Complement Evasion Contributes to Lyme borreliae-Host Associations

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Abstract (136 words)

Lyme disease or borreliosis is the most common vector-borne disease in the northern hemisphere and is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex. Lyme borreliae infect diverse vertebrate reservoirs without triggering apparent manifestations in these animals, however Lyme borreliae strains undoubtedly differ in their competent reservoir hosts. The mechanisms that drive those remarkable differences are largely unknown. To survive in their vertebrate hosts, Lyme borreliae require the ability to escape from host defense mechanisms, in particular complement. To facilitate complement evasion, Lyme borreliae produce a repertoire of structurally and functionally diverse proteins at different stages of infection, allowing them to persistently survive without being recognized by hosts and potentially resulting in host-specific infection. This review discusses the current

knowledge regarding the ecology and evolutionary mechanisms of Lyme borreliae-host associations driven by complement evasion.

Lyme borreliae and complement

Lyme disease (LD) or borreliosis is caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato (s.l.) complex [1]. (Note the genus of *Borrelia* also causes another disease, e.g. relapsing fever, we use the terminology Lyme borreliae here to represent the causative agent of LD). Lyme borreliae are transmitted from vertebrate **reservoir hosts** (see Glossary) to humans via hard ticks of the genus *Ixodes* [2]. More than 20 different genospecies of the complex have been identified so far of which six species are confirmed to cause human LD: *B. burgdorferi* sensu stricto (s.s.)*, B. afzelii*, *B. garinii*, *B. spielmanii, B. bavariensis* (formerly referred to as *B. garinii* OspA serotype 4), and *B. mayonii* [2]. Within a genospecies, the isolates of Lyme borreliae may differ in their genetic contents and have been genotyped using different methodologies [3-6]. These isolates often vary in their associations with particular host species [7, 8].

Common to *B. burgdorferi* s.l. species is their ability to counteract the innate immune defense mechanisms of diverse hosts. Some mammalian and avian reservoir hosts can be persistently infected by certain species for prolonged periods without suffering from disease manifestations. In contrast, the immune system of humans and other animals that are **non-reservoir hosts** (see Glossary) can develop disease manifestations, including arthritis, carditis, neurological symptoms (known as neuroborreliosis), and acrodermatitis chronica atrophicans [2, 9]. The ability of *B. burgdorferi* s.l. to be maintained in these hosts, or cause disease manifestations differ, but the mechanisms that drive such differences remain unclear.

Complement, as an important pillar of innate immunity, forms a powerful surveillance system that comprises a well-organized network of fluid-phase and membrane-bound regulatory proteins circulating in the blood. Upon recognition of invading microorganisms,

complement is immediately activated in a cascade-like manner. Despite the effectiveness of complement, Lyme borreliae develop strategies to circumvent this crucial, non-specific barrier of their hosts [10]. However, the heterogeneity in the ability of Lyme borreliae genospecies to survive in sera from different hosts lead to the hypothesis that such Lyme borreliae complement-inhibitory strategies do not necessarily protect spirochetes from killing by serum of every host species [11]. Additionally, the ability to evade complement appears to determines **host infectivity** (see Glossary) of these pathogens [10, 12, 13]. This review, thus focuses on the current knowledge of the molecular mechanisms utilized by Lyme borreliae to counteract complement and the potential role of complement evasion in the evolution of **host specialization** (see Glossary) for those bacteria.

