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BIOLOGICAL ASPECTS OF LYME DISEASE SPIROCHETES: UNIQUE BACTERIA OF THE *BORRELIA BURGDORFERI* SPECIES GROUP

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Background: *Borrelia burgdorferi* sensu lato is a group of at least twelve closely related species some of which are responsible for Lyme disease, the most frequent zoonosis in Europe and the USA. Many of the biological features of *Borrelia* are unique in prokaryotes and very interesting not only from the medical viewpoint but also from the view of molecular biology.

Methods: Relevant recent articles were searched using PubMed and Google search tools.

Results and Conclusion: This is a review of the biological, genetic and physiological features of the spirochete species group, *Borrelia burgdorferi* sensu lato. In spite of a lot of recent articles focused on *B. burgdorferi* sensu lato, many features of *Borrelia* biology remain obscure. It is one of the main reasons for persisting problems with prevention, diagnosis and therapy of Lyme disease. The aim of the review is to summarize ongoing current knowledge into a lucid and comprehensible form.

INTRODUCTION

Lyme disease is a chronic multi-system infectious disorder and the most frequent infectious arthropod-borne disease found both in Europe and the United States. The number of yearly reported cases continually increases in a number of geographical areas (Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report, 2004). This may be due to the actual spread of the disease or alternatively, to improved diagnostic methods. Its significance is shown by the frequency of publications in medical journals that focus on infectious conditions. From this point of view, Lyme's disease has assumed a position just behind acquired immunodeficiency syndrome (AIDS) in recent decades[1].

The disease is induced by spirochetes indicated totally as *Borrelia burgdorferi* sensu lato (*Borrelia burgdorferi* s. l.). This group of microorganisms comprises at least twelve species (Tab. 1). Descriptions of new species and variants arises continously, so the number of species is probably not final.

The disease has a vector character. Most of the time, it is transmitted to humans by infected ticks of the genus *Ixodes*, but *Borrelia* has also been found in the midgut of the mosquitoes, *Culex pipiens*[2, 3] and *Aedes vexans*[4]. The presence of *B. burgdorferi* s. s. was earlier described in the midgut of some species of mosquitoes and other blood sucking flies[5]. Hence transmission may be possible by other arthropods than ticks alone.

In about 70 % of patients, the clinical picture of the disease starts as a pruriginous erythema at the site of the tick bite. Over time the erythema spreads out of the initial focus. The acute phase of the disease presents a picture of non-specific flu-like symptoms. Untreated, the disease develops toward arthritis and/or dermal, neurological or cardiac complications. Involvement of the nervous system may manifest as diffuse or focal neuropathy, painful radiculopathy, or less frequently, meningitis or encephalomyelitis. Neurological complications are frequently associated with infections caused by *B. garinii* and *B. burgdorferi*[6]. Among dermatological manifestations, typical are erythema, mentioned above, and acrodermatitis chronica atrophicans, which is caused mostly by *B. afzelii*[7, 8]. Lymphocytoma and circumscribed scleroderma are less common. Ophthalmic disorders, most often intraocular inflammations, affected by *Borrelia* have been described as well[9–11]. A number of theories on the possible association between *Borrelia* infection and other poorly-understood syndromes have been published: sarcoidosis[12, 13], chronic fatigue syndrome[14] and neurodegenerative disorders, e. g. Alzheimer's disease[15, 16].

Borreliosis is not only a problem in human medicine. Clinical forms have been found in a number of species of domestic animals, e.g. dogs, horses, cats and cattle. Interesting is the lack of clinical symptoms in forest animals. This may be the result of the long-term co-evolution of *Borrelia* and its natural hosts, such as deer, hares and various rodents[17–19].

Basic microbiological characteristics

The genus *Borrelia*, Swellengrebel 1907 is a member of the family Spirochetaceae which contain three genera – *Borrelia*, *Leptospira* and *Treponema*. Three genospecies forming the species group *B. burgdorferi s. s.* commonly cause human disease. In the USA only *B. burgdorferi* s. s. cause the disease, in contrast to Europe where *B. affzeli* and *B. garinii* are the most common. Some other species are irregularly pathogenic (Tab. 1). These differences in *Borrelia* geographic distribution have not been explained to date. Some species of *Borrelia*, not belonging to *B. burgdorferi* s. l., are the cause of other human diseases, such as relapsing fever[33].

Borreliae are unicellular spiral microorganisms without a rigid cellular wall 10–30 μm in length, 0,2–0,3 μm in diameter (Fig. 1). About 7–14 flagella at each end of the bacterium are responsible for *Borrelia* mobility. They are localized in periplasmatic space (Fig. 2). The rarity is the *B. sinica* with only 2–4 flagella, which may be the reason for its immobility[30]. The flagella consist mainly of two types of flagellin proteins – minor FlaA (38 kDa) and major FlaB (41 kDa) (ref.[34]). The shape of *Borrelia* is formed by flagellae that are twisted around the protoplasmatic cylinder but, in contrast to other bacteria, the *Borrelia* cell wall does not have an active role in shape determination. Targeted inactivation of the FlaB gene induces loss of motility and the "rod-like" form of these mutants[35]. Comprehensive reviews of the motility and cytoskeleton of spirochetes were published by Charon and Goldstein in 2002 (ref.[36]) and Wolgemuth, Charon, Goldstein and Goldstein in 2006 (ref.[37]).

