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Citation for published version (APA):

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Tick-host-pathogen interactions in Lyme borreliosis

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Chapter 1

Abstract

*Borrelia burgdorferi*, the spirochetal agent of Lyme borreliosis, is predominantly transmitted by *Ixodes* ticks. Spirochetes have developed many strategies to adapt to the different environments that are present in the arthropod vector and the vertebrate host. This review focuses on *B. burgdorferi* genes that are preferentially expressed in the tick and the vertebrate host, and describes how selected gene products facilitate spirochete survival throughout the enzootic life cycle. Interestingly, *B. burgdorferi* also enhances expression of specific *Ixodes scapularis* genes, such as *TROSPA* and *salp15*. The importance of these genes and their products for *B. burgdorferi* survival within the tick, and during the transmission process, will also be reviewed. Moreover, we discuss how such vector molecules could be used to develop vector-antigen-based vaccines to prevent the transmission of *B. burgdorferi* and, potentially, other arthropod-borne microbes.
Ixodes–mouse–Borrelia interactions

Ticks are acarid ectoparasites that, while taking a bloodmeal, can transmit a variety of human and animal illnesses. Lyme borreliosis is a common tick-borne disease in parts of the USA, Europe and Asia. *Ixodes scapularis*, *Ixodes ricinus* and *Ixodes persulcatus* are the most important vectors for Lyme borreliosis in the USA, Europe and Asia, respectively. Lyme borreliosis is caused by spirochetes of the *Borrelia burgdorferi* sensu lato group [1]. In Europe and Asia, three major *Borrelia* genospecies (*B. burgdorferi* sensu stricto, *Borrelia garinii* and *Borrelia afzelii*) are the causative agents. By contrast, only *B. burgdorferi* sensu stricto strains are present in the USA. In humans, all three species frequently cause an expanding, migrating red skin lesion, classically accompanied by central clearing, and designated erythema migrans. When the infection is untreated, the spirochete can disseminate and cause disease that affects various organs, including the joints, central nervous system and skin. *B. burgdorferi* sensu stricto has been linked with arthritis, whereas *B. garinii* is often associated with neuroborreliosis and *B. afzelii* is the dominant cause of a late cutaneous disease manifestation – acrodermatitis chronica atrophicans [2-3].

The enzootic life cycle of *B. burgdorferi* sensu stricto, from here on referred to as *B. burgdorferi*, involves the tick and vertebrate host. Generally, uninfected *I. scapularis* larvae acquire the bacterium by feeding on infected small animals, such as the white-footed mouse, *Peromyscus leucopus*. Ticks remain infected during the molting period. Nymphs can then transmit spirochetes to other mammals, including mice and humans, while taking their next bloodmeal. Nymphs thereafter molt to become adults that can also transmit the spirochetes during feeding. After the final bloodmeal, adult female ticks (which have already mated) lay eggs. These eggs are not infected with *B. burgdorferi* because the spirochetes are usually not transovarially transmitted. *B. burgdorferi* has developed mechanisms to survive in both the arthropod vector and reservoir host, and differentially expresses certain genes depending on the environment. Some of these gene products have direct interactions with tick proteins, whereas others bind to, or interact with, reservoir host proteins. Interestingly, *B. burgdorferi* enhances expression of certain *I. scapularis* genes that are beneficial for either vector colonization or for spirochete transmission from the vector to the host. Both tick and *Borrelia* proteins that influence successful *Borrelia* infection represent potential candidates for vaccine and/or drug development, and are the focus of this review.

The *B. burgdorferi* genome

*B. burgdorferi* is an extracellular organism belonging to the order Spirochaetales. The genome is roughly $1.5 \times 10^6$ base pairs [4] comprising a linear chromosome and 21 linear and circular plasmids, together containing 1780 genes [5-6]. A large portion of the genome encodes lipoproteins [4], such as the well-studied outer surface proteins (Osp) A and C. Several *Borrelia* proteins have been identified that have interactions either with host or tick ligands and assist pathogen survival [7-14].
Chapter 1