Diversity in complement evasion of Lyme borreliae

Complement is a powerful component of vertebrates' immune defense against invading microorganisms. A Lyme borreliae strain's ability to evade complement has been determined by testing whether that a particular strain is able to survive in host sera (also described as serum resistance). *B. burgdorferi* s.l. varies in their ability to inhibit complement from humans and various animals (Table 1) [14-16]. A strain's ability to avoid complement-mediated killing by a particular host's serum is strongly correlated with the capability of that strain to survive in that host. For example, the ave-associated species *B. garinii* and *B. valaisiana* are generally able to survive in avian but not mammalian sera, while the mammal-associated species *B. afzelii, B. bavariensis*, *B. spielmanii*, *B. bissettiae*, and *B. japonica* can generally survive in mammalian but not avian sera (reviewed in [17] (Table 1). Additionally, *B. burgdorferi*, *B. afzelii*, *B. spielmanii*, *B. bavariensis*, and *B. mayonii*, which have been isolated from humans, are capable of surviving in human sera. Note that the pathogenicity of *B. valaisiana* and *B. lusitaniae* for humans remains unclear, but these strains are killed by human serum (reviewed in [18]) (Table 1). A notable exception is *B. garinii*, which has been

isolated from humans with neurological manifestations, yet some *B. garinii* strains are highly vulnerable to the killing by human sera. Despite several proteins derived from tick saliva were shown to contribute to the resistance of *B. burgdorferi* s.l. to complement attack [19], the correlation of host-specific serum resistance and the infectivity pattern among *B. burgdorferi* s.l. supporting the notion of bacterial factor(s) that determines host association.

The factors of Lyme borreliae involved in complement evasion.

Complement can be activated through three canonical routes: the classical (CP), the lectin (LP), and the alternative pathway (AP) (Fig. 1)[20]. The binding of antibody to antigen and C1 protein complex activates CP, whereas the association of mannan-binding lectin, ficolins, or collectins with carbohydrates on a pathogen's surface induces activation of the LP. Formation of the C3 convertase in the fluid phase, C3bBb and subsequent cleavage of C3 to C3a and C3b triggers the activation of the AP and leads to the deposition of C3b to the microbal or other target surface (Fig. 1). Activation of each of these pathways results in formation of two different types of C3 convertases: C3bBb formed by the AP, and C4b2a generated by the- CP and LP (Fig. 1). Both C3 convertases then promote formation of the central complement component, C3b, which leads to formation of the C5 convertase(s) to cleave C5 into C5a and C5b. C5b deposition on bacterial surfaces initiates the terminal sequence (TS), which recruits the late complement proteins C6, C7, and C8. The association of C5b, C6, C7, and C8 leads to the deposition of C9, which is multimerized to form the bacteriolytic terminal complement complex (TCC, also known as the membrane attack complex, MAC). To protect self surfaces from excessive activation, complement is tightly controlled by a number of soluble and membrane-anchored regulators. These regulators include, but not limited to, C1 esterase inhibitor (C1-INH) and C4b-binding protein (C4BP) that inhibit CP and LP, Factor H (FH) and Factor H-like protein 1 (FHL-1) that inhibit AP, and vitronectin that negatively modulates the formation of the MAC (Fig. 1)[20].

Lyme borreliae possess a number of structurally diverse outer surface proteins to inactivate complement at different stages of the infection cycle. These proteins target complement proteins/regulators that can modulate different arms of complement (reviewed in [13]). The proteins that inhibit AP include the collectively termed FH/FHL-1-binding Complement-Acquiring Surface Proteins (CRASP): CspA, CspZ, and OspE-related protein (members of a family of proteins collectively known as "Erp", which include ErpA, ErpC, and ErpP) [21-27](Table 2). The recruitment of FH and/or FHL-1 by these proteins onto the bacterial surface leads to inactivation of the AP, permitting Lyme borreliae to survival in host sera. Additionally, *B. burgdorferi* s.l. produce at least two additional outer surface proteins to inhibit complement: BBK32 and OspC (Table 2) [28, 29]. BBK32 binds to C1r and thereby inhibits the activation of the C1 complex, resulting in the termination of all downstream activation steps of the CP. OspC of *B. burgdorferi* s.l. binds to C4b to prevent the formation of C4b2a, the C3 convertase of CP and LP, and thus inhibits activation of those pathways [28, 29]. Of note, formation of the MAC can be down-regulated by several Lyme borreliae proteins [30, 31](Table 2) but the role of TS inhibition to contribute to Lyme borreliae infectivity is as yet unclear.

