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MicroReview

Outer surface protein polymorphisms linked to host–spirochete association in Lyme borreliae

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Summary

Lyme borreliosis is caused by multiple species of the spirochete bacteria *Borrelia burgdorferi* sensu lato. The spirochetes are transmitted by ticks to vertebrate hosts, including small- and medium-sized mammals, birds, reptiles, and humans. Strain-to-strain variation in host-specific infectivity has been documented, but the molecular basis that drives this differentiation is still unclear. Spirochetes possess the ability to evade host immune responses and colonize host tissues to establish infection in vertebrate hosts. In turn, hosts have developed distinct levels of immune responses when invaded by different species/strains of Lyme borreliae. Similarly, the ability of Lyme borreliae to colonize host tissues varies among different spirochete species/strains. One potential mechanism that drives this strain-to-strain variation of immune evasion and colonization is the polymorphic outer surface proteins produced by Lyme borreliae. In this review, we summarize research on strain-to-strain variation in host competence and discuss the evidence that supports the role of spirochete-produced protein polymorphisms in driving this variation in host specialization. Such information will provide greater insights into the adaptive mechanisms driving host and Lyme borreliae association, which will lead to the development of interventions to block pathogen spread and eventually reduce Lyme borreliosis health burden.

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Variability in host species association with Lyme borreliae

Lyme borreliosis is the most common vector-borne disease in the United States and Europe (Steere *et al.*, 2016). The disease is caused by the spirochetal bacteria *Borrelia burgdorferi* sensu lato (hereafter *B. burgdorferi* sl), which is vectored by *Ixodes* spp. ticks (Radolf *et al.*, 2012). Following a tick bite, the spirochetes can hematogenously disseminate from the tick bite site in the skin to distal tissues and organs within a host (Brisson *et al.*, 2012). In humans, the spirochete colonization of distal tissues leads to multiple pathologies, including arthritis, carditis and neuroborreliosis (Rosa *et al.*, 2005). In nature, ticks can acquire and transmit Lyme borreliae between multiple vertebrate reservoir hosts, including avian, reptile and mammalian hosts (Kurtenbach *et al.*, 2006). The ability of *B. burgdorferi* to survive in ticks, be transmitted to, and systemically infect hosts is essential for the maintenance of this spirochete in the enzootic cycle.

Borrelia burgdorferi sl is comprised of more than 15 genospecies (subspecific designation of species based on genotypes), each comprising multiple strains (Mead, 2015; Steere *et al.*, 2016). Interestingly, an association

between different classes of vertebrate hosts and some *B. burgdorferi* sl genospecies or strains has been observed (Kurtenbach *et al*., 2006) (Table 1). For example, *B. afzelii*, *B. bavariensis*, *B. bissettii*, *B. californiensis, B. carolinensis, B. japonica*, *B. kurtenbachii*, *B. mayonii*, *B. spielmanii* and *B. yangtzensis* have been found in rodents such as mice (field mice: *Apodemus flavicollis* and *A. sylvaticus*; wood/harvest mice: *Micromys minutus*) and voles (*Clethrionomys glareolus*, *Microtus arvalis*) (Kurtenbach *et al*., 1998a; Hanincova *et al*., 2003a; Richter *et al*., 2004b), while *B. garinii*, *B. valaisiana* and *B. turdi* have typically been isolated from avian hosts such as the ring-necked pheasant (*Phasianus colchicus*), the Atlantic puffin (*Fratercula arctica*), the common blackbird (*Turdus merula*) and numerous other passerine species (Humair *et al*., 1998; Kurtenbach *et al*., 1998a; Gylfe *et al*., 1999; Hanincova *et al*., 2003b; Comstedt *et al*., 2006). *Borrelia lusitaniae* was identified mainly in reptiles such as lizards (Richter and Matuschka, 2006; Amore *et al*., 2007). The host-specific infection of these spirochetes indicates that these species are specialists in the enzootic cycle. Unlike the specialists, *B. burgdoferi* sensu stricto (hereafter *B. burgdorferi*) has been isolated from multiple classes of vertebrate animals (e.g. mammalian and avian hosts) and thus could be considered a generalist species (Lane and Loye, 1989; Levin *et al*., 1996; Kurtenbach *et al*., 2006; Swanson and Norris, 2007). However, previous observations propose that some genotypes of *B. burgdorferi* are more prevalent in mammalian hosts such as small rodents whereas others are more widespread in avian hosts (Wang *et al*., 2002; Brisson and Dykhuizen, 2004; Brisson and Dykhuizen, 2006; Hanincova *et al*., 2006; Brisson *et al*., 2008; Brinkerhoff *et al*., 2010; Vuong *et al*., 2014; 2017; Mechai *et al*., 2016). These findings raise the possibility of interstrain variation of spirochete-host associations.

