Published in final edited form as:

Infect Dis Clin North Am. 2008 June ; 22(2): 217–234.

Biology of Infection with *Borrelia burgdorferi*

Kit Tilly, PhD^a, Patricia A. Rosa, PhD^b, and Philip E. Stewart, PhD^c

^aLaboratory of Zoonotic Pathogens, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, 903 South Fourth St., Hamilton, Montana 59840, Ph. 406-363-9239, FAX 406-375-9681, ktilly@niaid.nih.gov

^bLaboratory of Zoonotic Pathogens, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, 903 South Fourth St., Hamilton, Montana 59840, Ph. 406-363-9209, FAX 406-375-9681, prosa@niaid.nih.gov

^cLaboratory of Zoonotic Pathogens, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, 903 South Fourth St., Hamilton, Montana 59840, Ph. 406-363-9393, FAX 406-375-9681, pestewart@niaid.nih.gov

Keywords

Lyme disease; *Borrelia burgdorferi*; spirochete; *Ixodes*

The spirochete *Borrelia burgdorferi* is a tick-borne obligate parasite whose normal reservoir is a variety of small mammals [1]. Whereas infection of these natural hosts does not lead to disease, infection of humans can result in Lyme disease, as a consequence of the human immunopathological response to *B. burgdorferi* [2,3]. Consistent with the pathogenesis of Lyme disease, bacterial products that allow *B. burgdorferi* to replicate and survive, rather than true “virulence factors,” appear to be primarily what is required for the bacterium to cause disease in a susceptible host. In support of this idea, the genome sequence of B31, the type strain of *B. burgdorferi* sensu stricto [4,5], revealed that the bacterium lacks factors common to many bacterial pathogens, such as lipopolysaccharide, toxins, and specialized secretion systems. In this chapter, we will describe the basic biology of *B. burgdorferi*, and some of the bacterial components required to infect and survive in the mammalian and tick hosts.

Natural history of the Lyme disease spirochete

The causative agent of Lyme disease is a member of the eubacterial phylum *Spirochaetes*. Members of this group of organisms share a distinctive morphology that includes a spiral or wavelike body and flagella (organs of motility) enclosed between the outer and inner membranes. The spirochetes include several human pathogens, including *Treponema pallidum* (agent of syphilis), *Leptospira interrogans* (leptospirosis), and several *Borrelia* spp. that cause relapsing fever. Although these other spirochetes had long been known to medicine, it was relatively recently that the bacterial agent of Lyme disease was identified [1].

Lyme disease was clinically described as an infectious illness by Dr. Alan Steere and colleagues in 1977 and is currently the leading vector-borne disease in the United States [6,7]. Steere et

Correspondence to: Philip E. Stewart.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

al. suggested that the epidemiology of Lyme disease indicated transmission by an arthropod vector due to the geographic clustering of patients in rural areas and the seasonal occurrence of the symptoms [7]. Subsequently, Dr. Willy Burgdorfer and coworkers observed spirochetes in the midgut tissues from ticks collected in a Lyme disease endemic area [1]. These spirochetes produced a skin rash resembling erythema migrans when injected into rabbits, and sera from Lyme disease patients reacted with the bacteria in indirect immunofluorescence assays. In recognition of this discovery, the bacterium was named *Borrelia burgdorferi* [8].

In Europe and Asia, the three species of *Borrelia* responsible for most human cases of disease are *B. burgdorferi* sensu strictu (s.s.), *B. garinii* and *B. afzelii*, which are collectively referred to as *B. burgdorferi* sensu lato [9]. Lyme disease in the U.S. is caused by the single species *B. burgdorferi* s.s. The clinical manifestations of European and North American Lyme disease share some common features such as an erythema migrans rash and an influenza-like illness. Subsequently, other symptoms may develop that roughly correlate with the infecting species. Arthritis frequently accompanies *B. burgdorferi* s.s. infection, whereas neurological symptoms are correlated with *B. garinii*, and skin disorders with *B. afzelii*, although these clinical associations are not absolute [10].

Genome and molecular genetics of *B. burgdorferi*

Although the underlying genetic traits contributing to the differences in disease have not been identified, the release of three *Borrelia* genome sequences (one from each 21 species) should aid in this endeavor [5,11–13]. These genomes have several common features, including a linear chromosome and a large number of smaller DNA molecules (plasmids), some of which are linear and others circular [14–17]. The linear structure of the chromosome and many of the plasmids is unusual in the bacterial world, although the evolutionary advantage of this form of DNA is unknown. However, it likely confers some benefit to the genus *Borrelia*, as all characterized members retain linear DNA molecules.

Despite the atypical DNA form, the majority of genes encoded on the *B. burgdorferi* chromosome are commonly found in other bacterial genomes [4]. The genes encoded on the plasmid component of the genome are less recognizable and the majority of these appear to be unique to the genus *Borrelia* [4,5]. Additionally, several plasmid-encoded genes have been shown to be required for infectivity or persistence in the tick or the mammalian host (discussed below).

The genome sequence of the Lyme disease spirochete revealed several interesting features [4,5]. First, no classically-defined virulence factors were identifiable, probably because *B. burgdorferi* did not evolve to cause disease in mammals. However, a sizable number of putative lipoproteins were recognized and some have been shown to trigger components of the mammalian innate immune system (discussed below). Finally, the plasmids appear to be in a state of rapid evolution, as many genes appear to be mutationally inactivated, whereas others have been duplicated and may be functionally diverging from each other [5]. Perhaps reflecting this evolving state, the number of plasmids, their sizes, and the gene order varies substantially among strains and between species; in some cases, plasmids or portions of some plasmids are absent [18,19]. The implications of these variations in plasmid structure and content to infectivity or disease remain unclear.

Many genes are present across all three Lyme disease spirochetes but display sequence variation both between and within species. Genes required for infectivity or persistence within vertebrate hosts (such as *ospC* and *vlsE*, see below) may vary significantly at both the nucleotide and amino acid level [13,20–22]. The variability within a given gene product complicates the design of effective vaccines based on these proteins since the elicited immune response may not protect against all variants [23,24].

Analysis of the *B. burgdorferi* genome sequence found that greater than 6% of the chromosomal genes are involved in motility and chemotaxis [4]. The flagella of spirochetes traverse the length of the cell body and are “hidden” beneath the outer membrane, in contrast to other organisms that have external flagella radiating outward. A potential advantage to the flagellar arrangement of spirochetes is the shielding of the highly conserved and immunogenic flagella from the host immune system. Also, the morphology and motility of spirochetes allows these organisms to swim in highly viscous media that immobilize other bacterial species [25–28]. The structural form of spirochetes may aid pathogenic species in penetrating host tissues and disseminating throughout the host.

In contrast to those of free-living bacteria, the genome of *B. burgdorferi* is relatively small, probably reflecting its lifestyle as an obligate parasite. *B. burgdorferi* lacks the conventionally recognizable machinery for synthesizing nucleotides, amino acids, fatty acids, and enzyme cofactors, apparently scavenging these necessities from the host [4]. The limited metabolic capacity of *B. burgdorferi* requires a complex and chemically undefined growth medium for in vitro cultivation.

Over the last 15 years, substantial advances have been made in the ability to genetically modify *B. burgdorferi* (for a recent review see Rosa et al. [29]). Currently, targeted genes can be selectively inactivated and the resulting mutant strain can be tested for infectivity in an experimental mouse-tick cycle. In this way, putative virulence factors can be assessed in the laboratory. Using the opposite approach, transposon mutagenesis randomly inactivates a large number of genes, with each mutation occurring in an individual clone [30,31]. Potentially, batches of these random mutants can be tested together for infectivity in the mouse model and specific mutants that no longer infect or persist can be isolated. Use of this method on a limited scale identified several genes that appear to contribute to *B. burgdorferi* infectivity in the mouse [32].