Multiple regulatory mechanisms control expression of complement inhibitory proteins.

Lyme borreliae proteins that mediate resistance to host complement exhibit different patterns of expression during infection, indicative of several distinct regulatory pathways for the production of these proteins. Lyme borreliae within unfed ticks do not produce OspC, OspE-related proteins, CspA, or CspZ [32-35](Table 2). When an infected tick begins to feed on the blood of a vertebrate host, production of OspC is induced, so that transmitted bacteria possess this protein on their outer surface [32]. However, OspC production is repressed within a few days after establishment of infection [36] (Table 2). In contrast, OspE-related proteins are also induced during tick feeding, but these outer surface proteins continue to be produced

throughout vertebrate infection, and bacteria acquired by ticks from infected mammals produce all of their OspE-related proteins [33, 37] (Table 2). Production of CspA is also induced during tick feeding, repressed subsequently after the transmission begins, and the infection establishes at the tick biting site of the skin. The *cspA* expression is then induced when Lyme borreliae are transmitted from infected vertebrates to feeding ticks [34, 35] (Table 2). CspZ exhibits yet another pattern of expression: its production begins after transmission of bacteria from the tick into the vertebrate, persists throughout vertebrate infection, then is repressed during acquisition by feeding ticks [34, 35](Table 2).

Of the Lyme borreliae complement-resistance mediators, the regulatory networks of OspC and the OspE-related proteins are the most well studied. High-level expression of OspC is dependent upon an alternative sigma factor (RpoS), which has led to a hypothesis that RpoS directly controls *ospC* transcription [38]. However, *ospC* is transcribed at low levels in *rpoS*-deficient mutants, leading to an alternative hypothesis that the effect of RpoS is indirect [39, 40]. Consistent with that second model, a region of DNA 5' of the *ospC* promoter is required for RpoS-dependent induction of *ospC*, and is likely to be a binding site for a regulatory protein that is under control of RpoS [41, 42]. Additionally, *bbk32* is also regulated by such a RpoS-dependent mechanism in the similar fashion as *ospC* [43, 44]. While the operon of *ospE* is controlled in the RpoS-independent manner [39], this operon contains a highly-conserved operator region, and are under transcriptional regulation of three proteins that bind to *erp* operator DNA: the BpaB repressor, the BpuR co-repressor, and the EbfC anti-repressor [45-49]. Studies of BpuR and EbfC indicated that each protein regulates its own production, and that production of both proteins is also controlled by the DnaA protein (the master regulator of bacterial replication) [50-52]. In addition, our preliminary studies of CspZ found that a novel Lyme borreliae protein binds near the *cspZ* transcriptional promoter, which warrants further investigation.

Polymorphisms of complement-interacting proteins influencing Lyme borreliae-host association

CspA, a complement evasion factor operating in the ticks

The transcript encoding CspA is expressed by *B. burgdorferi* s.s. at the onset of tick feeding and during transmission to vertebrate hosts, and then repressed in the later stages of infection [35] (Table 2). The tick-specific expression profile of *cspA* is consistent with the previous finding that Lyme borreliae require CspA to survive in ticks' midgut upon blood feeding [53]. A recent observation indicates that CspA-mediated FH-binding activity is essential for these pathogens to evade complement in the ingested blood, permitting efficient tick-to-host transmission [53] (Fig. 2). The CspA polymorphisms are associated with variable FH-binding activity [53, 54], resulting in the strains that are either highly vulnerable (in the absence of FH) or highly resistant (upon binding of FH) to complement of vertebrate hosts [53, 54]. These findings indicate that CspA is one of the determinants that define host-specific infection. However, whether particular CspA variants that promote inefficient tick-borne transmission to mice have a role in facilitating transmission to other animals remains unknown. The evolutionary mechanisms and amino acid determinants of this protein to drive such host associations need further investigations.

