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Outer Surface Proteins of *Borrelia*: Peerless Immune Evasion Tools

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Abstract: Lyme borreliosis (LB), caused by *Borrelia burgdorferi* (*B.b.*), is the most frequently diagnosed tick-borne zoonosis in temperate zones of the Northern hemisphere. *Borrelia* is unique among bacteria in its ability to express a wide variety of lipoproteins on its surface, which play an essential role in pathogenesis. Surface proteins of spirochetes are important virulence determinants, immune evasion molecules and adaptation factors in the transmission and interaction with host tissues. Vast diversity in the expressed surface proteome of *Borrelia* in different niches and multifunctionality of proteins are the major strategies of *Borrelia* to avoid the destructive effect of immune system. In this review we provide deep insight into the protein:protein interactions that take place between different stages of life of *Borrelia*. Precise knowledge of surface proteins may help in improvement of the vaccines as well as for therapeutic agents against borreliosis.

Keywords: Borrelia, immune evasion, lyme disease, protein, protein interactions, surface proteins, ticks.

1. INTRODUCTION

Lyme disease (LD) is the most common tick-borne disease in the US and Europe [1-3]. It is a multistage multisystemic illness initiated upon infection with the spirochete *Borrelia burgdorferi* sensu lato (*B.b.*s.l.). *B.b.*s.l. complex is comprised of five genospecies with potential to infect human: *B.b.* sensu stricto (the only human pathogenic species presented in the US), *B. afzelii*, *B. garinii*, *B. spielmanii*, *B. bavariensis* [4, 5]. LD is associated with persistent infection, which can lead to chronic disease unless treated with antibiotics. Many of the mechanisms that lead to development of multisystemic LD, borrelial persistent and long-term infection are still not understood fully. After invasion of the skin, *Borrelia* can be heamatogenously disseminated to numerous host organs and results in early-disseminated disease characterized with development of dermatologic, cardiac, neurologic, and rheumatologic symptoms. Late-stage LB can be manifested as arthritis and/or neurological impairment [1]. The host-pathogen interaction of Borrelia is summarized in (Fig. **1**).

During transmission and dissemination in host, *Borrelia* has to overcome many defense mechanisms of host immune system. Borreliae are uniquely adapted to face immune system and thus survive efficiently in several host tissues. Immune evasion strategies include antigenic variations (gene conversion, mutation, recombination and variable expression of antigens), physical seclusion (formation of cysts and/or hiding in immunologically privileged sites) and active immune suppression. The active immune suppression includes complement inhibition, induction of anti-inflammatory cytokines, tolerance of monocytes and lymphocytes and sequestration of antibodies in immune complexes. Precise molecular basis of these mechanisms remains still an unresolved issue.

Compared to typical Gram-negative bacteria, borreliae lack highly immunogenic surface glycolipid LPS [6], however, they possess membrane-associated polypeptides, that act as pro-inflammatory agonists within the host [7]. The ability of *Borrelia* to adapt in different host environments is associated with an ability to express repertoire of outer surface proteins "Osps" and polypeptides. Diversity of Osps is further enhanced by mechanisms of antigenic variation (gene expression switching) (for example in *B.b.* switching of OspC, Bbf01 and Vmp-like sequence-expressed) [8, 9], plasmid recombination events mediating antigenic diversity (e.g., the erp and mlp genes, ospC gene) [10-12], and acquisition of new genetic information via transduction of a population of phage-like plasmids (the cp32 plasmid family) [13].

Antigenic variation is the first strategy of borreliae employed in the host immune evasion. Expression of genes coding surface proteins may be triggered globally by a variety of factors, including the surge from ambient to warm-blooded host body temperature, a decrease in blood meal pH, tick factors secreted, CO_2 tension, spirochete density after proliferation within the tick midgut and other factors related to the influx of host blood during tick feeding. A hypothetical model distinguishes the reciprocal two states and classifies genes coding surface proteins as "group I" (genes up-regulated in response to a temperature increase and pH decrease), with the remaining genes classified as "group II". This model allows predicting the regulation of other *Borrelia* genes that may be involved in spirochaete transmission, virulence or mammalian host immune responses.

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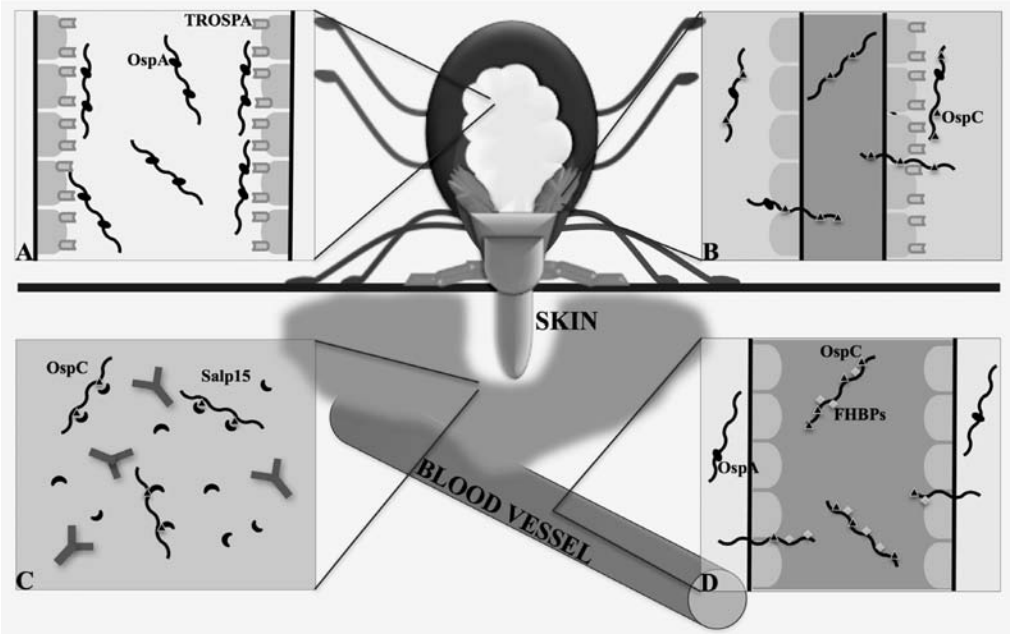