The composition of the cell envelope is similar to gram negative bacteria with some expressive differences, e. g. absence of lipopolysaccharide[38] and an abundance of lipoproteins in the outer cell membrane[39, 40]. As major lipid components of *Borrelia* membranes, identified are two phospholipids – phosphatidylcholine and phosphatidylglycerol and two atypical glycolipids – 1-O-palmitoyl-2-O-oleyl-3-O-α-D-galactopyranosyl-sn-glycerol and Cholesteryl 6-*O*-palmitoyl-β-D-galactopyranoside. Both glycolipids are able to elicit the production of specific antibodies in mouse models. It was assumed, that these antibodies can cross-react with gangliosides and thus can be involved in the pathogenesis of neuroborreliosis. This aside, the synthesis of glycolipid based vaccines has been published[41–43].

Cultivation of *Borrelia* requires unique formulation of growing media. Several modifications of Barbour-Stonner-Kely's medium (BSK) allow the cultivation of spirochetes in fluid medium at high densities[44]. BSK is very complex medium containing mainly amino acids, cofactors, salts, N-acetyl-D-glucosamine and a number of variable components such as bovine serum albumin, neopeptone, yeast extract and rabbit serum. Suppliers and a batch of a large number of the above ingredients can strongly affect growth rate, as well as the antigenic and morphologic characteristics of *Borreliae*[45–47]. Standardized medium, named BSK-H, is available commercially. Unique is the ability of *Borrelia* to grow in an iron ion-depleted environment, although iron level is limiting for a number of pathogenic bacteria. This feature of *Borrelia* is caused by elimination of most iron dependent matalloproteins and substituting Mn for Fe in the remaining metalloproteins[48].

For the cultivation of *Borrelia* on solid medium, the BSK solidified by agarose is commonly used in a one or two layer modification[49, 50]. The cultivation of *Borrelia* on solid medium enables the separation of individual colonies and thus selection of various genetic variants. Since some *Borrelia* strains do not grow in colonies on solid media, the limiting dilution method was developed for selection of *Borrelia* during cloning (D. S. Samuels, pers. comm.).

Another typical feature, which is not clearly understood until now is the formation of non-helical forms of *Borrelia*. Various cystic forms, blebs and L–forms have been repeatedly described during cultivation in medium

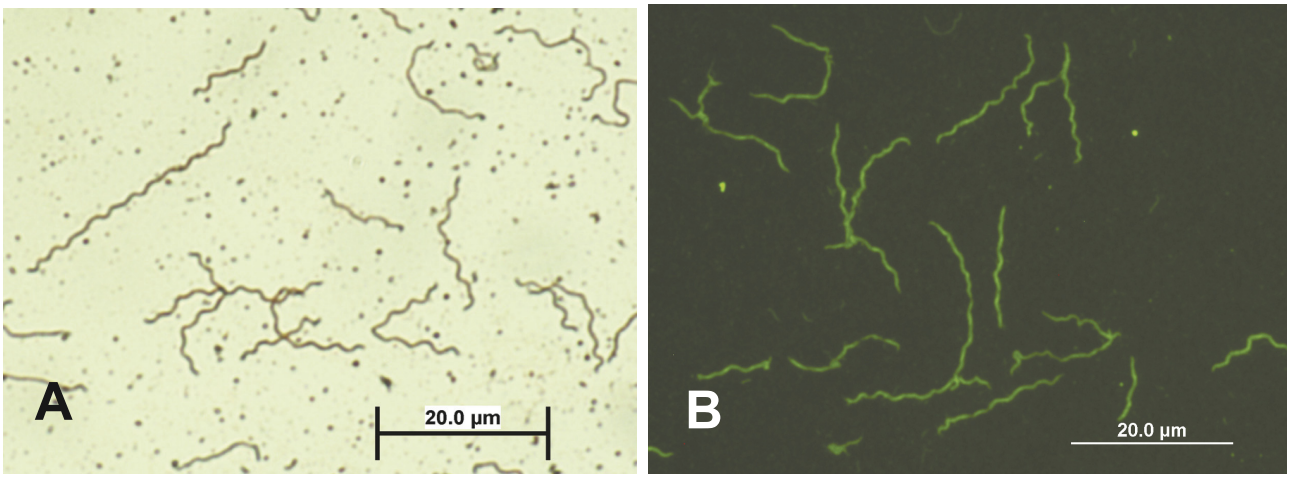

Fig. 1. Morphology of *Borrelia burgdorferi*:
A. Warthin-Starry silver nitrate staining.
B. Immunofluorescence – reaction with sera of needle-infected mouse. Magnification 1000x using immersion objective.

Table 1. Species of *B. burgdorferi* complex.