In support of this association, when different vertebrate hosts are infected by Lyme borreliae via ticks or needles, some spirochete species/strains preferentially infect small rodents (Matuschka and Spielman, 1992; Hu *et al*., 2001; Wang *et al*., 2002; Derdakova *et al*., 2004; Richter *et al*., 2004a; Hanincova *et al*., 2008; Craig-Mylius *et al*., 2009; Tonetti *et al*., 2015; Rynkiewicz *et al*., 2017), while others more efficiently colonize avian hosts (e.g. pheasant, *Coturnix* quail and American robins) (Isogai *et al*., 1994; Kurtenbach *et al*., 2002b; Ginsberg *et al*., 2005). Additionally, upon infection, Lyme borreliae species/strains differ in their ability to survive in the bloodstream or disseminate to distal tissues in *Mus musculus* (mice) or *Peromyscus leucopus* (white-footed mice) (Anderson *et al*., 1990; Barthold *et al*., 1991; Norris *et al*., 1995; Wang *et al*., 2002; Barbour *et al*., 2009; Baum *et al*., 2012; Chan *et al*., 2012). Consistent with this observation, the ability of hematogenous dissemination by these spirochetes and the severity of manifestations vary among spirochete species and strains during infection in humans (Anderson *et al*., 1990; Wang *et al*., 2002; Carlsson *et al*., 2003; Logar *et al*., 2004; Dykhuizen *et al*., 2008; Wormser *et al*., 2008; Craig-Mylius *et al*., 2009). These findings elucidate a spirochete strain-to-strain variation in the host-specific infectivity. Below, we discuss the potential mechanisms to drive the host tropisms of Lyme borreliae.

Hosts develop variable levels of innate and adaptive immune responses when infected with different species/strains of Lyme borreliae

The innate immune response is one factor that controls survival and disease severity of Lyme borreliae in vertebrate hosts (Barthold, 1999; Wang *et al*., 2001; Pachner *et al*., 2004; Steere and Glickstein, 2004). Upon tick bite, spirochetes can be engulfed by dendritic cells at the bite site in the skin, which permits host cells to produce antigens and activate naive T cells (Mason *et al*., 2014). Meanwhile, Lyme borreliae outer surface proteins recognized by multiple receptors (e.g. toll-like receptors) on the surface of macrophages lead to the activation of these cells (Talkington and Nickell, 2001; Alexopoulou *et al*., 2002; Wooten *et al*., 2002; Jacchieri *et al*., 2003; Soloski *et al*., 2014). This activation promotes the production of proinflammatory cytokines and chemokines and the phagocytosis of spirochetes (Rittig *et al*., 1992; Modolell *et al*., 1994; Montgomery *et al*., 1996). Effector molecules are then produced, which facilitates neutrophil infiltration of the infection site, resulting in disease manifestations in humans (Defosse and Johnson, 1992; Gebbia *et al*., 2001; Anguita *et al*., 2002). Non-reservoir mammalian hosts (e.g. humans or *M. musculus* mouse models) *in vivo*, cultivated macrophages, or dendritic cells *in vitro* develop distinct levels of cytokines and chemokines in response to different Lyme borreliae species/strains (Strle *et al*., 2009; 2011; Mason *et al*., 2015). The ability to trigger varying degrees of cytokine and chemokine production in different species/strains during infection is strongly correlated with the severity of resulting manifestations (Widhe *et al*., 2004; Jones *et al*., 2008; Strle *et al*., 2009; 2011). Additionally, complement has been demonstrated to prevent spirochetes from efficiently disseminating to distal tissues and appears to play a role in the differential clearance of numerous Lyme borreliae species *in vivo* (Lawrenz *et al*., 2003; Woodman *et al*., 2007). This is addressed in more detail in the following section.

The adaptive immune response also confers clearance of Lyme borreliae and may lead to clinical manifestations, such as arthritis. The B cell-mediated antibody

Table 1. Association of *Borrelia burgdorferi* sensu lato genospecies with vertebrate reservoir host species based on spirochetes previously isolated from particular hosts.

