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# IMMUNE EVASION OF THE LYME DISEASE SPIROCHETES

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## 1. ABSTRACT

The Lyme disease spirochetes, *Borrelia burgdorferi* sensu lato, have adapted very well to both surviving and persisting in the mammalian host despite a strong host antibody response. It appears that both temporal and spatial regulation of outer surface proteins have contributed to this persistence. The spirochetes are able to bind fH and FHL-1 to their surface, resulting in decreased complement activation. In addition, the organisms have taken advantage of components of tick saliva to aid in their initial immune evasion and dissemination. Studies leading to these conclusions are reviewed here.

## 2. INTRODUCTION

*Borrelia burgdorferi* sensu lato (s. l.) is a group of spirochetes responsible for causing Lyme disease in a variety of mammals. This group consists of the genospecies *Borrelia burgdorferi* sensu stricto (s. s.), *Borrelia afzelii,* and *Borrelia garinii. B. burgdorferi* s. s. is seen primarily in the United States while *B. afzelii* and *B. garinii* are found in Europe and Asia. These spirochetes are dependent on the *Ixodes* tick for their transmission to a mammalian host. During tick feeding, the spirochetes migrate from the tick gut to the salivary glands and enter the host through the saliva. Throughout their enzoonotic life cycle, the spirochetes demonstrate a very complex pattern of surface protein expression which is just beginning to be understood. Clinical signs of early stage Lyme disease are characterized by erythema migrans and fever. Following this, signs of infection of specific organs begin to arise, including disorders of the heart, joints, and nervous system, such as Bell's palsy. Late stage Lyme borreliosis, particularly in the United States, is characterized by non-purulent arthritis in larger joints which often leads to cartilage damage. Chronic neurologic abnormalities and acrodermatitis chronica atrophicans (ACA) are also seen in late stage Lyme disease. It seems that *B. garinii* is more often associated with neuroborreliosis, *B. burgdorferi* s. s. often manifests itself as arthritis, and *B. afzelii* causes more ACA than the other two genospecies (1, 2). Lyme disease is often a persistent infection, therefore, it is assumed that the *Borreliae* demonstrate multiple mechanisms for immune evasion. Research is being done to elucidate the mechanisms utilized by *B. burgdorferi* s. l. as the spirochete is able to persist in the host despite the induction and maintenance of a strong antibody response to surface antigens by the host (3). This antibody response in mice has been shown to be protective when serum from infected mice is passively transferred to naïve mice prior to inoculation (4). This review will cover several of the proposed immune evasion mechanisms of the *Borreliae,* focusing primarily on the role of outer surface proteins.

## 3. Osp PROTEINS

Outer surface proteins (Osp) are a group of lipoproteins produced by *Borrelia burgdorferi* s. l. which are surface expressed at various times throughout the spirochete life cycle. Among the best studied are OspA and OspC. OspA is expressed on the spirochete surface when they are present in the tick midgut, as the tick is resting. This lipoprotein appears to act as an adhesin, binding specifically to tick midgut epithelial cells keeping the organism lodged in this area during the blood meal. Near the end of the blood meal, OspA is downregulated and the spirochetes begin to move to the tick salivary glands where they will then be transmitted to the mammalian host. Several hours prior to the migration of the spirochetes from the midgut to the salivary glands, they begin to express OspC. As the spirochetes enter the host, they appear to suppress expression of OspC.