Species	Pathogenity for human	Geographical occurrence	Reference
B. burgdorferi sensu stricto (s. s.)	Yes	USA and Western Europe	Johnson et al.[20]
B. garinii	Yes	Europe and part of Asia	Baranton et al.[21]
B. afzelii	Yes	Europe and part of Asia	Canica et al.[22]
B. japonica	not confirmed	Japan	Marconi et al.[23]
B. andersonii	not confirmed	North America	Marconi et al.[23]
B. tanukii	not confirmed	Japan	Fukunaga et al.[24]
B. turdi	not confirmed	Japan	Fukunaga et al.[24]
B. valaisiana	described rarely [25]	Europe	Wang et al.[26]
B. lusitaniae	described rarely [27, 28]	Mediterranean and Middle Europe	Le Fleche et al. [29]
B. sinica	not confirmed	China	Masuzawa et al.[30]
B. bissettii	not confirmed	USA	Postic et al.[31]
B. spielmanii	not confirmed	Western Europe	Richter et al.[32]

Table 2. Replicons of *B. burgdorferi s. s.* strain B 31 MI [39, 63]

Replicon	Geometry	Size (bp)	G+C (%)	Coding (%)[a]
Chromosome	Linear	910 725	28,6	93
cp9	Circular	9 386	23,9	75
cp26	Circular	26 498	26,5	88
cp32-1	Circular	30 750	29,4	92
cp32-3	Circular	30 223	28,9	92
cp32-4	Circular	30 299	29,3	92
cp32-6	Circular	29 838	29,3	92
cp32-7	Circular	30 800	29,1	93
cp32-8	Circular	30 885	29,1	92
cp32-9	Circular	30 651	29,3	92
lp5	Linear	5 228	23,8	73
lp17	Linear	16 928	23,1	64
lp21	Linear	18 901	20,7	32
lp25	Linear	24 177	23,4	66
lp28-1	Linear	28 250	32,3	79
lp28-2	Linear	29 766	31,6	92
lp28-3	Linear	28 601	25	66
lp28-4	Linear	27 323	24,5	62
lp36	Linear	36 849	26,9	76
lp38	Linear	38 829	26,1	67
lp54	Linear	53 541	28,2	82
lp56	Linear	52 971	27,4	86
Plasmids total		610 694	27,6	81

[a] – Percent of plasmid occupied by genes and pseudogenes

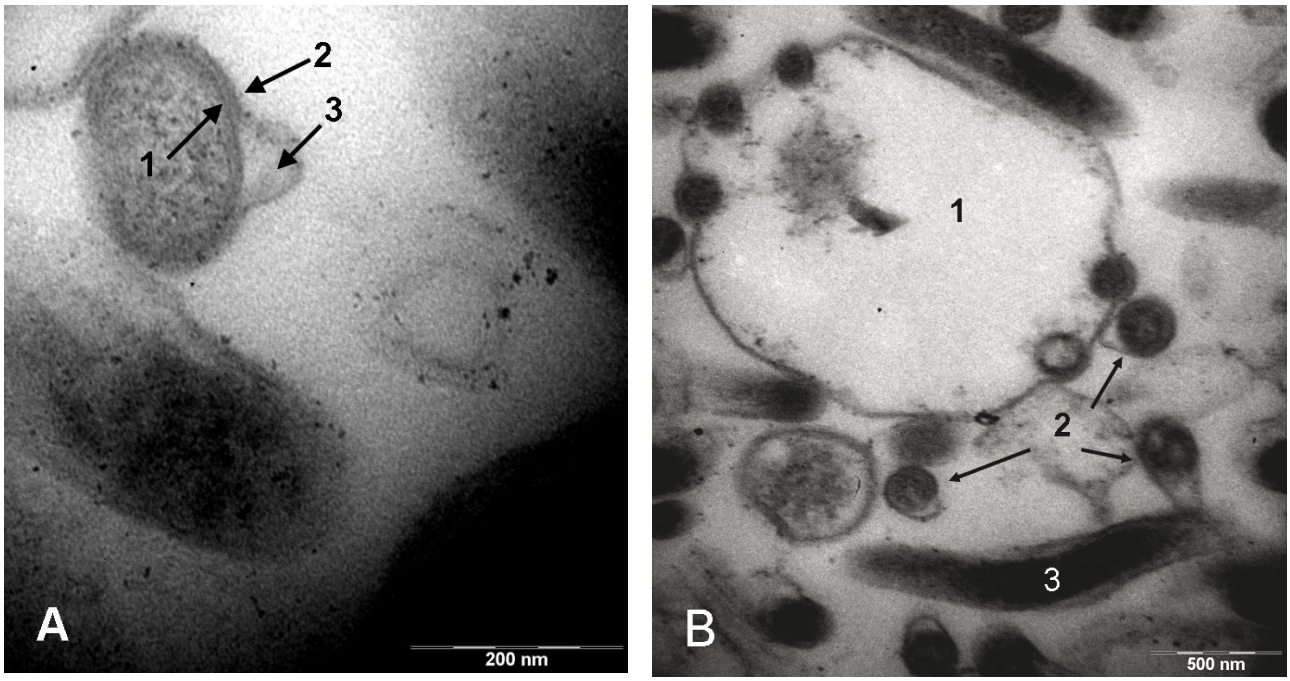

Fig. 2. *Borrelia burgdorferi* under Transmission Electron Microscope:
A. Transversal section, spiral form. 1 – inner membrane, 2 – outer membrane, 3 – periplasmatic space with flagella. B.
B. 1 – cystic form of *Borrelia,* 2 – transversal section, spiral form, 3 – longitudinal section, spiral form.

with non-optimal pH, absence of serum, presence of antibiotics and in aged cultures, or generally under stress conditions. It seems that at least some of these transformations are reversible. A number of authors propose that these alternations are significant in the pathogenesis, immune evasion and/or antibiotic resistance of at least *B. burgdorferi s. s.*[15, 16, 51–55].

