Lyme borreliosis (Lyme disease): molecular and cellular pathobiology and prospects for prevention, diagnosis and treatment

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Lyme borreliosis is a systemic infection caused by the spirochaete *Borrelia burgdorferi*, which is transmitted by tick bites and maintained in a delicately balanced ecological cycle. Recent increases in the population densities of tick hosts, the abundance of ticks and the proximity of man to natural tick habitats have led to an escalating worldwide incidence of Lyme borreliosis, and nonspecific clinical manifestations have yielded significant misunderstanding of the disease. After entry, *B. burgdorferi* activates local inflammation, yet evades host defences and facilitates dissemination by potentially masquerading with host components such as plasmin and complement. The extent of tissue injury is determined by the aggressiveness of host inflammation and immunological reactions, as well as by genetic attributes of the spirochaete. The clinical presentation can be highly varied, including early manifestations that are limited...
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Since the original description of Lyme disease in 1977 (Ref. 1), and the identification of the spirochaetal aetiology in 1982 (Ref. 2), infection caused by Borrelia burgdorferi sensu lato has become recognised as the most common vector-borne disease in the USA and Western Europe. The spirochaete is an obligate parasite of vertebrates and arthropods and, in the USA, is transmitted by the bite of Ixodes scapularis or Ixodes pacificus ticks; Ixodes ricinus is the main vector in Europe and Ixodes persulcatus in Asia, where other Borrelia species including Borrelia garinii and Borrelia afzelii also cause illness.

Although it is not a documented cause of death, Lyme disease has become a highly visible illness among the public because of fears and apprehension about its chronic disabling symptoms. This high visibility has been abetted by the misunderstood application of diagnostic testing during this phase of illness, as well as by frequent misunderstandings regarding the spectrum of the illness by patients and clinicians alike. Despite significant gains in scientific knowledge during the past decade, much remains unknown about this tick-borne infection, and existing advances in molecular-based methods of detection have not yet gained much traction in clinical practice.

Epidemiology and transmission

Lyme disease is a notifiable disease in the USA, and a record 17 730 reported cases occurred during 2000, more than in any year previously, with the majority of disease having been found in the north-eastern and Mid-Atlantic states from Maine to Maryland, and north-central states of Minnesota and Wisconsin, although some disease also occurred in the west from Northern California to Oregon (Refs 3, 4). Although it is described throughout Europe in especially arboreal regions, Lyme borreliosis is most frequently reported in Sweden, Germany, Austria and Slovenia (Ref. 3).

The upsurge of Lyme disease in the USA in the late 20th century appears to be due to several factors affecting the primary vector I. scapularis, whose life cycle includes larval, nymphal and adult stages that require bloodmeals. The adult ticks prefer feeding on the dramatically rising populations of white-tailed deer (Odocoileus virginianus), whereas the larval and nymphal forms favour the white-footed mouse (Peromyscus leucopus), which explains the tendency for disease to be acquired in temperate coastal and riparian environments (an area of land directly influenced by water). Infection is transmitted horizontally, and up to 95% of mice and 50% of I. scapularis ticks may harbour B. burgdorferi in areas of high endemnicity (Refs 4, 5). Humans are incidental hosts that have increased in number as the Ixodes spp. tick vector and its mammalian hosts have spread and increased in abundance, and as human habitation has spread from more-urban environments to rural environments where the tick vectors and hosts also reside (Refs 6, 7, 8). Although dependent on many factors, including the percentage of infected ticks and the length of tick attachment, the overall risk of acquiring the infection from a single tick bite is <3.5% even in these areas, and is significantly lower in most regions including Europe (Refs 9, 10).

The incidence of Lyme disease peaks from late spring through to early summer, correlating with the time of increased tick populations, especially with...
biting nymphal stages, as well as with the increase in human activity outdoors (Ref. 11). Transmission is discussed in further detail below with reference to disease pathogenesis.

**Microbiology, ecology and pathogenesis**

The spirochaete *B. burgdorferi* is a long and narrow spiral-shaped bacterium (15 × 0.5 µm); the spiral shape results from bundles of periplasmic flagella that attach to the ends and twist around the bacterium (Fig. 1). This distinctive morphology and bundling of flagella allow the bacterium to twist or ‘cork-screw’ through tissue and medium. Eleven closely related species have been identified in the *B. burgdorferi* sensu lato group since the original description of the first Lyme disease spirochaete, *B. burgdorferi* (now sometimes referred to as *B. burgdorferi* sensu stricto) (Ref. 12). Of these, at least four are associated with clinical manifestations in humans that are characterised as Lyme disease or Lyme borreliosis, including *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii* and *Borrelia bissetti*. 