### 3.1. OspC

It is unclear exactly what role(s) OspC plays in migration in the tick, establishment of persistent infection, and immune evasion of the spirochete. To this end, much

research has been conducted. The potential for antigenic variation of this protein has been investigated as infection persists despite a very good OspC antibody response in the host. Mice (C3H/HeN) were infected intradermally with $1x10^4$ spirochetes of a confirmed clonal population of either *B. burgdorferi* s. s. N40 or *B. afzelii* PKo grown in BSK II medium. Strain N40 was used as a negative control as antibodies directed at rOspC are not protective in mice, however, *B. afzelii* PKo rOspC elicits a protective response. After infection was established, the mice were hyperimmunized with the respective rOspC protein. At the completion of six months of persistent infection under hyperimmune pressure of OspC, spirochetes were isolated from the mice and OspC was evaluated for gene variation. PCR-amplified *ospC* DNA was subjected to both sequencing and RFLP analysis. When compared to the *ospC* of the original inoculum, no overt differences were observed by RFLP or sequence analysis for either *B. burgdorferi* s. s. N40 or *B. afzelii* PKo (5). The spirochetes used in these experiments were grown in culture medium. Culture grown spirochetes have very different gene expression profiles from those present in the tick (REF). This may have effected the outcome of these experiments. Barthold and co-workers speculate that these data support an alternative hypothesis to explain the genetic variation observed among different *B. burgdorferi* s. l. strains. Specifically, that the observed heterogeneity may be due to lateral gene transfer rather than exchange of genetic material within a clonal population (7). In addition, these authors did not evaluate expression of OspC, they only evaluated the genomic makeup of *ospC*. If OspC is not expressed on the spirochete surface throughout persistent infection, which appears to be the case (8,9), the presence of anti-OspC antibodies will not yield selective pressure. A later study by Liang, *et al.* addresses this issue, in which it is concluded that the regulation of OspC is likely to be an immune evasion mechanism through selection of surface-antigen non-expressers. In their study, *B. burgdorferi* s. s. B31 5A3 was grown in BSK II and intradermally injected into either C3H/HeN or C3H-SCID mice at $1x10^4$ spirochetes per animal. They found that 17d post-inoculation, spirochetes not expressing *ospC* were selected for. This selection was concomitant with the emergence of anti-OspC antibody in immunocompetent mice. The isolated spirochetes re-expressed *ospC* when either cultured *in vitro* or transplanted to naïve immunocompetent mice, but not OspC-immunized mice. Spirochetes isolated from SCID mice continued to express *ospC*, while spirochetes isolated from SCID mice passively immunized with OspC monoclonal antibody showed no *ospC* expression (9).

While the specific role(s) of OspC in migration in the tick and infection of the mammalian host remain somewhat controversial, two recent studies have addressed this issue using OspC mutant spirochetes. In the first study, OspC mutant spirochetes were generated in the *B. burgdorferi* s. s. strain 297 while the strain used as wild-type was *B. burgdorferi* s. s. N40. A strain was also created using a shuttle vector to complement the *ospC* mutation. In their studies, Pal, *et al.* determined that OspC binds efficiently to both soluble salivary gland extracts (SGE) and tissue sections of tick salivary glands. In addition, they found that OspC mutant spirochetes were deficient in their ability to migrate from the tick midgut to the salivary glands and, in turn, these mutants were not able to establish infection in the murine host after being fed on by infected ticks. Both wild-type and *ospC* complemented spirochetes were able to migrate from the midgut to the salivary glands and establish infection in the murine host. This group also evaluated the ability of wild-type spirochetes subjected to anti-OspC $Fab_2$ to both migrate from the tick midgut to the salivary glands and subsequently establish infection in mice. These spirochetes were unable to migrate to the salivary glands or establish infection in mice (10). The second study argues that OspC mutant spirochetes are still able to migrate to the salivary glands however, they cannot establish infection in mice. This group used *B. burgdorferi* s. s. B31-A3 to create an *ospC* mutant. The mice used were naïve RML mice, representing an outbred strain maintained at the Rocky Mountain Laboratories since 1937. SCID mice were B6.CB17-*Prkdc*$^{scid}$/SzJ from The Jackson Laboratories. The *ospC* mutant was able to colonize the tick midgut as well as migrate to the salivary glands, as determined by confocal microscopy with IFA staining. However, neither naïve immunocompetent or SCID mice fed on by ticks infected with the *ospC* mutant were infected while both wild-type and *ospC* complemented strains were able to infect mice. In addition, both inoculum from ground spirochete infected tick midguts and culture-grown inoculum were used to infect both immunocompetent and SCID mice. The *ospC* mutant spirochetes were unable to establish infection in either type of mouse while the wild-type and *ospC* complemented strains were able to establish infection (8). Grimm and co-workers conclude that while OspC is not required for migration from the tick midgut to the salivary glands, it is required for establishment of infection in mice. While upon inoculation into the murine host, *B. burdorferi* down-regulates expression of OspC, it is feasible that initial expression of OspC is somehow required for establishment of infection, perhaps in protecting the spirochetes from innate immune mechanisms. The crystal structure of OspC revealed that it is primarily an a-helical structure with a putative binding pocket for an as of yet unknown ligand (11, 12). Based on these collective data, it seems that the role of OspC in the *Borrelia* life cycle and immune evasion is very complex and as of yet not well understood. Future studies in this area elucidating and separating the various functions of OspC in migration in the tick, establishment of infection in the host, and immune evasion should yield important information. Discovery of an OspC ligand, or multiple ligands will also further this research.