B. burgdorferi gene expression in the mammalian host

Upon entry into the host, the first obstacle B. burgdorferi has to overcome is the host innate immune system. A crucial line of defense in the innate immune response against invading microorganisms is the complement cascade, consisting of the classical, lectin and alternative pathways. The three pathways merge at the common intersection of complement factor C3. C3 is cleaved by C3 convertase to give C3a and C3b. Binding of C3b to the surface of microorganisms generally results in phagocytosis. A fraction of C3b can bind to C5, which is the first step towards formation of the membrane attack complex (MAC). Formation of the MAC on the microorganism's surface causes cell lysis. Many bacteria, such as Streptococcus pneumoniae [15] and Neisseria meningitidis [16], have evolved mechanisms to inhibit complement-mediated killing by binding to host plasma factor H or factor-H-like (FHL) protein resulting in factor I-mediated degradation of C3b. B. burgdorferi utilizes complement regulating-acquiring surface proteins (CRASP) [9] and Osp E/F related proteins (Erp) [8, 17-18] to bind factor H or FHL protein, and consequently inhibit complement-mediated borreliacidal activity. The ability to inhibit complement varies between the different Borrelia genospecies [19]. Also, Erp proteins have different relative affinities for factor H proteins from various potential animal hosts. Stevenson et al. suggest this enables the bacterium to inhibit complement-mediated killing in the diverse hosts it encounters during its enzootic life cycle [20].

Early in infection, B. burgdorferi expresses several genes that might be important for survival. For instance, decorin-binding protein A and B (DbpA and B) [7] and the fibronectin-binding protein BBK32 [21] bind to the host extracellular matrix components decorin and fibronectin, respectively, as has been shown by biochemical studies [7, 12]. Adhesion to extracellular host components might assist in survival of the few initial spirochetes that are transmitted through a tick bite [22]. Decorin-deficient mice infected with B. burgdorferi show diminished Borrelia numbers and less severe arthritis than infected wild-type controls [23]. It has also been shown that immunization of mice with recombinant DbpA induced protective immunity against B. burgdorferi [24]. The significant attenuation of Lyme borreliosis in mice infected with BBK32-deficient B. burgdorferi would suggest the importance of BBK32 [25], although other studies suggest that BBK32 is not essential for spirochete infectivity and pathogenicity [26]. By contrast, other B. burgdorferi genes are downregulated during the course of infection. Liang et al. showed that of the 116 lipoprotein encoding genes, which B. burgdorferi expresses early in infection, less than 40 were expressed several weeks after infection [27]. Host immune pressure is likely to be involved in this selection [28].

Once a disseminated infection has been established, B. burgdorferi needs to evade adaptive host immune responses. One mechanism that might be important in immune evasion is recombination at the variable major protein-like sequence (vls) locus [29-30], which has also been described in other Borrelia genospecies [31]. The vls locus consists of an expression site for the lipoprotein VlsE and 15 unexpressed upstream silent cassettes [32]. Upon infection of the host, segments of
the silent vls cassettes randomly recombine into the vlsE gene, resulting in multiple variations of the VlsE protein during the course of infection [32-34]. In mice experimentally infected with B. burgdorferi [29-30, 34] and in human Lyme disease patients [35], strong antibody responses directed against conserved regions of the VlsE protein have been reported. In addition, in these experimentally infected mice, antibody responses against surface exposed [36] variable regions of VlsE protein variants were observed [34]. The ability of B. burgdorferi to survive in the presence of this robust anti-VlsE antibody response indicates that vls antigenic variation, resulting in changes of the variable regions and altered antigenicity [34] of the VlsE protein, protects the spirochete from destruction by anti-VlsE antibodies. Recombination seems to be promoted by host inflammatory responses [29]. In line with this, there is no detectable variation of VlsE in ticks [37].

We have discussed several B. burgdorferi genes that are differentially expressed in the mammalian host, but B. burgdorferi also differentially expresses selected genes in various tissues within the same host [38]. To study gene expression of B. burgdorferi in neuroborreliosis in primates, Narasimhan et al. modified a PCR-amplification strategy, differentiation expression using customized amplification libraries (DECAL), originally developed by Alland et al. to examine mycobacterial genes in vivo [38-39]. A large number of B. burgdorferi genes were found, both on the chromosome and plasmids, that were either up- or downregulated in the central nervous system (CNS) compared with other tissues. These genes are promising candidates for future research on the molecular interaction of the spirochete with the CNS.