CspZ, a complement evasion factor operating in the vertebrate host

In contrast to *cspA*, expression of *cspZ* occurs only in the vertebrate host [35] (Table 2). Lyme borreliae that lack *cspZ* or produce a mutant CspZ without FH-binding activity exhibit reduced colonization of distal tissues during mouse infection. Those results indicate that CspZ-mediated FH-binding activity contributes to spirochete dissemination [55, 56] (Fig. 2). Unlike CspA, the amino acid sequences of CspZ are largely conserved among different *B. burgdorferi* s.s. strains (> 95% identity) and species of the *B. burgdorferi* s.l. complex (>70% identity) [57, 58]. However, allelically different human FH-binding activity was observed in

188 CspZ from different *B. burgdorferi* s.s. strains [57, 58]. Comparisons of the solved structure
189 of CspZ of *B. burgdorferi* B31 with different *B. burgdorferi* s.s. strain showed variations in
190 the regions that are involved with FH-binding activity [59]. These results raise an intriguing
191 question: would this host-specific FH-binding activity of CspZ enable this protein as one of
192 the determinants that drive host association? Additionally, CspZ is not carried by every *B.*
193 *burgdorferi* s.s. strain, suggesting that additional genes encoding complement-inhibitory
194 proteins are co-expressed with *cspZ* [57, 60] (Table 2).
195
196 *OspE paralogs, additional complement evasion factors operating in the vertebrate host?*
197 *B. burgdorferi* s.l. produces multiple paralogs of OspE [61-63]. Consistent with
198 expression of *ospE* triggered by host-specific environmental cues (e.g. blood meal), a
199 previous study reported that passive transfer of anti-OspE IgG reduces the levels of
200 spirochetes transmission to mice [64]. A *B. burgdorferi* s.s. strain with transposon inserted
201 into *erpA* (one *ospE* paralog in *B. burgdorferi* s.s. strain B31-A3) displays a two-week delay
202 in the distal tissue colonization when co-infected with a population of mutant Lyme borreliae
203 strains with transposon inserting in different genes [65]. These findings suggest that OspE
204 promotes spirochetes' tick-to-host transmission and hematogenous dissemination (Fig. 2).
205 The *ospE* genes largely differ in the number of copies and sequences among different species
206 or strains of *B. burgdorferi* s.l. , raising a possibility that OspE determines host-specificity of
207 infection [66, 67].
208
209 *OspC and BBK32, complement evasion factors operating in the initial phase of infection*
210 OspC is one of the most studied outer surface lipoproteins in *B. burgdorferi* s.l. This
211 protein is not expressed when Lyme borreliae are in ticks prior to blood feeding but produced
212 upon the blood feeding of ticks and during transmission. After the entry into hosts, the
213 production of OspC remains until Lyme borreliae begin disseminating to distal tissues (Table

2). OspC binds to a tick salivary protein, Salp15, and the decoration of this tick protein on the surface of Lyme borreliae prevents **opsonophagocytosis** (see Glossary) at the tick biting site [68]. OspC also binds to human complement C4b to inactivate CP and LP. Consistent with these activities, OspC is required for Lyme borreliae to survive at infection initiation sites during the first 24 hours of pathogen inoculation and confers spirochetes' the ability to remain in the mammalian bloodstream [28, 69] (Fig. 2). Nonetheless, the molecular mechanisms leading to such phenotypes need further investigations. Furthermore, OspC is one of the most polymorphic proteins among different strains or species of *B. burgdorferi* s.l. [1]. However, whether this protein is a determinant of host-specific survival and if so, which mechanisms drive such survival is still unclear.