Classic microbiological technologies are unsatisfactory for identifying *Borrelia* species and hence, molecular biology approaches (PCR, RFLP-PCR) prevail today. For taxonomy studies the DNA typing of *OspA*, *OspC*, 16S rRNA and 5S – 23S rRNA spacer are frequently used approaches.

Genome structure

The spirochetes of the genus *Borrelia* are unique bacteria. One characteristic feature of this microorganism is its unusual genome which consists of one linear chromosome with an approximate size of one mega base and several circular and linear plasmids [39, 56–63]. The *Borrelia* chromosome is relatively small compared to other bacterial chromosomes whose size varies from 580 to 9 300 kbp[64]. In 1997 the genetic map of *B. burgdorferi s. s.* B31 was published[39], and later completed by Casjens et al.[63].

The *B. burgdorferi* s. s. B31 MI chromosome was investigated by Casjens et al.[63] and shown to contain 842 functional genes and only 1 pseudogene. Chromosomal genes code proteins necessary for replication, transcription, translation, energy metabolism, and transport of nutrients and ions. Neither chromosome nor plasmids contain genes for cellular biosynthetic reactions. This is the reason for the limited metabolic capacity of *B. burgdorferi s. l.,* which lacks enzymes necessary for the biosynthesis of aminoacids, fatty acids, nucleotides and cofactors. This also explains the need for a complex medium containing all these components for the growth of the Lyme disease spirochetes. Of the other chromosomal genes, the most important are genes for DNA repair (*mutS*, *mutL*, *uvrA*, *uvrB*, *uvrC* and *uvrD*), genes for homologous recombination *(recA*, *recBCD*, *sbsC*, *sbsD*, *recG*, *ruvAB, recJ*), genes for heat shock response (*groES, groEL, grpE, dnaJ, hslU, hslV, dnaK, htpG*), and chemotactic factors-coding genes (*cheR, cheW, cheA, cheY, cheB*) (ref.[39]). The ends of the chromosome are formed by covalently closed hairpin telomeres. This feature is unusual for prokaryotes, but it has been found on DNA of double stranded DNA viruses and on some eukaryotic mitochondrial DNA molecules[65].

In addition to the chromosome, 21 extrachromosomal replicons have been described in B31 strain: 9 circular plasmids (cp) and 12 linear plasmids (lp) (Tab. 2). This is the maximum number of plasmids described in bacteria to date[63]. Apart from this, two other plasmids (cp32–2 and cp 32–5) have been described in other subcultures of B31, which presumably means that the total number of plasmids in the original isolate B31 was 23 (ref.[66]). In other strains of *B. burgdorferi* s. s., the number of cp32 plasmids varies between 6 (strain N40) and 10 (strain Sh-2–82) (ref.[67]). Other unusual phenomena are the presence of a large number of pseudogenes on plasmids and low degree of identity between plasmid gene sequences

