

## REVIEW ARTICLE

# Complement evasion strategies of *Borrelia burgdorferi* sensu lato

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**Borreliosis (Lyme disease) is a spirochetal disease caused by the species complex of *Borrelia burgdorferi* transmitted by *Ixodes* spp. ticks. Recorded to be the most common tick-borne disease in the world, the last two decades have seen an increase in disease incidence and distribution, exceeding 360 000 cases in Europe alone. If untreated, infection may cause skin symptoms, arthritis, and neurological or cardiac complications. *Borrelia* spirochetes have developed strategies to evade the mammalian host immune system. These include the complement system, which is an important first-line defense mechanism against invading microbes. To evade the complement, spirochetes bind soluble complement regulators factor H (FH), factor H-like protein, and C4bp to their outer surfaces. *B. burgdorferi* spirochetes can inhibit the classical pathway of complement by the outer surface protein (Osp) BBK32, which blocks the activation of the C1 complex, composed of C1q, C1r, and C1s. The FH-binding proteins of borreliae include Osps OspE, CspA, and CspZ. Following repeated infections, antibodies against these proteins develop and may provide functional immunity against borreliosis. This review discusses critical immune evasion strategies, focusing on complement evasion by borreliae.**

**Keywords:** BBK32; *Borrelia*; complement system; CspA; CspZ; factor H; immune evasion; OspE

## Epidemiology and life cycle of *Borrelia burgdorferi*

Borreliosis is transmitted by ticks of *Ixodes* spp. [1]. It is a spirochetal disease caused by the species complex of *Borrelia burgdorferi* sensu lato (sl). Borreliosis, also called Lyme disease or Lyme borreliosis (LB), has been declared as the most common tick-borne disease in the world. Over the past few decades, the incidence of LB has increased to ~ 360 000 cases in Europe alone [2]. Of the *B. burgdorferi* sl spirochetes, *B. garinii* and *B. afzelii* are the most prevalent species in Europe [1,3]. More

recently, also *B. bavariensis* and *B. spielmanii* have been reported [4,5]. In North America, *B. burgdorferi* sensu stricto (ss) is the main species [6]. The different *Borrelia* species are connected to various clinical symptoms. *B. garinii* is the main species responsible for neurological symptoms. Skin symptoms are predominantly caused by *B. afzelii* and less frequently by *B. garinii* or other species [7]. In a systemic infection, *B. burgdorferi* ss usually causes joint symptoms.

## Abbreviations

ACA, acrodermatitis chronica atrophicans; CRASP, complement regulator-acquiring surface protein; Dbp, decorin-binding protein; EM, erythema migrans; FH, factor H; FHL-1, factor H-like protein; FHR, factor H-related protein; LB, Lyme borreliosis; MAC, membrane attack complex; Osp, outer surface protein.

The primary vectors of *B. burgdorferi* are ticks of *Ixodes* spp. These are *I. ricinus* in a large part of Europe, *I. pacificus* and *I. scapularis* in North America, and *I. persulcatus* in Asia and northeast Europe. The life cycle of *Ixodes* ticks involves the egg, larva, nymph, and adult tick stages. After hatching from an egg, the larva obtains its blood meal to be able to develop into a nymph [7]. The development period from an egg to an adult takes ~ 2 years (in *I. scapularis*). This depends on several factors, such as the availability of appropriate hosts and climatic conditions [8]. All the different forms of the tick, larvae, nymphs, and adults are able to carry borreliae, but only nymphs and adults transmit them to humans or other hosts (birds or reptiles). A developmental phase usually follows every blood meal. Several weeks after being hatched from eggs, the larva feeds on blood (for about 2–4 days) and molts (for several weeks). Subsequently, a nymph feeds on blood for 4–6 days and molts for several weeks before developing into an adult tick [9].

In order to survive within ticks, borreliae regulate their gene expression depending on the environmental conditions and various stimuli [10]. The spirochetes relocate from the animal host dermis to the tick gut lumen during a blood meal. They first adapt to the temperature change and stay alive within the tick [11–13]. In the tick, the spirochetes overexpress the outer surface proteins A and B (OspA and OspB) and attach to an OspA receptor that promotes survival of the bacteria [14,15]. In 24–48 h after attaching to a warm-blooded animal, the ratio of the Osps changes leading to downregulation of OspA and OspB and to the dominance of OspC, decorin-binding proteins A and B (DbpA and DbpB), and the P66 protein. Also, the complement factor H (FH)-binding protein OspE becomes upregulated [9,16]. Simultaneously, borreliae move from the salivary glands of the tick *via* saliva to the human host [17]. Borreliae can move from the tick to the human within hours, although the probability of the transmission increases during a prolonged duration of the tick attachment [18,19]. One reason for the delayed transmission is that it requires the upregulation of borrelial surface molecules for binding to host receptors and for immune evasion.