***B. burgdorferi* life cycle**

B. burgdorferi infects a wide range of vertebrate animals including small mammals, lizards, and birds [33–39]. Ticks of the genus *Ixodes* transmit *B. burgdorferi* between hosts and are the only natural agents through which humans have been shown to become infected [2,40]. Worldwide geographic distribution of Lyme disease correlates to the overlapping ranges of both a competent reservoir host for *B. burgdorferi* and the tick vector. In the northeastern and midwestern United States, the primary tick species for human disease is *Ixodes scapularis* (the black-legged tick) and in the western states *I. pacificus* (the western black-legged tick) is the main agent of dissemination [41]. European and Asian Lyme disease agents are primarily transmitted by *I. ricinus* (the European sheep tick) and *I. persulcatus* (the taiga tick), respectively [42,43].

Ticks most frequently acquire spirochetes from infected rodents during their larval feeding [36,44]. After molting to the nymphal stage, infected ticks feed on a broad range of animals, including rodents, which become a new reservoir perpetuating the cycle [40]. After the nymphs molt to the adult stage, they exclusively feed on larger mammals, which are often not competent hosts for *B. burgdorferi* [40]. The spirochetes are rarely, if ever, transmitted trans-ovarially [45,46], so the larval and nymphal feedings are crucial to maintaining the spirochete. Both nymphs and adults occasionally feed on humans, but the small size of the nymphs makes them difficult to detect and, hence, more likely to feed long enough to transmit the spirochete and cause Lyme disease.

The tick and mammalian hosts provide contrasting environments for bacterial growth. Notably, mammals regulate their body temperatures at about 37–39°C, whereas ticks vary with the ambient temperature, except when feeding on a mammal. Also, the pH of mammalian tissue

and blood is neutral, whereas the tick midgut is a more basic environment [47,48]. In order to cycle between two very different hosts, *B. burgdorferi* varies its gene expression, leading to different protein components and enabling physiological adaptation to these environments [49–53]. A number of studies have begun to delineate those changes.

Mammalian components affecting *B. burgdorferi* infection

Several different experimental animals have been used for laboratory studies of *B. burgdorferi* infection and disease (reviewed by Philipp and Johnson [54]). Hamsters become infected in multiple tissues but do not show signs of disease [55], although arthritis can be induced in vaccinated or irradiated hamsters [56,57]. Rabbits develop skin manifestations similar to the rash erythema migrans found in human disease, but clear the infection relatively rapidly [58,59]. Dogs develop arthritis and become persistently infected [60]. Infection of rhesus monkeys more closely resembles human infection, with some monkeys exhibiting skin rash, arthritis, and neuroborreliosis [61–64]. Because mice are small and have extensively characterized genetics, many scientists use a laboratory model of the natural infectious cycle involving mice and *Ixodes* ticks to assess host and bacterial components that allow perpetuation of the cycle and, in some cases, disease.

In the murine model, mice can be infected by needle inoculation or tick feeding. Most mouse strains, as well as the natural reservoir hosts, show no sign of disease, but do develop a serological response to *B. burgdorferi* proteins and become persistently infected [65,66]. Some mouse strains are considered to be disease-susceptible, in that they develop ankle swelling and inflammation that resembles arthritis in response to *B. burgdorferi* infection [66]. Other strains are disease resistant and considerable effort has been made to determine the factors and genes responsible for differences in susceptibility among mice [67–75]. In some cases, the differential severity of joint pathology exists despite similar numbers of spirochetes in the affected tissues [68]. These data suggest that disease is a consequence of host immunopathology, rather than a strategy of the bacterium to facilitate its persistence or transmission.

When *B. burgdorferi* enter a mouse, many components of the host innate immune response could potentially recognize the bacteria and help control their numbers. Among these are antigen presenting cells (such as macrophages and dendritic cells) in the peripheral tissues (e.g., at the site of the tick bite). The antigen presenting cells may subsequently migrate to lymph nodes, and stimulate T cell and B cell responses. Killing of *B. burgdorferi* by the phagocytic cells resident in the periphery and perhaps neutrophils attracted to the feeding lesion or inoculation site may also contribute to control of the initial inoculum. Complement may also help control *B. burgdorferi* numbers by opsonizing the bacteria (facilitating phagocytosis) or by direct killing via the alternative pathway.

To determine what components of the innate and acquired immune response recognize *B. burgdorferi*, and limit bacterial numbers and their ability to cause disease, infection has been characterized in mouse strains with mutations affecting a number of those components. These and other studies have identified a particular pattern receptor (TLR2) as key to mammalian recognition of *B. burgdorferi* lipoprotein antigens [76–78]. Mice deficient in TLR2 have increased spirochete loads and ankle swelling. However, mice lacking MyD88, an adapter required for signaling through TLR2 and several other pattern receptors, have even higher spirochete loads, suggesting multiple pathways respond to *B. burgdorferi* antigens [79–82]. Most likely, the phenotypes of these mutations result from poor recognition of *B. burgdorferi* components by phagocytic and antigen-presenting cells. Complement was first implicated in controlling spirochete numbers and perhaps in determining preferred reservoir hosts in a study that correlated sensitivity to various host sera with particular spirochete genospecies [83]. Complement was further implicated by infection of mice defective in

component C3 production, which results in somewhat higher numbers of spirochetes in tissues, especially at early times after infection [84,85]. In summary, several innate immune system components recognize spirochetes and control their numbers but, in most cases, are inadequate to completely clear an infection.

After the initial stage of *B. burgdorferi* infection, mice develop antibodies to numerous bacterial proteins [86,87]. When serum from infected mice was transferred to naïve mice, the recipient animals were protected from infection with the same strain of *B. burgdorferi* [88, 89]. Similar transfer of T cells did not confer protection [90], suggesting that T cells play a lesser role than antibody in protection. Despite the presence of neutralizing antibodies, the host acquired immune response limits spirochete numbers but does not eradicate *B. burgdorferi* infection and most mice become persistently infected after needle inoculation or the bite of an infected tick. Antibody does serve to limit disease and pathogenesis, since SCID mice, which lack both the cellular and antibody components of the acquired immune response, contain much higher spirochete loads in tissues and exhibit more severely arthritic joints than normal mice [91]. The ability of the bacteria to survive in the face of an antibody response suggests that either the bacteria “hide out” in sites protected from antibodies or that the bacteria evade antibody reactivity by varying antigens or otherwise masking reactive proteins. The low numbers of spirochetes typically found in infected mammalian blood limits direct detection of bacteria in human clinical samples, complicating diagnosis and analysis of the progress of an infection. Sensitive molecular techniques now allow amplification of bacterial targets, but the bacterial numbers are at the lower limit detectable by such methods, so they are difficult to apply in the clinical laboratory.

Studies of *B. burgdorferi* factors required for mammalian and tick infection began with identifying changes in protein composition as the spirochete alternated between these disparate environments [49,51,92]. As microarray analysis was developed, this technique was applied to measuring differences in gene expression between bacteria grown in conditions that mimic some characteristics of each host environment [93–95]. A variant on this has been to compare the gene expression of cultured spirochetes with that of bacteria grown in an immune-privileged chamber within a rat, allowing host-adaptation [96]. Other studies have used the polymerase chain reaction (PCR) to follow the in vivo expression of genes highlighted in the microarray analysis, using samples of infected mammalian and tick tissues [97–101]. These experiments demonstrated that the bacterial gene expression profile changes, not only between hosts, but also at different times within a single host. Finally, one group has corroborated some of the gene expression patterns identified in these studies by directly examining the proteins made by bacteria growing in SCID mouse and rabbit skin, taking advantage of the larger number of bacteria found in these models [102,103]. Using these methods, researchers have identified genes whose products are, or are likely to be, produced in mice or ticks, some of which have been tested for their contribution to bacterial survival in the corresponding host (Fig. 1).

***B. burgdorferi* products required for mammalian infection**

Efforts to identify *B. burgdorferi* products that play roles in mammalian infection began by serially passaging strains in culture and correlating loss of infectivity with loss of specific plasmids [55,104–108]. As genetic techniques have become available for *B. burgdorferi* analysis, other approaches have been applied to identifying genes that contribute to mammalian infectivity and the roles of some such genes have been rigorously defined by inactivation and restoration (complementation). The products of these genes can be divided into ones that play physiological roles and those that contribute to survival in the face of other aspects of the host environment, including normal defenses. Since *B. burgdorferi* has a limited biosynthetic capability, the bacteria rely on their host (or culture medium) for many nutrients that other bacteria can synthesize. Some enzymes shown to be crucial for bacterial survival in a

mammalian host (although dispensable for growth in rich culture medium) include PncA, a nicotinamidase involved in production of NAD [109], and two products involved in purine nucleotide synthesis [32,110]. Another unusual feature of *B. burgdorferi* is that it does not contain intracellular iron and, hence, does not use iron as a co-factor for enzymes [111], thereby facilitating survival in the iron-poor mammalian environment.