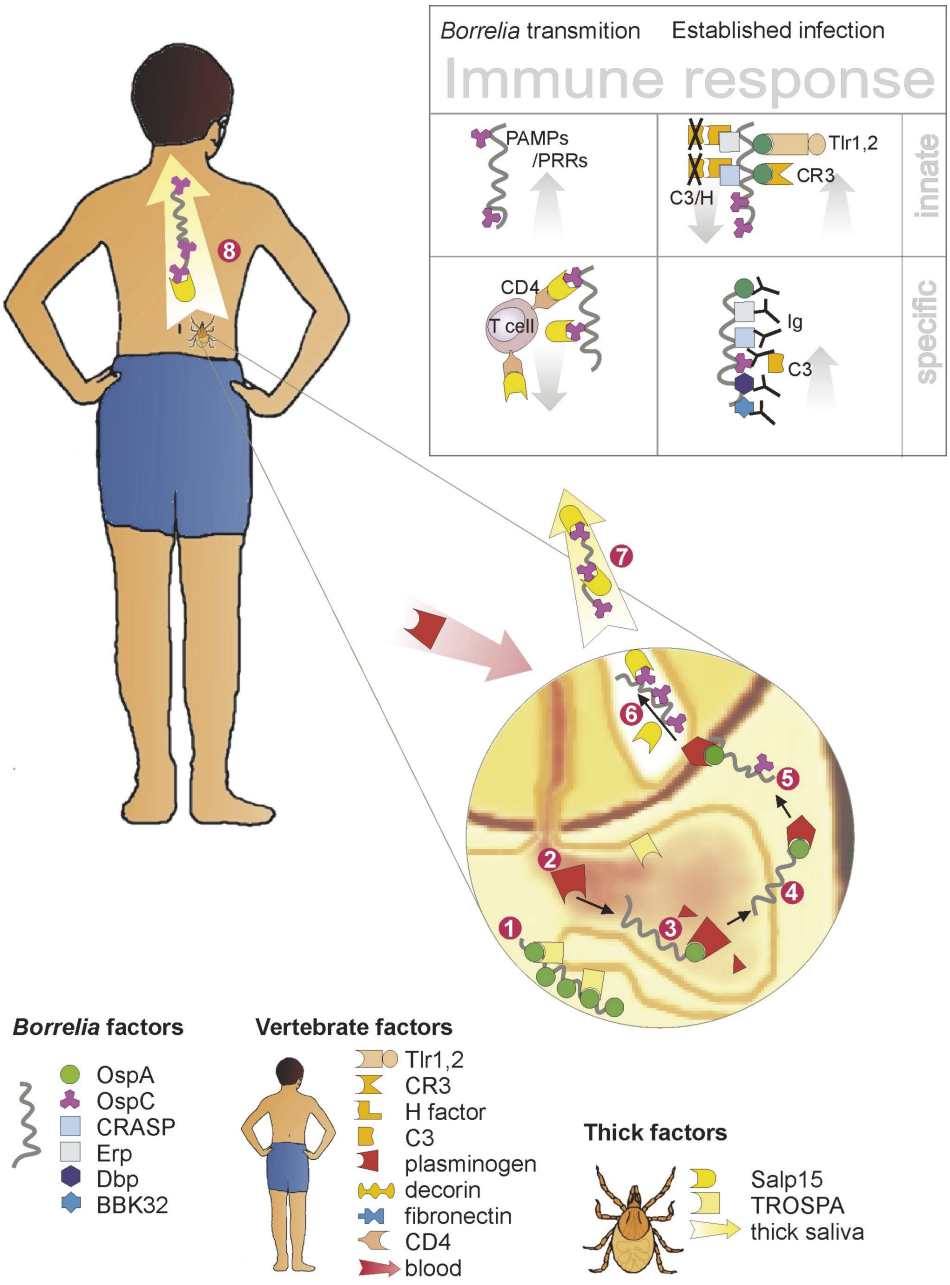

Fig. 3. Transmission of *Borrelia burgdorferi* from tick to human.
Borrelia binds tick gut epithelium via OspA-TROSPA interaction (1). Intake of human blood with plasminogen (2), which is subsequently bound on the OspA. Plasminogen after activation (3) helps *Borrelia* to pass through gut epithelium (4). Consequently *Borrelia* passes through haemocoel to salivary glands of tick (5). During this time, *Borrelia* changes its outer proteins profile and in the salivary glands binds immunosupresive Salp15 (6). "Armed" with this protein, *Borrelia* leaves tick (7) and enters to the human (8). Table in the upper right corner illustrates human immune reaction to *Borrelia*. Gray arrows indicate increasing or decreasing of immune response. Abbreviations are interpreted in the text.

and sequences of genes known from other organisms. Linear chromosomes end in hairpin telomeres similar to the chromosome telomeres[68]. This form, unusual for prokaryotes, was originally described in N15 prophage of *E. coli* – linear plasmid molecule with the above features[69]. Apart from *Borrelia*, linear plasmids have been described in *Streptomyces*, *Rhodococcus fascians*, *Nocardia opaca*, and in *Thiobacillius versutus* up to now[70]. Covalently closed hairpin telomeres have been found on DNA of double stranded DNA viruses and on some eukaryotic mitochondrial DNA molecules[65].

Interesting is the high degree of genetic redundancy between some plasmids. Cp32 plasmids share sequence homology with each other and with plasmids lp56, lp54 and cp9. It seems very likely that lp54 and lp56 arose from integration of cp32 into linear progenitors. On

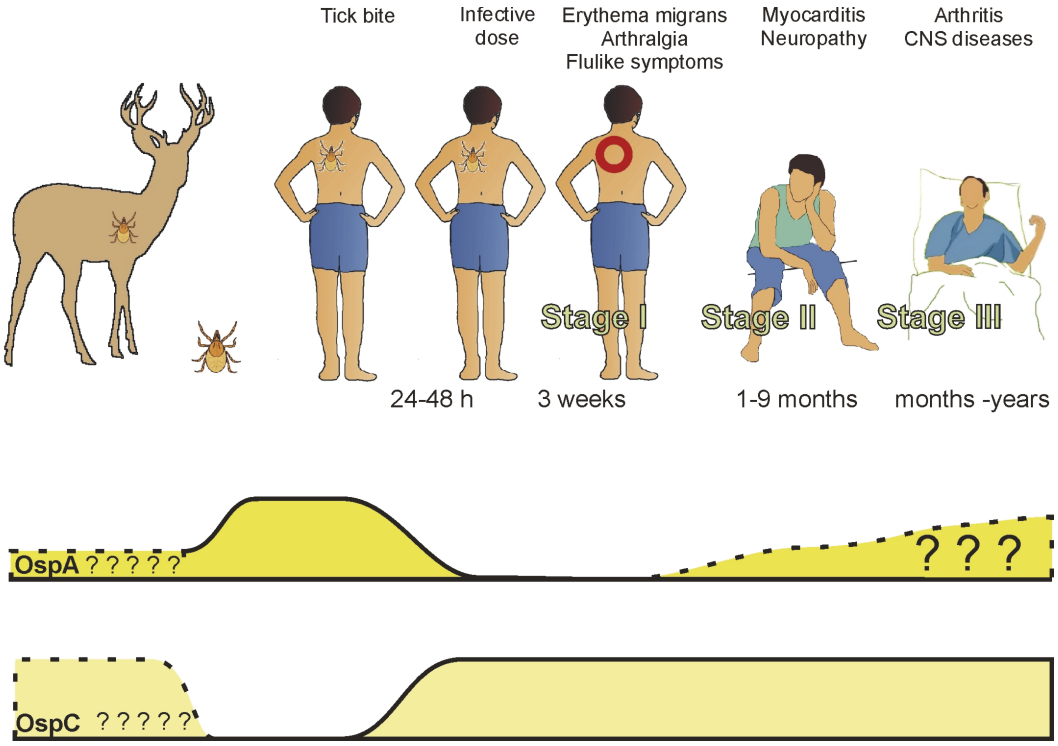

Fig. 4. Illustration of changing expression of OspA and OspC during Lyme disease transmission. Expression of Osp´s in the natural *Borrelia´*s hosts is poorly known. *Borrelia* express OspA during tick infection, where OspA acts as a tick specific adhesive molecule. During transmission to human, expression of OspA decreases and expression of OspC increases. Some reports proved expression of OspA during later stages of human infection.