	Vertebrate reservoir hosts		
Lyme borreliae	Class	Common name (Scientific name)	References
B. afzelii	Mammalia	Bank vole (*Clethrionomys glareolus*)	Humair *et al.* (1995; 1999)
		Edible dormouse (*Glis glis*)	Humair *et al.* (1999)
		Japanese field mouse (*Apodemus speciosus*)	Nakao *et al.* (1994)
		Microtinae vole (*Clethrionomys rufocanus bedfordiae*)	Ishiguro *et al.* (1996)
		Siberian chipmunk (*Tamias sibiricus barberi*)	Marsot *et al.* (2013)
		Wood mouse (*Apodemus sylvaticus*)	Humair *et al.* (1999); Marsot *et al.* (2013)
		Yellow-necked mouse (*Apodemus flavicollis*)	Humair *et al.* (1995; 1999)
B. bavariensis	Mammalia	Microtinae vole (*Clethrionomys rufocanus bedfordiae*)	Ishiguro *et al.* (1996); Takano *et al.* (2011)
B. bisettii	Mammalia	Deer mouse (*Peromyscus maniculatus*)	Schneider *et al.* (2000)
		Mexican woodrat (*Neotoma mexicana*)	Schneider *et al.* (2000)
		Prairie vole (*Microtus ochrogaster*)	Schneider *et al.* (2000)
		Zacatecan deer mouse (*Peromyscus difficilis*)	Schneider *et al.* (2000)
B. burgdorferi sensu stricto	Aves	American robin (*Turdus migratorius*)	Vuong *et al.* (2014)
		Veery (*Catharus fuscescens*)	Vuong *et al.* (2014)
		Wood thrush (*Hylocichla mustelina*)	Vuong *et al.* (2014)
	Mammalia	Bank vole (*Clethrionomys glareolus*)	Kurtenbach *et al.* (1998a)
		Eastern Chipmunk (*Tamias striatus*)	Hanincova *et al.* (2006); Brisson and Dykhuizen (2004)
		Gray squirrel (*Sciurus carolinensis*)	Hanincova *et al.* (2006); Brisson and Dykhuizen (2004)
		Mexican woodrat (*Neotoma mexicana*)	Maupin *et al.* (1994)
		Pine vole (*Microtus pinetorum*)	Hanincova *et al.* (2006)
		Raccoon (*Procyon lotor*)	Hanincova *et al.* (2006)
		Virginia opossum (*Didelphis virginiana*)	Hanincova *et al.* (2006)
		Short-tailed shrew (*Blarina brevicauda*)	Brisson and Dykhuizen (2004)
		Siberian chipmunk (*Tamias sibiricus barberi*)	Marsot *et al.* (2013)
		White-footed mouse (*Peromyscus leucopus*)	Hanincova *et al.* (2006); Brisson and Dykhuizen (2004)
		Wood mouse (*Apodemus sylvaticus*)	Kurtenbach *et al.* (1998a)
		Zacatecan deer mouse (*Peromyscus difficilis*)	Maupin *et al.* (1994)
		Southern red-backed vole (*Myodes gapperi*)	Stone *et al.* (2015)
B. californiensis	Mammalia	California kangaroo mouse (*Dipodomys californicus*)	Postic *et al.* (2007)
B. carolinensis	Mammalia	Cotton mouse (*Peromyscus gossypinus*)	Rudenko *et al.* (2009; 2011)
		Eastern woodrat (*Neotoma floridana*)	Rudenko *et al.* (2009; 2011)
		Amargosa vole (*Microsa californicus scirpensis*)	Foley *et al.* (2014)
B. garinii	Aves	Black guillemot (*Cepphus grylle*)	Olsen *et al.* (1995)
		Guillemot (*Uria aalge*)	Gylfe *et al.* (1999)
		Puffin (*Fratercula arctica*)	Gylfe *et al.* (1999)
		Razorbill (*Alca torda*)	Gylfe *et al.* (1999)
		Black-faced bunting (*Emberiza spodocephala*)	Nakao *et al.* (1994)
		Brown-headed thrush (*Turdus chrysolaus*)	Nakao *et al.* (1994)
		Common blackbird (*Turdus merula*)	Humair *et al.* (1998)
		Great tit (*Parus major*)	Hanincova *et al.* (2003b)
		Song thrush (*Turdus philomelos*)	Hanincova *et al.* (2003b)
		Black-browed albatross (*Thalassarche melanophris*)	Olsen *et al.* (1995)
		Fork-tailed storm petrel (*Oceanodroma furcata*)	Olsen *et al.* (1995)
		King penguin (*Aptenodytes patagonicus*)	Olsen *et al.* (1995)
B. japonica	Mammalia	Large Japanese field mouse (*Apodemus speciosus*)	Masuzawa *et al.* (1995)
		Smith's vole (*Myodes smithii*)	Masuzawa *et al.* (1995)
B. kurtenbachii	Mammalia	Meadow vole (*Microtus pennsylvanicus*)	Margos *et al.* (2010)
		Meadow jumping mouse (*Zapus hudsonius*)	Margos *et al.* (2014); Picken and Picken (2000)
		Eastern woodrat (*Neotoma floridana*)	Margos *et al.* (2014); Lin *et al.* (2001)
B. lusitaniae	Reptilia	Common wall lizard (*Podarcis muralis*)	Richter and Matuschka (2006)
		Green lizards (*Lacerta viridis*)	Majlathova *et al.* (2006)
		Large psammadromus (*Psammodromus algirus*)	Dsouli *et al.* (2006)
		Sand lizard (*Lacerta agilis*)	Richter and Matuschka (2006)
		Slow worm (*Anguis fragilis*)	Richter and Matuschka (2006)
B. mayonii	Mammalia	Red squirrel (*Tamiasciurus hudsonicus*)	Johnson *et al.* (2017)
		White-footed mouse (*Peromyscus leucopus*)	Johnson *et al.* (2017)