Fig. (1). Borreliae are primarily located in tick midgut where they are attached to gut endothelium via OspA and tick receptor TROSPA (Panel **A**). As soon as blood feeding begins, borreliae translocate the gut endothelium and disseminate through hemolymph to the tick salivary glands and saliva. The borrelial lipoprotein BBE31 interacts with tick receptor TRE31 in gut which is required for spirochete translocation into the hemolymph. During the blood meal *Borrelia* up-regulates expression of OspC, which strongly binds to tick salivary gland tissue (Panel **B**) from where spirochetes are inoculated into bite site on skin. Tick salivary protein Salp15 binds to OspC and protects spirochetes from antibody-mediated killing in the early stage of infection in skin (Panel **C**). After a couple of days of multiplication at the tick bite site, borreliae penetrate the wall of small arteries through interaction with ECM components and cell surface (BBK32 and host ligands Fn and GAGs, BBB07 interacting with RGD-independent integrins, OspA and host receptor CD40). To degrade ECM and disrupt intercellular junctions, borreliae may exploit host proteolytic enzymes (like plasminogen, MMP9). Borreliae disseminate hematogenously from the skin to the surrounding connective tissue in remote sites throughout host body. In the bloodstream it upregulates CRP binding proteins like factor H to evade complement mediated killing. Simultaneously it downregulates antigenic determinant OspA (Panel **D**).

Multiple functions of surface exposed proteins are not unusual among borreliae (examples of multifunctional proteins are P66, BBK32 and OspA). Borrelial surface proteins seem to play an important role in host:pathogen interactions and are essential for bacterial survival in multiple hosts (Table **1**).

A knowledge base of borrelial surface lipoproteins is therefore crucial for understanding of pathogenesis, development of effective vaccines and diagnostic tests. An insight into the role of lipoproteins and associated surface molecules of *Borrelia* in the pathogen life cycle, disease development and pathogenesis is presented here.

2. MAJOR SURFACE PROTEINS OF *BORRELIA* IN THE TICK VECTOR

Ticks acquire borreliae mostly from infected reservoir host (mammals, some birds and lizard species) during a blood meal. Spirochetes can be detected in the tick midgut within 24 hours of attachment to the rodent host [14]. Borreliae may also be found within flat or unfed nymphs [15]. During the first 24 hours of tick blood-feeding (low blood influx) number of residing borreliae in the midgut remains unchanged [15]. Blood influx (elevated temperature, reduced pH and increased borrelial cell density) dramatically alters borrelial transcriptome and surface proteome. The new environment activates Rrp2–RpoN–RpoS pathway that induce the transcription of more than 10% of all *Borrelia* genes [16]. When a tick attaches and engorges onto the host, spirochetes multiply to reach densities of 100,000 bacteria per tick. Nymphal feeding also induces the expression of genes coding outer-membrane-spanning protein p66 [17], and complement regulator-acquiring surface proteins (CRASPs), to avoid killing at the beginning of blood feeding.

Borrelial migration within the tick is a complex process beginning with blood feeding and replication of non-motile spirochetes attached onto the surface of epithelial cells. Spirochetal protein BBO250 is essential for proper cell division and cell envelope integrity [18]. While, borrelial outer surface protein (OspA), abundantly expressed in the tick midgut, mediates attachment to endothelium via interaction with tick receptor for OspA (TROSPA) and thus facilitates tick gut colonization. OspA might bind also itself, which could further facilitate OspA-based adherence in tick and might facilitate spirochete-spirochete aggregation *in vitro* and in the tick gut [19]. In addition to OspA, the surface lipoprotein BptA74 [20] and BB690 (Dps-like bacterioferritin orthologue 27) [21], are essential for prolonged residence of *Borrelia* in unfed tick. Under favorable conditions (in feeding ticks), non-motile borreliae transit into motile spirochetes, which translocate the basement membrane and enter the hemocoel [15]. The tick gut-to-salivary gland migration process involves diverse interactions between *Borrelia* outer-surface proteins and tick ligands. Lipoprotein BBE31 was

Table 1. Borrelial surface proteins (ligands), their receptors, site of expression and function.

Ligand	Alternative name	Receptor	Expression in	Process/Function	Reference
P66	Oms66, BBO603	αIIβ3, αvβ3	Host	Channel-forming porin	[55]
		Integrins		Adhesion to host cells	[56]
				Activation of host cells	[56]
BBK32		Fn	Vector/ host	Adhesion to ECM	[58]
OspA	BBA15	TROSPA	Vector and host	Adhesion to tick gut	[147]
		OspA		Spirochete-spirochete aggregation	[19]
		PLG/PL		Degradation of ECM	[73]
		CD40		Adhesion and activation of host cells	[64]
OspB		NA	Vector	Adhesion to tick gut	[148]
OspC		NA	Vector/Host	Adhesion to tick salivary glands	[26]
		Salp15		Establishment of early host infection	[25]
		PLG		ECM degradation	[74]
BB0690		NA	Vector	Persistence in tick during starvation	[21]
BptA	BBE16	NA	Vector	Persistence in tick	[20]
BBE31		TRE31	Vector/Host	Tick gut/salivary glands migration	[22]
BBA52		NA	Vector/host	Tick gut/salivary glands migration	[23]
BBE22	PncA	NA	Host	Nicotinamidase activity, Adaptation in host	[149]
BBA64	P35	NA	Vector/Host	Tick gut/salivary glands migration	[28]
				Persistent host infection	[27]
BBA65		NA	Vector/Host	Tick gut/salivary glands migration	[28]
BBA66		NA	Vector/Host	Tick gut/salivary glands migration	[28]
BBA73		NA	Host	Persistent host infection	[150]
BBA03		NA	Vector/Host	Tick/host transmission	[151]
BBO250		NA	Vector and host	Proper cell division and envelope integrity	[18]
BBA07		NA	Vector/Host	Tick/host transmission	[16]
FliG1		NA	Vector and host	Motility in highly viscous media	[152]
BBA74	Oms28	NA	Vector/Host	Tick environment adaptation	[153, 154]
CRASP-1	BBA68	FH	Host	Complement system evasion	[155]
		FHL-1		Complement system evasion	[156]
		PLG		ECM degradation	[77]
		BMP-2		Adhesion to host cells	[77]
		Col I, III, IV		Adhesion to ECM	[77]
		Fn		Adhesion to ECM	[27]
		Laminin		Adhesion to ECM	[77]
CRASP-2	BBH06	FH	Host	Complement system evasion	[96]
		FHL-1		Complement system evasion	[75]

(Table 1) contd….

