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Chronic Lyme Disease: Persistent Clinical Symptoms Related to Immune Evasion, Antibiotic Resistance and Various Defense Mechanisms of *Borrelia burgdorferi*

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Abstract

There are several factors involved in the ability of *Borrelia burgdorferi* to retain a persistent infection within a mammalian host. These factors of immune evasion include regulation of membrane proteins, variable epitopes of surface proteins, protection against the immune system through tick saliva, the ability to migrate to regions where it is not exposed to the immune system or antibiotics, invagination or invasion within various cells, pleomorphic forms, and the potential to produce biofilms. The window of conventional treatment for Lyme disease is short and has the potential to display different symptoms depending on the strain of *Borrelia bugdorferi*. These symptoms are dependent on the localization of *Borrelia burgdorferi* which correlates to the significance of diagnosing Lyme disease early to prevent such a spread throughout the body. Such complications of *Borrelia burgdorferi* may demand new clinical treatment discoveries for patient fighting the chronic form.

Keywords

***Borrelia burgdorferi*, Immune Response, Antibiotics, Surface Proteins, Pleomorphism, Ixode Tick**

1. Introduction

Borrelia burgdorferi (Bb) has one of the most complex genomes of any bacteria. Within this genome are genes that allow Bb to survive in two very different hosts: Ixode ticks and mammals. Its ability to adapt to these two very different environments is stunning. However, the capacity for evading the immune system in mammals is

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complicated and involves various factors. These factors range from regulation of virulent genes, changing the epitope of outer membrane proteins, to utilizing tick saliva to stave off the immune system during the initial infection. Although some methods of evading the immune system are still in conjecture or require more research to be certain, the capacity and potential for treating Lyme disease are quite difficult.

In addition to Bb's ability to protect itself from the immune system, many of the functional attributes and bacterial loci can influence the ability to treat Lyme disease with various antibiotics. Although Bb is susceptible to antibiotic treatment during early infection or treatment for Lyme disease, the bacteria is able to traverse the blood brain barrier, endothelial tissue, and imbed itself in joints. Bb can also enter certain cell intercellularly in addition to invaginating itself in a manner that reduces the potential exposure of antigens and limits the effectiveness of antibiotics.

Even after Bb has been cleared from the body, the effects of Lyme disease can remain. It is true that the diagnostic criteria for Lyme disease have the potential for either false positive or, more commonly, false negative diagnoses. The diagnostic criteria even require valuable time for the antibodies to present itself for an infection with Bb. However, the destructive impact from neuroburrliosis and arthritis caused by Bb is something that can not heal overnight even without detectable Bb in the body.

The following review is meant to understand why chronic Lyme disease is so difficult to treat. The capacity for Bb to stave off the immune system in addition to "hiding" itself in various tissues to avoid exposure to antibiotics are significant hurdles that from a clinical stand point are very hard to combat.

2. Outer Surface Proteins

There are various outer surface proteins (Osps) with varying functions with regard to immune evasion. Osps were originally organized in alphabetical order associated with the order of their discovery. These Osps include OspA [1], OspB, OspC [2], OspD [3], OspE, and OspF [3]-[8]. Eventually the names of these outer surface proteins were designated by their function. The induction of these genes depends on the environment where they are found (*i.e.* stomach of tick or serum of mammal). Of these induced Osps, many of these differentially expressed genes that encode Osps are found in one of the many plasmids associated within Bb [9] [10]. These outer membranes are associated with an inflammatory response within the host mammal due to their stimulatory events [11] without the presence of lipopolysaccharide [12].

OspA has been more thoroughly been examined than the other Osps since it has a key role in the resting (unfed) nymphal as well as its ability to be examined *in-vitro* culture [13]. The adherence of OspA during initial blood feeding within the Ixode tick is likely the reason for lack of internalization of OspA [14]. Detachment from the Ixode tick is likely caused by suppression or down regulation of OspA [15] [16] after a significant amount of blood has entered the mid gut. Suppression of the of OpsA after leaving the mid gut is likely the reason why mice immunized with OpsA are not protected against Bb infections [17].