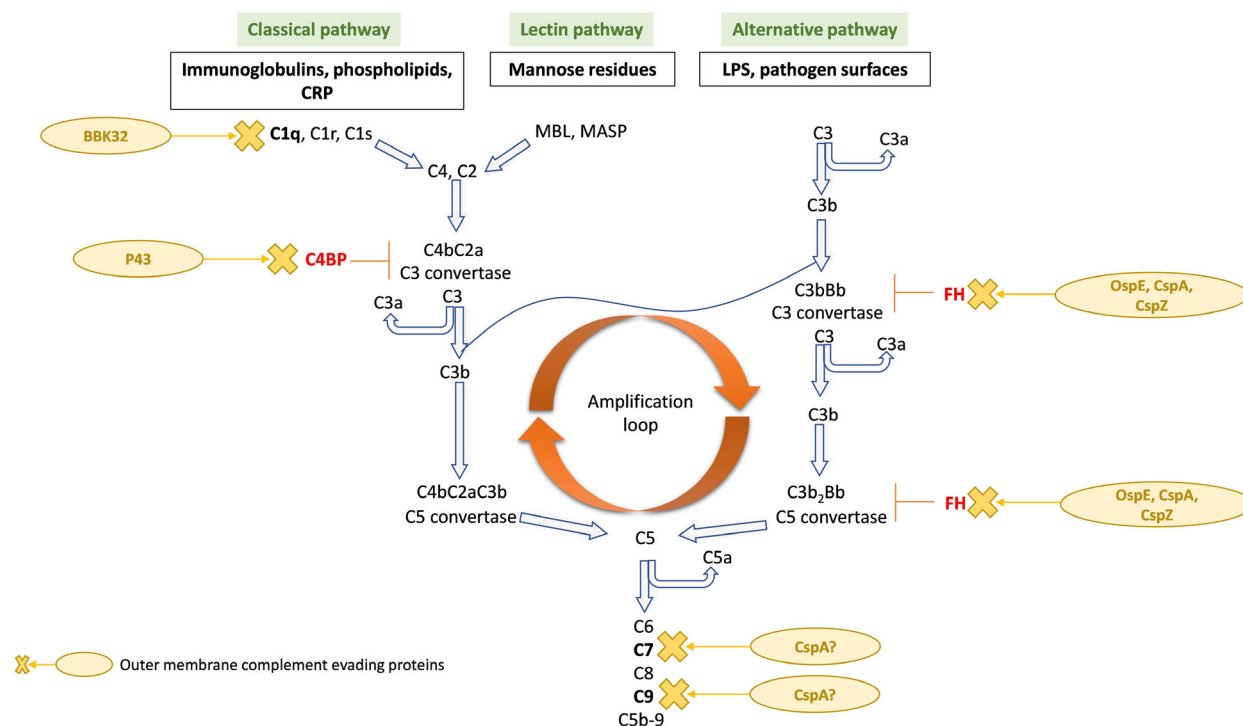
## The complement system and general mechanisms of microbial killing

The complement system is the first-line defense against any invading microbe, yet many pathogens can escape complement-mediated phagocytosis and killing. Complement protects the host animal from infection by

generating chemotactic molecules, recruiting and activating leukocytes, opsonizing targets for phagocytosis, and causing lysis of the target cells. The complement system includes ~ 30 serum proteins constituting 5–10% of total serum proteins (after removal of albumin). Another ~ 20 proteins act as complement regulators or receptors.

Three principal pathways are involved in complement activation (Fig. 1). They all converge at the activation of C3. C3 convertase enzymes, C4bC2a in the classical and lectin pathway or C3bBb in the alternative pathway (AP), cleave C3 into C3a and C3b. C3a functions as an anaphylatoxin and chemotxin and C3b, by covalent binding to the activating surface, opsonizes particles, and participates in the self-amplification loop of complement *via* the AP. The C3/C5 convertases also cleave C5 into C5a and C5b [20]. The association of C5b with C6, C7, C8, and multiple C9 molecules leads to the generation of C5b-9, the membrane attack complex of complement (MAC). MAC is a multimolecular barrel-like structure, which physically creates a pore on the target cell leading to cell lysis [21]. On human cell membranes, the main inhibitor of MAC is CD59 (protectin) [22]. *B. burgdorferi* has been suggested to display a functionally homologous 80 kDa protein that binds to C8b and C9 [23]. However, the nature and function of this molecule await further characterization. Figure 1 depicts the complement activation pathways and points of *Borrelia* interference that will be the focus of this article.