Ligand	Alternative name	Receptor	Expression in	Process/Function	Reference
CRASP-3	ErpP	FH	Host	Complement system evasion	[157]
		FHR-1,2		Complement system evasion	[156, 158]
		PLG		ECM degradation	[75]
CRASP-4	ErpC	FH	Host	Complement system evasion	[157]
		FHR-1,2,5		Complement system evasion	[156]
		PLG		ECM degradation	[75]
CRASP-5	ErpA	FHR-1,2	Host	Complement system evasion	[156, 158]
		PLG		ECM degradation	[75]
		FH	Host	Complement system evasion	[157]
		PLG		ECM degradation	[75]
Bgp	BBO588	GAG	Host	Adhesion to ECM	[50]
		Heparin		Adhesion to ECM	[159]
DbpA	BBA24	Decorin	Host	Adhesion to ECM	[52]
DbpB	BBA25	Decorin	Host	Adhesion to ECM	[52]
RevA		Fn	Host	Adhesion to ECM	[59]
		Laminin	Host	Adhesion to ECM	[59]
RevB		Fn	Host	Adhesion to ECM	[59]
ErpX		Laminin	Host	Adhesion to ECM	[62]
BmpA		Laminin	Host	Adhesion to ECM, purine transport	[61]
BmpB		Laminin	Host	Adhesion to ECM	[61]
BmpC		Laminin	Host	Adhesion to ECM	[61]
BmpD		Laminin	Host	Adhesion to ECM	[61]
BBB07		α3β1	Host	Stationary adhesion on endothelium	[89]
		Integrins	Host	Stationary adhesion on endothelium	

ECM – extracellular matrix, FH – factor H, FHL – factor H like protein, FHR – factor H related protein, Fn – fibronectin, GAG – glycosamino glycan, NA – data not available, PL – plasmin, PLG – plasminogen

found to interact with tick receptor TRE31 in the gut which is required for spirochete translocation into the hemolymph [22]. The spirochete surface protein BBA52 also facilitate migration of *Borrelia* to salivary glands [23].

OspC, in contrast to above described abundant proteins, is not present in significant concentration in the gut of an unfed tick [14]. However, when a tick starts to feed on a mammalian host, *Borrelia* upregulates the expression of OspC and BBA64 [24]. The OspC strongly binds to tick salivary gland tissue, suggesting its role in gland invasion, which is a critical step in vector-host transmission cycle [25]. Tick salivary protein Salp15 binds to OspC and protects spirochetes from antibody-mediated killing in the early stage of infection [26]. Spirochetes generally continue with OspC expression in the host. This protein, therefore, has been proposed to have function not only in transmission from the vector but also in early mammalian infection. It was demonstrated that BBA64 is also necessary for mammalian infection via tick transmission [27, 28].

3. PROTEINS INVOLVED IN SURVIVAL STRATEGIES OF *BORRELIA* WITHIN THE HOST

3.1. Proteins of *Borrelia* Involved in its Survival in the Skin and Early Stage of Infection

To cope with different environments, within tick and host, *Borrelia* expresses different proteins in different life stages. Once *Borrelia* enters the host body, a whole set of new surface proteins may be needed to adapt to the new niche, evade the host immune system, and disseminate from the site of deposition (skin) to other sites, including the joint, heart, central and peripheral nervous system. Outer surface proteins A and B (OspA/OspB) are abundantly expressed on borrelial surface within an unfed tick. During the feeding process OspA/B is downregulated and disappears from borrelial surface and *Borrelia* instead express OspC. *Borrelia* then continues OspC expression in the host and not OspA/B. This is reflected in the absence of anti-OspA/B antibodies during early infection. OspA is indeed highly immunogenic,

potent stimulator of neutrophils [29] and stimulates production of cytokines. However, during persistent infection OspA antibodies are detectable in serum of some patients, which indicates that OspA plays a significant role in the establishment of chronic infection [30]. Several factors in tick saliva also facilitate borrelial survival and evasion of various immune responses. Tick proteins have anti-hemostatic, anti-inflammatory and immunomodulatory effect on innate and adaptive immunity. Salivary proteins ISL929 and ISL1373 downregulate polymorphonuclear PMN integrin expression (CD18) and inhibit production of O_2^- at the site of tick bite [31]. *Ixodes scapularis* salivary protein 20 (Salp20) and ISAC protein inhibit the alternative complement pathway [32]. The OspC:Salp15 dyad inhibits OspC:TLR2 interaction and thus protects *Borrelia* from antibody-mediated killing. Tick salivary proteins inhibit production of defensin and chemokines, which act as chemotactic molecules active on immune cells. This local inhibition of cellular infiltrate could allow *Borrelia* to multiply locally before dissemination to the rest of the body (including joints, heart and central nervous system) [26, 33]. Tick saliva affects not only the proliferation but also on distribution of borreliae in the host [34]. Inhibitory effect of tick proteins provides spirochaetes with a competitive advantage during the first stages of the host infection process.

Skin is the first target for the spirochetes in the early stage (erythema migrans, EM) of Lyme borreliosis, as well as in the course of borrelial lymphocytoma and acrodermatitis chronica atrophicans (ACA). The innate immune system, constituting the first line of defense, recognizes *Borrelia* lipoproteins through pattern-recognition receptors (PRRs) like Toll-like receptors (TLRs) mainly TLR2 [35] and NOD-like receptors on surface of keratinocytes, Langerhans cells and dermal fibroblasts [36]. The receptor TLR2 forms a heterodimer with TLR1 to recognize triacylated lipopeptides of borrelial lipoproteins, which leads to the induction of different inflammatory chemokines and cytokines [37]. Interaction of *Borrelia* with dermal fibroblasts induces the proinflammatory chemokine IL-8, along with the antimicrobial peptides defensin and cathelicidin [33]. Induction of IL-8 in DCs is in similar manner to induction by LPS and does not require engagement of OspA and OspB molecules [38].

