Complement Evasion Contributes to Lyme Borreliae–Host Associations

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Review

Complement Evasion Contributes to Lyme Borreliae–Host Associations

Yi-Pin Lin,[1,2,*] Maria A. Diuk-Wasser,[3] Brian Stevenson,[4,5] and Peter Kraiczy[6,*]

Lyme disease 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 differ in their reservoir hosts. The mechanisms that drive those differences are unknown. To survive in vertebrate hosts, Lyme borreliae require the ability to escape from host defense mechanisms, in particular complement. To facilitate the evasion of complement, Lyme borreliae produce 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 of the *Borrelia burgdorferi* sensu lato (s.l.) complex (Note that bacteria of the genus *Borrelia* also cause other diseases, e.g., relapsing fever; here we use the term 'Lyme borreliae' to represent the causative agent of LD) [1]. 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].

One common feature 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** can develop disease manifestations, including arthritis, carditis, neurological symptoms (known as neuroborreliosis), and acrodermatitis chronica atrophicans [2,9]. Strains of *B. burgdorferi* s.l. differ in their ability to be maintained in these hosts, and to cause disease manifestations, 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, nonspecific barrier of their hosts [10]. However, the heterogeneity in the ability of Lyme borreliae genospecies to survive in sera from different hosts leads to the hypothesis that Lyme borreliae have complement-inhibitory strategies that do

Highlights

Emerging and re-emerging zoonotic diseases have a major impact on global public health, including tick-transmitted illnesses such as Lyme disease. Lyme disease-causing pathogens develop a range of sophisticated strategies to overcome the innate immune system of various vertebrate hosts to accomplish their –enzootic cycle.

Inactivation of the host´s complement in the tick's blood meal, and in the host's bloodstream, are crucial steps to prevent the spirochetes from being killed during their transmission and dissemination.

Strain-to-strain variation in the spirochetes' ability to evade complement may contribute to variation in the range of Lyme borreliae–host associations.

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not necessarily protect them from the killing action of serum in every host species [11]. Additionally, the ability to evade complement appears to determine the **host infectivity** of these pathogens [10,12,13]. This review thus focuses on the current knowledge of the molecular mechanisms used by Lyme borreliae to counteract complement and the potential role of complement evasion in the evolution of **host specialization** in these 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 a particular strain is able to survive in host sera (also described as serum resistance). Strains of *B. burgdorferi* s.l. vary 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 avian-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 [13]; 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 [17]; 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 killing by human sera. Although several proteins derived from tick saliva were shown to contribute to the resistance of *B. burgdorferi* s.l. to complement attack [18], the correlation of host-specific serum resistance with the infectivity pattern among strains of *B. burgdorferi* s.l. supports the notion that bacterial factor(s) determine host association.

The Factors of Lyme Borreliae Involved in Complement Evasion

Complement can be activated through three canonical routes: the classical pathway (CP), the lectin pathway (LP), and the alternative pathway (AP) (Figure 1) [19]. The binding of antibody to antigen and the 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 on the microbial or other target surface (Figure 1). Activation of each of these pathways results in the formation of two different types of C3 convertase: C3bBb formed by the AP, and C4b2a generated by the CP and LP (Figure 1). Both C3 convertases then promote formation of the central complement component, C3b, which leads to the 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 are 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 (Figure 1) [19].

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 [13,17]). The proteins that inhibit AP include the collectively termed FH/FHL-1-binding complement-

Glossary

Host generalist: able to infect a wide range of hosts.
Host infectivity: the efficiency with which infection is transmitted from a tick host population to feeding ticks.
Host specialism/specialization (in contrast to host generalism): an ecological and evolutionary process in which a pathogen becomes differentially adapted and thus restricts its host range to a subset of potential hosts. The fitness variation of *B. burgdorferi* s.l. strains in vertebrate host species is generally cited as evidence of host specialization.
Lyme borreliae speciation: the evolution of a new species of Lyme borreliae.
Lyme borreliae–host association: hosts from which specified Lyme borreliae species/strains have been isolated. 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 of this paper) as well as other mechanisms.
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.
Opsonophagocytosis: identification of an invading microorganism by opsonins followed by phagocytosis.
Reservoir hosts: natural hosts that the vector (e.g., tick) becomes infected by when feeding on such hosts

Table 1. Serum Susceptibility Pattern of *Borrelia burgdorferi* s.l. to Human and Diverse Animal Sera[a]

Species[b]	*B. burgdorferi* s.s.	*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
Wolf	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