### 3.2. OTHER Osps

Osp family members that may be contributing to persistence in the host through some form of recombination or high mutation rate that would lead to antigenic diversity have been studied. OspF and OspE, along with a group of OspE/F related proteins (Erp) have been investigated for these properties. One study conducted by Hefty, *et al.* used *B. burgdorferi* s. s. 297 to infect C3H/HeJ mice by feeding ticks. Spirochete populations from hyperimmunized mice

were evaluated two weeks post-infection. Various combinations of recombinant Erp and Elp (proteins with OspE/F leader peptides) proteins were used as antigens for hyperimmuization. Spirochete populations from unimmunized mice were evaluated eight months post-infection. At both time points, no mutations were detected either by RFLP or sequence analysis, leading to the conclusion that the genes for the OspE-related, OspF-related and Elp lipoproteins are stable during infection. Although after eight months, the genes for these proteins remained stable, it was found that cellular localization of many of these proteins was in the periplasm rather than surface exposed and that host-derived environmental cues were often responsible for altering expression or cellular localization of these proteins (13). Interestingly, this could lead to antigenic variation although these genes remain stable during infection. OspF was evaluated by McDowell, *et al.* for demonstration of antigenic variation through recombination events or mutations due to genetic instability. *B. burgdorferi* s. s. B31MI was used to inoculate C3H/HeJ mice by needle inoculation. Serum was taken every two weeks and *B. burgdorferi* were isolated from infected mice twelve weeks post-infection. The spirochetes were analyzed by SNP (ddATP only) and sequencing for indications of genetic instability for three genes in the *ospF* family, BBR42, BBM38, and BBO39. The sequence/SNP analysis showed these loci to be genetically stable and identical to the sequence/SNP analysis of the original clonal isolates. However, antibody analysis revealed that the paralogs appeared to be temporally regulated. BBO39 was weakly reactive at week 4 and strongly reactive by week 6, BBR42 was only weakly reactive, and BBM38 was not immunoreactive (14). These data indicate that BBO39 is expressed and probably surface exposed early in the infection, while BBR42 is expressed further into the infection, and BBM38 is expressed much later, if even at all. Alternatively, these proteins may be weakly immunogenic, however, this is not likely based on previous work that determined the OspE/OspF family of proteins to be highly immunogenic in mice (15). Again, temporal regulation of surface expressed lipoproteins is potentially aiding in immune evasion by the Lyme disease spirochetes.