**Genes, proteins and molecular biology of *B. burgdorferi***

Among bacteria, *B. burgdorferi* is unusual in that it contains a small (910 kb) linear chromosome and up to 21 plasmids; 12 of these plasmids are linear and nine are circular, and together they account for an additional 610 kb (Ref. 13). The complete genomic sequence and the sequences of all the plasmids have been determined for *B. burgdorferi* s.s. strain B31 (Refs 13, 14). The genome of *B. burgdorferi* includes at least 132 genes that encode lipoproteins, and make up ~15% of all functionally complete open reading frames. The cell membranes have a simple ultrastructure and appear to have a thin layer of peptidoglycan, but are devoid of lipopolysaccharides. Most surface-exposed proteins on *B. burgdorferi* are lipoproteins, including outer surface proteins (Osp) A, B and C, among many others. Much attention has been given to both OspA and OspC, as each appears to be differentially regulated depending on the host type (tick versus mammal) and other factors such as temperature and pH (Ref. 15). OspA is relatively phylogenetically conserved among even distant genospecies, and is predominantly expressed in the tick vector, potentially as an adhesin by which the bacterium binds to midgut epithelial cells before the next tick bloodmeal (Refs 16, 17). Conversely, OspC is highly heterogeneous and is transiently expressed during and after the interval when the tick vector obtains a bloodmeal; thus, one speculation is that OspC has a direct role in the ability of the spirochaete to penetrate through the tick midgut and into the tick salivary tissues (Refs 15, 18). The recognition that only a few OspC genotypes are associated with bloodstream infection in humans also suggests a role for this lipoprotein in mammalian invasion and dissemination (Refs 19, 20). Invasiveness has also been linked to the restriction fragment length polymorphism patterns in the *rrs-rrl* (16S-23S ribosomal RNA gene) spacer, a non-coding region that is unlikely to be subjected to the environmental pressures that exist for OspC (Ref. 21). Definitive evidence of the functions of OspA and OspC still needs to be provided; however, with the advent of the genetic transformation of *B. burgdorferi*, it should be possible to produce strains that are specifically deficient in these proteins and ultimately elucidate Osp function more precisely (Refs 22, 23). The natural reservoir hosts for *B. burgdorferi* vary with genospecies, but small mammals...
such as mice and voles are often implicated (Refs 6, 24, 25). In North America, the white-footed mouse is an important reservoir that develops a persistent, life-long infection (Refs 26, 27). Because *B. burgdorferi* can persist in wild rodents for up to several years and the average life span of wild rodents may be only 1–2 years, the bacterium must have acquired mechanisms to evade the host immune response (Refs 15, 28, 29). Although many *B. burgdorferi* genes that code for lipoproteins such as Erps (OspEF family), MLPs and OppAV are upregulated with the increased temperature expected with mammalian infection, others are not, perhaps suggesting they play an important role in the bacterium’s survival in vectors that live at lower ambient temperatures (Refs 15, 30, 31, 32). One important family of genes, the variable membrane-like sequence genes (*vls*), which are found as a locus on linear plasmid 28 (lp28), undergo a high degree of genetic recombination in the first 30 days of mammalian infection, but not in vitro (Refs 28, 33). *B. burgdorferi* that lack lp28 are poorly able to infect mice, suggesting a potentially important role for the *vls* family (Refs 34, 35). The gene locus contains 15 silent gene cassettes that may be recombined by gene conversion into an active expression site (*vlsE*) (Ref. 36) (Fig. 2). Because each cassette possesses conserved flanking 5’ and 3’ regions surrounding a variable internal region, the potential to generate diverse VlsE proteins that would allow immune evasion has been suggested (Ref. 28). Similar rearrangements have been observed in other Osp families, suggesting that environmental signals of the host may significantly alter the antigenic profile and potential biological responses of the spirochaete (Refs 29, 37).

Other proteins and lipoproteins of *B. burgdorferi* s.l. that have been studied in some depth include the 34-kDa OspB protein, decorin-binding proteins of 19 kDa and 20 kDa, a fibronectin-binding protein (BBK32), and the OspEF or Erp family proteins that are encoded by 17 genes in 10 distinct loci and bind complement inhibitor factor H (Refs 38, 39, 40, 41, 42).