OspC is thought to have a major role in the migration of Bb from the mid gut to the salivary gland [18]. The expression of OspC depends on the whether or not the Ixode tick is fed or feeding since the expression of OspC only occurs when a tick ingests blood form the host [19]. OspC is then down regulated once Bb reaches the salivary glands. Although OspC is down regulated once it reaches the salivary glands, there is still potential for some Bb to respectively induce antibody resistance toward OspC [20]. OspC also differs among strains of Bb and have the capacity to be attacked from adaptive immune response in a way that selects which Bb strain is viable to spread within the host [21]. The adaptive immune response toward Bb is geared toward the initial infection which has the potential to inadequately respond to emerging variants [21]. In addition to targeting Bb during the initial infections, transposon elements within Bb's chromosome and plasmids allows Bb to mutate during an infection in a way that has potential cause further variation within Bb; thus potentially giving Bb an advantage against host immunity.

3. VlsE

The humoral response was investigated to determine why Bb has the potential to cause the host immune response to be inadequate for the elimination of Bb. Although increased numbers of B cells were found independent of T cells in the lymph nodes with a short lived germinal center, the phase of germinal center did not have clear zones of T cell and B cells [22]. This respectively led to the discovery of short lived plasma cells and a high level of long lived antibody secreting plasma cells [22]. This suggests an increase in humoral antibodies

that are ineffective in targeting foreign cells like Bb. Such a discovery suggests that Bb has the potential to create antigenic variations that limit the recognition of the humoral response.

Variable major protein like sequence (vls) aids in the survival of Bb within infected mammals by altering the epitope the outer surface proteins associated with the vls gene locus [23] [24]. The expression site of vls (vlsE) involves 2 constant regions and an internal variable segment that comprise a 34 kDa surface protein [24] [25]. The various antigenic protein structures obtain variability via the exchange of various DNA cassettes associated with altered recombination. Silent cassettes are involved in various gene conversions and generate new epitopes of vlsE variants [26]. Such recombination "confuses" the adaptive immune response since the variable region is likely to have changed before the antibodies can reach Bb. The vlsE locus is stimulated by ixode ticks during feeding [20]. The outer most region of the protein is the variable region. The gene expression for vlsE is down regulated in Ioxode ticks and up regulated after the infection has been established in the mammalian host [27].

4. Complement Regulatory-Acquiring Surface Proteins

Bb needs to be able to avoid the host immunity long enough to find a niche environment where they can grow and proliferate without significant interference from various immune pathways. There are 3 pathways that involve circulating protein precursors that can trigger membrane attack complexes (MACs). MACs have the potential to create membrane channels that have the capacity to lyse cells. The three pathways include the classic pathway, the alternative complement pathway, and the lectin pathway. Ixode ticks contain a protein called Salp 15 that has the capacity to inhibit complement-mediated killing *in vitro* [28]. However, there is evidence that the classical pathway was present in patients with neuroborreliosis when the cerebral spinal fluid (CSF) was analyzed [29]. These findings suggest that despite the activation of the complement pathways, Bb has the capacity to evade them long enough to find a niche environment.

Complementation systems involve the use of opsonizing molecules, including C1q, C3b, and iC3b, to coat microorganisms after their entry in the host. Antibody-independent mechanisms initiate this process by either direct or alternative pathways. Microorganisms have the ability to evade these systems by directly binding the complement regulators associated with the alternative pathway to the cell surface [30] [31]. This process inhibits the complement activation cascade [30] [31]. The following 5 CRASP proteins can be found on the surface of Bb: CRASP-1, CRASP-2, CRASP-3, CRASP-4, and CRASP-5. Each one of these CRASPs has the ability to bind to different host immune regulators via their unique properties on the cellular surface [32]. This includes binding of CRASPs to factor H and factor H like protein-1 (FHL-1). To be more specific BbCRASP-3, BbCRASP-4 and BbCrasp-5 bind to factor H and FHL-1 [31]. In addition to CRASPs, Erp proteins also have the ability to complementarily bind and inhibit factor H. These proteins that can interact with complement regulators with various affinity include Elps [33], ErpA, ErpP [34], p21 [34], OspE, and OspF [35]-[37].