Factors important for bacterial survival in a mammalian host can be subdivided into two groups. Some are involved in early infection, such as OspC [112–116], which are presumably required for host colonization or resistance to innate immunity. Others are involved in resistance to acquired immunity, such as VlsE [13,109,117,118].

The OspC product has been shown to be required for an early stage of mammalian infection [113–116] and conflicting data have been presented regarding its importance in tick transmission [113,114,119,120]. OspC production begins in feeding ticks (or immediately after needle inoculation, Fig. 1) and lasts for the first couple of weeks of mammalian infection [49,51,103,121–123]. Once the bacteria are established in a host, OspC production is not required for persistence [114]. The molecular function of the OspC protein is undefined, although the crystal structure of the protein predicts that it binds a small ligand [124,125]. The gene product contains a highly variable region that has allowed characterization of OspC types [126–128]. Studies of the significance of OspC type have reached contradictory conclusions. Some studies have found a correlation between OspC type and wild rodent host specificity [129], but others found no such link [130]. The OspC types of human clinical isolates have also been correlated with invasive and non-invasive phenotypes [128,131]. However, similar studies demonstrated greater diversity among invasive types than previously recognized, calling into question any causative effect of OspC sequence on invasion [130,132]. Carefully controlling for variables other than OspC has not been possible in such studies, since additional genome components of these isolates also differ. Recently, the invasive OspC protein types were shown to bind plasminogen [131], a trait shared with other *B. burgdorferi* proteins [133,134] that was suggested to facilitate *B. burgdorferi* infection of mammals and ticks [135].

Considerable attention has been paid to several *B. burgdorferi* proteins that co-opt the complement regulators factor H and factor H-like proteins, which normally protect mammalian cells from attack by their own complement by blocking activation of the alternative pathway [136–141]. Surface coating with factor H could protect the bacteria from killing or opsonization by host complement, facilitating infection. Surprisingly, however, *B. burgdorferi* infection of mice deficient in factor H production did not differ from infection of wild type mice, suggesting that any protection conferred by factor H binding was unimportant or redundant [85]. Because of these findings, the significance of the factor H-binding proteins for *B. burgdorferi* remains unknown.

VlsE is a *B. burgdorferi* protein that is required for persistent mammalian infection [13,109,117,118] and whose synthesis begins around the time that OspC production ceases (Fig. 1) [103]. Although its function is unknown, this lipoprotein has an elaborate system for variation [22]. Variation at the VlsE locus appears to be required for persistence [118,142], probably because its (unknown) essential function requires its presence on the bacterial surface, where it will be targeted by the adaptive immune response of the mammalian host.

Since *B. burgdorferi* can survive in the face of a neutralizing antibody response, the idea that the bacteria shelter in tissues with little exposure to antibodies by interacting with the host extracellular matrix (ECM) has been investigated (reviewed by Cabello *et al.* [143] and Coburn *et al.* [144]). Among the *B. burgdorferi* proteins that bind ECM components are DbpA and DbpB, which bind decorin [145], BBK32, which binds fibronectin [146], Bgp, which binds

proteoglycans [147], and P66, which binds integrins [148]. These proteins may help *B. burgdorferi* migrate through mammalian tissue and persist in joints and skin, where the spirochetes may be inaccessible to circulating antibodies. Genetic studies, however, have shown that spirochetes lacking DbpA have little if any defect in mammalian infectivity [149]. Two studies of mutants lacking BBK32 also found a small [150] or no [151] decrease in infectivity. These studies call into question whether interaction with host ECM protects the bacteria. Alternatively, the multiplicity of ECM-binding proteins may ensure such interaction by providing redundant binding proteins so that the loss of any single protein may fail to yield a noticeable phenotype.

An accumulating body of evidence demonstrates that a cascade of transcriptional regulators, encoded by the *rpoS* and *rpoN* genes, controls the production of a number of lipoproteins in response to changing environmental factors [152,153]. A sensor-regulator pair of proteins also contributes to this regulation [154]. When *B. burgdorferi* mutants were tested in the infection model, *rpoS* and *rpoN* expression were found to be required for infecting mice [153,155], demonstrating the importance of this pathway for survival in a mammal.

***B. burgdorferi* infection of ticks**

While infected nymphal ticks feed, spirochetes in the midgut respond in several ways to the incoming blood and increased temperature. The population of spirochetes expands [156–158] and their protein synthesis alters [49,99,122,159,160]. Then, spirochetes migrate from the midgut to the salivary glands, allowing transmission into a new host. The *B. burgdorferi* outer surface protein OspA was shown to be abundant on the surface of bacteria resident in ticks (Fig. 1), but down-regulated during tick feeding and transmission to a mammal [49, 160]. Subsequent studies suggest that OspA is an adhesin, important for retaining spirochetes in the tick midgut until feeding [161–163]. OspB, another potential midgut adhesin [164], BptA, a lipoprotein of unknown function, and the product of the BB0690 gene, which is probably involved in resistance to oxidative stress [165], also appear to contribute to bacterial survival in ticks [166–168]. The RpoN-RpoS regulatory cascade appears to be required for migration of spirochetes to the salivary glands during transmission, but not for survival within the tick environment [153]. Since the tick and mammalian environments differ significantly from each other, there are likely to be other *B. burgdorferi* proteins that carry out important roles during bacterial growth and survival in ticks.

Complementary studies have begun to elucidate tick proteins that contribute to *B. burgdorferi* infection and transmission. Ribeiro and colleagues identified transcripts that were differentially regulated between *B. burgdorferi*-infected and uninfected *I. scapularis* salivary glands [169]. A recent genetic study showed that the midgut protein TROSPA is a receptor for OspA binding, whose presence enhances colonization by *B. burgdorferi* [170]. The salivary gland protein Salp15 is immunosuppressive and may facilitate infection by the low numbers of spirochetes that are transmitted during tick feeding [171,172]. Recently, a tick antioxidant was shown to facilitate tick acquisition of spirochetes from infected animals [173]. The *Ixodes scapularis* genome project, which is in progress, should yield additional gene candidates whose influences, positive or negative, on *B. burgdorferi* infection remain to be elucidated.

Conclusions and Perspectives

B. burgdorferi produces a number of products that allow it to colonize and persist in its natural mammalian and tick hosts. Although the functions of only a few *B. burgdorferi* products have been clearly defined, some (such as OspC) are required for the bacteria to survive the initial attack of the mammalian innate immune system, while others (like VlsE) contribute to resisting the subsequent acquired immune response. Bacterial factors such as RpoS and RpoN are

components of signaling cascades regulating gene expression for survival in different environmental conditions. With the powerful genetic techniques now available for manipulating the spirochete, the mouse, and even the tick, the interactions among these three that lead to infection and disease are beginning to emerge.

Acknowledgements

We thank Catharine Bosio, Claire Checroun, and Mollie Jewett for comments on the manuscript, and Gary Hettrick and Anita Mora for graphical expertise. This research was supported by the Intramural Research Program of the NIAID, NIH.