BBK32 was initially identified as an adhesin that binds to extracellular matrix molecules fibronectin and glycosaminoglycans on the host cell surface and later demonstrated as a C1r-binding protein to inactivate CP [29]. In agreement with a blood meal-induced expression profile of *bbk32* (Table 2), BBK32 contributes to the ability to survive in mouse bloodstream at short-term and disseminate to joints at early stages of infection [69, 70] (Fig. 2). Though BBK32 is conserved (close to 90% similarity among strains or species of *B. burgdorferi* s.l.), the orthologs from *B. afzelii* and *B. garinii* differ in their capability to bind to human C1r [71]. Assuming that C1r-binding activity plays a role in conferring spirochete survival in vertebrate bloodstream and promoting dissemination at infection onset, such a strain-to-strain variation of BBK32-mediated C1r-binding activity may support the notion that this protein drives host-specific infectivity.

Host specialism of LD spirochetes at a glance

The spirochetes of the *B. burgdorferi* s.l. complex are maintained in an enzootic cycle between ticks of the *Ixodes ricinus* species complex and reservoir hosts, including small and medium-sized mammals, birds and reptiles [9]. In most Lyme disease endemic regions, there

240 is a diverse community of co-circulating Lyme borreliae and an association between different
241 classes of vertebrate hosts and some *B. burgdorferi* s.l. genospecies has been observed [9, 72,
242 73]. Some of these observed associations may be due to extrinsic factors such as geographic
243 co-occurrence of hosts with specific *B. burgdorferi* s.l. genospecies. However, there is strong
244 evidence that at least some of these genospecies differ intrinsically in transmissibility across
245 hosts, i.e. they are "host specialized" [11, 12, 72]. The strongest evidence is provided by
246 experiments demonstrating increased fitness for *B. afzelii* in mice and *B. garinii* in birds [11,
247 12, 73] and, to some extent, field studies demonstrated greater genospecies infection
248 prevalence in certain hosts compared to the background infection prevalence in local
249 populations of *Ixodes* spp [74].

250 In contrast to the other genospecies in the *B. burgdorferi* s.l. complex, *B. burgdorferi*
251 s.s. is considered a host generalist, as it has been isolated from multiple classes of vertebrate
252 animals (e.g. mammalian and avian hosts) [[72] and summarized in [9]]. However, multiple
253 studies indicate that some genotypes of *B. burgdorferi* s.s. have higher fitness in some hosts in
254 laboratory studies [75] and are more prevalent in certain mammalian or avian host species [9,
255 76-82]. Evidence of within-genospecies association of specific genotypes of *B. burgdorferi*
256 s.l. and certain hosts has also been described for *B. garinii* and *B. afzelii* in laboratory
257 experiments [9, 73, 83] and some field studies [84, 85], but not in others [86]. A limitation of
258 field studies is that they represent only snapshots of population structures that are spatially
259 and temporally variable due to stochastic effects or other forces, making inferences of host
260 association difficult [72].

261

262 **Eco-evolutionary mechanisms driving *B. burgdorferi*-host specialism**

263 Despite evidence for some level of association between *B. burgdorferi* s.s. strains and hosts
264 from laboratory infections and field studies [5, 87], the extent to which host adaptation drives
265 the genome-wide diversification in *B. burgdorferi* s.l. is currently under debate. Particular

attention has focused on factors driving polymorphism in OspC, one the most diverse Lyme borreliae antigens that is heavily targeted by the vertebrate immune system [88-90]. Balancing selection has been proposed to maintain *ospC* alleles at intermediate frequencies, with high sequence diversity within a population [91]. Genome-wide linkage to this single locus may then be responsible for maintaining genetic variation at linked loci [92-94]. It is currently debated which specific mode of balancing selection drives the OspC polymorphism in *B. burgdorferi* s.s.. Some authors have proposed that, similarly to the process operating across *B. burgdorferi* s.l. species, **host specialization** (see Glossary) via multiple-niche polymorphism (with hosts acting as different 'niches' for *B. burgdorferi*) could lead to diversification within *B. burgdorferi* s.s. [72, 80, 81, 95].