Table 1. *Continued*

Lyme borreliae	Vertebrate reservoir hosts: Class	Common name (Scientific name)	References
B. spielmanii	Mammalia	Garden dormouse (*Eliomys quercinus*)	Richter *et al.* (2004b)
		Hazel dormouse (*Muscardinus avellanarius*)	Richter *et al.* (2004b)
		European hedgehog (*Erinaceus europaeus*)	Skuballa *et al.* (2012)
		Northern white-breasted hedgehog (*Erinaceus roumanicus*)	Skuballa *et al.* (2012)
B. turdi	Aves	Common blackbird (*Turdus merula*)	Norte *et al.* (2013)
		Song thrush (*Turdus philomelos*)	Norte *et al.* (2013)
B. valaisiana	Aves	Common blackbird (*Turdus merula*)	Hanincova *et al.* (2003b); Norte *et al.* (2013)
		Song thrush (*Turdus philomelos*)	Hanincova *et al.* (2003b)
B. yangtzensis	Mammalia	Chestnut white-bellied rat (*Niviventer fulvescens*)	Margos *et al.* (2015)
		Striped field mouse (*Apodemus agrarius*)	Margos *et al.* (2015)
		Black rat (*Rattus rattus*)	Margos *et al.* (2015)
		Lesser Ryukyu shrew (*Crocidura watasei*)	Margos *et al.* (2015)
		Asian house shrew (*Suncus murinus*)	Margos *et al.* (2015)
		Ryukyu mouse (*Mus caroli*)	Margos *et al.* (2015)
		Norway rat (*Rattus norvegicus*)	Margos *et al.* (2015)

immune response plays a major role for pathogen clearance (Steere and Glickstein, 2004; Blum *et al.*, 2018). This B cell immunity is enhanced by *B. burgdorferi*-specific CD4$^+$ T helper cell (T_H1) response, in which interferon-γ is the marker (Keane-Myers and Nickell, 1995; Kang *et al.*, 1997; Zeidner *et al.*, 1997). In fact, humans infected with different Lyme borreliae strains generate distinct levels of interferon-γ (Strle *et al.*, 2011). When *P. leucopus* or *M. musculus* hosts were infected with different *B. burgdorferi* strains, the levels of antibodies against specific *B. burgdorferi* outer surface proteins and the spirochete burdens varied at heart and joint tissues (Wang *et al.*, 2001; Baum *et al.*, 2012). These findings thus raise the possibility that the variation in antibody-mediated clearance induced by Lyme borreliae species/strains results in different levels of host competence. Further, invariant natural killer T cells (iNKT cells) recognize the lipids on the surface of *B. burgdorferi* to eradicate spirochetes, which limits their dissemination to joints and prevents Lyme disease-associated arthritis (Kinjo *et al.*, 2006; Tupin *et al.*, 2008; Lee *et al.*, 2010; 2014). However, whether this iNKT cell-mediated lipid binding activity, pathogen clearance and alleviation of manifestations is strain-specific remains unclear and warrant further investigations.

Lyme borreliae develop host-specific serum resistance activity to evade the complement

Complement, composed of numerous serum proteins, is one of the innate immune responses in the vertebrate bloodstream (Figure 1) (Zipfel and Skerka, 2009;

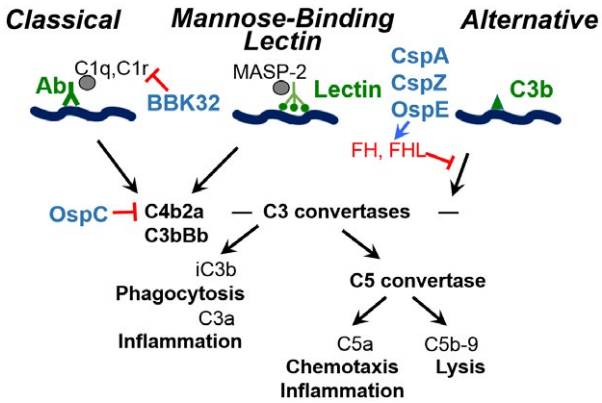

Fig. 1. Complement activation and control. The host complement is activated via classical, mannose-binding lectin (MBL) and alternative pathways. The classical pathway is activated by the binding of C1q, C1r and antibodies to the pathogen antigens. The MBL pathway is initiated by the binding of lectins and MASP-2 to the pathogen's carbohydrates. Finally, the alternative pathway is triggered by C3b binding to the pathogen's surface structure. Host complement regulators, factor H (FH) and FH-like protein 1 (FHL), are targeted by Lyme borreliae surface proteins, CspA, CspZ and OspE, which then inhibits the formation of C3bBb. *Borrelia burgdorferi* sl outer surface protein BBK32 binds to C1r to inhibit the activation of the classical pathway. *B. burgdorferi* sl outer surface protein OspC binds to C4b and prevents the creation of C4b2a. The inhibition of C3bBb and C4b2a hinders the generation of C3a, iC3b and C5a leading to phagocytosis, inflammation and the prevention of C5b-9 formation on the surface of *B. burgdorferi* and ultimately spirochete lyses.