**Ecology and pathogenesis**

The ecology of *B. burgdorferi* and pathogenesis of Lyme disease are complex and have multiple stages, and are determined largely by the specific adaptations of the bacterium to the diverse host niches that it encounters. Persistent infection must be present in the tissues, interstitial fluids and, potentially, blood of small mammals, if an uninfected immature tick (larval or nymphal stage) is to acquire *B. burgdorferi* from a host in the bloodmeal. With the lower temperature of the tick, the spirochaete initially binds to the midgut epithelium of the tick and to other borreliae after the upregulated expression of OspA (Refs 16, 18). It may persist in this location for several weeks to months, even through molts; this is known as trans-stadial passage, which indicates its passage to each subsequent stage of vector life. However, tick progeny are not infected by *B. burgdorferi* because transovarian transmission is highly inefficient. Once the next stage has emerged, either as a nymph or adult that has the spirochaete in its midgut, the tick will seek a bloodmeal generally from another animal, occasionally humans. With the entry of warm blood and a consequent drop in pH from 7.4 to 6.8, among other potential factors that prompt replication, the spirochaete replicates >300-fold over several days; replication is accompanied by a burst of gene conversion, recombination and rearrangement (Refs 15, 43, 44, 45) (Fig. 3). Changes include genetic recombination and transcriptional regulation of several important lipoprotein genes (Refs 30, 31). In particular, the expression of OspA protein, which is predominantly expressed on spirochaete surfaces in the tick midgut and is believed to enable long-term adherence to midgut epithelial cells, is now downregulated, and the spirochaete disattaches (Refs 15, 18). The increased production of OspC, which peaks at 48 hours after tick attachment, is closely associated with the penetration of the spirochaete through the midgut limiting membrane, into the haemocele, and into tick tissues, including the salivary gland (Refs 15, 18). The entire process of transcriptional switching from OspA to OspC and the passage of spirochaetes from midgut to salivary gland requires ~48 hours, which is consistent with the known ‘grace period’ during which many ticks that harbour *B. burgdorferi* are ineffective at transmission (Ref. 46). The result is a population of *B. burgdorferi* in the salivary gland that no longer expresses OspA. The occasional lack of OspC expression in salivary spirochaetes may indicate that it is not needed for mammalian infection; however, further study is required (Refs 15, 18).

In addition to the changes in OspA and OspC regulation, the burst of gene regulatory and
transcriptional activity results in many different spirochaete phenotypes that enter into the tick haemocele and salivary glands, as well as into the mammalian host (Refs 15, 18, 47). Presumably, the advantage of such a heterogeneous population that has undergone diverse gene recombination is that some phenotypes will be fitter for survival in the mammalian environment. These may then initiate local infection and disseminate (Refs 19, 48). Although unproven, this phenotypic diversity could result in populations of *B. burgdorferi* that are better able to penetrate into...
Figure 3. Superimposition of the changes in *Borrelia burgdorferi* protein and lipoprotein expression during acquisition from an infected mammalian host and penetration into tick tissues and the spirochaete-vector-host cycle.

Superimposition of the changes in *Borrelia burgdorferi* protein and lipoprotein expression during acquisition from an infected mammalian host and penetration into tick tissues and the spirochaete-vector-host cycle. The patterns and changes in protein and lipoprotein expression are listed in association with each stage; proteins shown in bold have the highest expression levels. *B. burgdorferi* present in the interstitial dermal fluids of the infected mammalian reservoir host are depleted from the tick-induced inflammatory site and enter into the tick midgut (top). A large variety of lipoproteins and other proteins are expressed to yield a heterogeneous population, illustrated by the presence of multicoloured spirochaetes (9 o’clock). With time after digestion of the bloodmeal and after moulting, *B. burgdorferi* lipoprotein expression reduces, with the exception of OspA, which is believed to act as an adhesin for the spirochaete onto tick midgut epithelial cells (illustrated by single coloured spirochaetes at 7 o’clock). With the subsequent bloodmeal, *B. burgdorferi* in the midgut are activated and display a marked increase in gene transcription, lipoprotein expression, and genetic heterogeneity (5 o’clock). The expression of OspC is believed to be related to penetration of the midgut and dissemination into the tick haemocele and perhaps salivary gland (4 o’clock). The lack of both OspA expression in tick salivary gland and the marked heterogeneity of lipoprotein expression (2 o’clock) allows many different populations to be inoculated into the dermis of the new mammalian host. The process is then repeated as the nymph moults into an adult. *B. burgdorferi* do not penetrate the tick ovaries; thus, eggs and newly emerged larvae are not infected.
dermal tissues and interstitial fluids, where another tick may again acquire them and continue the natural maintenance.