References

1. Burgdorfer W, Barbour AG, Hayes SF, et al. Lyme disease - a tick-borne spirochetosis? *Science* 1982;216:1317–1319. [PubMed: 7043737]
2. Steere AC. Lyme Disease. *N Engl J Med* 2001;345(2):115–125. [PubMed: 11450660]
3. Wooten RM, Weis JJ. Host-pathogen interactions promoting inflammatory Lyme arthritis: use of mouse models for dissection of disease processes. *Curr Opin Microbiol* 2001;4(3):274–279. [PubMed: 11378478]
4. Fraser CM, Casjens S, Huang WM, et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 1997;390:580–586. [PubMed: 9403685]
5. Casjens S, Palmer N, van Vugt R, et al. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. *Mol Microbiol* 2000;35(3):490–516. [PubMed: 10672174]
6. Centers for Disease Control and Prevention. Lyme Disease - United States, 2003–2005. *MMWR* 2007;56(23):573–576. [PubMed: 17568368]
7. Steere AC, Malawista SE, Snyderman DR, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis Rheum* 1977;20:7–17. [PubMed: 836338]
8. Johnson RC, Schmid GP, Hyde FW, et al. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. *Int J Syst Bacteriol* 1984;34:496–497.
9. Baranton G, Postic D, Saint Girons I, et al. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *Int J Syst Bacteriol* 1992;42:378–383. [PubMed: 1380285]
10. van Dam AP, Kuiper H, Vos K, et al. Different genospecies of *Borrelia burgdorferi* are associated with distinct clinical manifestations of Lyme borreliosis. *Clin Infect Dis* 1993;17:708–717. [PubMed: 7903558]
11. Fraser CM, Norris SJ, Weinstock GM, et al. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* 1998;281(5375):375–388. [PubMed: 9665876]
12. Glöckner G, Lehmann R, Romualdi A, et al. Comparative analysis of the *Borrelia garinii* genome. *Nucleic Acids Res* 2004;32(20):6038–6046. [PubMed: 15547252]
13. Glöckner G, Schulte-Spechtel U, Schilhabel M, et al. Comparative genome analysis: selection pressure on the *Borrelia vls* cassettes is essential for infectivity. *BMC Genomics* 2006;7:211–220. [PubMed: 16914037]
14. Barbour AG, Garon CF. Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. *Science* 1987;237:409–411. [PubMed: 3603026]
15. Baril C, Richaud C, Baranton G, et al. Linear chromosome of *Borrelia burgdorferi*. *Res Microbiol* 1989;140:507–516. [PubMed: 2696058]
16. Ferdows MS, Barbour AG. Megabase-sized linear DNA in the bacterium *Borrelia burgdorferi*, the Lyme disease agent. *Proc Natl Acad Sci USA* 1989;86:5969–5973. [PubMed: 2762306]
17. Hyde FW, Johnson RC. Characterization of a circular plasmid from *Borrelia burgdorferi*, etiologic agent of Lyme disease. *J Clin Microbiol* 1988;26:2203–2205. [PubMed: 3183006]
18. Palmer N, Fraser C, Casjens S. Distribution of twelve linear extrachromosomal DNAs in natural isolates of Lyme disease spirochetes. *J Bacteriol* 2000;182(9):2476–2480. [PubMed: 10762248]

19. Terekhova D, Iyer R, Wormser GP, et al. Comparative genome hybridization reveals substantial variation among clinical isolates of *Borrelia burgdorferi* sensu stricto with different pathogenic properties. *J Bacteriol* 2006;188(17):6124–6134. [PubMed: 16923879]
20. Jauris-Heipke S, Fuchs R, Motz M, et al. Genetic heterogeneity of the genes coding for the outer surface protein C (OspC) and the flagellin of *Borrelia burgdorferi*. *Med Microbiol Immunol* 1993;182:37–50. [PubMed: 8098841]
21. Livey I, Gibbs CP, Schuster R, et al. Evidence for lateral transfer and recombination in OspC variation in Lyme disease *Borrelia*. *Mol Microbiol* 1995;18:257–269. [PubMed: 8709845]
22. Zhang JR, Hardham JM, Barbour AG, et al. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. *Cell* 1997;89:275–285. [PubMed: 9108482]
23. Wilske B, Preac-Mursic V, Jauris S, et al. Immunological and molecular polymorphisms of OspC, an immunodominant major outer surface protein of *Borrelia burgdorferi*. *Infect Immun* 1993;61:2182–2191. [PubMed: 8478108]
24. Wilske B, Busch U, Fingerle V, et al. Immunological and molecular variability of OspA and OspC. Implications for *Borrelia* vaccine development. *Infection* 1996;24(2):208–212. [PubMed: 8740124]
25. Kaiser GE, Doetsch RN. Enhanced translational motion of *Leptospira* in viscous environments. *Nature* 1975;255(5510):656–657. [PubMed: 1134561]
26. Greenberg EP, Canale-Parola E. Motility of flagellated bacteria in viscous environments. *J Bacteriol* 1977;132(1):356–358. [PubMed: 410784]
27. Berg HC, Turner L. Movement of microorganisms in viscous environments. *Nature* 1979;278(5702):349–351. [PubMed: 370610]
28. Kimsey RB, Spielman A. Motility of Lyme disease spirochetes in fluids as viscous as the extracellular matrix. *J Infect Dis* 1990;162:1205–1208. [PubMed: 2230247]
29. Rosa PA, Tilly K, Stewart PE. The burgeoning molecular genetics of the Lyme disease spirochaete. *Nature Rev Microbiol* 2005;3:129–143. [PubMed: 15685224]
30. Morozova OV, Dubytska LP, Ivanova LB, et al. Genetic and physiological characterization of 23S rRNA and *ftsJ* mutants of *Borrelia burgdorferi* isolated by mariner transposition. *Gene* 2005;357(1):63–72. [PubMed: 16023305]
31. Stewart PE, Hoff J, Fischer E, et al. Genome-wide transposon mutagenesis of *Borrelia burgdorferi* for identification of phenotypic mutants. *Appl Environ Microbiol* 2004;70(10):5973–5979. [PubMed: 15466540]
32. Botkin DJ, Abbott A, Stewart PE, et al. Identification of potential virulence determinants by *HimarI* transposition of infectious *Borrelia burgdorferi* B31. *Infect Immun* 2006;74(12):6690–6699. [PubMed: 17015459]
33. Anderson JF, Magnarelli LA. Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut. *Yale J Biol Med* 1984;57(4):627–641. [PubMed: 6516460]
34. Levine JF, Wilson ML, Spielman A. Mice as reservoirs of the Lyme disease spirochete. *Am J Trop Med Hyg* 1985;34:355–360. [PubMed: 3985277]
35. Anderson JF, Johnson RC, Magnarelli LA, et al. Involvement of birds in the epidemiology of the Lyme disease agent *Borrelia burgdorferi*. *Infect Immun* 1986;51(2):394–396. [PubMed: 3943893]
36. Donahue JG, Piesman J, Spielman A. Reservoir competence of white-footed mice for Lyme disease spirochetes. *Am J Trop Med Hyg* 1987;36:92–96. [PubMed: 3812887]
37. Brown RN, Lane RS. Lyme disease in California: a novel enzootic transmission cycle of *Borrelia burgdorferi*. *Science* 1992;256:1439–1442. [PubMed: 1604318]
38. Levin M, Levine JF, Yang S, et al. Reservoir competence of the southeastern five-lined skink (*Eumeces inexpectatus*) and the green anole (*Anolis carolinensis*) for *Borrelia burgdorferi*. *Am J Trop Med Hyg* 1996;54(1):92–97. [PubMed: 8651379]
39. Clark K, Hendricks A, Burge D. Molecular identification and analysis of *Borrelia burgdorferi* sensu lato in lizards in the southeastern United States. *Appl Environ Microbiol* 2005;71(5):2616–2625. [PubMed: 15870353]
40. Lane RS, Piesman J, Burgdorfer W. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. *Annu Rev Entomol* 1991;36:587–609. [PubMed: 2006870]