Alternatively, the OspC polymorphism could be maintained by negative frequency dependent selection mediated by adaptive immunity, such that bacterial populations carrying rare genotypes have a selective advantage over common genotypes and are thus maintained in the population [91, 95, 96]. Theoretical studies predict that frequency dependent fitness leads to fluctuations in the abundance of spirochete genotypes, which would result in temporal shifts in the population structures; however evidence for these fluctuations is limited [97, 98].

An intriguing question is whether the partial and regionally constrained host associations observed in *B. burgdorferi* s.s. represent an incipient evolutionary process of host specialization (Fig. 3). That is, is *B. burgdorferi* s.s. on an evolutionary path to diversify into species-associated ecotypes similar to the *B. burgdorferi* s.l. genospecies in Europe? *B. burgdorferi* s.s. generalism, i.e. the ability to infect multiple hosts, has in fact been proposed as a key property allowing it to spread across the northeastern United States following large-scale habitat destruction in the course of the post-Columbian settlement and during the industrial revolution [81]. The more recent geographic expansion of *B. burgdorferi* s.s may provide additional opportunities for adaptation to different host niches, resulting in the development of species-associated ecotypes similar to the *B. burgdorferi* s.l. genospecies in

Europe [85]. The recent redefinition of *B. bavariensis* from a genotype of *B. garinii* to a novel genospecies, after it was shown to infect mice in contrast to *B. garinii* (a bird-adapted genospecies), provides a glimpse of potential future processes of **host specialization** and **Lyme borreliae speciation** (see Glossary) by *B. burgdorferi* s.l. linked to vector or host association [85].

Concluding Remarks

Here we summarize the evidence that supports the proteins that contribute to complement evasion-mediated infectious phenotypes in a host-specific manner, leading a question if complement evasion activity of *B. burgdorferi* s.l. confers the host association (see Outstanding questions). The fact that some of these proteins are functionally redundant and produced simultaneously in the infection cycle, raising the hypothesis that these proteins play in concert in promoting host association of *B. burgdorferi* s.l. (see Outstanding Questions). Furthermore, the ability of complement to eliminate Lyme borreliae differs among diverse animal species falling in the same taxonomic class (e.g. aves or mammalia) appears to differ. This leads to an intriguing question if complement plays a role in defining the different levels of competence for the hosts within the same taxonomic classes (see Outstanding Questions). In addition, though a spirochete-host association has been clearly defined for different Lyme borreliae genospecies, whether this association also applies to different genotypes of spirochetes within the same genospecies (e.g. *B. burgdorferi* s.s.) is unclear. Teasing apart this question could examine an incipient evolutionary process of *B. burgdorferi* s.l. toward a more complete host association (see Outstanding Questions). Future investigations of the above-mentioned questions will undoubtedly contribute to an insight about the factors contributing in the pathobiology of spirochetes and their diversity in host association.

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Glossary

Lyme borreliae-host association: Hosts from which the specified Lyme borreliae species/strains has been isolated, i.e. the species/strain is capable of infecting (surviving and disseminating) in the host. These associations represent a pattern (compare with host specialization) that may be due to multiple processes, including differential susceptibility or resistance to serum complement (the topic in this paper) as well as other mechanisms.

Reservoir hosts: Nature hosts that the vector (e.g. ticks) become infected by feeding on such hosts.

Non-reservoir hosts: Hosts that may have contact with infected ticks and may or may not develop a long-lasting infection but are incapable of transmitting the infection to ticks.

Host infectivity: Efficiency with which infection is transmitted from a tick host population to to feeding ticks.

Host specialism/specialization (with host specialization as the process and host specialized as the adjective): Ecological and evolutionary *process* by which a pathogen becomes differentially adapted and thus restricts its host range to a subset of potential hosts. Intrinsic fitness variation of *B. burgdorferi* s.l. strains in vertebrate host species is generally cited as evidence of host specialization.