Once in the tick salivary gland, *B. burgdorferi* have ready access to the host dermis via the salivary secretions of the tick. Ticks generate an inflammatory blood pool in the mid-dermis of the host, from which erythrocytes, plasma and interstitial fluids are imbibed. In turn, the tick delivers a series of biologically active compounds that have anaesthetic, anti-inflammatory, anti-complementary and anti-coagulative properties, among others (Refs 49, 50). The specific genetic and molecular factors that regulate whether *B. burgdorferi* can infect human skin and cause Lyme disease are not fully understood (Ref. 51). However, it appears that the ability to infect and disseminate may be regulated in part by bacterial genetics and in part by the host. Several studies have shown genetic linkages between *B. burgdorferi* genes and infectivity in human skin and dissemination in humans. At least 21 *B. burgdorferi* OspC genotypes can be identified; eight OspC genotypes are identified among patients with erythema migrans (EM) but no evidence of dissemination, whereas only four OspC genotypes are found in patients with bloodstream dissemination (Ref. 19). Similarly, a genetic association of specific *B. burgdorferi* genotypes, based on the sequence of the 16S–23S rRNA intragenic spacer region, with bloodstream dissemination and fever was also identified in humans, and confirmed with controlled experiments in mice (Refs 48, 52).

*B. burgdorferi* that access the dermis of a human host after a tick bite may initiate a local infection. The spirochaetes initially spread through interstitial connective tissue matrix by virtue of several adaptations. The collagen-rich dermis provides ample decorin motifs to which the *B. burgdorferi* decorin-binding proteins DbpA and DbpB presumably facilitate binding and dissemination (Refs 53, 54). Likewise, the presence of a fibronectin-binding protein in *B. burgdorferi* and its ability to bind to glycosaminoglycans and integrins might also enhance local spread (Refs 40, 55, 56, 57). The hallmark features of the local lesion, EM (Fig. 4), are attributable predominantly to the host inflammatory and early immune response as the spirochaetes spread radially in the dermis from the site of the tick bite.

*B. burgdorferi* has several adaptations that could allow it to penetrate through tissue. It is well documented that the surface of the spirochaete can bind human plasminogen and that, in the presence of urokinase-type plasminogen activator (uPA), plasminogen can...
be cleaved into proteolytically active plasmin (Refs 58, 59, 60, 61, 62). Although plasmin is typically associated with the cleavage of proteins that are involved in coagulation, including clot resolution, it is a broadly active protease with numerous potential substrates. Plasmin stabilised on the surface of the spirochaete becomes resistant to the effects of its major regulator, α2-antiplasmin, as well as regulators of uPA, plasminogen activator inhibitor 1 (PAI-1) and 2 (PAI-2) (Ref. 63). Bacterial-surface-stabilised plasmin may degrade fibronectin, and release and activate the matrix metalloproteases (MMPs) gelatinase B (MMP-9) and collagenase 1 (MMP-1) (Refs 64, 65). In vitro, B. burgdorferi also stimulates the production of the urokinase receptor (CD87; uPAR) on cell surfaces, a potentially important virulence mechanism (Ref. 66). In turn, the activation of plasminogen to plasmin by uPA after B. burgdorferi upregulation of cellular uPAR facilitates penetration through the extracellular matrix or through microvasculature and might allow haematogenous dissemination (Refs 62, 67). Binding of plasmin is a critical factor because it is associated not only with dissemination in ticks but also with enhancement of spirochaetaemia in murine models of Lyme disease (Ref. 67).

Clinical manifestations, diagnosis and treatment

The characteristic presentations of Lyme disease are often placed into three different phases of infection based on the location and duration of the infection: early localised, early disseminated and late disease. There is no characteristic progression, and some cases of infection clearly have overlapping features. For reporting purposes in the USA, Lyme disease has been defined by the Centers for Disease Control and Prevention as outlined in Box 1, although these criteria are neither 100% sensitive nor specific. The diagnosis of Lyme disease remains heavily reliant on clinical manifestations (Table 1) and any available laboratory test must be interpreted in the context of a clinical history and physical examination.

Early localised disease

The transmission of B. burgdorferi appears to require a tick to be attached for at least 36–48 hours, otherwise the risk of infection appears to be low (Ref. 68). Within several days to a month afterwards, EM, a skin lesion that forms at the site of the tick attachment, is characteristically observed in early Lyme disease. The rash is the best clinical indicator of Lyme disease and is present in 90% of cases, although it can occasionally go unnoticed (Ref. 69). An estimated 10% of patients in North America, and perhaps >10% of those infected in Europe, may experience asymptomatic infection (Ref. 70). Most patients experience additional symptoms such as headache, muscle soreness, fever and malaise, although up to 20% may have only the rash. Over time, the rash enlarges to sizes often >15–20 cm.