Factor H and factor H-like protein-1 (FHL-1), an alternatively spliced product of the FH gene, control C3b formation. They inhibit the formation and activity of the AP C3 convertase enzyme C3bBb and act as cofactors for factor I-mediated inactivation of C3b. The ‘regulators of complement activation’-gene cluster in chromosome 1 encodes also five FH-related (FHR) proteins: FHR1, FHR2, FHR3, FHR4, and FHR5. They are secreted mostly from the liver into the plasma, although local synthesis at various body sites also occurs. The FHRs, akin to FH and FHL1, are made up of SCR (short consensus repeats) domains. However, with the possible exception of FHR5, FHRs lack domains that are directly involved with complement regulation. There is a high structural similarity between the C-terminal domains of FH and FHR proteins, which allows the FHRs to bind to similar surface ligands. Toward this, FHR1 is known to bind to the C3d part of C3b *via* SCRs 4–5 and to surfaces coated with polyanions such as FH SCRs 19–20. Thus, FHR1 and certain other FHRs can compete with FH for binding to C3b/d and ‘deregulate’ complement activation. This is more efficient, if FHRs occur in dimers, naturally or as a consequence of



**Fig. 1.** The complement activation pathways and interference points by borrelial surface proteins. For details, see text.

genetic alterations [24]. This activity may have evolved upon the need to counteract microbial FH-binding proteins that the bacteria use for their complement evasion. Antibodies reacting with FH, especially those against the C terminus, can also bind to some FHR proteins [25]. These antibodies have been observed in the autoimmune form of atypical hemolytic uremic syndrome [26].

Factor H is a plasma protein of 155 kDa. It is the most important soluble regulator of the complement cascade. It is made up of 20 SCRs and has several functions and binding sites spread along the length of the protein [27]. It acts as a cofactor for the inactivation of C3b-mediated by factor I and promotes the decay of C3bBb, the AP C3 convertase. FH has a higher affinity for C3b, when it is attached to sialylated lipo-oligosaccharides on the surfaces of pathogens, such as *Neisseria gonorrhoeae* [28]. Through the binding of FH to the surface of pathogenic microorganisms, the amplification of complement activation is prevented, thereby protecting the invading pathogenic bacteria from complement-mediated killing [29].

## Expression of surface proteins and motility help invasion

The *B. burgdorferi* sl share common structural features of spirochetes. They are microaerophilic motile

bacteria with spiral-shaped cells of 20–30 µm length and 0.2–0.3 µm in diameter [30,31]. The cell envelope is made of an inner cell membrane, a periplasmic peptidoglycan layer, and an outer membrane made of lipoproteins [32]. The outer cell membrane lacks lipopolysaccharide (LPS) [33,34]. *Borreliae* possess low molecular weight glycolipids anchored to the membrane and many lipoproteins (e.g., OspA, OspB, OspC, OspD, OspE, OspF, CspA, BptA, variable-like sequence expressed, DbpA, DbpB, BBK32, and BBA64) [33]. The glycolipids are localized to the periplasmic side of the outer membrane, as well. *Borrelia* spirochetes can change the location of these glycolipids at different points of infection between the outer membrane surface and the periplasmic side [32,33]. The glycolipids in the outer membrane form lipid-raft-like microdomains. They vary in size and order in response to temperature changes, which are vital environmental cues during transmission of *borreliae* between the tick vector and the mammalian host [35]. Like other spirochetes, also *Lyme borreliae* have periplasmic flagellas. They direct the movement of the spirochetes toward chemoattractants (e.g., nutrients such as serum proteins) and away from repellents (e.g., ethanol and butanol) [36–38]. The insidious invasion and mild immune reactions to invading spirochetes suggest that *borreliae* suppress strong inflammatory reactions, at least during the early stages of infection.

Chemotaxis and motility are known to be vital for the survival of borreliae in both ticks and vertebrate hosts [39]. It has been reported that the spirochetes might have an ability to penetrate through epithelial cells [40].