[a]Data shown were derived from [13]; R, serum-resistant; I, intermediate serum-resistant; S, serum-sensitive, ND, no data available.
[b]*B. burgdorferi* s.s., *B. afzelii, B. bavariensis*, *B. japonica*, *B. bissettiae*, and *B. andersonii* are (mainly) rodent-associated species; *B. garinii* and *B. valaisiana* are bird-associated species; and *B. lusitaniae* is a reptile-associated species.
[c]Variations in the serum susceptibility pattern have been reported for the heterogeneous genospecies *B. garinii* [14]. Of note, *B. garinii* OspA serotype 4 was thereafter referred to as *B. bavariensis* as it was known to display a resistant phenotype to human serum. *B. mayonii* and *B. spielmanii* have not been included due to the lack of available data but both species resist complement-mediated killing by human serum [98,99].
[d]Eur., European.

acquiring surface proteins (CRASPs): CspA, CspZ, and OspE-related proteins (members of a family of proteins collectively known as 'Erp', which include ErpA, ErpC, and ErpP) [20–26] (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 survive in host sera. Additionally, strains of *B. burgdorferi* s.l. produce at least two additional outer-surface proteins to inhibit complement: BBK32 and OspC (Table 2) [27,28]. 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 [27,28]. Of note, formation of the MAC can be downregulated by several Lyme borreliae proteins [29,30] (Table 2) but the role of TS inhibition in contributing 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