**B. burgdorferi gene expression in the tick and B. burgdorferi-induced tick gene expression**

While infected *Ixodes* ticks engorge, the antigenic composition of B. burgdorferi changes drastically. B. burgdorferi genes upregulated in engorging *I. scapularis* nymphs include those encoding putative lipoproteins and periplasmic proteins [40]. The biological function of OspA, a B. burgdorferi protein produced while the spirochete resides in the *I. scapularis* gut [41-43], but downregulated during tick engorgement, has been established. A strain of B. burgdorferi deficient for OspA and OspB was able to infect mice and cause arthritis [44], but could not colonize the tick gut [45]. This strongly suggests that OspA and/or OspB have an important role in persistence of the spirochete within the vector. Indeed, Pal et al. identified a tick receptor for OspA, designated the tick receptor for OspA (TROSPA) [11] (Figure 1). TROSPA was highly expressed in the guts of tick larvae and nymphs, and, to a lesser extent, in the gut of adult ticks. In infected nymphs TROSPA was expressed more abundantly than in uninfected controls. It might be advantageous for the spirochete to induce the tick to produce high levels of TROSPA so that the spirochete can persist in the tick gut during the long interval between bloodmeals. In line with this, TROSPA mRNA levels in flat ticks were significantly higher than in engorged ticks. Recently, it was shown that an OspB-deficient B. burgdorferi strain had impaired ability to adhere to the tick gut [10]. Neelakanta et al. suggest a synergistic interaction of OspA, OspB and TROSPA [10].
While ospA is downregulated within the gut of the engorged tick, the spirochete produces OspC during migration to the salivary glands and initial mammalian infection. Pal et al. have shown that *B. burgdorferi* recombinant OspC binds to *I. scapularis* salivary gland extracts [46]. Moreover, IgG F(ab)2 fragments from polyclonal OspC antisera reduce invasion of the salivary gland by *B. burgdorferi*. They also created an OspC-deficient spirochete that migrated poorly to the salivary glands and transmission of the OspC mutant to the murine host was dramatically impaired. This is in line with data from Grimm et al. that showed that OspC mutants were unable to infect severe combined immunodeficient mice (SCID); however, they showed (by immunofluorescence assays (IFA) and confocal microscopy) that OspC mutants were able to invade the tick salivary gland [47].

**Figure 1.** Schematic overview of tick–host–pathogen interactions important in the development of Lyme borreliosis. In the gut of *Ixodes scapularis* ticks, *Borrelia burgdorferi* upregulates ospA expression. OspA binds to the tick receptor for OspA (TROSPA) [11]. During tick feeding, *B. burgdorferi* downregulates ospA expression, starts producing OspC and migrates to the salivary gland. OspC binds to a tick salivary protein, Salp15. Salp15 has immunosuppressive properties, such as inhibiting CD4+ T-cell activation [48], and immunoprotective effects, such as inhibiting antibody-mediated killing of *B. burgdorferi* by the host [13]. For other tick–host–pathogen factors that are of importance in Lyme borreliosis see the text. Unbroken arrows represent processes that take place within the tick, and broken arrows represent processes that take place at the tick–host–pathogen interface. Abbreviations: APC, antigen presenting cell; MHC, major histocompatibility complex; OspA and OspC, outer surface protein A and C; TCR, T cell receptor. For color figure see page 258.
The spirochete enhances expression of certain tick genes, among which salp15, a gene encoding for a 15 kDa feeding-induced salivary gland protein. Salp15 inhibits activation of T lymphocytes by binding to the CD4 co-receptor on the surface of T lymphocytes [14, 48]. A recent study showed that Salp15 also interacts with B. burgdorferi by binding to OspC [13]. This binding protects the spirochete from antibody-mediated killing (Figure 1). Syringe infection of naive mice with B. burgdorferi and recombinant Salp15 resulted in significantly higher Borrelia numbers compared to infection with Borrelia alone. Moreover, mice with an acquired immune response to B. burgdorferi – mice infected with B. burgdorferi and thereafter treated with ceftriaxone – could not be re-infected with B. burgdorferi given as an intradermal challenge. However, these mice could be infected when they were (re-)infected with a cocktail of B. burgdorferi and recombinant Salp15. RNA interference-mediated repression of salp15 in I. scapularis ticks drastically reduced the capacity of these ticks to transmit spirochetes to mice [13]. In nature, increased Salp15 levels could be beneficial for both vector and spirochete, because the tick might use Salp15’s immunosuppressive capacities to avoid rejection and engorge more effectively, whereas the spirochete, by binding to Salp15, is capable of invading hosts that have previously encountered B. burgdorferi. Interestingly, Salp15 homologues were recently also identified in the European vector for Lyme borreliosis, Ixodes ricinus [49].