### ***Borrelia* species and their relation to different clinical symptoms in humans**

Clinical symptoms of LB are divided into three phases: early local, early disseminated, and late phase. Different *Borrelia* species are connected to various clinical symptoms. *B. garinii* is mainly (66.7%) responsible for neurological symptoms, but also *B. afzelii* (27.5%), *B. burgdorferi* ss (5%), and rarely *B. bavariensis* (0.8%) can cause them [41,42]. The neurological symptoms include facial palsy and meningitis as an early disseminated disease and encephalomyelitis or chronic neuropathy as a late disease [41,42]. Of the skin symptoms, erythema migrans (EM) in Europe is predominantly caused by *B. afzelii* (70–90%), less frequently by *B. garinii* (10–20%), rarely by *B. burgdorferi* ss (0.4%), and only exceptionally by other species such as *B. spielmanii* [7]. *B. burgdorferi* ss can cause joint symptoms. Because it is the main subspecies in North America, the clinical symptoms there are usually EM as a skin symptom and arthritis.

The skin lesion EM represents the early local sign of borreliosis. It appears days to weeks after the tick bite. EM can also be multiple with several erythema lesions [43–45]. Lymphocytoma occurs more frequently in children (7%) than in adults (2%). It can persist from weeks to months. Typical sites for lymphocytoma are ear lobes, nipples, and scrotum in males [46,47]. The skin manifestation of the late phase is acrodermatitis chronica atrophicans (ACA) appearing months to years from the tick bite. It occurs more frequently in elderly people and women. An edematous infiltrative stage with erythema as well as atrophic stage with skin atrophy, red-bluish-brownish color, and sometimes juxta-articular fibroid nodules and peripheral neuropathy manifest usually at the extremities [45,48,49]. Lymphocytoma and ACA are caused by *B. afzelii*. Therefore, they are not seen in North America [50,51]. Various skin manifestations of borreliosis are shown in Fig. 2.

It is likely that the clinical manifestations are caused partially by the spirochetes directly and partially by the host immune system, including complement as an effector mechanism. The ability to escape complement may also explain the paucity of symptoms, such as the absence of granulomas or abscesses, and chronic nature of some of the manifestations. Therefore, the

interactions of the spirochetes with the complement system are of particular interest.

### **Complement evasion by *Borrelia burgdorferi* sensu lato**

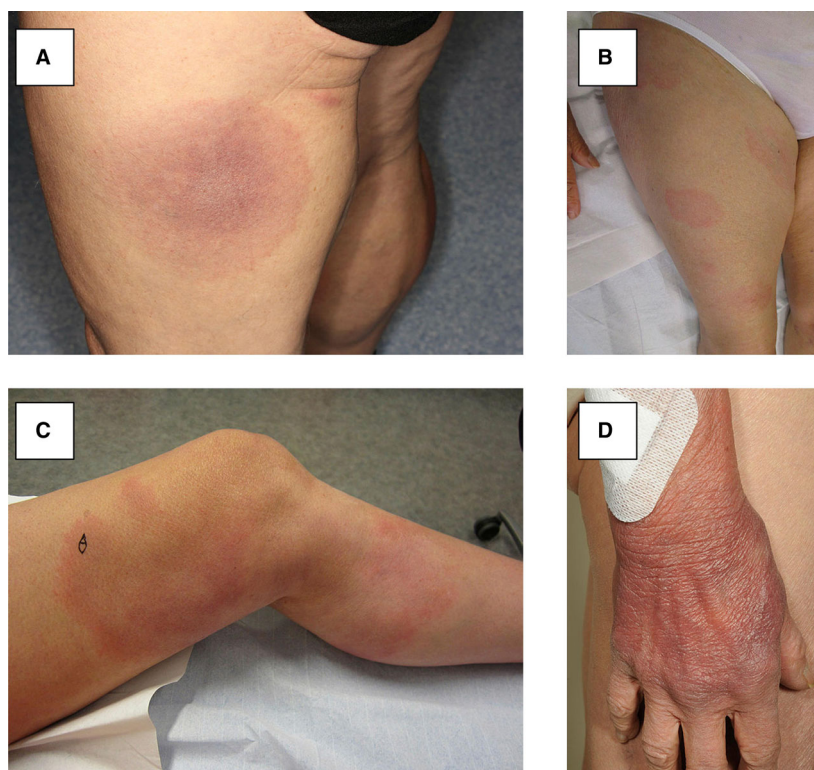
The mechanisms of complement evasion by borreliae have been intensively studied during the last two decades. The spirochetes have to deal with all the three different complement activation pathways or with a common pathway to be able to invade and cause a systemic disease. Since borreliae are widely distributed in different animals in nature, including many vertebrates and ticks, they must have multiple means to escape complement [52–54]. *In vitro* studies have shown that some species of borreliae (*B. burgdorferi* ss, *B. bavariensis*, *B. afzelii*, and *B. spielmanii*) are serum-resistant in humans, while at least *B. garinii* and *B. lusitaniae* are—to a certain extent—serum-sensitive. However, the degrees of serum sensitivities differ among strains of certain species [52–54]. The spirochetes have been reported to evade complement pathways by three main means: (a) recruiting mammalian host complement regulators, (b) possessing factors that affect the complement system of the host, and (c) exploiting tick proteins [55]. Points (a) and (b) are schematically presented in Fig. 3 and will be discussed below.