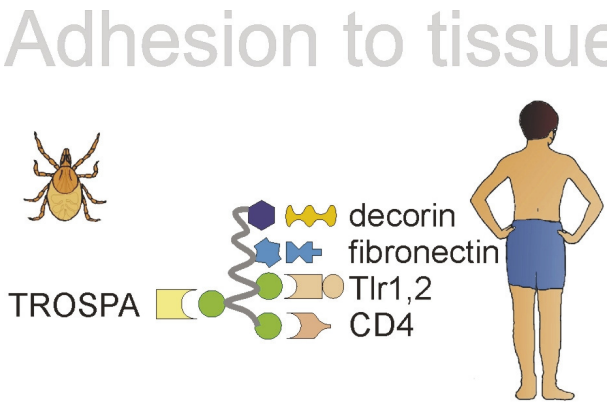

Fig. 5. Adhesion of *Borrelia* to tick and human tissues. Symbols are explained in Fig. 4.

the other hand, cp9 is probably a fragment of ancestral cp32. Another truncated member of cp32 family, cp18 has been described in *B. burgdorferi* s. s. isolates N40 and 297(ref.[63, 66, 71, 72]). Other highly similar plasmids are lp5 and lp 21 (ref.[63]).

Eggers et al.[73, 74] proved that cp32 plasmids contains prophage genome and they described bacteriophage mediated lateral gene transfer in *B. burgdorferi* s. s. This may be an important mechanism in *Borrelia* genome variability.

Glockner et al.[75] compared genome of *B. burgdorferi* s. s. B31 with genomes of *B. garinii* strain PBi (isolated from cerebrospinal fluid) and *B. afzelii* strain BKo (skin isolate). Chromosomes of these three species have a high degree of similarity. Only three regions show greater differences – *ori* replicator and genes for membrane proteins *bmp* and *lmp*. Apart from this, plasmid content varies widely between these species, whereas *B. garinii* and *B. afzelii* are more similar to each other than to *B. burgdorferi s. s.* Plasmids of these three species were divided into 6 orthologous groups. Higher similarity was found in cp26 analogs (*B. garinii* cp28, *B. afzelii* cp27) and in lp54 analogs (*B. garinii* lp59, *B. afzelii* lp60–1). Similarity in the other groups was significantly lower.

Some *B. burgdorferi s. s.* plasmids are not essential for *in vitro Borrelia* growth and thus can be easily lost when passaged out of its natural host. However, some of those genes are necessary during natural infection course and for *Borrelia* persistence in the host as demonstrated by Labandeira-Rey et al.[76, 77]. They examined the infectivity of mutants lacking lp25 and lp28–1 in C3H mice and demonstrated that the loss of infectivity in lp25$^-$ and elimination of lp28–1$^-$ mutants from tissues coincident with the onset of specific IgM immune response. In immune-deficient SCID mice, the lp28$^-$ mutants normally persist. This observation reveals the importance of some genes located on these plasmids: the gene for nicotinamidase (protein

BBE22) substantial for bacteria metabolism, located on plasmid 25 and the gene for VlsE (Vmp-like sequence, expressed) important for immune evasion[76–80] located on plasmid 28–1. Abberation in *vls* was associated with loss of infectivity of *B. garinii*[75]. Purser and Norris[81] tested 19 clonal isolates of *B. burgdorferi s. s.* of known plasmid pattern. They divided plasmids into three groups: the first group – plasmids required for infectivity (lp25, lp28–1), the second group – plasmids which were missing randomly in infective or non-infective isolates, thus not required for infectivity (cp9, cp32–3, lp21, lp28–2, lp28–4, lp56), and a third group – plasmids presented in all 19 isolates (cp26, cp32–1, cp32–7, cp32–4, cp32–6, cp32–8, cp32–9, lp17, lp28–3, lp36, lp38 and lp54). A few years later, clinical *B. Burgdorferi s. s.* isolates lacking lp38, lp56, cp32–1, cp32–8, and segment of 28–1 were described[82]. In contrast to the majority of bacteria, some *Borrelia* plasmids carry genes essential for survival, as was demonstrated for plasmid cp26 (ref.[83, 84]) coding at least three necessary proteins – GMP synthase (*guaA*), IMP dehydrogenase (*guaB*) and telomere resolvase (*resT*), the enzyme responsible for capping linear replicons with hairpin telomeres[85]. This is another speciality of the *Borrelia* genome as plasmids often carry genes that confer a selective advantage, but not genes necessary for the life cycle. Moreover, cp26 plasmid carries a gene coding for outer surface protein C (OspC), which is necessary for early stage of vertebrate host infection but is not required during growth in the tick vector or during *in vitro* growth[86–88].