EM is characterized by a lack of neutrophils in the skin lesion. It can be associated to the fact that borreliae can bind on its surface host proteins that protect spirochetes against host immune response and borreliae stay undetected by the immune system in the skin. [39]. In the skin, spirochetal lipopeptides elicit cellular infiltrates comprising neutrophils, activated macrophages, monocytoid and plasmacytoid dermal denritic cells (DCs), memory and memory/effector T cells [40] and induce expression of CCR5 by monocytes, macrophages and DCs [41, 42]. The EM skin lesions show higher level of neutrophil chemoattractant CXCL1, the macrophage chemoattractants, CCL2, CCL3, and CCL4, and the T-cell chemoattractants, CXCL9 and CXCL10 in comparison to normal skin [43].

DCs uptake borreliae by manner of coiling phagocytosis and phagocytised spirochetes are processed into small fragments inside DCs [38]. Live borreliae can induce maturation of DCs, which allows the cells to develop their Ag-presenting potential and thus induce immune responses in naive T cells. Presence of OspA or OspB is not a prerequisite for *Borrelia*-induced maturation of DCs. TLR2 and borrelial molecules, which include other surface lipoproteins and flagellin, can play a role in the *Borrelia*-induced DCs maturation process (Hertz *et al.*, 2001) [38]. *Borrelia* is also able to elicit production of pro-inflammatory cytokines TNF-α and IL-12 in host cells. TNF-α has an eminent role in the early eradication of *Borrelia* [44]. Whether or not, TNF-α production in DCs is unique for borreliae is still not clear [44]. Surprisingly, the same level of TNF-α production was found in patients with chronic neuroborreliosis and in asymptomatic patients. This finding supports the theory, that chronic patients may have a defect at another level of the immune response, which leads to chronicity [44]. Spirochetal lipoproteins also induce expression of chemokine receptor CCR5 on surface of monocytes/macrophages and DCs in the skin.

Borrelia and its lipoprotein OspA can induce an upregulation of suppressors of cytokine signalling SOCS1 and SOCS3 expression in macrophages and thus prevent the expression of pro-inflammatory cytokines (IL-6, IL-12p40, IL-1β, IL-18, and TNF-α) and regulate TLR signaling in immune cells. SOCS mediated macrophage deactivation (enhanced production of IL-10 and the lessened production of proinflammatory cytokines) during the early immune response might explain diminished inflammation and significantly reduce the onset and progress of antigen mediated arthritis in mouse model of Lyme disease (suppression of synovial fibroblast proliferation and IL-6 production) [45].

Borreliae were found to specifically bind stress hormones epinephrine and norepinephrine. Catecholamines are induced by the combined mechanical and biochemical stimulation of skin tissue during tick feeding and production of these hormones upregulates expression of OspA. Recognition of catecholamines and further OspA upregulation prepares borreliae for re-entry into a tick from a mammalian host [46].

3.2. *Borrelia* in Extracellular Matrix (ECM)

After a couple of days (~ 2 days) of multiplication at the site of tick bite, borreliae penetrate the walls of small arteries, disseminate hematogenously and gain entrance to the surrounding connective tissue in remote sites throughout the body, where they interact with components of the extracellular matrix (ECM) [47, 48]. At the same time, some spirochetes repopulate the infection site by 4-7 days post-infection [49]. Colonization of host tissues by *Borrelia* is a key step in the pathogenesis of Lyme borreliosis. Borreliae adhere to cell surface and ECM, however the mechanisms by which borreliae penetrate ECM and evade distant tissues is still poorly understood. Borreliae possess several surface molecules that mediate adhesion to various ECM components such as type I collagen, fibronectin and decorin. One ECM/cell binding pathway is mediated by glycosaminoglycans (GAGs), present on the mammalian cell surface and in the ECM. GAG-binding protein (Bgp, BB0588) expressed on the borrelial surface promotes the establishment of an initial infection in host tissues by inactivating toxic metabolites during borrelial growth in host. Bgp also binds heparin, agglutinates erythrocytes and can be secreted into the medium. The GAG-binding property of Bgp is shared with decorin binding proteins, thus constant expression of Bgp is

not required throughout the infection [50]. Decorin binding protein A (DbpA; BBA24) and B (DbpB; BBA25) bind a collagen associated glycoprotein, decorin. Decorin binding has an important role in mammalian infection, dissemination and is associated with arthritis and acrodermatitis chronica atrophicans evolvement [51]. Thus DbpA and DbpB each play central but distinct roles in cell-specific binding by spirochetes [52].

A previous study has reported that *Borrelia* binds type I collagen (a major protein of connective tissue of ECM) only in the presence of associated molecule 'decorin' [53], which leads to the formation of native protein fibers facilitating direct attachment, invasion and finally formation of microcolonies of *Borrelia* in ECM [54]. *Borrelia* can also adhere to surface of host cells through P66 protein (BB0603, Oms66), which interacts with αIIβ3 and αvβ3 integrins. P66 was also identified as a channel-forming porin [55], which activates downstream signaling pathways in several cell types [56] and is used as a diagnostic antigen in serological testing [57]. The P66 surface localization, channel-forming activity, integrin-binding and cell induction ability indicate that P66 is an important borrelial virulence factor.