Box 1. Lyme disease case definition for reporting in the USA as recommended by the Centers for Disease Control and Prevention

Erythema migrans, characteristically slowly enlarging over days to weeks into a large, round lesion sometimes with central clearing. In single-lesion presentations, it must be at least 5 cm (2 in) in diameter.

Or at least one later manifestation and laboratory evidence of infection in the following:

- nervous system: lymphocytic meningitis, cranial neuritis, radiculoneuropathy (a disease of the spinal nerve roots and nerves) or encephalomyelitis;
- cardiovascular system: advanced heart block (2nd or 3rd degree);
- musculoskeletal system: arthritis usually of large weight-bearing joints;
- laboratory evidence: serodiagnostic evidence of Borrelia burgdorferi by the two-tier testing approach of enzyme-linked immunosorbent assay followed by western blot.

Adapted from recommendations of the Centers for Disease Control and Prevention (Refs 142, 143).

If <1 month of symptoms, use IgM and IgG antibody responses. If >1 month, use only IgG responses, as positive IgM titres alone in this situation are likely to be false. IgM western blot is positive if two out of three bands (23, 39 and 41 kDa) are present; IgG western blot is positive if at least five out of ten bands (18, 23, 28, 30, 39, 41, 45, 58, 66 and 93 kDa) are present. May not be applicable for infections outside of North America.
with central clearing (Fig. 4a), earning the well-recognised moniker ‘bull’s eye rash’. The bull’s eye rash appears to be a minority presentation that is seen mostly in older and larger rashes, and one recent study suggested that a homogeneous centrally red rash (Fig. 4b) is seen most commonly in patients with microbiologically confirmed EM who sought medical attention at an early stage (Ref. 71).

Recent advances in laboratory diagnosis include skin and blood cultures for the isolation of \( B. \) burgdorferi and polymerase chain reaction (PCR) identification of \( B. \) burgdorferi nucleic acids in plasma or skin (Refs 72, 73, 74). Currently, no single diagnostic method is uniformly accurate in the assessment of early Lyme disease; most new techniques are not widely available and conventional serology has a low sensitivity. Clinical assessment by experienced healthcare personnel remains the most accurate method in endemic areas.

The treatment of early localised disease in adults involves the use of \( \beta \)-lactam antibiotics (such as amoxicillin or penicillin) or a member of the tetracycline class (e.g. doxycycline); tetracycline-based antibiotics are frequently employed by clinicians to cover the possibility of infection or co-infection with other tick-borne pathogens such as \( Ehrlichia \), \( Anaplasma \) or \( Rickettsia \) species. Regardless of antibiotic choice, >90% of patients have good outcomes with oral therapy (Ref. 75). The use of parenteral agents has not proven to be superior to oral medication in the treatment of early Lyme disease (Ref. 76).

<table>
<thead>
<tr>
<th>Assay</th>
<th>Specimen type</th>
<th>Clinical manifestation or phase</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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</thead>
<tbody>
<tr>
<td>PCR or other molecular diagnostic test</td>
<td>Skin</td>
<td>Erythema migrans</td>
<td>71</td>
<td>100</td>
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<tr>
<td></td>
<td></td>
<td>Acrodermatitis chronica atrophicans</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma/serum</td>
<td>Early localised or disseminated</td>
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<td>100</td>
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<td></td>
<td>Synovial fluid</td>
<td>Arthritis</td>
<td>65</td>
<td>99</td>
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<td></td>
<td>Cerebrospinal fluid</td>
<td>Meningitis/encephalopathy</td>
<td>24</td>
<td>100</td>
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<tr>
<td>Whole-cell ELISA</td>
<td>Serum</td>
<td>Early</td>
<td>40–60</td>
<td>72–94</td>
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<td>Early</td>
<td>75</td>
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<td>C6 peptide VlsE ELISA</td>
<td>Serum</td>
<td>Late†</td>
<td>100</td>
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<td>Serum</td>
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<td>Serum</td>
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<tr>
<td></td>
<td></td>
<td>Late</td>
<td>80–100</td>
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Abbreviations: ELISA, enzyme-linked immunoabsorbent assay; PCR, polymerase chain reaction; VlsE, variable membrane-like sequence E protein.