Strother et al.[89] tried to identify plasmids of *B. burgdorferi* required for tick infection. They demonstrated that plasmids lp5, lp28–1, and cp9 are not required for tick infection, whereas loss of lp25 and lp28–4 is associated with reduced tick infectivity. Moreover it was discovered that absence of lp28–1 reduces transmission of *Borrelia* to tick salivary glands, the organ from which spirocheta is released to vertebrate host.

These data show that plasmids of *B. burgdorferi s. l.*, especially linear ones, have some characteristics exceptional for common definition of "plasmid" – the redundant, mainly circular DNA molecule, conferring antibiotic resistance or other selective advantage and present in cells in variable number. In addition, linear plasmids have similar features to linear chromosome which supports the idea that at least some plasmids of *B. burgdorferi* s. l. should be regarded as "minichromosomes"[90].

Outer membrane proteins and their significance in the pathogenesis and transmission of the disease

Members of *B. burgdorferi* species group have a complex life cycle. All these species circulate between arthropod vector and vertebrate host. As mentioned above, the most frequent vectors are ticks of the genus *Ixodes* and vertebrate hosts originate from a broad range of avian and mammalian species.

During its life cycle *Borrelia* must adapt to two very different environments – tick and vertebrate organisms. *Borrelia* must be able to adhere and survive in the tick gut, pass by gut epithelium to the haemolymph and transport through salivary glands to the host bloodstream, must avoid the immune reaction and disseminate to the target tissue. During *Borrelia* transition between *Ixodes* and vertebrate, a key role is played by several outer surface proteins. Their expression is regulated in separate phases of *Borrelia* infection[91–94].

Outer surface protein A

The most known is antagonism between expression of outer surface protein A (OspA) and OspC in *B. burgdorferi* s. s. The OspA was the first described member of outer surface proteins – more precisely lipoproteins group[95]. OspA has molecular weight of 31 kDa. The gene for this protein is part of the bicistronic operon on plasmid lp54 expressed together with the gene for another outer surface protein – OspB, with a molecular weight 34 kDa[96]. These proteins are expressed in the midgut of tick, or in cultured *Borrelia* and are downregulated during transition to the vertebrate host[97]. The main function of OspA consists in binding to glycoprotein TROSPA present in tick gut epithelium (tick receptor for OspA). The biological function of TROSPA glycoprotein in tick is unknown. Using quantitative RT-PCR, expression of TROSPA was shown to be significantly increased in *Borrelia* infected ticks. Another purported function of *Borrelia* OspA is the binding of plasminogen from the host blood during tick feeding. The phenomenon of plasminogen binding is described as the virulency factor in some other bacteria, e.g. *Streptococcus, Salmonella, Haemophilus* and *Yersinia*[99]. The presumed sense of OspA-induced plasminogen activation toward active plasmin is the misuse of its proteolytic activity for *Borrelia* invasion into the host tissues. Plasmin proteolytic activity is presumed to be important during *Borrelia* transit through tick gut epithelium to the haemocoel[100]. Consequently *Borrelia* pass through haemocoel to tick salivary glands from where spirochetes are exported to the bloodstream of vertebrate host. Garcia et al.[101] demonstrated that both OspA and OspB directly bind to vertebrate host complement receptor 3 (CD11b/CD18) and so induce activation of immune system cells. OspA also activate immune system via binding to Toll-like receptors 1 and 2 (reviwed in Singh and Girschick[102]). This may be one of the reasons for the downregulated of OspA/B expression during transmission to vertebrate host which minimizes the immune inflammatory reaction in the early stage of disease. On the other hand, Crowley and Huber[103] discovered that expression of OspA in vertebrate host is stimulated in an inflammatory environment, particularly after zymosan-induced inflammation. Because the expression of *B. burgdorferi* s. s. OspA is noted in later stages of mammalian host infection, usually accompanied by arthritis[104]. It is not clear whether increased OspA expression during chronic infection is just the *Borrelia* response to otherwise induced host inflammatory response to *Borrelia* or if *Borrelia* OspA expression is necessary for chronic infection. One explanation for active *Borrelia* OspA expression during chronic infection is the potential

protective function of OspA for *Borrelia* cells. Bunkis & Barbour[105] cited OspA as the protease and acid resistant structure on *Borrelia* surface which shields *Borrelia* against proteases and low pH in such "unfriendly" places as the tick gut or inflamed mammalian tissue.

In contrast to an inflammatory environment – antibodies downregulate OspA expression, irrespective of their specificity[106, 107]. Thus the regulation of OspA expression resembles positive feedback (inflammation – protection) which must be regulated in *Borrelia* due to the stage of disease development by as yet not well-understood mechanisms.