5. Tick Saliva

Initial protection from the immune system is critical for the survival of Bb after leaving the Ixode tick and entering into a mammalian host. As discussed earlier, OspC is up regulated upon Ixode tick feeding and aids in allowing Bb to enter the salivary glands. Once there, OspC binds to tick salivary protein Salp15 which essentially temporarily protects Bb from antibody mediated cell lysis [38]. More specifically, tick salivary proteins are known to inhibit the proliferation and activation of CD4+ T cells via the binding of the CD4 receptor [39] [40] in addition to inhibiting natural killer cells [41], dendritic cells [42], neutrophils [43] and macrophages [44]. B cell interfering protein (BIP) is a protein found in salivary gland extract (SGE) has shown to inhibit the ability of B cells to recognize OspA and OspC at the site of the tick bite [45]. BIP does not have the same effect on T lymphocytes when examined under the same concentration as that used in BIP inhibition of B cells *in vitro* studies [45].

6. Motility

The capacity for Bb to swim through liquid environments including blood, lymph, and CSF in addition to being able to tunnel through epithelial cell matrix (ECM) makes Bb agile enough to find various niche environments in addition to scattering itself within the body. The ability to move allows Bb to follow chemoattractants and by modifying the coordination of Bb's flagella [46]. The motility of Bb is different than many other organisms since it has two flagella motors in the front and the back of bacteria. Each of the flagella of Bb has a motor-

hook-filament structure [47] in addition to filaments that have distinctive ribbon within the periplasm [48]. The front and rear motor interact with each other by their ribbon which overlaps in the center of Bb. These filaments coordinate either a clockwise or counter clockwise motion of both flagella motors.

7. Survival and Immune Escape of *B. Burgdorferi* by Tissue Localization

Immune clearance and chemotherapeutic resistance of Bb is likely caused by invagination of cellular membranes or potentially the cytosol of joint cells or other bradyotrophic tissue [49]. Bb is also known to invaginate itself by binding to fibrocytic cells *in vitro* to avoid being phagocytized [50] [51]. Bb ability to hide from immune cells gives it a survival advantage [50]. Invagination decreases the surface area of exposed surface antigenic proteins. Bb cells can be recovered even after ceftriaxone treatment suggesting its ability to survive antibiotic treatment in addition to hiding from immune cells [52]. It is likely that Bb will pass through the endothelial cells to reach synovial cells [53] [54]. Bb is also capable of internalization within endothelial cell [55] and respectively macrophages [56]. Survival of Bb within macrophages has the potential to provide a reservoir for Bb in chronic or reoccurring Lyme disease [56]. It is important to note that finding intracellular Bb is quite rare.

8. Intracellular Localization in Nerve Cells

Nearly 15% of those infected with Bb experience neurological syndromes including cranial neuropathy, meningitis, and encephalitis [57]. Inflammation of the central nervous system (CNS) is a hallmark of neuroborreliosis. The ability of Bb to evade the immune system has its effects on the CNS. The inflammation caused by the immune system's attempt to eliminate Bb from the body is instead aggravating the CNS. In addition to the other mentioned methods of immune evasion, the ability for Bb to evade the host immune system may also include Bb's intracellular invasion within human neuronal and glial cells [58]. Intracellular Bb cells, within human cortical neuronal cells, were able to be protected from gentamicin treatment as indicated by Bb's ability to be cultured after the fact [58]. This could mean that intracellular habitats might be preferentially sought by Bb. The mechanism for this action is still in conjecture but is thought to involve Bb's ability to bind to various components of mammalian cells including integrins, decorin, fibronectin, and glycosaminoglycans [59].

9. Pleomorphism

Under harmful conditions, like dramatic temperature changes, Bb has the potential to change from a spirochete form to atypical forms including cystic and to fine single granules [60]-[62]. Osmotic shock by presenting Bb with cold distilled water or heat shock caused the same pleomorphic behavior as other spirochetes [63] [64]. Filamentous, ring shaped, and cystic forms of Bb have the capacity to survive immune attack while Bb is intracellular. Atypical and cystic forms of Bb were present in the cerebral cortex of three patients with Bb indicates that Bb can survive in cystic forms within brain tissue [65]. Atypical and cystic forms of Bb were also found intracellularly within the host's neurons. Cystic forms of Bb have the capacity to revert into vegetative forms [66] [61] which suggests that some of the pleomorphic forms might actually be in a vegetative state. Vegetative and cystic forms of Bb still retain their surface proteins indicating that they can still illicit an inflammatory response in addition to complement activation [67]. Pleomorphic forms of Bb can persist in the brain; indicating that latent stage of Lyme neuroborreliosis might involve various forms of Bb within the brain.