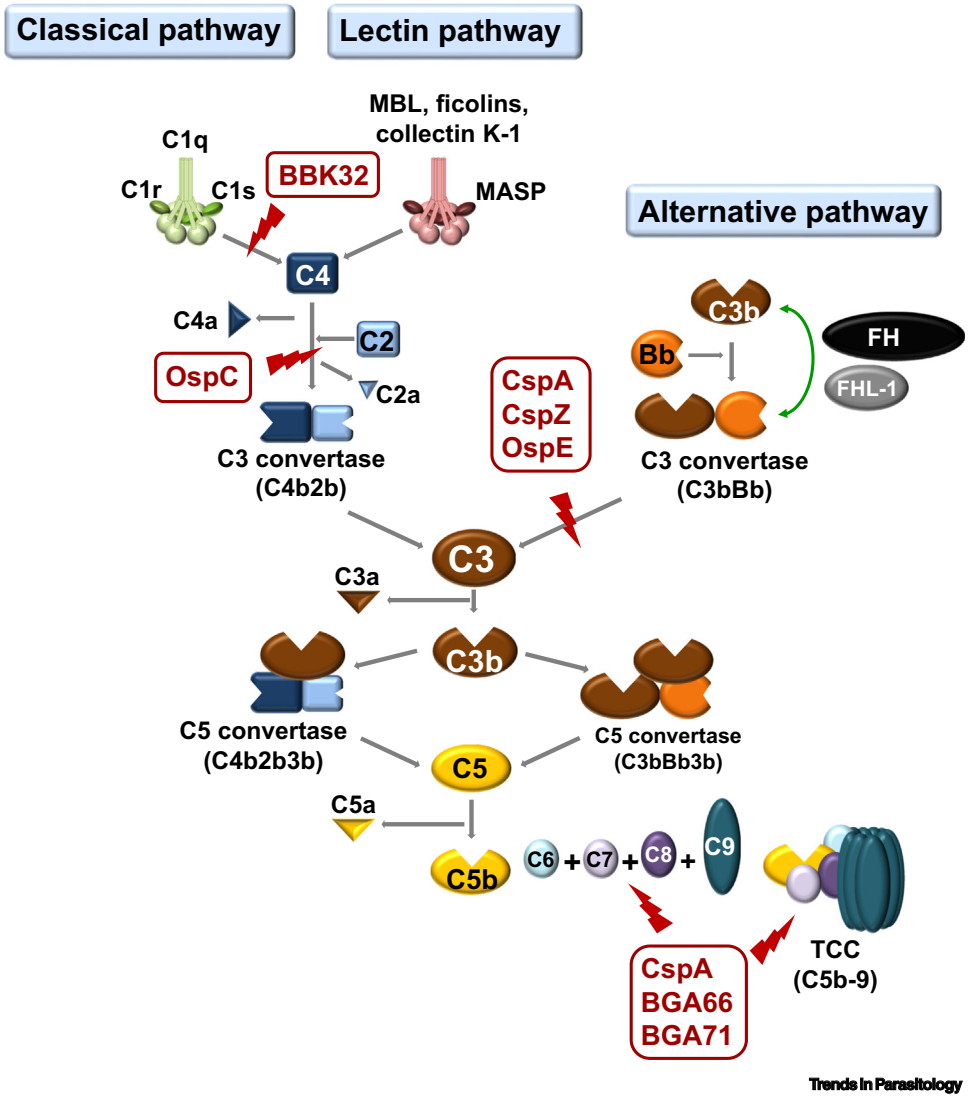

Figure 1. Schematic Diagram of Vertebrate Complement Cascades and the Particular Steps at Which Lyme Borreliae Anti-Complement Proteins Interact. The CspA, CspZ, and OspE-related proteins of *Borrelia burgdorferi* s.l. target the host complement regulator factor H (FH) by inhibiting the formation of C3bBb in order to inactivate AP. Lyme disease 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 the terminal complement complex (TCC) by preventing the formation of C5b–9 on the surface of spirochetes (part of the figure is adapted from [13]). AP, alternative pathway; CP, classical pathway; LP, lectin pathway; TS, terminal sequence.

these proteins. Lyme borreliae within unfed ticks do not produce OspC, OspE-related proteins, CspA, or CspZ [31–34] (Table 2). When an infected tick begins to feed on the blood of a vertebrate host, the production of OspC is induced, so that transmitted bacteria possess this protein on their outer surface [31]. However, OspC production is repressed within a few days after establishment of infection [35] (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 [32,36] (Table 2). Production of CspA is also induced during tick feeding,

Table 2. Characteristics of Complement-Inhibitory Proteins of Lyme Borreliae[a]

	BBK32	OspC	CspA	CspZ	OspE paralogs			BGA66	BGA71	p43
					ErpP[b]	ErpC[b]	ErpA[b]			
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[c]	Unclear[c]	Unclear[c]	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

[a]CRASP, complement-regulator-acquiring surface protein; Erp, OspE/F-like protein; FH, Factor H; FHL, Factor H-like protein, FHR, FH-related protein; TCC, terminal complement complex; Bb, *B. burgdorferi* s.s.; Bba, *B. bavariensis*; Ba, *B. afzelii*; Bs, *B. spielmanii*; Bm, *B. mayonii*; AP, alternative pathway; CP, classical pathway; LP, lectin pathway; TS, terminal sequence; ND, no data available; HE, high expression; LE, low expression.
[b]Binding of Factor H (FH) has been confirmed only for recombinant proteins.
[c]Confers serum resistance only when ErpP and ErpA are expressed under *flaB* promoter in a *cspA*-deficient strain of *B. burgdorferi* in the infectious background.

but is 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 [33,34] (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, and is then repressed during acquisition by feeding ticks [33,34] (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 [37]. However, *ospC* is transcribed at low levels in *rpoS*-deficient mutants, leading to an alternative hypothesis that the effect of RpoS is indirect [38,39]. 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 [40,41]. Additionally, *bbk32* is also regulated by such an RpoS-dependent mechanism in a fashion similar to that of *ospC* [42,43]. While the operon of *ospE* is controlled in an RpoS-independent manner [38], this operon contains a highly conserved operator region, and is under the transcriptional regulation of three proteins that bind to *erp* operator DNA: the BpaB repressor, the BpuR corepressor, and the EbfC antirepressor [44–48]. 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) [49–51]. 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-Inhibitory Proteins Influence Lyme Borreliae–Host Association

CspA, a Complement-Evasion Factor Operating in 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 is then repressed in the later stages of infection [34] (Table 2). The tick-specific expression profile of *cspA* is consistent with the previous finding that Lyme borreliae require CspA to survive in the tick's midgut upon blood feeding [52]. 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 [52] (Figure 2, Key Figure). The CspA polymorphisms are associated with variable FH-binding activity [52,53], 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 [52,53]. These findings suggest 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 which drive such host associations need further investigation.

CspZ, a Complement-Evasion Factor Operating in the Vertebrate Host

In contrast to *cspA*, expression of *cspZ* occurs only in the vertebrate host [34] (Table 2). Blood-treated 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 [54,55] (Figure 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) [56,57]. However, allelically different human FH-binding activity was observed in CspZ from different *B. burgdorferi* s.s. strains [56,57]. Comparisons of the solved structure of CspZ of *B. burgdorferi* B31 with different *B. burgdorferi* s.s. strains showed variations in the regions that are involved with FH-binding activity [58]. These results raise an intriguing question: would such a host-specific FH-binding activity of CspZ enable this protein to be one of the determinants that drive host association? Additionally, CspZ is not carried by every *B. burgdorferi* s.s. strain, suggesting that additional genes encoding complement-inhibitory proteins are coexpressed with *cspZ* [56,59] (Table 2).