41. Burgdorfer W, Lane RS, Barbour AG, et al. The western black-legged tick, *Ixodes pacificus*, a vector of *Borrelia burgdorferi*. *Am J Trop Med Hyg* 1985;34:925–930. [PubMed: 3898886]
42. Gern, L.; Humair, P-F. Ecology of *Borrelia burgdorferi* sensu lato in Europe. In: Gray, JS.; Kahl, O.; Lane, RS., et al., editors. *Lyme Borreliosis: Biology, Epidemiology and Control*. Wallingford: CABI Publishing; 2002. p. 149-174.
43. Korenberg, EI.; Gorelova, NB.; Kovalevskii, YV. Ecology of *Borrelia burgdorferi* sensu lato in Russia. In: Gray, JS.; Kahl, O.; Lane, RS., et al., editors. *Lyme Borreliosis: Biology, Epidemiology and Control*. Wallingford: CABI Publishing; 2002. p. 175-200.
44. Mather TN, Wilson ML, Moore SI, et al. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Am J Epidemiol* 1989;130(1):143–150. [PubMed: 2787105]
45. Burgdorfer W, Hayes SF, Corwin D. Pathophysiology of the Lyme disease spirochete, *Borrelia burgdorferi*, in ixodid ticks. *Rev Infect Dis* 1989;11:S1442–S1450. [PubMed: 2682956]
46. Patrican LA. Absence of Lyme disease spirochetes in larval progeny of naturally infected *Ixodes scapularis* (Acari:Ixodidae) fed on dogs. *J Med Entomol* 1997;34(1):52–55. [PubMed: 9086711]
47. Balashov YS. Bloodsucking ticks (Ixodoidea) - vectors of diseases of man and animals. *Misc Publ Entomol Soc Am* 1972;8:161–376.
48. Ribeiro JMC. The midgut hemolysin of *Ixodes dammini* (Acari: Ixodidae). *J Parasitol* 1988;74:532–537. [PubMed: 3397814]
49. Schwan TG, Piesman J, Golde WT, et al. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc Natl Acad Sci USA* 1995;92:2909–2913. [PubMed: 7708747]
50. Carroll JA, Garon CF, Schwan TG. Effects of environmental pH on membrane proteins in *Borrelia burgdorferi*. *Infect Immun* 1999;67:3181–3187. [PubMed: 10377088]
51. Schwan TG, Piesman J. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. *J Clin Microbiol* 2000;39(1):382–388. [PubMed: 10618120]
52. Carroll JA, Cordova RM, Garon CF. Identification of 11 pH-regulated genes in *Borrelia burgdorferi* localizing to linear plasmids. *Infect Immun* 2000;68(12):6677–6684. [PubMed: 11083781]
53. Yang X, Goldberg MS, Popova TG, et al. Interdependence of environmental factors influencing reciprocal patterns of gene expression in virulent *Borrelia burgdorferi*. *Mol Microbiol* 2000;37(6):1470–1479. [PubMed: 10998177]
54. Philipp MT, Johnson BJB. Animal models of Lyme disease: pathogenesis and immunoprophylaxis. *Trends Microbiol* 1994;2:431–437. [PubMed: 7866701]
55. Johnson RC, Marek N, Kodner C. Infection of Syrian hamsters with Lyme disease spirochetes. *J Clin Microbiol* 1984;20:1099–1101. [PubMed: 6520220]
56. Schmitz JL, Schell RF, Hejka A, et al. Induction of Lyme arthritis in LSH hamsters. *Infect Immun* 1988;56:2336–2342. [PubMed: 3410540]
57. Lim LC, England DM, DuChateau BK, et al. Development of destructive arthritis in vaccinated hamsters challenged with *Borrelia burgdorferi*. *Infect Immun* 1994;62(7):2825–2833. [PubMed: 8005672]
58. Burgdorfer W. The New Zealand white rabbit: an experimental host for infecting ticks with Lyme disease spirochetes. *Yale J Biol Med* 1984;57(4):609–612. [PubMed: 6393612]
59. Kornblatt AN, Steere AC, Brownstein DG. Infection in rabbits with the Lyme disease spirochete. *Yale J Biol Med* 1984;57(4):613–616. [PubMed: 6393613]
60. Appel MJ, Allan S, Jacobson RH, et al. Experimental Lyme disease in dogs produces arthritis and persistent infection. *J Infect Dis* 1993;167:651–654. [PubMed: 8440936]
61. Philipp MT, Aydinoglu MK, Bohm RP Jr, et al. Early and early disseminated phases of Lyme disease in the rhesus monkey: a model for infection in humans. *Infect Immun* 1993;61(7):3047–3059. [PubMed: 8514412]
62. Pachner AR, Delaney E, O'Neill T, et al. Inoculation of nonhuman primates with the N40 strain of *Borrelia burgdorferi* leads to a model of Lyme neuroborreliosis faithful to the human disease. *Neurology* 1995;45(1):165–172. [PubMed: 7824109]

63. Roberts ED, Bohm RP Jr, Cogswell FB, et al. Chronic lyme disease in the rhesus monkey. *Lab Invest* 1995;72(2):146–160. [PubMed: 7853849]
64. Roberts ED, Bohm RP Jr, Lowrie RC Jr, et al. Pathogenesis of Lyme neuroborreliosis in the rhesus monkey: the early disseminated and chronic phases of disease in the peripheral nervous system. *J Infect Dis* 1998;178(3):722–732. [PubMed: 9728541]
65. Schwan TG, Burgdorfer W, Schrupf ME, et al. The urinary bladder, a consistent source of *Borrelia burgdorferi* in experimentally infected white-footed mice (*Peromyscus leucopus*). *J Clin Microbiol* 1988;26:893–895. [PubMed: 3290239]
66. Barthold SW, Beck DS, Hansen GM, et al. Lyme borreliosis in selected strains and ages of laboratory mice. *J Infect Dis* 1990;162:133–138. [PubMed: 2141344]
67. Kang I, Barthold SW, Persing DH, et al. T-helper-cell cytokines in the early evolution of murine Lyme arthritis. *Infect Immun* 1997;65(8):3107–3111. [PubMed: 9234761]
68. Ma Y, Seiler KP, Eichwald EJ, et al. Distinct characteristics of resistance to *Borrelia burgdorferi*-induced arthritis in C57BL/6N mice. *Infect Immun* 1998;66(1):161–168. [PubMed: 9423853]
69. Brown CR, Reiner SL. Genetic control of experimental lyme arthritis in the absence of specific immunity. *Infect Immun* 1999;67(4):1967–1973. [PubMed: 10085044]
70. Weis JJ, McCracken BA, Ma Y, et al. Identification of quantitative trait loci governing arthritis severity and humoral responses in the murine model of Lyme disease. *J Immunol* 1999;162(2):948–956. [PubMed: 9916719]
71. Kumar H, Belperron A, Barthold SW, et al. Cutting edge: CD1d deficiency impairs murine host defense against the spirochete, *Borrelia burgdorferi*. *J Immunol* 2000;165(9):4797–4801. [PubMed: 11046002]
72. Roper RJ, Weis JJ, McCracken BA, et al. Genetic control of susceptibility to experimental Lyme arthritis is polygenic and exhibits consistent linkage to multiple loci on chromosome 5 in four independent mouse crosses. *Genes Immun* 2001;2(7):388–397. [PubMed: 11704805]
73. Crandall H, Ma Y, Dunn DM, et al. Bb2Bb3 regulation of murine Lyme arthritis is distinct from Ncf1 and independent of the phagocyte nicotinamide adenine dinucleotide phosphate oxidase. *Am J Pathol* 2005;167(3):775–785. [PubMed: 16127156]
74. Guerau-de-Arellano M, Alroy J, Bullard D, et al. Aggravated Lyme carditis in CD11a^{-/-} and CD11c^{-/-} mice. *Infect Immun* 2005;73(11):7637–7643. [PubMed: 16239568]
75. Crandall H, Dunn DM, Ma Y, et al. Gene expression profiling reveals unique pathways associated with differential severity of Lyme arthritis. *J Immunol* 2006;177(11):7930–7942. [PubMed: 17114465]
76. Hirschfeld M, Kirschning CJ, Schwandner R, et al. Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J Immunol* 1999;163(5):2382–2386. [PubMed: 10452971]
77. Wooten RM, Ma Y, Yoder RA, et al. Toll-like receptor 2 is required for innate, but not acquired, host defense to *Borrelia burgdorferi*. *J Immunol* 2002;168:348–355. [PubMed: 11751980]
78. Wang G, Ma Y, Buyuk A, et al. Impaired host defense to infection and Toll-like receptor 2-independent killing of *Borrelia burgdorferi* clinical isolates in TLR2-deficient C3H/HeJ mice. *FEMS Microbiol Lett* 2004;231(2):219–225. [PubMed: 14987768]
79. Bolz DD, Sundsbak RS, Ma Y, et al. MyD88 plays a unique role in host defense but not arthritis development in Lyme disease. *J Immunol* 2004;173(3):2003–2010. [PubMed: 15265935]
80. Liu N, Montgomery RR, Barthold SW, et al. Myeloid differentiation antigen 88 deficiency impairs pathogen clearance but does not alter inflammation in *Borrelia burgdorferi*-infected mice. *Infect Immun* 2004;72(6):3195–3203. [PubMed: 15155621]
81. Behera AK, Hildebrand E, Bronson RT, et al. MyD88 deficiency results in tissue-specific changes in cytokine induction and inflammation in interleukin-18-independent mice infected with *Borrelia burgdorferi*. *Infect Immun* 2006;74(3):1462–1470. [PubMed: 16495516]
82. Bockenstedt LK, Liu N, Schwartz I, et al. MyD88 deficiency enhances acquisition and transmission of *Borrelia burgdorferi* by *Ixodes scapularis* ticks. *Infect Immun* 2006;74(4):2154–2160. [PubMed: 16552045]
83. Kurtenbach K, Sewell H-S, Ogden NH, et al. Serum complement sensitivity as a key factor in Lyme disease ecology. *Infect Immun* 1998;66(3):1248–1251. [PubMed: 9488421]