† A gradually progressive late skin manifestation comprising erythematous plaques appearing first on feet, hands, elbows or knees.

Table 1. Sensitivity and specificity of assays for the diagnosis of Lyme disease

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Early disseminated EM

Early localised EM may often progress to haematogenous dissemination within the first month, although end-organ effects can occur up to one year after primary infection in untreated patients. Multiple EM is an early sign of disseminated disease. Common presentations of disseminated infection include neurological disease that can afflict up to 15% of untreated patients mostly within 4–8 weeks of initial infection (Ref. 77). When investigated, the presence of fever, headache and neck stiffness might represent lymphocytic meningitis, which is the leading neurological finding of Lyme disease. Cranial neuritis affecting the peripheral seventh nerve (Bell’s Palsy) can occur in unilateral or bilateral fashion. Other manifestations described include mild encephalitis, cerebellar dysfunction, motor or sensory radiculoneuritis, mononeuropathy multiplex and myelitis (Ref. 78). Even if left untreated, most neurological deficits improve within weeks to months. Cardiac involvement occurs within several weeks of infectious onset, affecting 5–10% of untreated patients and most commonly manifesting as first-degree asymptomatic atrioventricular (AV) block; less frequently, an advanced AV nodal block produces symptoms that may require a temporary pacemaker to be fitted until antibiotic therapy corrects the inflammation causing electrical dysfunction (Ref. 79).

Suggested treatment of acute neurological disease that is associated with early disseminated Lyme disease involved a 2–4-week course of parenteral antibiotics. Therapy for cardiac disease secondary to Lyme disease, especially with AV nodal impairment, is often initiated with intravenous antibiotics until improvement occurs. After that, the course can be completed by oral therapy and there is no need to fit a permanent pacemaker. The problems of early disseminated Lyme disease tend to abate quickly, generally within weeks.

Late disease

Late manifestations of Lyme disease occur within weeks to months or even years after initial infection. The spectrum of disease is perhaps most varied in comparison between typical North American and European/Asian presentations.

Late dermatological disease has mostly been observed in Europe, where elderly women are especially prone to developing reddish-blue plaques called acrodermatitis chronica atrophicans (ACA) over dorsal aspects of upper and lower limbs in sun-exposed regions (Ref. 80). These lesions, almost exclusively due to B. afzelii, later turn atrophic or sclerotic, and may be associated with a late sensory polyneuropathy (Ref. 81). Culture of B. burgdorferi is unusual in many patients with late Lyme disease; however, spirochaetes have been cultured from the ACA lesions even 10 years after onset (Ref. 82).

Joint involvement may occur months after the onset of illness in up to 60% of untreated patients (Ref. 83). Large weight-bearing joints such as the knee are classically afflicted in a migratory fashion. Attacks are self-limiting even without treatment after a period of weeks or months, although persistent inflammation may develop after multiple episodes. Most patients afflicted with remitting untreated arthritis due to B. burgdorferi have positive B. burgdorferi IgG Western blots. In Europe and Asia, oligoarticular presentations of Lyme disease are observed less often than in North America, but this may be due to fewer intensely inflammatory joints and presentations being more varied (Refs 70, 84). Oral therapy is frequently employed first with arthritis, although parenteral antibiotics may be preferred if there is concern about the concurrent or subsequent development of neuroborreliosis (Ref. 85). Persistent joint inflammation due to Lyme disease is rare in Europe, whereas ~10% of Lyme arthritis patients in the USA, especially with HLA-DRB1*0401 or related alleles, may have ongoing arthritis despite robust courses of antibiotic therapy (Ref. 70). The lack of PCR detection of B. burgdorferi in these patients suggests there is no active infection, and it is possible that autoimmune mimicry may have developed (Ref. 86).

Chronic neurological disease can occur in up to 5% of untreated patients. Radiculopathic pain or sensory polyneuropathy has been well described in both Europe and the USA. B. garinii in Europe has been implicated as a cause of a severe chronic encephalomyelitis that may result in cognitive impairment, cranial nerve paralysis or paraparesis with easily detectable intrathecal antibodies securing the diagnosis (Ref. 78). In the USA, encephalopathic involvement causes less-severe disturbances, usually manifesting as mild neurocognitive dysfunction or depression often with demonstrable cerebrospinal-fluid-specific antibody production (Ref. 87). Chronic
neuroborreliosis requires 2–4 weeks of parenteral drug therapy and can take weeks to months for improvement to occur.