Implications for tick-antigen-based vaccine development to prevent Lyme borreliosis

A large number of human pathogens are transmitted by specific arthropod vectors. Understanding the interactions between vector and pathogen might help in developing strategies to combat arthropod-borne infections. The rationale for a tick-antigen-based vaccine is that repeated exposure of animals to tick bites results in an inability of ticks to successfully take a bloodmeal from these animals, as measured by impaired attachment, lower post engorgement weights, increased levels of tick mortality and lower fertility rates [48]. Acute basophil hypersensitivity, altered cytokine production profiles and circulating antibodies against tick antigens might all contribute to this phenomenon, also known as ‘tick immunity’ [50]. Interestingly, tick immune animals are less susceptible to transmission of several pathogens, including B. burgdorferi [51].

Several tick-antigen-based vaccines have been developed with mixed success and some of them also affect pathogen transmission [52]. It was shown that mice vaccinated with a recombinant Rhipicephalus appendiculatus cement protein, 64TRP, and challenged with tick-borne encephalitis virus (TBEV)-infected I. ricinus, were largely protected from lethal infection [53]. De la Fuente et al. identified an I. scapularis protein, sobulesin, that is involved in tick feeding and reproduction, and is highly conserved among a broad range of tick species [54]. They showed that ticks fed on mice vaccinated with recombinant sobulesin were unable to efficiently acquire Anaplasma phagocytophilum [52]. Recently, 500 new predicted salivary gland proteins were identified by comparing random clones from salivary gland cDNA expression libraries derived from fed, unfed,
Chapter 1

*B. burgdorferi*-infected, uninfected, nymphal and adult *I. scapularis* ticks [55]. Understanding the function of these novel proteins in tick biology is crucial for development of new tick-antigen-based vaccines.

Because the interactions of TROSPA and Salp15 with *B. burgdorferi* have been established, in the next paragraphs, we will focus on TROSPA and Salp15 as potential candidates for tick-antigen-based vaccines to prevent Lyme borreliosis. A TROSPA vaccine could lead to decreased *B. burgdorferi* colonization and survival in the tick gut, and might diminish pathogen transmission to subsequent hosts. Previously, it had been shown that vaccinating wild mice with an OspA-based vaccine results in a reduction of the prevalence of *B. burgdorferi*-infected ticks, thus potentially lowering the risk of pathogen transmission to humans [56-58]. In theory, uninfected *I. scapularis* ticks feeding on *B. burgdorferi*-infected, yet also TROSPA-vaccinated, mice, will not acquire the spirochetes because TROSPA antibodies could interfere with *B. burgdorferi* binding to the tick gut. Therefore, vaccination of wild mice in endemic areas with a TROSPA-based vaccine, alone or in combination with an OspA vaccine, could lead to decreased *B. burgdorferi* infection rates of *I. scapularis* populations. Appropriate vaccine delivery systems would need to be developed. However, oral OspA-based preparations have been shown to protect laboratory mice against *B. burgdorferi* infection [59].

Interfering with OspC and Salp15 binding could, theoretically, result in diminished transmission of spirochetes from the vector to the host. Salp15 was originally identified by screening an *I. scapularis* salivary gland cDNA expression library with tick immune rabbit sera, suggesting that antibodies against Salp15 participate in tick rejection [60]. A vaccine against Salp15 could inhibit or diminish pathogen transmission from the tick to the host in two distinct ways. Salp15 antibodies could neutralize the immunosuppressive effects of Salp15 and thereby impair tick engorgement, making the tick–host–pathogen interface a more hostile environment for both tick and *B. burgdorferi*. In addition, Salp15 antibodies could bind to Salp15 that has previously bound to OspC on the surface of *B. burgdorferi* in the tick salivary gland, and thereby enhance clearance by host phagocytotic immune cells. The Salp15 antibodies would need to recognize a different Salp15 epitope than the epitope that is required for binding of Salp15 to OspC.

**Summary**

We have reviewed some of the interactions between *B. burgdorferi*, *I. scapularis* and the mammalian host. *B. burgdorferi* genes that are preferentially expressed in the tick and the mammalian host are crucial for spirochete survival throughout the enzootic life cycle. *B. burgdorferi* might also enhance the production of *I. scapularis* proteins that are crucial for *Borrelia* survival within the tick and transmission to the host. Future studies should focus on the identification of both vector and pathogen proteins that are important in pathogen transmission or vector colonization because these could be potential candidates for novel vaccination or therapeutic strategies for Lyme...
borreliosis. The mechanism by which *I. scapularis* TROSPA and Salp15 interact with *B. burgdorferi* could serve as a model to understand the interaction of other vectors and pathogens, and lead to new prevention strategies.

**Acknowledgments**

We are grateful to Christian Stutzer who assisted in the design of the figure and Marcel Schouten and K.E. Hovius for critically reading the manuscript.
Chapter 1

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Introduction


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Chapter 1

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