#### **Binding of FH/FHL-1**

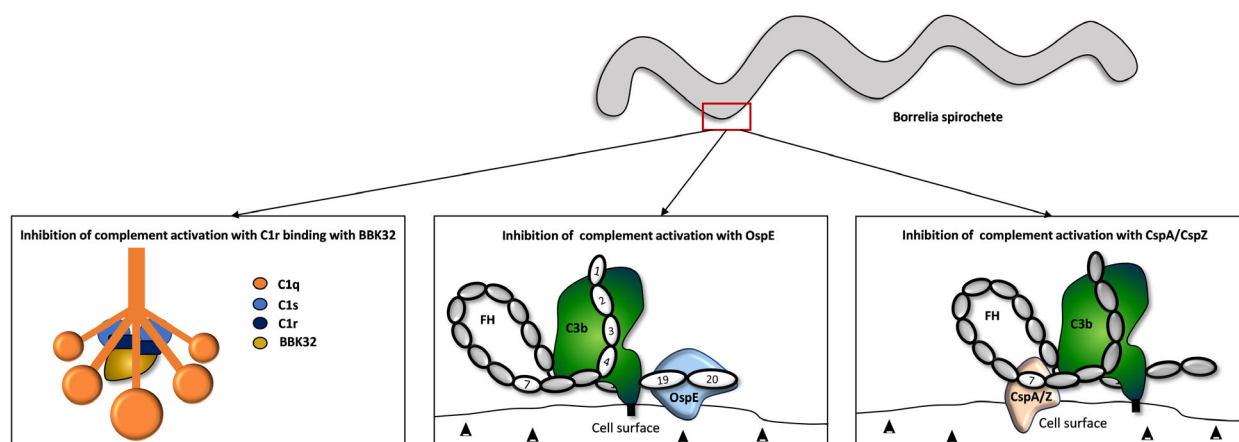
To protect themselves and to avoid attack by the complement system, borreliae bind soluble complement regulatory proteins to their surfaces. Initial studies measuring bacterial killing by the loss of their motility showed that *B. burgdorferi* ss and *B. afzelii* could resist killing in nonimmune serum, whereas *B. garinii* were killed within 2 h, when exposed to 40% normal human serum [56,57]. *B. garinii* strains were adapted to growth in the laboratory, which may have contributed to a possible loss of complement evading capacity. *In vivo*, their ability to cause neuroborreliosis suggests that they must be capable of penetrating through the tissues and avoid complement, as well.

#### **OspE**

The initial hint for the complement escape mechanism came, when we found that the *B. burgdorferi* ss and *B. afzelii* strains promoted C3b inactivation on their surfaces [56]. Subsequently, the serum-resistant *B. burgdorferi* ss and *B. afzelii* were found to bind complement inhibitors FH and FHL-1 [56,58,59]. This



**Fig. 2.** Skin manifestations of borreliosis patients. Solitary erythema migrans (A and C), multiple EM (B), and ACA (D).



**Fig. 3.** Schematic illustration of inhibition of complement activation at the C1 and C3 steps by known borrelian outer surface proteins. For details, see text.

mechanism was first discovered by using a ligand-blotting assay, where detergent-solubilized lysates of bacterial outer membranes were run on SDS/PAGE gels, transferred to a nitrocellulose membrane, and blotted with radiolabeled FH or FHL-1 [56]. By this method, two different FH-binding proteins were detected. The first FH-binding protein was identified as the OspE, which was found to bind to the C-terminal domains 19–20 of FH [58]. Binding of FH/FHL-1 was

confirmed by immunofluorescence microscopy and the surface plasmon resonance technique [58,60]. When added to normal human serum in a soluble form, OspE was able to increase killing of borrelia spirochetes apparently because it prevented FH binding to the bacteria coated with C3b. OspE constitutes a family of proteins encoded by genes in the circular cp32 plasmid. A single bacterium can have multiple cp32 plasmids and thereby multiple OspE proteins, as well.