Ricklin *et al.*, 2010). The formation of enzymatic complement complex proteins, termed C3 convertases, is a critical control point in the complement cascade. Two distinct C3 convertases, C4b2a and C3bBb (named for the complement components that make them up) are

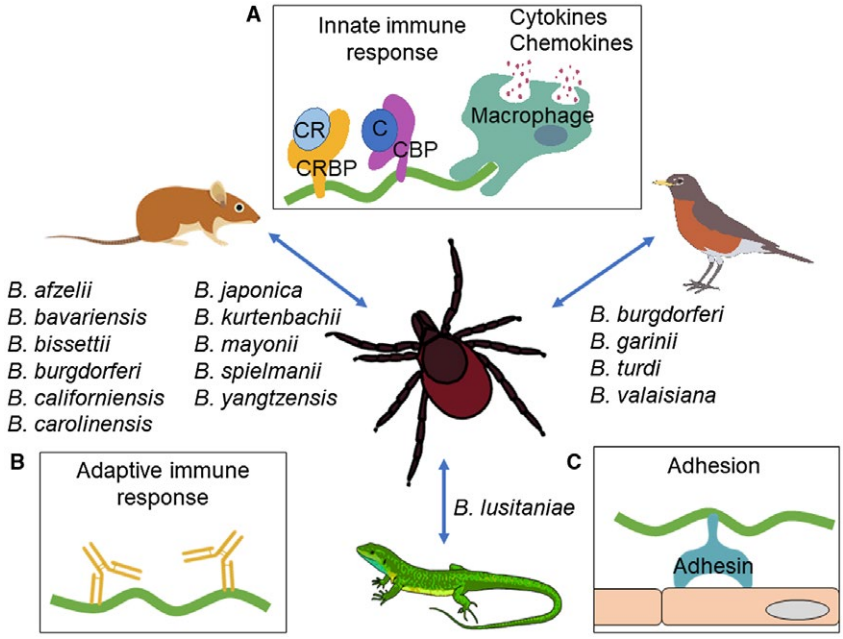

Fig. 2. Potential mechanisms that drive vertebrate reservoir host and Lyme borreliae species association. The indicated *B. burgdorferi* sensu lato species are acquired and transmitted between *Ixodes scapularis* ticks and different vertebrate reservoir hosts, including mammals, birds and reptiles. The potential mechanisms that drive this spirochete-host association include strain-to-strain differences in the induced.
A. Innate immune responses such as the activation of macrophages leading to phagocytosis and cytokine/chemokine release, and the binding of spirochete complement regulator-binding proteins (CRBP) to complement regulators (CR) and complement binding proteins to complement.
B. Adaptive immune response such as antibody production.
C. Polymorphic spirochete adhesins facilitate Lyme borreliae binding to cells and colonizing tissues.

formed from the activation of three pathways: the classical pathway, the mannose-binding lectin (MBL) pathway, and the alternative pathway (Ricklin *et al.*, 2010; Merle *et al.*, 2015). C4b2a is generated by both the classical pathway, which is initiated by the binding of antibody, antigen, and complement C1qrs complexes, and the MBL pathway, initiated by microbial recognition via the formation of MBL-microbial carbohydrate complexes (Ricklin *et al.*, 2010; Merle *et al.*, 2015). C3bBb is formed by the alternative pathway, which is initiated by binding of the complement component, C3b, to the microbial surface. C4b2a (consisting of C4b and C2a) and C3bBb (consisting of C3b and Factor Bb) then recruit other complement components to generate C5 convertases. This leads to downstream effects, including the release of proinflammatory peptides, the activation of phagocytic clearance and the formation of a membrane attack complex that can lyse pathogens (Ricklin *et al.*, 2010; Merle *et al.*, 2015). Vertebrate hosts also produce complement regulators that bind to complement components (Zipfel and Skerka, 2009). These complement regulators include factor H (FH) as well as FH-like protein 1 (the truncated form of FH), both of which bind to C3b (Zipfel *et al.*, 2002). These complement regulators recognize and lead to the degradation of other complement proteins, eventually inhibiting the complement system (Meri, 2016). The complement components and their regulators exhibit sequence variation among vertebrate hosts (approximately 60% to 70% sequence identity among different classes of vertebrate animals) (Ripoche *et al.*, 1988a; 1988b). The sequence variation of these proteins suggests a host-to-host difference of complement. Consistent with the amino acid variation in different host complement proteins, different Lyme borreliae species/strains differ in their ability to survive in vertebrate host sera (Kurtenbach *et al.*, 1998b; 2002a; Ullmann *et al.*, 2003) (Fig. 2). This difference in spirochete survival in the serum has been correlated with the spirochetes' capability to inactivate particular hosts' complement (Kurtenbach *et al.*, 1998b; 2002a; Kuo *et al.*, 2000; Nelson *et al.*, 2000).