84. Lawrenz MB, Wooten RM, Zachary JF, et al. Effect of complement component C3 deficiency on experimental Lyme borreliosis in mice. *Infect Immun* 2003;71(8):4432–4440. [PubMed: 12874322]
85. Woodman ME, Cooley AE, Miller JC, et al. *Borrelia burgdorferi* binding of host complement regulator factor H is not required for efficient mammalian infection. *Infect Immun* 2007;75(6):3131–3139. [PubMed: 17420242]
86. Magnarelli LA, Anderson JF, Hyland KE, et al. Serologic analyses of *Peromyscus leucopus*, a rodent reservoir for *Borrelia burgdorferi*, in northeastern United States. *J Clin Microbiol* 1988;26(6):1138–1141. [PubMed: 3384925]
87. Schwan TG, Kime KK, Schrupf ME, et al. Antibody response in white-footed mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). *Infect Immun* 1989;57:3445–3451. [PubMed: 2807530]
88. Barthold SW, Bockenstedt LK. Passive immunizing activity of sera from mice infected with *Borrelia burgdorferi*. *Infect Immun* 1993;61:4696–4702. [PubMed: 8406868]
89. Barthold SW, deSouza M, Feng S. Serum-mediated resolution of Lyme arthritis in mice. *Lab Invest* 1996;74(1):57–67. [PubMed: 8569198]
90. Schaible UE, Wallich R, Kramer MD, et al. Protection against *Borrelia burgdorferi* infection in SCID mice is conferred by presensitized spleen cells and partially by B but not T cells alone. *Int Immunol* 1994;6(5):671–681. [PubMed: 8080839]
91. Barthold SW, Sidman CL, Smith AL. Lyme borreliosis in genetically resistant and susceptible mice with severe combined immunodeficiency. *Am J Trop Med Hyg* 1992;47:605–613. [PubMed: 1449201]
92. Schwan TG, Karstens RH, Schrupf ME, et al. Changes in antigenic reactivity of *Borrelia burgdorferi*, the Lyme disease spirochete, during persistent infection in mice. *Can J Microbiol* 1991;37:450–454. [PubMed: 1913349]
93. Revel AT, Talaat AM, Norgard MV. DNA microarray analysis of differential gene expression in *Borrelia burgdorferi*, the Lyme disease spirochete. *Proc Natl Acad Sci USA* 2002;99(3):1562–1567. [PubMed: 11830671]
94. Ojaimi C, Brooks C, Casjens S, et al. Profiling temperature-induced changes in *Borrelia burgdorferi* gene expression using whole genome arrays. *Infect Immun* 2003;71(4):1689–1705. [PubMed: 12654782]
95. Tokarz R, Anderton JM, Katona LI, et al. Combined effects of blood and temperature shift on *Borrelia burgdorferi* gene expression as determined by whole genome DNA array. *Infect Immun* 2004;72(9):5419–5432. [PubMed: 15322040]
96. Brooks CS, Hefty PS, Jolliff SE, et al. Global analysis of *Borrelia burgdorferi* genes regulated by mammalian host-specific signals. *Infect Immun* 2003;71(6):3371–3383. [PubMed: 12761121]
97. Narasimhan S, Santiago F, Koski RA, et al. Examination of the *Borrelia burgdorferi* transcriptome in *Ixodes scapularis* during feeding. *J Bacteriol* 2002;184(11):3122–3125. [PubMed: 12003955]
98. Liang FT, Nelson FK, Fikrig E. Molecular adaptation of *Borrelia burgdorferi* in the murine host. *J Exp Med* 2002;196(2):275–280. [PubMed: 12119353]
99. Hodzic E, Feng S, Freet KJ, et al. *Borrelia burgdorferi* population kinetics and selected gene expression at the host-vector interface. *Infect Immun* 2002;70(7):3382–3388. [PubMed: 12065476]
100. Hodzic E, Feng S, Freet KJ, et al. *Borrelia burgdorferi* population dynamics and prototype gene expression during infection of immunocompetent and immunodeficient mice. *Infect Immun* 2003;71(9):5042–5055. [PubMed: 12933847]
101. Narasimhan S, Caimano MJ, Liang FT, et al. *Borrelia burgdorferi* transcriptome in the central nervous system of non-human primates. *Proc Natl Acad Sci USA* 2003;100(26):15953–15958. [PubMed: 14671329]
102. Crother CR, Champion CI, Wu XY, et al. Antigenic composition of *Borrelia burgdorferi* during infection of SCID mice. *Infect Immun* 2003;71:3419–3428. [PubMed: 12761126]
103. Crother TR, Champion CI, Whitelegge JP, et al. Temporal analysis of the antigenic composition of *Borrelia burgdorferi* during infection in rabbit skin. *Infect Immun* 2004;72(9):5063–5072. [PubMed: 15321999]