Despite appropriate treatment for Lyme disease, a few patients complain of persistent fatigue, musculoskeletal aches and neurocognitive impairments that appear to share features with chronic fatigue syndrome or fibromyalgia. This is sometimes referred to as ‘chronic Lyme disease’ and is a poorly understood phenomenon but might occur more frequently in untreated patients who have a history of nervous system involvement in early disseminated disease (Ref. 88). Importantly, these chronic symptoms do not respond to additional antibiotic therapy, although some are vocal advocates of chronic antibiotic therapy (Refs 89, 90). Moreover, especially in patients who have questionable histories or variable Lyme serological testing patterns, the link with spirochaetal infection must be questioned as the frequencies of pain and fatigue do not appear to differ from uninfected, age-matched controls from the general population (Ref. 91).

**Immunological mechanisms in Lyme disease**

**Immune reactions stimulated by* B. burgdorferi***

Several components of *B. burgdorferi* are known to elicit inflammatory responses, including OspA and OspB; however, neither of these proteins is substantially present in the *B. burgdorferi* bacteria that initially enter the host (Refs 18, 92). Other lipoproteins are also capable of stimulating the release of proinflammatory cytokines and substances that allow the activation of endothelial cells and recruitment of inflammatory cells (Refs 93, 94). Recent studies suggest that initial inflammation occurs as a result of innate immunity being activated by *B. burgdorferi* lipoprotein binding to toll-like receptor 2 (TLR-2) on macrophages, and is possibly also dependent on a macrophage CD14-associated mechanism (Refs 93, 95, 96). However, signalling of inflammation through TLR-2 does not account for all responses, because *B. burgdorferi* non-lipoprotein components can also initiate inflammatory and adaptive immunity (Ref. 97).

Local and disseminated infection with *B. burgdorferi* stimulates inflammatory and immune reactions, the latter evidenced by active humoral and cellular immunity. Based on animal models, it is widely believed that some aspects of protective immunity result from antibodies that opsonise and allow *B. burgdorferi* to be engulfed and destroyed by phagocytes or by complement-mediated lysis. The resolution of *B. burgdorferi*-induced arthritis in mice is dependent on T cells and specific IgG; in contrast, the resolution of carditis in the mouse model requires the participation of CD4 cells, notably interferon-γ (IFN-γ), and not antibody (Ref. 98). The earliest evidence of humoral immunity is ordinarily present within several weeks of a patient receiving a tick bite, and ~7 days or more after the recognition of EM. Both IgG and IgM antibodies can be detected early in infection, and the most frequent targets of antibody response at this time include FlaB (flagellin; 41 kDa) and OspC (21–23 kDa). In time, an increasing spectrum of *B. burgdorferi* antigens are targets for antibodies, occasionally including both OspA and OspB (which are rarely expressed during mammalian infection) and several lipoprotein antigens that may be associated with antigenic diversity and immune evasion or even autoimmunity (see below). The paradoxical efficacy of the recombinant OspA lipoprotein vaccine in humans is thought to lie in the ability of preformed antibodies in the bloodmeal to kill spirochaetes in the tick midgut, but not by a mechanism that is completely dependent on complement (Refs 99, 100, 101).

Most early disseminated and late manifestations of human Lyme disease are associated with inflammation at sites to which the spirochaetes have migrated. In humans with inflammatory sequelae such as arthritis or carditis, high levels of proinflammatory cytokines can be detected, including interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor α (TNF-α) (Refs 102, 103, 104). Such responses confirm an abundance of T helper 1 (Th1) cells versus T helper 2 (Th2) cells in the synovial fluid of patients who have chronic Lyme arthritis (Ref. 105). Experimental studies using a murine model of Lyme disease showed that arthritis and severe inflammatory reactions occurred in C3H mice and were less frequent or absent in BALB/c or C57/BL6 (B6) mice (Refs 106, 107, 108). The severity of the inflammatory reactions is attributed to the stimulation of Th1 immunity in C3H mice, characterised by high levels of interleukin 12 (IL-12), IFN-γ and IL-2; by contrast, resistance in BALB/c and B6 mice is attributable to the...
predominant Th2 responses, characterised by the presence of interleukin 4 (IL-4) and interleukin 10 (IL-10), and presumably dominated by the production of protective, complement-fixing or -opsonising antibodies (Refs 108, 109). The availability of gene-disrupted (knockout) mice has allowed an intensive investigation of specific immunological parameters that are associated with disease in the murine models. Infected B-cell-deficient mice develop severe arthritis, and adoptive transfer of naive B and T cells into infected B6-Rag1-knockout mice that lack functional B, T and natural killer (NK) cells resolves infection and inflammatory lesions (Ref. 108). Paradoxically, reconstitution of infected B6-Rag1-knockout mice with T cells results in severe destructive arthritis and myocarditis, suggesting that under some circumstances, T-cell-mediated immunopathology may be a part of Lyme disease. Thus, the human polymorphisms of T-cell response probably play a significant role in protective or deleterious inflammatory responses to B. burgdorferi.