OspE-Related Proteins – Additional Complement-Evasion Factors Operating in the Vertebrate Host?

Strains of *B. burgdorferi* s.l. produce multiple OspE-related proteins [60–62]. Consistent with the expression of *ospE* triggered by host-specific environmental cues (e.g., a blood meal), a previous study reported that passive transfer of anti-OspE IgG reduces the levels of spirochete transmission to mice [63]. A *B. burgdorferi* s.s. strain with a transposon inserted into *erpA* (one *ospE* paralog in *B. burgdorferi* s.s. strain B31-A3) displays a 2-week delay in the distal tissue colonization when coinfected with a population of mutant Lyme borreliae strains with transposons inserted into different genes [64]. These findings suggest that OspE-related proteins promotes the spirochetes' tick-to-host transmission and hematogenous dissemination (Figure 2). The *ospE* genes largely differ in the number of copies and sequences among different species or strains of *B. burgdorferi* s.l., raising the possibility that OspE-related proteins determines host specificity of infection [65,66].

Key Figure

Complement-Inhibitory Proteins and Their Potential Roles in the Infection Route

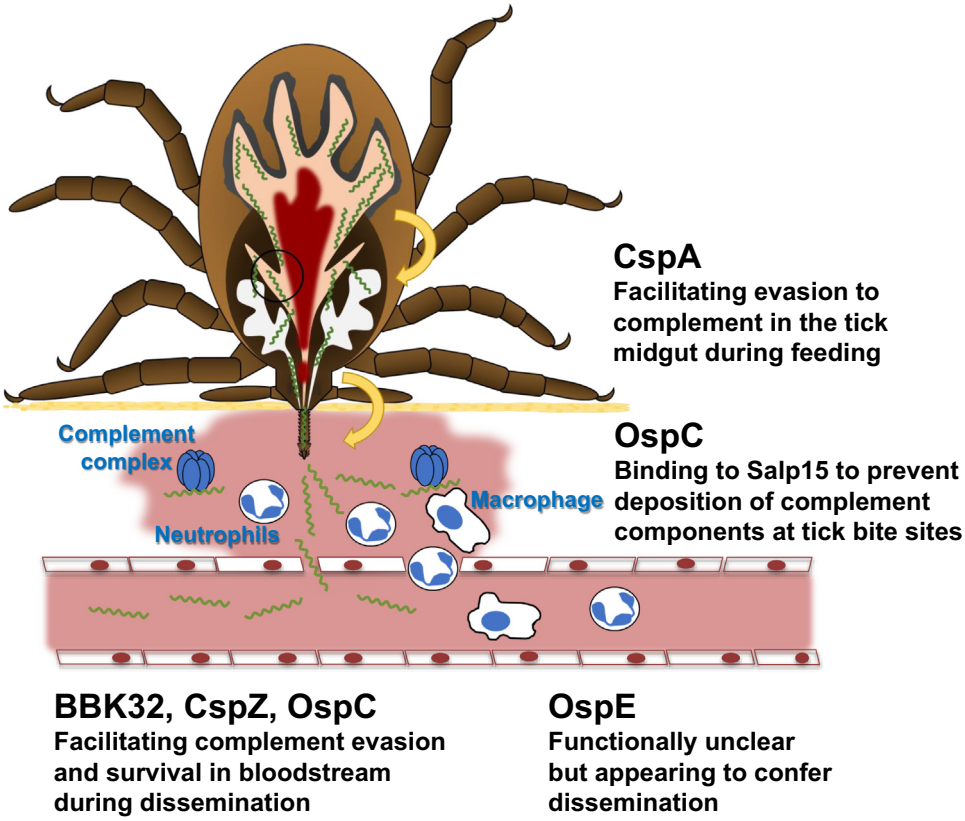

Figure 2. When ticks feed on hosts, Lyme borreliae produce CspA to facilitate spirochete escape from complement-mediated killing in the blood meal. After transmission to a host, the tick salivary protein, Salp15, binds to OspC on the spirochete surface to prevent opsonophagocytosis at tick-bite sites. Additionally, Lyme borreliae produce OspC, BBK32, and CspZ to promote complement evasion and bloodstream survival of spirochetes. The cell types and complement complex are indicated on the figure. Though the function of OspE-related proteins during infection remains unclear, current evidence supports the notion that this protein may confer spirochete dissemination in vertebrate animals. (Part of the figure is adapted from [26].)