The antigenic variation of OspE has been fairly well studied. It has been shown that during infection in C3H/HeJ mice, new variants of the *B. burgdorferi* s. s. B31 *ospE* gene family arise after three months of infection. Some of these new variants were predicted to be antigenically distinct from the parent clone based on sequencing data obtained from these experiments. Several of them demonstrated these changes in the predicted hypervariable regions, which coincide with the predicted antigenic regions. It is important to note that spirochetes cultured in the laboratory (*B. burgdorferi* Sh-2-81 – passage 10 and 187 and *Borrelia garinii* Pbi – passage 7 and 311) over the long-term do not demonstrate any genetic variation, indicating that this is an *in vivo* phenomenon, used for host immune evasion during infection with *Borrelia* species. When humoral immune response was analyzed, it was found that both IgM and IgG antibodies to parental OspE existed in these mice, starting at weeks 2 and 4 respectively. It was then investigated as to whether or not the new variants of OspE family members were also able to elicit an immune response. Three of the new variants were analyzed, two of the three reacted with antibodies starting at week 4, the third variant did not react with antibodies until week 12. This demonstrates that at least one of the variants was antigenically distinct and likely arose later in the infection process (16).

## 4. VlsE

VlsE is an approximately 35-kD protein found surface exposed on the Lyme disease spirochetes. The *vlsE* locus is very interesting, composed of one expression site and up to 15 silent cassettes depending on the strain. *VlsE* of *B. burgdorferi* consists of 6 variable regions and segmental gene conversion can occur as early as 4 days in murine infection and continue throughout the duration of the infection. In addition, no segmental gene conversion occurs in spirochetes cultured *in vitro* for up to 84 days, indicating that this conversion is specific to the mammalian host (17). These silent cassettes share a very high sequence homology to the expressed copy. The silent cassettes are involved in gene conversion in a unidirectional manner, thus leaving them untouched and in their original state (18). Elucidation of the crystal structure of VlsE (19) has revealed that the six variable regions predominate on the surface exposed regions of the protein, seemingly protecting the non-variable regions from exposure to host immune cells and antibodies. It was presumed that this protein would be an important part of the immune evasion strategy of *B. burgdorferi.* One study attempted to address what these changes in the variable regions meant in an actual infection. In this study, they isolated five antigenic variants of *vlsE* that arose during spirochete infection in a murine host. They used these variants as antigens and used antisera from mice infected with a clonal population of spirochetes with *vlsE* genes of known sequence, not matching that of the test antigens. All of the antisera reacted with the test antigens, including antisera from mice infected with *B. burgdorferi* of different strains. Additionally, this group tested human antisera from patients with established *B. burgdorferi* infections and found that these antisera reacted with all of the variants as well. These results indicate that the antibodies generated in these infections are reactive towards non-variable regions of the VlsE protein. The study was able to demonstrate that there is at least some antibody response to the antigenic variation of VlsE through experiments in which they pre-absorbed the sera to a specific recombinant VlsE and were able to see specificity following absorption (20). These data indicate that while common antigenic epitopes present on VlsE are generating an antibody response, the antigenic variation of the variable regions is also effecting antibody response and may aid the spirochete in host immune evasion. Additionally, the protective qualities of these antibodies, particularly those recognizing common epitopes, was not assessed. It could be that although these antibodies are present, they are not protective and therefore averting the antibody response.

## 5. SERUM RESISTANCE

Serum resistance in the Lyme disease spirochetes involves the surface expression of several Factor H (fH)