Spirochetes produce polymorphic outer surface proteins that facilitate different levels of host complement evasion

A number of Lyme borreliae polymorphic proteins may be involved in host-to-host differences in complement evasion. The main candidates are five Lyme borreliae FH-binding proteins termed CRASPs (Complement Regulator Acquiring Surface Proteins), including CspA (also termed CRASP-1), CspZ (CRASP-2), ErpP (CRASP-3), ErpC (CRASP-4) and ErpA (CRASP-5)

Table 2. Lyme borreliae outer surface proteins that confer allelic variable functions *in vitro* and/or *in vivo*.

Lyme borreliae Protein	Ligands[a]	Allelic variable functions	
		In vitro	***In vivo***
Complement regulator-binding proteins			
CspA	Factor H	FH binding, Serum resistance, Complement inactivation	Survival in ticks' blood meal, Tick-to-host transmission
CspZ	Factor H	FH binding	ND[b]
OspE (ErpP, ErpC, ErpA)	Factor H	FH binding	ND
Complement-binding protein			
OspC	C4b	C4b binding	Early bloodstream survival
Adhesins			
DbpA	Dermatan Sulfate, Decorin, biglycan	Dermatan Sulfate/Decorin/biglycan binding, Attachment to cells	Tissue colonization
OspF	Heparan Sulfate	Heparan Sulfate binding	ND

[a]The ligands that particular Lyme borreliae proteins bind in an allelic variable fashion.
[b]Not determined.

(Table 2) (Kraiczy and Stevenson, 2013). CspA is unique among the five CRASP proteins in that it is only expressed when the spirochetes are in the tick vector and at the biting site of host skin (Bykowski *et al.*, 2007; Hart *et al.*, 2018). The lack of *cspA* expression results in the inability of spirochetes to survive in vertebrate host sera (Brooks *et al.*, 2005; Kenedy *et al.*, 2009; Hart *et al.*, 2018). Additionally, a *cspA*-deficient *B. burgdorferi* is cleared from nymphal ticks feeding on mice, eventually leading to a dearth of spirochetes transmitted from ticks to mice (Hart *et al.*, 2018). These defects *in vitro* and *in vivo* have been attributed to the lack of FH-binding activity of the *cspA*-deficient spirochetes to evade complement in a tick's blood meal (Hart *et al.*, 2018). Further, CspA is highly conserved within each Lyme borreliae species, but exhibits variation at the interspecific level (Wallich *et al.*, 2005; Hammerschmidt *et al.*, 2014). These CspA variants differ in their ability to facilitate FH binding and serum survival in a host-specific manner and promote distinct levels of *B. burgdorferi* transmission from ticks to mice. This suggests CspA may play a role in promoting host-specific transmission of Lyme borreliae (Kraiczy *et al.*, 2001; Wallich *et al.*, 2005; Bhide *et al.*, 2009; van Burgel *et al.*, 2010; Hammerschmidt *et al.*, 2014; Hart *et al.*, 2018).

CspZ, when produced on the surface of a serum-sensitive spirochete, allows for binding to human FH and confers spirochete survival in human serum (Hartmann *et al.*, 2006; Siegel *et al.*, 2008). Unlike *cspA*, *cspZ* is mainly expressed when spirochetes are in vertebrate hosts (Bykowski *et al.*, 2007). A *cspZ*-deficient *B. burgdorferi* strain has the ability to colonize mice at the same levels as its wild-type parental strain (Coleman *et al.*, 2008). However, Marcinkiewicz and colleagues (2019) incubated wild-type *B. burgdorferi* with human blood to induce the production of CspZ. They discovered that this wild-type spirochete displayed greater levels of bacteremia and dissemination in laboratory mice compared to a *cspZ* deletion mutant under the blood treatment condition (Marcinkiewicz *et al.*, 2019). These findings suggest that spirochetes do not require CspZ to survive in mammalian hosts, but its presence may enhance the infectivity of *B. burgdorferi*. Additionally, CspZ is not carried by all Lyme borreliae species/strains (Rogers and Marconi, 2007; Rogers *et al.*, 2009). Despite its high sequence conservation, i.e. 98% in *B. burgdorferi* strains, the ability of these strains to bind to human FH varies (Rogers and Marconi, 2007). This finding implies that the 2% sequence difference may contribute to this variable human FH-binding activity and human complement evasion by *B. burgdorferi* (Brangulis *et al.*, 2014).