Interestingly, only paralogs of OspE, but not of the related OspF or Elp proteins, bound FH [60]. The OspE family includes proteins referred to as ErpA, ErpP, ErpC, and p21. Erp refers to OspE/F-related proteins. Thus, it is important to note that among the Erp proteins it is only the OspE proteins that bind FH. Despite the fact that these represent paralogs of the same OspE protein, these have been later called also as complement regulator-acquiring surface protein (CRASP)-3, CRASP-4, and CRASP-5 [61].

### OspE expression

Interestingly, the expression of OspE proteins was found to be induced in ticks fed with blood, but not by bacteria in nonfed ticks [16,60,62]. A similar upregulation was found in the host adaptation model developed by Akins *et al.*, where bacteria were incubated in a dialysis chamber within the rat peritoneum [60,63] (Fig. 4). *Borrelia* spirochetes can bind also other members of the FH family, namely FHR proteins 1, 2, and/or 5, but the functional relevance of these interactions is not well known [58,64]. FHRs are 'pseudoinhibitors' of complement that resemble FH, but do not have its functional activities in controlling complement activation. Potentially, FHR binding could compete out FH binding and thereby promote complement attack against the bacteria.

OspE-FH interaction studied on *B. garinii* showed that while *B. garinii* carries *ospE* genes, it has decreased OspE protein expression *in vitro* and

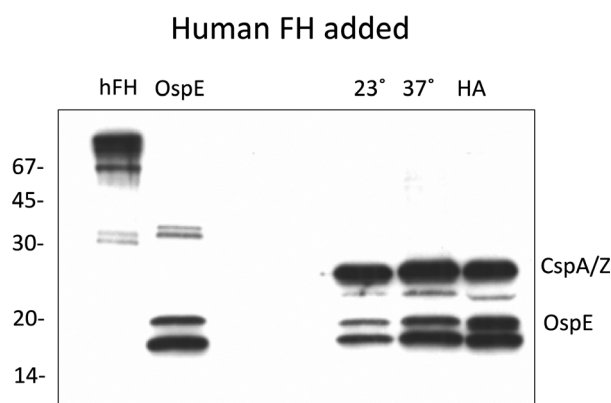
therefore, a decreased capability for FH binding, especially in long-term cultures. However, neuroinvasive *B. garinii*, the leading cause of neuroborreliosis, can express FH-binding proteins *in vivo*, thereby contributing its virulence [65]. Expression of OspE *in vivo* in borreliosis patients has also been concluded from the fact that neuroborreliosis patients with *B. garinii* infections have generated antibodies against OspE [66]. Furthermore, the anti-OspE antibodies are capable of preventing FH binding to the borrelial surfaces. A peculiar feature of *B. garinii* is that most of the OspE sequences derived from strains causing neuroborreliosis were found to be identical, whereas from all other borrelia strains a variety of different sequences were derived [67]. It may thus be that the OspE with this sequence has particular properties that assist the virulence of *B. garinii* in causing neuroborreliosis.

### Structure of OspE-FH19-20

The X-ray crystal structure of the complex between OspE and the FH C terminus, that is, domains 19 and 20, showed that OspE binds the domain 20 of FH similarly as our own cells do [68]. Thus, borreliae functionally mimic the essential self-protection mechanism that the host cells also use. In fact, the specific binding site on FH domain 20 is exploited by multiple pathogenic bacteria such as *Haemophilus influenzae*, *Bordetella pertussis*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*, relapsing fever spirochetes, and even by the yeast *Candida albicans* [69]. In evolution, it has thus been remarkable that various pathogenic microbes have developed the same mechanism, but different proteins, for complement escape.

### CspA

The second identified FH-binding protein on *Borrelia* was identified as CspA, known as BBA68 on the basis of its gene's open reading frame number, and also previously referred to as CRASP-1 [70]. *cspA*-encoding sequences, as well as those of *ospE*, have been found to occur only in the genus *Borrelia* [64]. The mature form of CspA from *B. afzelii* was found to be encoded by the linear plasmid lp54 and has a molecular weight of 26.4 kDa [71]. CspA bound more strongly to FHL-1 than to FH. A *B. burgdorferi* mutant that did not express CspA was serum sensitive *in vitro*, a property that could be reversed in a complementation mutant [72]. Subsequently, the binding site on FH/FHL-1 was mapped to domain 7, which, in addition to domains 19–20 in FH, is another 'hot spot' for binding of microbial FH-binding proteins [70]. A homodimer or