104. Schwan TG, Burgdorfer W, Garon CF. Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of *in vitro* cultivation. *Infect Immun* 1988;56:1831–1836. [PubMed: 3397175]
105. Norris SJ, Howell JK, Garza SA, et al. High- and low-infectivity phenotypes of clonal populations of *in vitro*-cultured *Borrelia burgdorferi*. *Infect Immun* 1995;63(6):2206–2212. [PubMed: 7768600]
106. Xu Y, Kodner C, Coleman L, et al. Correlation of plasmids with infectivity of *Borrelia burgdorferi* sensu stricto type strain B31. *Infect Immun* 1996;64(9):3870–3876. [PubMed: 8751941]
107. Purser JE, Norris SJ. Correlation between plasmid content and infectivity in *Borrelia burgdorferi*. *Proc Natl Acad Sci USA* 2000;97(25):13865–13870. [PubMed: 11106398]
108. Labandeira-Rey M, Skare JT. Decreased infectivity in *Borrelia burgdorferi* strain B31 is associated with loss of linear plasmid 25 or 28-1. *Infect Immun* 2001;69(1):446–455. [PubMed: 11119536]
109. Purser JE, Lawrenz MB, Caimano MJ, et al. A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of *Borrelia burgdorferi* in a mammalian host. *Mol Microbiol* 2003;48(3):753–764. [PubMed: 12694619]
110. Jewett MW, Lawrence K, Bestor AC, et al. The critical role of the linear plasmid lp36 in the infectious cycle of *Borrelia burgdorferi*. *Mol Microbiol* 2007;64(5):1358–1374. [PubMed: 17542926]
111. Posey JE, Gherardini FC. Lack of a role for iron in the Lyme disease pathogen. *Science* 2000;288(5471):1651–1653. [PubMed: 10834845]
112. Liang FT, Jacobs MB, Bowers LC, et al. An immune evasion mechanism for spirochetal persistence in Lyme borreliosis. *J Exp Med* 2002;195(4):415–422. [PubMed: 11854355]
113. Grimm D, Tilly K, Byram R, et al. Outer-surface protein C of the Lyme disease spirochete: A protein induced in ticks for infection of mammals. *Proc Natl Acad Sci USA* 2004;101(9):3142–3147. [PubMed: 14970347]
114. Tilly K, Krum JG, Bestor A, et al. *Borrelia burgdorferi* OspC protein required exclusively in a crucial early stage of mammalian infection. *Infect Immun* 2006;74(6):3554–3564. [PubMed: 16714588]
115. Stewart PE, Wang X, Bueschel DM, et al. Delineating the requirement for the *Borrelia burgdorferi* virulence factor OspC in the mammalian host. *Infect Immun* 2006;74(6):3547–3553. [PubMed: 16714587]
116. Tilly K, Bestor A, Jewett MW, et al. Rapid clearance of Lyme disease spirochetes lacking OspC from skin. *Infect Immun* 2007;75(3):1517–1519. [PubMed: 17158906]
117. Labandeira-Rey M, Seshu J, Skare JT. The absence of linear plasmid 25 or 28-1 of *Borrelia burgdorferi* dramatically alters the kinetics of experimental infection via distinct mechanisms. *Infect Immun* 2003;71(8):4608–4613. [PubMed: 12874340]
118. Bankhead T, Chaconas G. The role of VlsE antigenic variation in the Lyme disease spirochete: persistence through a mechanism that differs from other pathogens. *Mol Microbiol* 2007;65(6):1547–1558. [PubMed: 17714442]
119. Pal U, Yang X, Chen M, et al. OspC facilitates *Borrelia burgdorferi* invasion of *Ixodes scapularis* salivary glands. *J Clin Invest* 2004;113(2):220–230. [PubMed: 14722614]
120. Fingerle V, Goettner G, Gern L, et al. Complementation of a *Borrelia afzelii* OspC mutant highlights the crucial role of OspC for dissemination of *Borrelia afzelii* in *Ixodes ricinus*. *Int J Med Microbiol* 2007;297(2):97–107. [PubMed: 17267282]
121. Montgomery RR, Malawista SE, Feen KJM, et al. Direct demonstration of antigenic substitution of *Borrelia burgdorferi* ex vivo: exploration of the paradox of the early immune response to outer surface proteins A and C in Lyme disease. *J Exp Med* 1996;183:261–269. [PubMed: 8551229]
122. Fingerle V, Liegl G, Munderloh U, et al. Expression of outer surface proteins A and C of *Borrelia burgdorferi* in *Ixodes ricinus* ticks removed from humans. *Med Microbiol Immunol* 1998;187(2):121–126. [PubMed: 9832327]
123. Liang FT, Yan J, Mbow ML, et al. *Borrelia burgdorferi* changes its surface antigenic expression in response to host immune responses. *Infect Immun* 2004;72(10):5759–5767. [PubMed: 15385475]

124. Kumaran D, Eswaramoorthy S, Luft BJ, et al. Crystal structure of outer surface protein C (OspC) from the Lyme disease spirochete, *Borrelia burgdorferi*. EMBO J 2001;20(5):971–978. [PubMed: 11230121]
125. Eicken C, Sharma V, Klabunde T, et al. Crystal structure of Lyme disease antigen outer surface protein C from *Borrelia burgdorferi*. J Biol Chem 2001 March 30;276:10010–10015. [PubMed: 11139584]
126. Wilske B, Jauris-Heipke S, Labentanzer R, et al. Phenotypic analysis of outer surface protein C (OspC) of *Borrelia burgdorferi* sensu lato by monoclonal antibodies: relationship to genospecies and OspA serotype. J Clin Microbiol 1995;33:103–109. [PubMed: 7699024]
127. Wang IN, Dykhuizen DE, Qiu W, et al. Genetic diversity of *ospC* in a local population of *Borrelia burgdorferi* sensu stricto. Genetics 1999;151(1):15–30. [PubMed: 9872945]
128. Seinost G, Dykhuizen DE, Dattwyler RJ, et al. Four clones of *Borrelia burgdorferi* sensu stricto cause invasive infection in humans. Infect Immun 1999;67(7):3518–3524. [PubMed: 10377134]
129. Brisson D, Dykhuizen DE. *ospC* diversity in *Borrelia burgdorferi*: different hosts are different niches. Genetics 2004;168(2):713–722. [PubMed: 15514047]
130. Alghaferi MY, Anderson JM, Park J, et al. *Borrelia burgdorferi ospC* heterogeneity among human and murine isolates from a defined region of northern Maryland and southern Pennsylvania: lack of correlation with invasive and noninvasive genotypes. J Clin Microbiol 2005;43(4):1879–1884. [PubMed: 15815012]
131. Lagal V, Portnoi D, Faure G, et al. *Borrelia burgdorferi* sensu stricto invasiveness is correlated with OspC-plasminogen affinity. Microbes Infect 2006;8(3):645–652. [PubMed: 16513394]
132. Earnhart CG, Buckles EL, Dumler JS, et al. Demonstration of OspC type diversity in invasive human Lyme disease isolates and identification of previously uncharacterized epitopes that define the specificity of the OspC murine antibody response. Infect Immun 2005;73(12):7869–7877. [PubMed: 16299277]
133. Fuchs H, Wallich R, Simon MM, et al. The outer surface protein A of the spirochete *Borrelia burgdorferi* is a plasmin(ogen) receptor. Proc Natl Acad Sci USA 1994;91:12594–12598. [PubMed: 7809084]
134. Hu LT, Perides G, Noring R, et al. Binding of human plasminogen to *Borrelia burgdorferi*. Infect Immun 1995;63(9):3491–3496. [PubMed: 7642282]
135. Coleman JL, Gebbia JA, Piesman J, et al. Plasminogen is required for efficient dissemination of *B. burgdorferi* in ticks and for enhancement of spirochetemia in mice. Cell 1997;89:1111–1119. [PubMed: 9215633]
136. Kraiczy P, Skerka C, Brade V, et al. Further characterization of complement regulator-acquiring surface proteins of *Borrelia burgdorferi*. Infect Immun 2001;69(12):7800–7809. [PubMed: 11705962]
137. Hellwage J, Meri T, Heikkilä T, et al. The complement regulator factor H binds to the surface protein OspE of *Borrelia burgdorferi*. J Biol Chem 2001;276(11):8427–8435. [PubMed: 11113124]
138. Alitalo A, Meri T, Ramo L, et al. Complement evasion by *Borrelia burgdorferi*: serum-resistant strains promote C3b inactivation. Infect Immun 2001;69(6):3685–3691. [PubMed: 11349031]
139. Stevenson B, El-hage N, Hines MA, et al. Differential binding of host complement inhibitor factor H by *Borrelia burgdorferi* Erp surface proteins: a possible mechanism underlying the expansive host range of Lyme disease spirochetes. Infect Immun 2002;70(2):491–497. [PubMed: 11796574]
140. Kraiczy P, Hellwage J, Skerka C, et al. Complement resistance of *Borrelia burgdorferi* correlates with the expression of BbCRASP-1, a novel linear plasmid-encoded surface protein that interacts with human factor H and FHL-1 and is unrelated to Erp proteins. J Biol Chem 2004;279(4):2421–2429. [PubMed: 14607842]
141. Hartmann K, Corvey C, Skerka C, et al. Functional characterization of BbCRASP-2, a distinct outer membrane protein of *Borrelia burgdorferi* that binds host complement regulators factor H and FHL-1. Mol Microbiol 2006;61(5):1220–1236. [PubMed: 16925556]
142. Lawrenz MB, Wooten RM, Norris SJ. Effects of *vlsE* complementation on the infectivity of *Borrelia burgdorferi* lacking the linear plasmid lp28-1. Infect Immun 2004;72(11):6577–6585. [PubMed: 15501789]