OspC and BBK32, Complement-Evasion Factors Operating in the Initial Phase of Infection

OspC is one of the most studied outer-surface lipoproteins in *B. burgdorferi* s.l. This protein is not expressed when Lyme borreliae are in ticks prior to blood feeding but is produced upon the blood feeding of ticks and during transmission. After entry into hosts, the 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** at the tick-biting site [67]. 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 h of pathogen inoculation, and it gives spirochetes the ability to persist in the mammalian bloodstream [27,68] (Figure 2). Nonetheless, the

molecular mechanisms leading to such phenotypes need further investigation. 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 the extracellular matrix molecules fibronectin and glycosaminoglycans on the host cell surface and was later demonstrated to be a C1r-binding protein that inactivates CP [28]. In agreement with a blood-meal-induced expression profile of *bbk32* (Table 2), BBK32 contributes to the ability to survive in the mouse bloodstream for a short time and disseminate to joints at early stages of infection [68,69] (Figure 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 [70]. Assuming that C1r-binding activity plays a role in conferring spirochete survival in the vertebrate bloodstream, and promotes dissemination at infection onset, such a strain-to-strain variation in 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* complex and reservoir hosts, including small and medium-sized mammals, birds, and reptiles [9]. In most Lyme disease-endemic regions, there is a diverse community of cocirculating Lyme borreliae, and an association between different classes of vertebrate hosts and some *B. burgdorferi* s.l. genospecies has been observed [9,71,72]. Some of these observed associations may be due to extrinsic factors such as geographic co-occurrence of hosts with specific *B. burgdorferi* s.l. genospecies. However, there is strong evidence that at least some of these genospecies differ intrinsically in transmissibility across hosts, that is, they are 'host specialized' [11,12,71]. The strongest evidence is provided by experiments demonstrating increased fitness for *B. afzelii* in mice and *B. garinii* in birds [11,12,72] and, to some extent, field studies demonstrating greater genospecies infection prevalence in certain hosts compared to the background infection prevalence in local populations of *Ixodes* spp vectors [73].

In contrast to the other genospecies in the *B. burgdorferi* s.l. complex, *B. burgdorferi* s.s. is considered to be a **host generalist** as it has been isolated from multiple classes of vertebrate animals (e.g., mammalian and avian hosts) [71] and is summarized in [9]. However, multiple studies indicate that some genotypes of *B. burgdorferi* s.s. have a higher fitness in some hosts in laboratory studies [74] and are more prevalent in certain mammalian or avian host species [9,75–81]. Evidence of within-genospecies association of specific genotypes of *B. burgdorferi* s.l. and certain hosts has also been described for *B. garinii* and *B. afzelii* in laboratory experiments [9,72,82] and in some field studies [83,84] but not in others [85]. A limitation of field studies is that they represent only snapshots of population structures that are spatially and temporally variable due to stochastic effects or other forces, making inferences of host association difficult [71].

Ecoevolutionary Mechanisms Driving *B. burgdorferi*–Host Specialism

Despite evidence for some level of association between *B. burgdorferi* s.s. strains and hosts from laboratory infections and field studies [5,86], the extent to which host adaptation drives the genome-wide diversification in *B. burgdorferi* s.l. is currently under debate. Particular attention has focused on factors driving polymorphism in OspC, one of the most diverse Lyme borreliae antigens that is heavily targeted by the vertebrate immune system [87–89]. Balancing selection has been proposed to maintain *ospC* alleles at intermediate frequencies, with high

sequence diversity within a population [90]. Genome-wide linkage to this single locus may then be responsible for maintaining genetic variation at linked loci [91–93]. It is currently debated which specific mode of balancing selection drives the OspC polymorphism in *B. burgdorferi* s.s. Some authors have proposed that, similar to the process operating across *B. burgdorferi* s.l. species, host specialization via multiple-niche polymorphism (with hosts acting as different 'niches' for *B. burgdorferi* s.s.) could lead to diversification within *B. burgdorferi* s.s. [71,79,80,94].

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 [90,94,95]. 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 [96,97].