Opsonophagocytosis: Identification of an invading microorganism by opsonins following phagocytosis.

Lyme borreliae speciation: The evolution of a new Lyme borreliae species.

Figure Legends

Figure 1. Schematic diagram of vertebrate complement cascades and the particular steps Lyme borreliae anti-complement protein interact. The CspA, CspZ, and OspE of *B. burgdorferi* s.l. target the host complement regulator, FH, by inhibiting the formation C3bBb to inactivate AP. LD spirochetes also produce BBK32 and OspC that bind to C1r and C4b, respectively. These proteins inhibit CP (for BBK32 and OspC) and LP (for OspC). Additional proteins of *B. burgdorferi* s.l. (e.g. CspA, BGA66, and BGA71) inactivate TCC by preventing the formation of C5b-9 on the surface of spirochetes (Part of the figure is adapted from [18]). FH, Factor H; AP, alternative pathway; CP, classical pathway; LP, Lectin pathway; TS, terminal sequence; LD, Lyme disease; TCC, terminal complement complex

Figure 2, Key Figure. Complement inhibitory proteins and their potential roles in the infection route. When ticks feed on hosts, *B. burgdorferi* s.s. produce CspA to facilitate spirochete escape from complement-mediated killing in the blood meal. After transmission to a host, the tick salivary protein, Salp15, binds to OspC on the spirochete surface to prevent opsonophagocytosis at tick bite sites. Additionally, *B. burgdorferi* s.s. produces OspC, BBK32, and CspZ to promote complement evasion and bloodstream survival of spirochetes. The cell types and complement complex have been indicated on the figure. Though the function of OspE during infection remains unclear, the current evidence supports that this protein may confer spirochete dissemination in vertebrate animals (Part of the figure is adapted from [27]).

Figure 3. The host-pathogen association for *B. burgdorferi* s.l. genospecies. The indicated *B. burgdorferi* s.l. genospecies are acquired and transmitted between ticks and different vertebrate hosts including humans, small mammals, reptiles, and aves. Shown is the

625 vertebrate hosts that have been demonstrated or suspected to carry respective species of the *B.*

626 *burgdorferi* s.l. complex (Part of the figure is adapted from [9]).

627 **Table 1. Serum susceptibility pattern of *B. burgdorferi* s.l. to human and diverse animal sera[a]**

Species[b]	*B. burgdorferi*	*B. afzelii*	*B. bavariensis*	*B. japonica*	*B. bissettiae*	*B. andersonii*	*B. garinii*[c]	*B. valaisiana*	*B. lusitaniae*
Human	R	R	R	R	I	S	S	S	S
Mouse	R	R	R	R	R	ND	S	S	ND
Rat	S	R	R	ND	ND	ND	S	ND	ND
Hamster	R	R	R	R	ND	ND	S	S	S
Squirrel	R	R	R	R	ND	ND	S	S	ND
Rabbit	I	S	ND	ND	I	ND	S	ND	ND
Cat	I	R	R	R	ND	ND	I	R	ND
Lynx	I	I	R	S	R	I	I	R	S
Dog	I	R	R	I	R	R	S/I	I	S
Wolve	I	S	R	S	R	I	S/I	S	S
Mouflon	I	R	R	R	R	I	R/I	R	R
Pheasant	I	S	S	S	ND	ND	R	R	S
Blackbird	I	S	S	S	ND	ND	R	R	S
Sheep	I	S	S	R	S/R	I	S	S	R
Horse	I	S	S	S	ND	ND	S	S	S
Pig	I	S	S	S	ND	ND	S	S	S
Goat	S	S	ND	ND	ND	ND	S	ND	ND
Bovine	S	S	S	S	S	S	S	S	S
Deer	S	S	S	S	S	S	S	S	S
Eur. Bison[d]	S	S	S	S	S	S	S	S	S
Lizard	S	S	S	S	S	ND	R	R	R
Quail	R	ND	ND	ND	S	ND	ND	ND	ND