Outer surface protein C

Another outer surface protein which is tightly linked to the vertebrate stadium of *Borrelia* infection is OspC. The *OspC* gene is located on cp26 plasmid and encodes a 23 kDa lipoprotein, previously known as pC[108]. *Borreli*ae lacking this protein are not pathogenic but they can be cultured[88]. The function of this protein was unknown for a long time. Recently the ligand for OspC – tick salivary immunosuppressive protein Salp15 – was described[109]. It is a soluble component of tick saliva which suppresses the vertebrate host immune response against components of tick saliva during tick engorgement. Salp15 inhibits proliferation of $CD4^+$ T cells by binding to extracellular domains of the CD4 molecule and inhibits calcium signals needed for activation of transcription factors NF-κB and NF-AT during T cell receptor mediated signaling *in vivo* as well as *in vitro*[110, 111]. In this way *Borrelia* acquires an immunosuppressive coat of OspC-binded Salp15, which make them undetectable to the vertebrate immune system during the early stage of host colonization. Interestingly, the expression of Salp15 is elevated in infected ticks[109] as in the case of TROSPA mentioned above. The evolutionary reason for this tick cooperation with its own pathogen is open to question. Newly published data attribute to OspC plasminogen binding activity as was described for OspA above[112].

However, OspC is not the only protein involved in immune evasion. An essential role is attributed to VlsE, the lipoprotein mentioned in the previous section. The gene for this protein consists of 15 silent *vls* cassetes, segments of which are recombined into the *vlsE* region during mammalian infection[113]. This results in antigenic variability of expressed lipoprotein and thus evasion of specific immune reaction. Recombination of silent fragments into *vlsE* region occurs only *in vivo.* Nevertheless signals involved in recombination are unknown. It was found that the number of amino acid changes is significantly higher in immunocompetent C3H mice than in SCID mice and this suggests an influence of selective pressure[114, 115].

OspEF related proteins and CRASP

Borrelia is also armed against the activity of complement. And this arm again posses to the host of this bacteria. It has been demonstrated, that one *B. burgdorferi* s. s. outer surface lipoprotein – OspE, is responsible for *Borrelia* surface-binding of factor H in human blood. Factor H is a human regulatory protein, inhibiting the activity of complement component C3b by means of facilitating its cleavage by factor I and competing with factor B required for formation of C3 convertase in an alternative pathway of complement activation. Factor H-like protein (FHL), the truncated form of factor H, has a similar function[116]. These mechanisms are used by mammalian cells to protect themselves against non-specific complement activation which could otherwise result in autoimmune damage. Thus *Borrelia* utilizes the host's own cell surface protective mechanisms. OspE is a member of so-called Erp's (OspEF related proteins), together with proteins coded by genes *OspF*, *erpAB, erpCD, erpG, erpHY, erpIJ, erpK, erpLM, erpNO, erpPQ*, and *erpX*. These genes are localized on cp32 plasmids. The exception is the *erpX* localized on plasmid lp56 (ref.[117, 118]). The similarities of predicted products of these genes vary between 17–100 % and protein products have diverse ability to bind factor H in various animal species[119]. Apart from this observation, Kraiczy et al.[120, 121] described a group of proteins capable of binding factor H and factor H – like protein (FHL) and designated them CRASPs (Complement regulator-acquiring surface protein). From analysis of differences between CRASP members, the authors explain the variable sensitivity of *B. burgdorferi* s. s., *B. afzeli* and *B. garinii* to the complement observed earlier by Breitner-Ruddock et al.[122], who divided Lyme disease *Borreliae* into three groups: complement resistant (*B. afzeli*), complement sensitive (*B. garinii*) and complement partially sensitive (*B. burgdorferi s. s.*). CRASPs appear to be a heterogenic group, because later research showed that some CRASPs members belong to erp family[123] and at least one, CRASP-1 of *B. burgdorferi* s. s., does not[124].

Adhesive proteins

Other *B. burgdorferi* s. s. group of outer surface proteins included in the pathogenesis of Lyme disease are adhesive molecules which bind vertebrate tissue structures. Decorin binding protein A and B (DbpA, DbpB) bind collagen-associated proteoglycan decorin. ,*Borrelia* glycosaminoglycans binding protein (Bgp) binding glycosaminoglycans and Fibronectin binding protein (BBK32) binding extracellular protein fibronectin and glycosaminoglycans[125, 126]. The mediation of *B. garinii* adhesion to neuronal cell proteoglycans during neuroborreliosis has been recently attributed to OspA, the protein mentioned above[127]. Not fully undersood is role of hemagglutinating lectin B31LEC, but its function in host-pathogen interactions is supposed[128, 129]. *Borrelia* adhesion is discussed in detail by Coburn et al.[130] and Grubhoffer et al.[131].

Regulation of expression

The mechanisms of transcriptional regulation of these proteins are not fully understood but known is the essential role of alternative sigma factors – RpoN (σ^{54}) and RpoS (σ^{38}, σ^{s}). RpoS expression is activated by RpoN cooperating with response regulatory protein (Rrp2) under conditions such as increased temperature or decreased pH. RpoS induces expression of temperature inducible

genes, like *OspC*, *OspF* and *DbpA*. In contrast, expression of OspA is dependent on housekeeping factor RpoD (σ^{70}) and is downregulated by the RpoS dependent pathway[132–134].

CONCLUSIONS

In spite of considerable research progress on the biology of *Borrelia* in the most recent years, some features of this bacteria remain obscure. Further, most publications focus only on one species – *Borrelia burgdorferi* s. s. This may be due to the concentration of *Borrelia* research groups in the USA where *Borrelia burgdorferi* s. s. is the only pathogenic species of this group while the situation in Europe is different. Continued research in this field is important not only from the medical point of view but also because it yields valuable outcomes for understanding a number of general biological questions such as plasmid plasticity, antigenic variation and vector-pathogen-host interactions.

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