143. Cabello FC, Godfrey HP, Newman SA. Hidden in plain sight: *Borrelia burgdorferi* and the extracellular matrix. *Trends Microbiol* 2007;15(8):350–354. [PubMed: 17600717]
144. Coburn J, Fischer JR, Leong JM. Solving a sticky problem: new genetic approaches to host cell adhesion by the Lyme disease spirochete. *Mol Microbiol* 2005;57(5):1182–1195. [PubMed: 16101994]
145. Guo BP, Brown EL, Dorward DW, et al. Decorin-binding adhesins from *Borrelia burgdorferi*. *Mol Microbiol* 1998;30(4):711–723. [PubMed: 10094620]
146. Probert WS, Johnson BJ. Identification of a 47 kDa fibronectin-binding protein expressed by *Borrelia burgdorferi* isolate B31. *Mol Microbiol* 1998;30(5):1003–1015. [PubMed: 9988477]
147. Parveen N, Cornell K, Bono J, et al. Bgp, a secreted GAG-binding protein of *Borrelia burgdorferi* strain N40, displays nucleosidase activity and is not essential for infection of immunodeficient mice. *Infect Immun* 2006;74(5):3016–3020. [PubMed: 16622242]
148. Coburn J, Cugini C. Targeted mutation of the outer membrane protein P66 disrupts attachment of the Lyme disease agent, *Borrelia burgdorferi*, to integrin $\alpha_v\beta_3$. *Proc Natl Acad Sci USA* 2003;100(12):7301–7306. [PubMed: 12748384]
149. Shi Y, Xu Q, Seemanapalli SV, et al. The *dbpBA* locus of *Borrelia burgdorferi* is not essential for infection of mice. *Infect Immun* 2006;74(11):6509–6512. [PubMed: 16954404]
150. Seshu J, Esteve-Gassent MD, Labandeira-Rey M, et al. Inactivation of the fibronectin-binding adhesin gene *bbk32* significantly attenuates the infectivity potential of *Borrelia burgdorferi*. *Mol Microbiol* 2006;59(5):1591–1601. [PubMed: 16468997]
151. Li X, Liu X, Beck DS, et al. *Borrelia burgdorferi* lacking BBK32, a fibronectin-binding protein, retains full pathogenicity. *Infect Immun* 2006;74(6):3305–3313. [PubMed: 16714558]
152. Hübner A, Wang X, Nolen DM, et al. Expression of *Borrelia burgdorferi* OspC and DbpA is controlled by a RpoN-RpoS regulatory pathway. *Proc Natl Acad Sci USA* 2001;98(22):12724–12729. [PubMed: 11675503]
153. Fisher MA, Grimm D, Henion AK, et al. *Borrelia burgdorferi* sigma54 is required for mammalian infection and vector transmission but not for tick colonization. *Proc Natl Acad Sci USA* 2005;102(14):5162–5167. [PubMed: 15743918]
154. Yang XF, Alani SM, Norgard MV. The response regulator Rrp2 is essential for the expression of major membrane lipoproteins in *Borrelia burgdorferi*. *Proc Natl Acad Sci USA* 2003;100(19):11001–11006. [PubMed: 12949258]
155. Caimano MJ, Iyer R, Eggers CH, et al. Analysis of the RpoS regulon in *Borrelia burgdorferi* in response to mammalian host signals provides insight into RpoS function during the enzootic cycle. *Mol Microbiol* 2007;65(5):1193–1217. [PubMed: 17645733]
156. Piesman J, Oliver JR, Sinsky RJ. Growth kinetics of the Lyme disease spirochete (*Borrelia burgdorferi*) in vector ticks (*Ixodes dammini*). *Am J Trop Med Hyg* 1990;42:352–357. [PubMed: 2331043]
157. de Silva AM, Fikrig E. Growth and migration of *Borrelia burgdorferi* in *Ixodes* ticks during blood feeding. *Am J Trop Med Hyg* 1995;53:397–404. [PubMed: 7485694]
158. Piesman J, Schneider BJ, Zeidner NS. Use of quantitative PCR to measure density of *Borrelia burgdorferi* in the midgut and salivary glands of feeding tick vectors. *J Clin Microbiol* 2001;39(11):4145–4148. [PubMed: 11682544]
159. de Silva AM, Telford SR III, Brunet LR, et al. *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. *J Exp Med* 1996;183:271–275. [PubMed: 8551231]
160. Ohnishi J, Piesman J, de Silva AM. Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. *Proc Natl Acad Sci USA* 2001;98(2):670–675. [PubMed: 11209063]
161. de Silva AM, Fish D, Burkot TR, et al. OspA antibodies inhibit the acquisition of *Borrelia burgdorferi* by *Ixodes* ticks. *Infect Immun* 1997;65(8):3146–3150. [PubMed: 9234767]
162. Pal U, de Silva AM, Montgomery RR, et al. Attachment of *Borrelia burgdorferi* within *Ixodes scapularis* mediated by outer surface protein. *A. J Clin Invest* 2000;106(4):561–569.
163. Yang XF, Pal U, Alani SM, et al. Essential role for OspA/B in the life cycle of the Lyme disease spirochete. *J Exp Med* 2004;199(5):641–648. [PubMed: 14981112]

164. Fikrig E, Pal U, Chen M, et al. OspB antibody prevents *Borrelia burgdorferi* colonization of *Ixodes scapularis*. *Infect Immun* 2004;72(3):1755–1759. [PubMed: 14977984]
165. Boylan JA, Posey JE, Gherardini FC. *Borrelia* oxidative stress response regulator, BosR: a distinctive Zn-dependent transcriptional activator. *Proc Natl Acad Sci USA* 2003;100(20):11684–11689. [PubMed: 12975527]
166. Neelakanta G, Li X, Pal U, et al. Outer surface protein B is critical for *Borrelia burgdorferi* adherence and survival within *Ixodes* ticks. *PLoS Pathog* 2007;3(3):e33. [PubMed: 17352535]
167. Revel AT, Blevins JS, Almazan C, et al. *bptA* (*bbe16*) is essential for the persistence of the Lyme disease spirochete, *Borrelia burgdorferi*, in its natural tick vector. *Proc Natl Acad Sci USA* 2005;102(19):6972–6977. [PubMed: 15860579]
168. Li X, Pal U, Ramamoorthi N, et al. The Lyme disease agent *Borrelia burgdorferi* requires BB0690, a Dps homologue, to persist within ticks. *Mol Microbiol* 2007;63(3):694–710. [PubMed: 17181780]
169. Ribeiro JM, Alarcon-Chaidez F, Francischetti IM, et al. An annotated catalog of salivary gland transcripts from *Ixodes scapularis* ticks. *Insect Biochem Mol Biol* 2006;36(2):111–129. [PubMed: 16431279]
170. Pal U, Li X, Wang T, et al. TROSPA, an *Ixodes scapularis* receptor for *Borrelia burgdorferi*. *Cell* 2004;119(4):457–468. [PubMed: 15537536]
171. Anguita J, Ramamoorthi N, Hovius JWR, et al. Salp15, an *Ixodes scapularis* salivary protein, inhibits CD4+ T cell activation. *Immunity* 2002;16:849–859. [PubMed: 12121666]
172. Ramamoorthi N, Narasimhan S, Pal U, et al. The Lyme disease agent exploits a tick protein to infect the mammalian host. *Nature* 2005;436(7050):573–577. [PubMed: 16049492]
173. Narasimhan S, Sukumaran B, Bozdogan J, et al. A tick antioxidant facilitates the Lyme disease agent's successful migration from the mammalian host to the arthropod vector. *Cell Host Microbe* 2007;2:7–18. [PubMed: 18005713]

Fig. 1.

B. burgdorferi outer surface proteins are differentially regulated in response to host conditions. Spirochetes remodel their outer surface in different host environments, represented above by different colors. In the unfed tick, *B. burgdorferi* (represented in blue) produce a variety of proteins, such as OspA, to persist within the tick midgut for extended periods of time. Once a tick has attached to a vertebrate host, *B. burgdorferi* (now represented in red) expresses other proteins (e.g. OspC) in preparation for transmission to the new host. During infection of the mammalian host, *B. burgdorferi* (colored in yellow) expresses a variety of other proteins (including VlsE), presumably to survive attack by the host immune system, disseminate to distant sites within the host, and acquire specific nutrients. This figure shows representative proteins and is not meant to be a comprehensive list.