10. Persistent Symptoms of Chronic Lyme Arthritis

Symptoms of Lyme arthritis can persist after the ability to detect Bb in serum. Although a clear picture of what causes Lyme arthritis has yet to be fully revealed, it appears that Bb has the capacity to alter connective tissue and joints by cleaving aggrecans [68]. Aggrecans are proteoglycan that can be cleaved by an aggrecanase called BbHtrA. BbHtra is found on the surface of the spirochete and can effectively destroy the utility of proteoglycans. Other studies have shown that Bb can express ADAMTS-4; an aggrecanase that cleaves the proteoglycan in mice and human joints [69]. The regulation of ADAMTS-4 appears to involve inflammatory cytokines including IL-6, IL-1β, and TNF-α [70]. Due to the destructive nature of aggrecanases, these enzymes have been targeted as means to treat connective tissue disease and arthritis [71]-[73]. The evolutionary advantage of Bb's aggrecanase has yet to be established.

11. Biofilms

Biofilms are a complex concoction of aggregates of plaktonic microorganisms that are utilized by microorganisms to defend itself against a hostile environment [74] [75]. Extreme changes in pH and high temperatures, as wells as the presence of metals, xenobiotics, antimicrobial agents, and in some species oxygen can induce the formation of biofilms [76] [77]. These microbes secrete extracellular polymeric substances (EPS) to shield the bacteria from environmental stressors and/or therapeutics interventions [77]. *In vitro* cultures of Bb in early log phase (1×10^7 cells/ml) begin to aggregate [78]. Bb aggregates are comprised of extracellular polysaccharides which are consistent with other microorganisms that produce biofilms [77]. Bb's biofilm also contained channel like structures similarly to those observed in other biofilms [79] including biofilms produced by *Leptosira* spp. [80]. Such findings suggest similar function where the channels provide nutrients and oxygen in addition to waste removal of imbedded cells [79].

12. Discussion

After reviewing all of the potential mechanisms associated with the survival of Bb, in particular Bb infections in mammals, it is clear that Bb is a highly evolved species. Bb is unique in that it has the capacity to have all of these features available to protect itself from various stages of immune attack in addition to reducing its exposure to antibiotics in chronic patients.

In contrast, acute Lyme disease can be easily treated if caught early. Early detection often requires a patient to have the characteristic bull's eye on their skin with the center being the location of the tick bite. Although 80% of patients develop erythema migrans (EM), of that 80% only 19% acquire the characteristic bull's eye rash [81] [82]. However, there is potential for people not realizing that we were in fact bit by a tick. Since the symptoms of Lyme disease can mimic other diseases and the molecular diagnostics being neither very specific or sensitive, there is an opportunity for the window of acute Lyme disease, before it becomes chronic, to be missed and not treated in time. Since blood in the gut of Ixode ticks changes the pH and temperature, which is associated with OpsA and OpsC to detach from the ticks gut and transfer Bb to the salivary glands, there is still a window of time to remove the tick before Bb has an opportunity to infect the patient. Too often the symptoms of Lyme disease are misdiagnosed, leading to a greater opportunity for disease progression to chronic Lyme disorder as a consequence of not treating the disease at an early stage. At that point, common antibiotic treatment becomes either ineffective or significantly attenuated by various methods of immune and antibiotic evasion.

Novel treatment options for those with chronic Lyme disease are direly needed. This might include unconventional methods including drug delivery to directly attack Bb in regions that are hard to reach by conventional methods. Late antibiotic treatment of chronic Lyme disease has been shown to be no more effective than a placebo and has the potential to expose the patient with adverse side effects. Enhancing the body's ability to fight infection by either nutritional means or perhaps even immunotherapy might be an avenue worth exploring. Vaccines that recognize outer surface proteins displayed by Bb in early infections could be a valid form of preventing Lyme disease.

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