 2 - 4 weeks. IDSA and EUCALB recommend oral administration of doxycycline or IV administration of penicillin, ceftriaxone, or cefotaxime respectively. Guidelines associated with the treatment of neuroborreliosis with peripheral neuropathy and acrodermatitis chronica atrophicans (ACA) by EFNS in Europe [157] recommends 3 weeks of either oral doxycycline or ceftriaxone by IV administration. EFNS also recommends 3 weeks of IV ceftriaxone for patients with chronic neuroborreliosis if there are manifestations from the central nervous system. The evaluation of the effectiveness of antibiotic treatment should occur 3 - 6 months after complete administration of the antibiotics. Retreatment for refractory arthritis may involve an additional 2 - 4 weeks of treatment.

11. Discussion

There are many challenges in the diagnosis and treatment of Lyme disease. These challenges arise particularly when a patient develops chronic Lyme disease in a region that is typically not considered in the endemic region. The diagnostic criteria can also be problematic leading to type I and type II errors depending on which kind of diagnostic method is used. It is possible for a patient to diagnostically be considered as a host for Bb even after successful treatment since the antibodies associated with Bb might still be in circulation. There is also an opportunity for many of the symptoms, especially neurological symptoms, to persist after Bb has been eradicated from the host. Arthritis symptoms may also persist after the infection has been cleared.

Table 1. The following diagram is to better explain why certain conditions can be confused with symptoms associated with chronic Lyme disease. The symptoms found in chronic fatigue, fibromyalgia, rheumatoid arthritis, and Parkinson's disease are not exhausted in this table since the goal is to document the overlap of symptoms. Of the diseases listed in this table, the symptoms of fibromyalgia are more closely related to chronic Lyme disease. Issues in diagnosing Lyme disease over fibromyalgia is complicated by poor serologic diagnostic criteria associated with detecting Bb.

	Chronic Lyme Disease	Chronic Fatigue	Fibromyalgia	Rheumatoid Arthritis	Parkinson's Disease
Fatigue	X	X	X	X	X
Loss of Concentration/Short Term Memory Loss	X	X	X		X
Joint Pain	X	X	X	X	
Poor Sleep	X	X	X	X	X
Mood Problems/Depression, Anxiety, etc.	X	X	X	X	X
Muscle Skeletal Pain	X	X	X	X	X
Neurological Presentation	X	X	X		X
Muscle Stiffness	X		X		X

Better diagnostic methods are sorely needed to enhance the diagnosis of Bb infections. This is particularly true with early detection of Bb to specifically reduce the disease progression before many of the major symptoms have emerged. Although recognizing a tick bite may inform the doctor for early treatment of Lyme disease, there are many patients who do not recall being bitten by a tick that does not have the luxury of identifying the early stages of infection.

The potential for coinfections with *Babasi microti*, *Bartonella henselae*, *Ehrlichia rickettsiales*, *Coltivirus*, *Mycoplasma*, *Powassan encephalitis* virus, *Coxiella burnetii*, *Rickettsia slovaca*, *Rickettsia helvetica*, and *Francisella tularensis* can complicate an infection with Bb. These bacteria and viruses can be found in the Ixode ticks and although they are phylogenetically distant from each other, they transmit themselves from tick to vertebrates via tick bite like Bb. Further screening to determine if an infection is actually a coinfection should be examined by clinicians to make sure the treatment protocol is sound for the patient.

Novel forms of antibiotics have recently been lagging with regard to drug discovery. There is potential for new treatments that have a higher success rate to be discovered. Treatment of Bb neurological symptoms during and after infection is also sorely needed to enhance the quality of life of patients. Since Bb scavenge for nutrients that it cannot produce on its own, there is potential for novel drugs that mimic many of these nutrients in a manner that could prevent disease progression.

More research is necessary with regard to the promotion of cancer from infected patients with Bb. A better understanding of the mechanism is important and recognizing or predicting patients with a higher likelihood of developing cancer may play a role in treatment alongside the antibiotic treatment that is currently used.

The complex nature of Bb's genome and the potential for horizontal gene transfer could lead to new strains of Bb. Such new strains may present antigens that may not be detected by current methods of diagnosis. Research of Bb that is not readily identified by the current diagnostic criteria should be more closely examined to determine if this is the case.

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