The CRASP genes *erpP*, *erpC* and *erpA* are encoded on highly homologous cp32-derived plasmids and are co-expressed when *B. burgdorferi* is in vertebrate hosts (Bykowski *et al.*, 2007). The proteins derived from these genes belong to the OspE-related protein family (OspE proteins) because of their sequence similarity (77–90% of sequence similarity) (Marconi *et al.*, 1996; Stevenson *et al.*, 1996; 2002; Akins *et al.*, 1999; Kraiczy *et al.*, 2004; Brissette *et al.*, 2008). These OspE proteins, though able to bind to human FH, do not promote human serum survival when they are individually produced on the surface of serum-sensitive borreliae (Siegel *et al.*, 2010; Hammerschmidt *et al.*, 2012). However, simultaneously producing ErpP and ErpA in a serum-sensitive spirochete enables this strain to survive in human serum (Kenedy and Akins, 2011). Transposon-inserted *erpA* mutant spirochetes co-infected with other transposon mutants exhibit decreased levels of colonization in C3H/HeN mice (Lin *et al.*, 2012). These results suggest a nonessential but important function of OspE proteins in

facilitating mammalian infection, consistent with the finding that not all Lyme borreliae species/strains encode these proteins (Alitalo *et al.*, 2005). Variation in OspE proteins has been observed among *B. burgdorferi* sl species/strains (Marconi *et al.*, 1996; Stevenson *et al.*, 1996; 2002; Akins *et al.*, 1999; Metts *et al.*, 2003; Alitalo *et al.*, 2005; Hovis *et al.*, 2006; Brissette *et al.*, 2008). OspE variants differ in their FH-binding ability in humans (Stevenson *et al.*, 2002; McDowell *et al.*, 2003; Alitalo *et al.*, 2005) and other vertebrate hosts (Hellwage *et al.*, 2001; Stevenson *et al.*, 2002; McDowell *et al.*, 2003; Alitalo *et al.*, 2004; 2005), implying a possibility that polymorphic OspE proteins may drive host-specific infection.

Additional Lyme borreliae proteins including BBK32 and OspC have been recently identified to promote host complement inactivation and/or facilitate the spirochete bloodstream survival and dissemination (Caine and Coburn, 2015; Garcia *et al.*, 2016; Caine *et al.*, 2017). BBK32, for example, binds to C1r to inhibit the initiation of the classical pathway, but high sequence identity of the variants among Lyme borreliae (greater than 70%) suggests that this protein is less likely to confer allelic variable and/or host-specific complement inactivation (Probert *et al.*, 2001; Garcia *et al.*, 2016). OspC binds to C4b to prevent the formation of C4b2a, resulting in spirochete evasion of classical and MBL pathways (Table 2) (Caine *et al.*, 2017). In addition, an *ospC*-deficient *B. burgdorferi* exhibits the defects of bloodstream survival during early stages of murine infection, suggesting that OspC facilitates hematogenous dissemination (Caine and Coburn, 2015; Caine *et al.*, 2017). OspC has been known as one of the most polymorphic proteins produced in Lyme borreliae (approximately 60% sequence identity among *B. burgdorferi* sl) (Wilske *et al.*, 1993). This polymorphic protein also displays variable binding activity to human C4b (Caine *et al.*, 2017). These findings thus encourage further investigations into the potential role of OspC in promoting the adaptive divergence of *B. burgdorferi* sl host-specific infection at the species and strain level.

Polymorphic spirochete adhesins are potential contributors of host–Lyme borreliae association