A BBK32 protein, fibronectin-binding protein and BBK50 proteins are also differentially expressed surface lipoproteins of *Borrelia* (during tick feeding and mammalian infection) and their expression is controlled by the transcription regulating network RpoN-RpoS [58]. The BBK32 protein is highly immunogenic and level of anti-BBK32 IgG response correlates with less sever disease. Antibodies to BBK32 can partially protect mice from *Borrelia* infection and affect borrelial survival within tick, therefore anti-BBK32 may serve as vaccine destroying borreliae at several time points [58]. Borrelial fibronectin-binding protein, unrelated to BBK32, was further identified as outer surface protein RevA/RevB. Expression of BBK32 and RevA are differentially regulated, which suggests that these proteins are expressed differentially in various host niches or at different stages of Lyme disease. The RevA also binds mammalian laminin, however with a significantly lesser extent than fibronectin [59]. Antibodies against RevA are typical for initial stages of Lyme disease, which makes RevA another useful target for preventative or curative therapies [60]. Apart from RevA, surface exposed ErpX (the first identified borrelial laminin-binding protein), BmpA and its three paralogs, BmpB, BmpC and BmpD, may also serve as laminin-binding ligands [61, 62]. Moreover, BmpA and BmpB were found to play important role in course of Lyme arthritis [63].

Ability of borrelial proteins to bind multiple host receptors/adhesins is not unusual. For example, borrelial BBK32 binds host GAGs and fibronectin, whereas ErpACP/OspE/CRASP3-5 proteins can simultaneously bind both human factor H and plasminogen. Not all ECM-binding proteins are expressed at the same level, thus certain synergy is required in the expression of adhesion molecules to colonize host tissues [63]. Borreliae often compensate lack of one adhesin by increasing expression of another adhesin. Further, it is noteworthy that OspA can bind CD molecule (CD40) [64], mimic LFA-1 [65] and exploits host's proteolytic system by binding plasminogen [66].

Several bacteria express their own proteases or bind and use the host proteolytic system that digests ECMs in order to invade peripheral tissues. *Borrelia* is a successful extracellular pathogen that exploits host proteins for its own advantage. To degrade ECM and disrupt intercellular junctions, *Borrelia* exploits host's proteolytic enzymes (like plasminogen, MMP9) [67-70]. *Borrelia* is not known to produce toxins and proteolytic enzymes [71, 72]. However, borreliae possess on their cell surface both high and low-affinity plasminogen-binding proteins (Erps, Osps and enolases) that might be exploited at different life cycle stages and can enhance plasminogen/plasmin binding [66]. *Borrelia* is able to bind both, human plasminogen and plasmin via various binding structures like OspA [73], OspC [74], ErpA/C/P [75], 70 kDa protein (PBPB) [76] and CRASP-1 [77]. Expression of OspA is down-regulated almost immediately after a tick blood meal [78], however, OspA expression *in vivo* can be significantly induced if the spirochetes are kept in an inflammatory environment [79]. Plasminogen bound on bacterial surface can be converted into plasmin by host activators and is stabilized and protected against inactivation by α_1- and α_2 antiplasmin [80]. Such protection of plasmin from physiological inhibitors allows spirochete to traverse tissue barriers and to propagate pathological processes within the invaded tissues. *Borrelia* with bound plasmin is able to degrade fibronectin, penetrate the endothelium, and activate matrix metalloprotease-9 (MMP-9) and MMP-1 [81, 82].

Borrelia induces the expression and secretion of the urokinase-type plasminogen activator (uPA) and expression of the uPA receptor (uPAR; CD87) in a variety of cell types, including monocytes [68, 83]. The uPAR synthesis can be induced through CD14 and TLR2 signaling, which establishes a new functional link between the plasminogen activation system (PAS) and the innate immune system [68]. PAS can directly digest components of the extracellular matrix [84] and activate other proteases, including matrix metalloproteinases (MMPs). *Borrelia* is capable of upregulating and activating the human inflammatory cell MMPs [82]. Mononuclear cells and neutrophils may express many different MMPs that are often involved in host tissue destruction in various inflammatory diseases. These molecules could be used to penetrate various host barriers by enhanced penetration across collagen I, laminin and collagen IV, which are important constituents of blood-brain barrier [82]. The pathway for *Borrelia* to elicit an increase in the release of MMP-9 by host cells could be either direct or indirect [85], i.e. either by acting directly on cells via receptors to increase production of MMPs or by evoking the release of other mediators, such as cytokines, which ultimately stimulates the release of MMPs [70]. The up-regulation of MMP-9 by *Borrelia* could involve a CD14 signalling pathway. *Borrelia* can induce MMP-9 (but not MMP-1) in monocytic cells mediated by TLR2 [85].

Although borrelial enolases are essentially glycolytic enzymes, they possess additional function as plasminogen receptors [66]. Interestingly, enolases lack a traditional signal peptide and thus the mechanism how these proteins become surface exposed is still unknown. Enolases can be both cytoplasmic and surface exposed [86]. The cell-surface localization is confirmed by the fact that specific antibodies were detected during animal and human infection [87].

The ECM is a protected niche for prolonged persistence of *Borrelia* in host despite strong humoral immune responses

to borrelial antigens. Therefore, interaction between *Borrelia* and ECM proteins (fibrous and non-fibrous proteins, proteoglycans, collagen and its associated molecules) seems to be essential for chronic infection [88]. It can be hypothesized, that borrelial residing in ECM can be influenced by borrelial surface proteome and its interaction with antibodies, complement and immune cells.

3.3. Borrelial Proteins and Modulation of Cell Signalling

Another strategy of *Borrelia* to establish persistent infection in the host is the alteration of the host cell response to the pathogen via ligand:receptor mediated signaling. Interplay between *Borrelia* and host is complex and employs several molecules from both sides. The most characterized interaction is the binding of borrelial ligands to host integrins. Integrins are divalent, heterodimeric transmembrane proteins involved in cell-to-cell contact, inflammatory responses, actin dynamics, and other processes. The final effect depends on the particular integrin heterodimer on the cell surface and interacting ligand. The P66:αIIβ3 or αvβ3 interaction activates endothelial and epithelial cells and upregulates multiple cell surface adhesion molecules, which increase availability of cell receptors for pathogen and decreases immune response in cells, which may play role in the establishment of persistent infection in immunocompetent host. Alterations in the actin cytoskeleton may have a double impact during infectious process. Cytoskeleton rearrangement may allow increased space for penetration of *Borrelia* through cell junctions of various barrier and alter the migration of immune cells to sites of infection [56]. Moreover, interactions between integrins and borrelial surface proteins lead to vague inflammatory cell response. For example, αIIIβ1 integrin interaction with BBB07 and P66 cause release of inflammatory mediators with massive difference in their intensity and kinetics [89], which may lead to the immune response with chaotic behavior.