**Fig. 4.** Regulation of two main types of complement FH-binding proteins by temperature and host adaptation of *B. burgdorferi* strain B31. Spirochetes were incubated at 23 °C, 37 °C, or in dialysis membrane chambers in the peritoneum of rats as described by Akins *et al.* [63]. Bacterial lysates, purified FH (control for secondary antibody), and borrelial OspE were run in an SDS/PAGE gel and transferred to nitrocellulose. After incubation with FH, the FH-binding proteins were detected by an anti-FH antibody. (Alitalo A, Hefty S, Akins D and Meri S, unpublished).

another multiple order structure of CspA was required for the interaction with FH/FHL-1 [73]. Although CspA binds FH/FHL-1, it is expressed by the *Borrelia* spirochetes in the mammalian host only at the tick biting site (Fig. 4), but not during later stages of the infection.

### CspZ

The third candidate molecule that binds FH is CspZ [74]. Its gene is located on the linear plasmid lp28-3. In contrast to CspA, the CspZ protein has been shown to be expressed by the spirochetes only during mammalian infection, whereas, as mentioned above, CspA is being expressed particularly during the tick–host and host–tick transmissions [75]. Like CspA, also CspZ binds to the domain 7 of FH and FHL-1. However, mutant strains lacking CspZ are still resistant to complement killing, which suggests that this molecule has an additional role. Furthermore, CspZ is expressed by *B. burgdorferi* ss and *B. spielmanii*, but not by *B. garinii* nor by *B. afzelii* strains, although CspZ genes have been detected in these strains [64]. Immunization of mice with CspZ does not protect them from borreliosis. However, a recent study suggested that a CspZ variant without the ability to bind FH could be more useful in this respect [76]. The same principle of removing the FH-binding activity of FH-binding proteins for vaccine purposes [77] has been exploited in the case of group B meningococcal vaccine development [78].

Thus, the complement resistance of borreliae is associated with the expression of OspE, CspA, and CspZ proteins. Their activities are synergistic, because they bind to different regions of FH, their temporal expression patterns differ, and their expression levels vary in the different borrelia species. In addition, it is likely that their activities toward FH molecules from different host animal species vary, as well [79].

### Binding of C4bp

The classical and lectin pathways of complement are inhibited by the C4b-binding protein C4bp. The various types of Lyme borreliae (*B. burgdorferi*, *B. afzelii*, and *B. garinii*) bind C4bp, but, interestingly, the strongest binding was observed by *B. garinii* strains, which only showed a weak binding of FH [55,80]. The receptor for C4bp was observed to be a protein with a molecular weight of 43 kDa (P43). The molecular characteristics of this protein have not yet been further elucidated. Inhibition of the classical pathway (CP) is particularly important for the bacteria, when patients have either

natural antibodies against the bacteria or antibodies acquired during infections. Because of an ability to inhibit complement even in the presence of antibodies, the borrelia spirochetes are capable of causing repeated or prolonged infections. Functionally, C4bp binding leads to inhibition of the classical/lectin pathway C3 convertase C4bC2a and promotion of the inactivation of C4b in an analogous fashion as FH does for C3b in the AP. C4bp binding is not unique for the *B. burgdorferi* species/group. We observed that relapsing fever borreliae, such as *B. recurrentis* and *B. duttonii*, are particularly strong binders of C4bp [81].

### Inhibition of C1 activation

In addition to the above described mechanisms, *B. burgdorferi* ss can inhibit the CP of the complement by the Osp BBK32, which blocks the activation of the C1 complex composed of C1q, C1r, and C1s [82]. BBK32 was originally described as a fibronectin-binding protein (P35) that can mediate attachment of the bacteria and induce potentially protective immunity [83]. BBK32 is known to initiate the tethering of *B. burgdorferi* to the vascular surface. BBK32–glycosaminoglycan interaction is responsible for further stabilizing the vascular interaction, whereafter borreliae transmigrate across the endothelium to disseminate into tissues [84].

BBK32 was described to bind to the C1r protein in the C1 complex with high affinity and prevent its autoactivation or proteolytic activity toward C1s [82]. The activity was located to the C-terminal domain of BBK32 (BBK32-C). Orthologues of BBK32 have been identified and found to have ~90–70% overall amino acid sequence identity in *B. afzelii* BAD16 and *B. garinii* BGD19 strains, respectively. The orthologues of BBK32 in *B. afzelii* (strain PGau) and *B. garinii* (strain IP90) showed also specific binding to purified C1r and to the C1 complex and inhibition of the CP. Structurally, BBK32-C contains an antiparallel four-helix bundle fold with a fifth alpha-helix projecting from the helical core. The latter part contains three residues available for C1r binding and inhibition. The binding of BBK32 to C1r was found to be calcium-dependent [85]. Binding and inhibition of C1r are a novel type of anticomplement activity by bacteria. Whether it is unique to Lyme borreliae remains to be established.