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 (Figure 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, that is, the ability to infect multiple hosts, has in fact been proposed as a key property allowing it to spread across the northeastern USA following large-scale habitat destruction in the course of the post-Columbian settlement and during the industrial revolution [80]. The

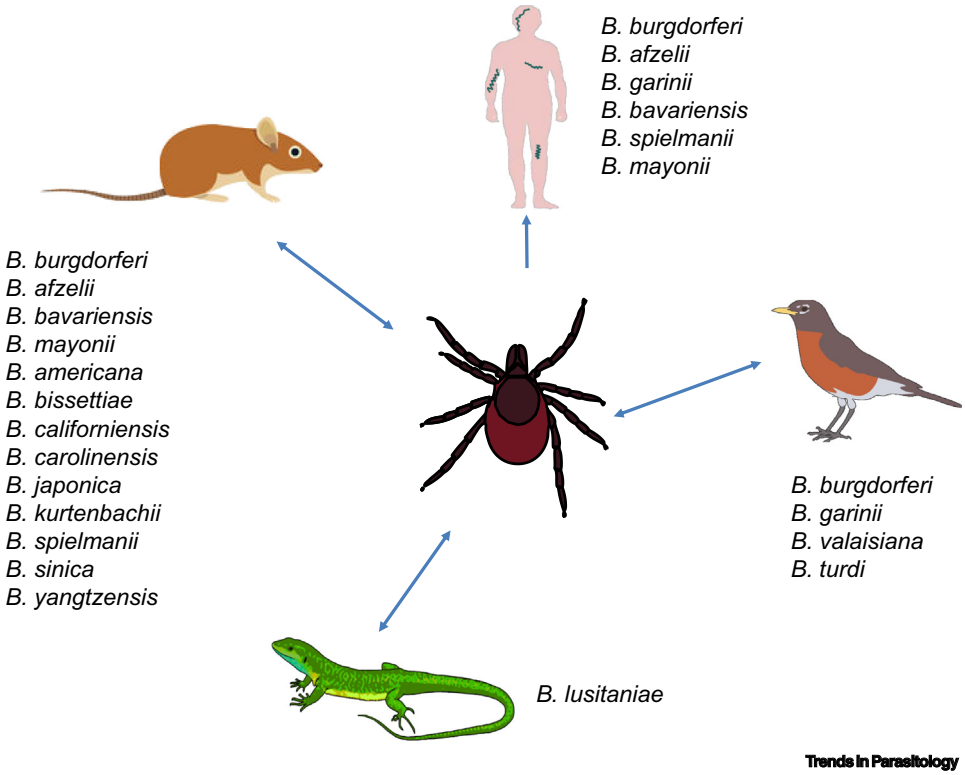

Figure 3. The Host–Pathogen Association for *Borrelia burgdorferi* s.l. Genospecies. The indicated *Borrelia burgdorferi* s.l. genospecies are acquired and transmitted between ticks and different vertebrate hosts, including humans, small mammals, reptiles, and songbirds. (Part of the figure is adapted from [9].)

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 [84]. 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** by *B. burgdorferi* s.l. linked to vector or host association [84].

Concluding Remarks

Here we summarize evidence supporting the contribution of proteins to host-specific complement evasion-mediated infection phenotypes. Does the complement-evasion activity of *B. burgdorferi* s.l. confer its host-association phenotype (see Outstanding questions)? The fact that some of these proteins are functionally redundant, and are produced simultaneously in the infection cycle, raises the hypothesis that they act in concert to promote the host association of *B. burgdorferi* s.l. (see Outstanding Questions). Furthermore, the ability of complement to eliminate Lyme borreliae appears to differ among diverse animal species in the same taxonomic classification (e.g., birds or mammals). This leads to an intriguing question: does complement play a role in defining the different levels of competence for the hosts within the same taxonomic classification (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 elucidate an incipient evolutionary process of *B. burgdorferi* s.l. toward host specialization (see Outstanding Questions). Future investigations of the aforementioned questions will undoubtedly contribute to insight about the factors contributing to the pathobiology of spirochetes and their diversity in host associations.

Outstanding Questions

Does the species-specific polymorphism of complement- or complement-regulator-binding activity play a key role in driving the host association of *B. burgdorferi* s.l.?

Why would *B. burgdorferi* s.l. produce multiple proteins displaying redundant complement-inhibitory activity (e.g., CspZ, OspE, BBK32, and OspC) when they are in the vertebrate hosts? Would they all contribute to the Lyme borreliae–host association?

Within the same taxonomic classifications (e.g., birds or mammals), would there be a difference in complement evasion for different species/strains of the *B. burgdorferi* s.l. complex leading to different levels of Lyme borreliae–host association?

Does the partial and regionally constrained host-specific infectivity observed in *B. burgdorferi* s.s. represent an incipient evolutionary process toward a more complete Lyme borreliae–host association?

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