In addition to the evasion of the host immune response, spirochete infectivity may also be driven by its ability to colonize host tissues (Coburn *et al.*, 2005; 2013). Such ability is partly attributed to the binding of Lyme borreliae to the extracellular matrix (ECM) components, including proteoglycans (Coburn *et al.*, 2005; Brissette and Gaultney, 2014). Glycosaminoglycans (GAGs), including dermatan sulfate and heparin sulfate, are the components of proteoglycan (Lin *et al.*, 2017). *Borrelia burgdorferi* colonizes mouse tissues less efficiently in mice deficient in decorin, a proteoglycan composed of GAGs (Brown *et al.*, 2001). This observation is consistent with a positive correlation of the levels of GAG at mouse joints and the severity of arthritis during Lyme disease infection (Bramwell *et al.*, 2014). In fact, Lyme borreliae produce outer surface proteins (known as adhesins) that contribute to spirochete binding to GAGs and proteoglycans, resulting in cell adhesion and tissue colonization (Lin *et al.*, 2017). Decorin-binding protein A (DbpA) binds to proteoglycan components, including dermatan sulfate, decorin and biglycan (Guo *et al.*, 1998; Parveen *et al.*, 2003; Lin *et al.*, 2014) (Table 2). *Borrelia burgdorferi* strains that lack *dbpA* (and its functional paralog *dbpB*) are unable to infect mice (Blevins *et al.*, 2008; Shi *et al.*, 2008; Weening *et al.*, 2008). This infectivity defect of the *dbpBA*-deficient mutant has been correlated with an inability of this strain to bind to decorin and dermatan sulfate (Benoit *et al.*, 2011). DbpA variants are extremely polymorphic among *B. burgdorferi* sl (58% sequence identity) (Roberts *et al.*, 1998) and variants from different Lyme borreliae species/strains differ in their ability to bind to human decorin/dermatan sulfate/biglycan (Benoit *et al.*, 2011; Salo *et al.*, 2011; Lin *et al.*, 2014). Further, the spirochetes producing each of these DbpA variants colonize mouse tissues at different levels (Lin *et al.*, 2014). Because the lengths of GAGs vary among different vertebrate hosts (Thunell *et al.*, 1967; Barry *et al.*, 1994), these findings raise the possibility that DbpA may promote host-Lyme borreliae association by facilitating distinct levels of tissue colonization in different hosts. Additionally, Lyme borreliae produce OspF-related proteins (OspF proteins) that bind to heparan sulfate to promote spirochete attachment to mammalian cells (Antonara *et al.*, 2007; Lin *et al.*, 2015) (Table 2). A recent study indicated that OspF variants from different *B. burgdorferi* strains display slightly different affinity in binding to porcine heparin sulfate (Lin *et al.*, 2015). This finding illuminates the potential role of OspF as a contributor to host-Lyme borreliae association. Overall, these variations in protein production among species/strains have allowed the spirochetes to effectively infect their specific classes of vertebrate hosts, thus reinforcing the host specialization and contributing to the divergence of *Borrelia burgdorferi* sl.

Barriers to investigate host–Lyme borreliae association: Application of appropriate spirochete strains and animal models

Investigating the host-specific roles of many Lyme borreliae proteins poses difficult challenges. *Borrelia*

burgdorferi sl encodes nearly 100 outer surface proteins, many with redundant functions and/or expressed in a similar manner (Fraser *et al.*, 1997; Dowdell *et al.*, 2017), which makes it difficult to delineate the phenotype promoted by each of these proteins and protein variants during infection. Thus, identifying the appropriate spirochete background strains with required defects, such as susceptibility to different hosts' sera, lack of infectivity in different hosts or lack of adhesion to different hosts' cells, is needed to study the influence of the protein variants on host competence. In addition, the major hurdle to studying the host-pathogen association of Lyme borreliae is that no well-established animal models for nonmammalian hosts are currently available. Though previous efforts using nonmammalian animals for Lyme borreliae infection have been documented (for birds, see Burgess, 1989; Bishop *et al.*, 1994; Isogai *et al.*, 1994; Olsen *et al.*, 1996; Piesman *et al.*, 1996; Richter *et al.*, 2000; Kurtenbach *et al.*, 2002b; for reptiles see Lane, 1990; Lane and Quistad, 1998), obtaining and maintaining wild-caught animals in the laboratory is often prohibitive. An additional challenge is that not all vertebrate hosts are able to persistently maintain Lyme borreliae (Burgess, 1989; Lane, 1990; Olsen *et al.*, 1996; Piesman *et al.*, 1996; Richter *et al.*, 2000). Furthermore, interspecies variation within animal orders such as rodents (Rodentia) and songbirds (Passeriformes) in Lyme borreliae competence have been observed (see Table 1 for references). These findings raise a general issue about which animal species appropriately represents a particular category of hosts. These difficulties warrant further investigations, as establishing nonmammalian Lyme borreliosis models would permit us to replicate the patterns of host competence seen in the field in a more controlled laboratory environment.

Conclusion and future work

Lyme borreliae are comprised of numerous strains and species that are maintained in an enzootic cycle by surviving in *Ixodes* ticks and various vertebrate hosts. Variation among spirochete species/strains in their ability to infect different hosts has been documented, but the cause of this variation remains unknown. Here, we discussed the possibility of variability of host immune response to different species of Lyme borreliae, resulting in variable infectivity. We also listed potential polymorphic Lyme borreliae proteins that could facilitate host-specific infection. Future work is needed to further define these mechanisms using different laboratory animals such as avian and mammalian hosts. This line of investigation will help design targeted intervention strategies against these mechanisms to block the infection route and ultimately reduce the burden of Lyme borreliosis.

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Author contributions

DMT, TMH and YL wrote the manuscript. All authors critically reviewed and accepted this manuscript.

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