A series of experiments and reports have shown us that OspA mediate activation of CD40 on endothelial cells, which leads to production of pro-inflammatory cytokines [90], enhanced expression of adhesins ICAM-1, E-selectin, VCAM-1 with a consequent increase in cell binding [91, 92] and altered expression of MMPs [64]. Such orchestral signaling events also take place during the translocation of leukocytes across endothelial barrier. Thus it is tempting to speculate that *Borrelia* evoke and mimic orchestral signaling events to cross various cell barriers.

3.4. *Borrelia* in the Bloodstream (*Borrelia and Complement Proteins*)

Borrelia penetrate cell barriers, leave the ECM and gain entry to the bloodstream, where the innate and adaptive immune systems pose a major threat for circulating borreliae. Complement system is the first challenge that spirochetes have to face in bloodstream. Antibody independent alternative pathway of complement activation is an important component of innate immunity, which outlines phagocytosis and evokes cell membrane damage in pathogens. To avoid complement-mediated destruction, host cells and many pathogens express on thier surfaces complement control proteins or ligands that can bind complement regulatory factors like factor H, FHL-1 protein, C4bp etc. The most effective mechanism that may contribute to immune evasion is the binding of the complement regulatory protein factor H on the cell surface. This protein factor is a complement regulatory subunit that serves as a cofactor for the factor I–mediated cleavage of C3b. By increasing the local concentration of factor H at the cell surface, binding bacteria can promote more efficiently the degradation of C3b and thereby decrease the efficiency of complement-mediated killing [93-96]. In the last decade extensive work has been done on the binding of human and animal factor H by *Borrelia*. *Borrelia* produce an array of distinct surface exposed factor H binding proteins (FHBPs). Among them FHBPs are OspE, P21, ErpA, ErpC, ErpP, ~19 kDa hypothetical protein, FhbA (only in *B. hermsii*) and various complement regulator-acquiring surface proteins (CRASPs) [97-103]. Hitherto, CRASPs were identified from different *Borrelia* species, for example: BbCRASP-1 to BbCRASP-5 (*B.b.* sensu stricto CRASP), BaCRASP- 1 to BaCRASP-5 (*B. afzelii* CRASP) and *B. garinii* – BgCRASP-1 (*B. garinii* CRASP). It is interesting to note that numerous FHBPs are expressed by *Borrelia*, albeit very few possess strong factor H binding ability. We [97] and others [100, 104-107] have shown apparently stronger binding affinity of human factor H to BbCRASP-1, BaCRASP-1, BgCRASP-1, FhbA and novel Bg19 kDa proteins than to other FHBPs. In addition to the factor H binding affinity, an expression of the given protein on borrelial surface in given niche is equally important aspect. A temporal analysis of FHBPs expression throughout the mammal–tick infection cycle indicates that these major FHBPs are expressed differentially in vector and host, however the shift in the expression level is rapid enough to protect the spirochete in quickly changing environments i.e. tick gut – tick saliva – skin – blood stream [108-110].

It is well known that members of the *B.b.s.l.* complex can infect a wide range of host species, however, certain host specificity exists for each *Borrelia* species. The host specificity matches with complement resistance of the given *Borrelia* species, while complement resistance depends on the binding affinity of FHBPs to factor H [93, 111]. Binding affinities of FHBPs of various *Borrelia* strains to human and animal factor H are not equal [97]. For example, cattle and horses are not suitable hosts for Lyme disease related *Borrelia*, which strongly correlates with the susceptibility of *Borrelia* to bovine and equine complement and inability of borrelial proteins to bind bovine and equine factor H [111]. On the other hand, binding of bovine factor H by *B. coriaceae* (mediated through ~40kDa & ~58kDa proteins) suggests that *B. coriaceae* can evade bovine complement system and establish an infection (well known bovine borreliosis in Africa). A 58 kDa *B. coriaceae* protein was identified as BESBP (borrelial extracellular solute binding protein) [97]. Another example of entangled relationship between factor H binding, complement sensitivity and host specificity is *Borrelia*-rodent model. Surface proteins of *B. bissettii*, *B. afzelii* and *B. japonica* readily bind murine factor H, which correlate with the presence of these *Borrelia* species in rodents [112, 113]. It is noteworthy that expression of FHBPs by given species of *Borrelia* differ from host to host depending on the chemical signalling [110]. In a nutshell, host-dependent expression and binding affinity of FHBPs to factor H from various mammalian species may contribute to the differential host selectivity and pathogenicity of *Borrelia*

species. Interactions and binding affinities between FHBPs of different genospecies of *Borrelia* and factor H from various hosts are presented briefly in (Table **2**).

Factor H:FHBPs interactions are among the most thoroughly studied protein:protein interactions, while a plethora of the work has been performed to unfold molecular basis of factor H:FHBP interfaces [98, 100, 106, 114, 115]. It was shown that the factor H binding domain is conformational or discontinuous and dependent on the coiled-coil structures [116, 117]. Coiled-coil domains, as heptad repeats – ***abcdefg.n*** (*a* to *g* denote position of amino acid in heptad repeat, while *n* indicate residue/s out of repeat) , consist of two or more right handed α helices that form interface with factor H. On the other side, secreted form of human factor H is composed of 20 repetitive units known as short consensus repeats (SCRs) or sushi domains [118]. Structural assays showed a central role of sushi 19–20, which encodes heparin, sialic acid and C3b-binding domains, while sushi 7 serves as a binding site for heparin and c-reactive protein [119]. Microorganisms can bind factor H through various sushi domains, for example: *Streptococcus pyogenes* (M protein) and *Candida albicans* bind to sushi 7, *Streptococcus pneumoniae* (Hic/PspC proteins) interacts with sushi 8–15, and *B. burgdorferi* FHBPs exploit sushi 7 and sushi 16–20 [119-121].