binding proteins (FHBPs) which are also referred to as CRASPs (complement regulator-acquiring surface proteins) (21-23). Some of these FHBPs/CRASPs are also able to bind to Factor H-like protein (FHL-1), a protein derived from the same transcript as fH. fH and FHL-1 act as cofactors for degradation of C3b by factor I, resulting in decreased C3 convertase complex which leads to decreased complement-mediated killing. By binding fH and/or FHL-1 to their surface, the spirochetes are able to evade complement activation. While it is known that there are several of these FHBPs/CRASPs, we revisit OspE and a more recently identified protein, BbCRASP-3, also a member of the Erp family of proteins, as the two which have been identified to the genetic level (22, 23). Among the *B. borgderferi* s. l. group, *B. burgdorferi* s. s., *B. afzelii*, and *B. garinii* have all been evaluated for their ability to evade complement activation. It was found that both *B. burgdorferi* s. s. and *B. afzelii* are complement resistant as facilitated by increased factor I-mediated degradation of radiolabeled C3b. Additionally, it was observed that *B. garinii* is not able to evade complement activation. This study by Alitalo, *et al.* also elucidated the binding of both fH and FHL-1 by the complement resistant spirochetes from normal human sera (24). A recent study by Metts, *et al.* assessed the ability of the three paralogs of OspE in *B. burgdorferi* s. s. B31MI to bind fH as well as antibodies in sera from immunized mice. In addition, this group evaluated whether or not these binding regions were on the same site of OspE. Their data indicate that full length OspE is necessary for optimal binding of both anti-OspE antibody as well as fH, however, these sites were distinct from each other as demonstrated by competitive ELISA. It was also reported that all OspE paralogs are able to bind fH and that this binding region is likely located on a conserved region. They observed that paralog-specific antibodies were produced during infection in C3H/HeJ mice and that these antibodies are likely directed at areas within the two predicted hypervariable regions (25). It is important to note that specific binding residues were not identified in these studies.

## *6. IXODES* TICK VECTOR CONTRIBUTIONS

Using the tick as a transmission vector has a number of advantages for *B. burgdorferi.* Tick saliva has been shown to have multiple immunomodulating functions, particularly in *Ixodes scapularis*. This is of benefit to the spirochetes as it takes >53 hours of tick attachment/feeding for an infection to be transmitted to the host, therefore, if the tick were rejected by the host prior to the completion of >53 hours of feeding the spirochetes would not be effectively transmitted. In addition, the spirochetes enter into the host surrounded by saliva, which potentially aids in protecting the organisms from the host immune system. Tick saliva contains prostaglandins which may act as vasodilators in the host and help in dissemination of the spirochetes from the site of inoculation (26). Gillespie, *et al.* has identified a protein in tick saliva which acts as an IL-2 binding protein and is able to decrease the response of T-cells to mitogen stimulation (27). Experimental inoculation of C3H/HeN mice with *B. burgdorferi* s. s. N40 either with or without salivary gland extract (SGE) from *I. scapularis* showed that mice inoculated with both the spirochete and SGE had a significantly higher spirochete burden in target organs (heart, skin, bladder, and femurorbital joint) than those inoculated with the spirochete alone. This study also showed that the increased spirochete load was species specific as SGE from *Ixodes ricinus* was unable to cause this phenomenon. However, *I. ricinus* was able to cause this phenomenon when co-inoculated with *Borrelia lusitaniae* PotiB2, a Portuguese strain, whereas SGE from *I. scapularis* was unable to increase spirochete load using this same spirochete strain (28). Importantly, both *I scapularis* and *B. burgdorferi* are both present in the United States (29-32) while *I. ricinus* and *B. lusitaniae* are both located in Europe (33) and North Africa (34). Of note, a recent report by Collares-Pereira and co-workers identified the first isolate of *B. lusitaniae* in a patient (35). The data from Zeidner, *et al.* demonstrate a very specific relationship between spirochete and tick with a mechanism to increase spirochete load in target organs, potentially through assistance in dissemination and/or host evasion. Another, more recent set of data demonstrated that when human polymorphonuclear cells (PMNs) were incubated with *I. scapularis* saliva they had reduced adhesion and spreading via ß-2 integrin downregulation. In addition, they have a reduced ability to uptake and kill *B. burgdorferi* s. s. N40 as compared to PMNs not incubated with tick saliva (36). These effects of tick saliva on PMNs are thought to aid the spirochetes in initial survival in the host and establishment of infection.

## 7. CONCLUSION

It can be concluded that the *B. burgdorferi* s. l. complex of organisms is very well adapted to both host and vector. While several mechanisms for host immune evasion have been demonstrated, it is not likely that these can completely explain the success of these spirochetes in persistent infection. Several of these mechanisms have only slightly been uncovered as of yet and much more research is needed in this field to sort out the complex surface protein expression patterns and what they mean in both the tick and the mammal.

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