### Interaction of C4b with OspC

OspC is dimeric outer membrane lipoprotein of borreliae. It is produced in the midgut of a feeding tick [86].

OspC is one of the crucial proteins involved in causing infection in mammals in the initial stages of borreliosis. It shows high variability in the central region of the protein across different strains and species of borreliae [87]. Therefore, based on these differences, OspC has been classified into 22 classes, called A-U. The variability of alleles is < 2% in the nucleotide sequence within a class and > 8% across different classes [88]. Strains linked to disseminated infection in mice or humans produce A-D, I, K, or N classes of OspC. Hence, due to the need of OspC in the survival of *B. burgdorferi* sl in the early stages of infection and the variation in the invasiveness and the disease as observed with borreliae having different classes of OspC, it is hypothesized to be a factor in innate immune evasion. Survival capabilities of *in vivo* ospC deletion in mouse models have led way toward the identification of a role for OspC in complement escape. Pull-down assays using human serum incubated with a resin saturated with GST-OspC<sub>B31</sub> (purified OspC protein fused to GST from *B. burgdorferi* ss strain B31-A3) displayed an abundance of the  $\beta$ -chain of C4b (~ 75 kDa) identified with 32% sequence coverage. Other proteins found include full-length C4 and its shorter cleavage products. OspC-B31 bound C4b in ELISA with a  $K_D$  of 414 nM. In functional complement tests, using serum pools and an ELISA with wells coated with IgM, mannan, or LPS, GST-OspC was found to inhibit the classical and lectin pathways. *In vitro* studies on ELISA indicated relatively low-affinity binding of C4b with OspC<sub>Pbr</sub> (from *B. garinii*) as compared to OspC<sub>B31</sub> and OspC<sub>N40</sub> (both from *B. burgdorferi* ss strains) [89]. Because of the wide genetic polymorphism of C4, the possibility remains that it could influence the susceptibility to borreliosis or to its individual clinical manifestations.

### Serum resistance of *Borrelia bavariensis* is mediated by two surface proteins BGA66 and BGA71

*Borrelia bavariensis* (formerly referred to as *B. garinii* OspA serotype 4) has shown tropism for nervous tissues. Thus, it could cause neurological symptoms in humans. *B. bavariensis* isolates do not bind complement regulators of the AP or the CP or proteolytically inactivate complement components. However, it has been indicated that all *B. bavariensis* isolates can resist complement-mediated killing by borrelial Osps BGA66 and BGA71 [90]. These molecules can bind complement components C7, C8, and C9, thereby blocking C9 polymerization and preventing the MAC assembly.

The complement inhibitory activity of these proteins has several differences, mainly in their capacity to inhibit C9 polymerization, or in their affinity for C7, C8, and C9. The complement inhibitory region was proposed to be located in the N-terminal region of the BGA71 protein [90].

## Conclusions and Perspectives

*Borrelia burgdorferi* uses multiple mechanisms to evade the mammalian host immune and complement systems. These include differential gene expression depending on environmental cues. Complement, being the first line of defense against any invading pathogen, plays an important role in the sequestration and killing of pathogens. Unsurprisingly, Borreliae have developed several mechanisms to evade the human complement. Some of them have been well characterized over the last three decades. FH is an important complement inhibitor, which several *Borrelia* species exploit for their evasion. The FH-binding proteins OspE, CspA, and CspZ work together to prevent opsonization via the alternative pathway and the amplification loop at different stages of the borrelial cycle. Also, several spirochetal proteins regulate the CP of complement. C4BP is acquired by P43, while BBK32 sequesters the C1r protease of the CP component C1. These bacterial surface proteins contribute to the virulence of the bacteria to cause borreliosis. Therefore, the mentioned bacterial Osps could potentially be used as vaccine components. Currently, a preparation referred to as VLA15 is the only active vaccine being developed against the LB. VLA15 targets OspA present on the outer surface of borreliae. It could render active immunity against the spirochetes prevalent both in Europe and in North America [91]. However, more candidates are needed. The current knowledge about spirochetal evasion strategies and molecular mechanisms provides a basis for vaccine candidate identification and development.

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