Apart from factor H, *Borrelia* readily exploits complement regulatory factor H-like protein (FHL-1). Some Erp proteins of *Borrelia* do preferably bind FHL-1, while CRASPs-1 and -2 are able to bind both FHL-1 and factor H. Interestingly borrelial CRASP-1 is a multifunctional protein that also binds several human extracellular matrix proteins and plasminogen [77]. Rodents lack FHL-1 so ability to bind FHL-1 might have consequences for disease in human but not for disease in rodents [119]. Factor H related proteins (FHR proteins) form another complement regulator family. ErpC producing borreliae bind FHR-1, 2 and 5. Despite FHR binding, borreliae are susceptible to complement, which indicate subordinate role of FHR binding in borrelial serum resistance [122].

During prolonged infection, antibodies against borreliae can activate the classical (antibody dependent) pathway of complement. Lyme disease related borreliae can bind C4bp (C4-binding protein, one of the major complement regulators) from human serum with ~ 43kDa major ligand [123]. Bound C4bp downregulates complement activation, opsonization and phagocytosis [124], which allow *Borrelia* to persist in chronic stages even in the presence of specific antibodies [123]. Some *Borrelia* species also carry a specific receptor CihC for the serum-derived complement inhibitor, C1 esterase inhibitor, which strengthens the versatility of borreliae to evade classical pathway of complement activation [125].

Another complement evasion tool of *Borrelia* is mimickry of complement regulatory protein CD59. Borrelial CD59-like protein inhibits formation of membrane attack complex (MAC) and allows *Borrelia* to evade complement-mediated killing [126]. CD59-like protein was further identified as VlsE of *B.afzelii* and OspA of *B. parkeri* (our unpublished data). Furthermore, a series of experiments revealed that *B. afzelii* can mimic CD46 molecule and show resistance to the complement mediated lysis (own unpublished data).

3.5. Proteins Involved in the Translocation of *Borrelia* Across Vascular Endothelium

Pattern of borrelial crossing of the vascular endothelium (paracellular versus transcellular) remains controversial. Comstock *et al.* (1993) [127] demonstrated that *B. burgdorferi* translocate human umbilical vein endothelial cells (HUVECs) monolayer across the cytoplasm, albeit the majority of researchers support a transcellular route of crossing. Research team of Szczepanski has demonstrated the presence of *Borrelia* in the intercellular junctions of endothelial cells, as well as beneath the monolayers, as evidence that spirochetes actually pass between the cells [128]. Penetration of blood vessel wall is progressive multistage process including short-term dragging interactions, tethering (surface of endothelial cells) and stationary adhesion (intercellular junctions) on endothelium followed by extravasation. Interactions with endothelium are mediated through borrelial binding of several host molecules like fibronectin (Fn), GAG and integrins. Hitherto, 19 adhesive protein candidates of *Borrelia* have been identified [129]. Initiation of transient and dragging interactions is mediated through BBK32 and host ligands Fn (independently or via a fibronectin bridge) and GAGs, although their participation in stationary adhesion of *Borrelia* on endothelial cells cannot be ruled out [129]. Recent experiments show that OspA of *Borrelia* and CD40 on the brain-microvascular endothelial cells also take part in the transient tethering-type association mainly during crossing of spirochete across blood-brain barrier (BBB) [130]. The BBK32:Fn interactions aggregate soluble Fn into superfibronectin network, which act as a molecular anchor, Which allows the formation of more stable BBK32:GAG interactions (dragging) between circulating borreliae and vascular endothelium [131]. Stationary adhesion is mechanistically distinct and additional borrelial molecules may be exploited during this step [129]. Adhesion might also be mediated by borrelial protein BBB07 interacting with RGD-independent integrins such as α3β1 [89], which is expressed at the site of intercellular junctions.

3.6. Borrelial Proteins in Neuroborreliosis

Borrelial infection affects several organs and is followed by bacterial dissemination. Although borreliae elicit strong antibody response, spirochetes are able to evade the immune defenses, cross the blood-brain barrier and colonize the central nervous system (CNS) (neuroborreliosis). Borreliae possess several mechanisms of immune system evasion including sequestration into immune privileged sites such as CNS. Damage caused by association of *Borrelia* to cells in central nervous system could be the basis of neurological manifestations, however mechanisms by which *Borrelia* affects the nervous system are not yet known. Moreover there is a controversy about the localization of the spirochetes in CNS tissue. Viable *Borrelia* have been observed intracellularly in CNS, which provides a putative mean of host immune response evasion [132]. Mechanisms of cell penetration and the determinants for borrelial survival within cells are yet to be determined. It can be hypothesized that chronic neuroborreliosis (NB) may be due to a deregulation of the initial innate immune response, which in turn affects the following adaptive response. CNS infection results in the proliferation and apoptosis of astrocytes, which then leads to a

Table 2. Major FHBPs expressed by various *Borrelia* genospecies and their factor H binding affinities in various animals and human [97].

Borrelia species (strain)	OspA Serotype	MW	Estimated pI[a]	Human and animal factor H binding affinity							
				Human	Mouse	Rat	Guinea pig	Cattle	Horse	Dog	Cat
B. burgdorferi s.s. (SKT2)	1	~26 kDa[c]	8.0-8.1	+++[b]	+++	-	-	-	-	-	-
B. afzelii (SKT4)	2	~15 kDa	4.0-5.2	-	+++	-	-	-	-	+++	++
		~26 kDa	6.8-7.1	+++	++	-	-	-	-	-	-
B. bavariensis (PBi)	4	~19 kDa	6.0-7.0	+++	-	-	-	-	-	-	-
		~28 kDa	5.0-5.3	-	+++	-	-	-	-	-	-
B. garinii (G117)	5	~26 kDa	5.6-6.2	-	++	-	-	-	-	-	-
B. garinii (T25)	7	~17 kDa	5.0-5.5	++	-	-	-	-	-	-	-
B. valaisiana (VS116)	NA[c]	~17 kDa	4.2-5.0	+++	-	-	-	-	-	+++	-
B. andersonii (21123)	NA	~15 kDa	5.5-6.1	+	-	-	-	-	-	-	-
		~17 kDa	8.0-8.8	+	-	-	-	-	-	+	-
		~23 kDa	6.1-6.6	+	++	-	-	-	-	-	-
		~26 kDa	8.0-8.3	-	++	-	-	-	-	-	-
B. bissettiii (DN127)	NA	~25 kDa	8.0-8.5	+	-	-	-	-	-	-	-
		~28 kDa	5.0-5.5	-	+++	-	-	-	-	-	-
		~40 kDa	6.9-7.8	-	+	-	-	-	-	-	-
B. japonica (HO14)	NA	~15 kDa	4.8-5.2	-	+++	++	-	-	-	-	-
		~19 kDa	4.5-5.0	-	-	++	-	-	-	-	-
		~22 kDa	6.0-6.8	+	-	-	-	-	-	++	++
		~24 kDa	4.5-4.9	-	-	++	-	-	-	-	-
		~26 kDa	7.9-8.3	-	+++	-	-	-	-	-	-
B. hermsii (HS1)	NA	~20 kDa	8.0-8.3	+++	+++	+++	+++	-	-	-	-
B. parkeri (M3001)	NA	~23 kDa	8.0-8.5	++	-	-	-	-	-	-	-
B. coriaceae (Co53)	NA	~40 kDa ~58 kDa	7.0-8.0 5.5-5.7	- -	+++ +++	- ++	- -	+++ +++	- -	- -	- -