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629 [a]Data shown were derived from [13]; R, serum-resistant; I, intermediate serum-resistant; S, serum-sensitive, ND, no data available

630 [b]*B. burgdorferi, B. afzelii, B. bavariensis*, *B. japonica*, *B. bissettiae*, and *B. andersonii* are (mainly) rodent-associate species, *B. garinii* and *B.*

631 *valaisiana* are bird-associate species, and *B. lusitaniae* is a reptile-associate species.

[c]Variations in the serum susceptibility pattern have been reported for the heterogenous genospecies *B. garinii* [14]. Of note, *B. garinii* OspA serotype 4 was thereafter referred to as *B. bavariensis* known to display a serum-resistant phenotype. *B. mayonii* and *B. spielmanii* has not been included due to the lack of available data but both species resist complement-mediated killing by human serum [99, 100].

[d]Eur., European.

637 **Table 2. Characteristics of complement interacting proteins of LD spirochetes**
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	BBK32	**OspC**	**CspA**	**CspZ**	**OspE paralogs**			**BGA66**	**BGA71**	**p43**
					ErpP[a]	**ErpC[a]**	**ErpA[a]**			
synonyms and other designations	none	none	CRASP-1 BBA68	CRASP-2 BBH06	CRASP-3 BBN38	CRASP-4	CRASP-5 ErpI ErpN BBP38 BBL39 OspE	none	none	none
gene name	*bbk32*	*ospC*	*cspA*	*cspZ*	*erpP*	*erpC*	*erpA*	*bga66*	*bga71*	ND
origin	Bb	Bb	Bb, Ba, Bs, Bm	Bb	Bb	Bb	Bb	Bba	Bba	Bb
confers serum resistance	yes	yes	yes	yes	unclear[b]	unclear[b]	unclear[b]	yes	yes	ND
interaction with complement regulators / components	C1r	C2	FH FHL-1 C7, C8, C9, TCC	FH FHL-1	FHR-1 FHR-2 FHR-5	FHR-1 FHR-2	FHR-1 FHR-2 FHR-5	C7, C8, C9, TCC	C7, C8, C9, TCC	C4BP
affected complement pathways	CP	CP	AP, TS	AP	ND	ND	ND	TS	TS	CP/LP(?)
Fed larvae	-	-	+	+ (LE)	+ (HE)	+ (HE)	+ (HE)	ND	ND	ND
Unfed nymphs	-	-	+ (HE)	-	-	-	-	ND	ND	ND
Fed nymphs	+	+	+ (LE)	+ (LE)	+	+	+	ND	ND	ND
Tick biting sites	+	+	+	+ (HE)	+ (HE)	+ (HE)	+ (HE)	ND	ND	ND
Distal sites	+	-	-	+ (HE)	+ (HE)	+ (HE)	+ (HE)	ND	ND	ND
			+	+ (LE)	+ (HE)	+ (HE)	+ (HE)	ND	ND	ND

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640 [a]Binding of FH has only been confirmed for recombinant proteins.

641 [b]Confers serum resistance only when ErpP and ErpA are expressed under *flaB* promoter in a *cspA*-deficient *B. burgdorferi* in the infectious

642 background; CRASP, complement-regulator acquiring surface protein; Erp, OspE/F-like protein; FH, Factor H; FHL, Factor H-like protein, FHR,

643 FH-related protein; TCC, terminal complement complex; Bb, *B. burgdorferi*; Bba, *B. bavariensis*; Ba, *B. afzelii*; Bs, *B. spielmanii*; Bm, *B. mayonii*;

644 AP, alternative pathway; CP, classical pathway; LP, lectin pathway; TS, terminal sequence, ND; no data available, HE; high expression, LE; low

645 expression