[a] pI: isoelectric point observed on 2D gel; [b] fH binding strength. +++: high; ++: medium; +: weak; -: negative; [c] NA: not applicable. ~26 kDa proteins of *B. burgdorferi* s.s. and *B. afzelii* are well known BbCRASP-1 and BaCRASP-1 respectively, while ~20 kDa protein of *B. hermsii* is FhbA. Other proteins are not yet well characterized. MW – molecular weight

dysregulation of inflammatory cytokine production. [133, 134]. Stimulation of astrocytes by borrelial lipoprotein OspA increases production of glial fibrillary acidic protein (GFAP) and TNFα [133, 134]. Elevated level of GFAP in CSF is typical for astrogliosis associated with neurodegenerative diseases such as Alzheimer disease or prion disease. Lipoprotein-induced TNF-α appeared to be the sole determinant of astrocyte apoptosis. While lipoprotein-induced increased CSF level of IL-6 contributes to stimulating of astrocyte proliferation and correlate with disease activity in NB and other neurological disorders [134, 135].

Borreliae were shown to interact with several host cell surface molecules within the blood-brain barrier and CNS. Host cell surface molecules such as decorin, fibronectin, integrins and GAGs, and borrelial adhesive molecules like P66, BBK32, DbpA, DbpB and Bgp seems to be essential for successful CNS invasion. Decorin is expressed in the central nervous system (CNS) and cerebral endothelial cells. A strong affinity of neuroinvasive *Borrelia* to decorin suggests that this protein might have a central role in the colonization of the CNS. High affinity of both DbpA and DbpB to decorin under constant blood flow is essential for the adherence to cerebral endothelium cells and for the initiation of CNS colonization. It can be hypothesized that DbpA forms a "catch bond" with decorin and needs shear force to induce the binding, as has been shown for certain other bacterial adhesins [136].

Additionally, OspA was found to be upregulated in the unique environment of the cerebrospinal fluid (CSF), but not in the serum [137] and all neuronal cell-adherent borreliae were shown OspA positive and OspC negative [138]. These findings supports the theory, that OspA plays an important role in binding to neuronal cells and indicate that OspA must be upregulated during the CNS invasion and probably acts as an important adhesion factor which is essential in the pathogenesis of Lyme neuroborreliosis [137].

Traditional hypothesis is that borreliae have to penetrate BBB to cause the CNS infection. Recent studies confirm that pro-inflammatory bacterial product (Vsp1) released from abundant spirochetes in the peripheral sites may disseminate to brain and cause widespread cerebral inflammation, however pattern of Vsp1 dissemination into brain is still unknown [139].

3.7. Borrelial Lipoproteins as Molecular Mimicry

Variability of individual clinical manifestations of infection depends on the combination of host immune response and pathogen properties. This may give rise to infection-triggered autoimmunity-like phenomenon in case of LD. Molecular mimicry is one of autoimmunity mechanisms following infection due to the persistent release or presentation of immunogenic material in affected individuals and may have relevance to the arthritis and carditis in LD. Alternatively, arthritis may be due to the triggering of anti-self-reactivity from cross-reactive antibodies (Abs) which recognize both the pathogen and self-components.

The anti-OspA response may have both beneficial and potentially harmful effects. OspA has been shown to cause polyclonal activation of B cells [140], which may result in increased production of polyreactive IgM antibodies which cross-react with self-components in *B. burgdorferi*-infected host and lead to autoimmune tissue reactivity or impairment of function. *In silico* analysis revealed common motifs between *B. burgdorferi* and human alpha myosin heavy chain [141, 142].

Borrelia flagellar basal rod protein (FBRP) may be implicated in the pathogenesis of sporadic schizophrenia at conception [143, 144]. The FBRP shares an epitope with the human IL-1 receptor antagonist (IL-1ra). Such homology between *Borrelia* and its host potentially induces and misdirects anti-IL-1ra antibodies. The immune response against IL-1ra might therefore activate a cascade of pro-inflammatory cytokines and enhances inflammation. Conversely, dysbalance in favour of IL-1ra versus IL-1 reportedly mitigates this reaction in Lyme disease [145, 146].

CONCLUSION

Expression of wide range of surface proteins is crucial for *Borrelia* to survive and propagate in different niches. In a nutshell, unique features of borrelial lipoproteins can be characterized in four points: 1. Expression of borrelial proteins and its level can be altered to adapt to different environments and host niches, 2. Borrelial lipoproteins have a synergic effect and compensate each other in their function, 3. A single *Borrelia* protein may have multiple functions, 4. Many borrelial lipoproteins can successfully subvert signaling pathways in the host cells and bind host molecules (ECM components, complement, proteolytic enzymes) to evade the immune system. Deep insight into the borrelial protein´s function and vector/host interactions is necessary to understand basic principles of pathogenesis of Lyme disease. Indeed, it takes two to dance a tango: both host receptors and pathogen ligands. Continuous efforts are necessary to unfold the enigma of borrelial surface proteins. Only then we will understand quiet entrance and prolonged persistence of *Borrelia* in the host.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest

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