Recently, evidence has emerged to suggest that B. burgdorferi might stimulate autoimmune responses that mediate some aspects of treatment-resistant Lyme disease (Refs 110, 111, 112). B. burgdorferi is rarely detected in patients who have arthritis or neurological manifestations after appropriate therapy, yet in some patients, inflammatory sequelae continue for many years despite the apparent eradication of the spirochaete (Refs 89, 113, 114). One potential explanation is the occurrence of molecular mimicry between specific epitopes of OspA and leukocyte function-associated antigen 1 (LFA-1) because synovial fluid T cells from some patients with treatment-resistant arthritides responded to both OspA and LFA-1 (Refs 111, 112). Additionally, components of B. burgdorferi flagellin might also induce antibodies with specificity for certain heat shock proteins (e.g. HSP60) that are expressed within neurons in vitro, and these antibodies might block spontaneous and peptide-hormone-stimulated neuritogenesis (Ref. 110). In fact, sera from patients who have neurological manifestations of Lyme disease have antibodies that react with neurons in a manner identical to that of a monoclonal antibody specific for the suspected component of HSP60. This latter mechanism might not be associated with an inflammatory response yet could still mediate clinical manifestations. In spite of this emerging data, few other convincing arguments that Lyme disease is in part autoimmune have emerged, and convincing arguments against autoimmunity have been advanced (Ref. 115).

Evasion of host immune mechanisms
Under natural circumstances, B. burgdorferi must persist in its small-mammal host to allow subsequent transmission into a tick via the bloodmeal. In fact, most of the early disseminated manifestations and some of the late-disseminated manifestations of human Lyme disease are known to result from persistent infection (Refs 116, 117). Given that infection stimulates an active protective antibody response in addition to potentially damaging T-cell responses, the spirochaete must have developed specific adaptations for survival. Aside from the masking of surface lipoproteins and other antigenic components by coating with host plasmin and other proteins (Refs 58, 59), B. burgdorferi evades immune destruction by several mechanisms.

It has recently been recognised that certain surface proteins of B. burgdorferi s.s., such as OspE and related protein families (Erps), bind the complement inhibitor factor H (Refs 42, 118), and that strains of B. burgdorferi s.l. possess other surface lipoproteins called complement-regulatory-acquiring surface proteins (CRASP)s that also bind complement regulatory components (Ref. 119). The net effect of binding factor H or factor H-like protein-1 (which is also known as reconectin) imparts resistance to deposition of the complement factors C5b to C9, which comprise the complement membrane attack complex that would otherwise disrupt the membrane and destroy B. burgdorferi. As the presumed major mechanism of the recombinant OspA vaccine (see below) is to fix complement and to lyse B. burgdorferi in the tick midgut before haemocoele penetration (Refs 99, 100), abrogation of complement lysis is potentially a significant adaptive advantage for the spirochaete (Ref. 120). One speculation is that the host specificity of some strains of B. burgdorferi s.l. depends on CRASP that have higher specificity for the complement regulatory proteins of other mammalian hosts, thus potentially explaining why only three or four genospecies infect humans (Ref. 42).

Another significant mechanism for persistence has recently been suggested. Antigenic variation that requires an ongoing genesis of host responses is now a well-recognised phenomenon among
bacteria, fungi and protozoan pathogens. The related relapsing fever borreliae possess a series of orthologous genes, called variable membrane protein (vmp) genes, which allow for ongoing recombination about every 10^3 or 10^4 replications (Ref. 121). Infection and clinical manifestations, chiefly fever, are observed in a remitting fashion during several weeks; each relapse is associated with the emergence of a population of relapsing fever borreliae that possesses new vmp genes after gene conversion or recombination and express new surface antigens. Each new population that emerges stimulates a concurrent protective immunological response that diminishes clinical signs. Eventually, protective immunity directed towards conserved domains in the Vmp surface antigens develops and the infection is controlled. The vls family of B. burgdorferi probably functions similarly in Lyme disease, except that the spirochaemia is much lower and does not generate the severe clinical signs observed in relapsing fever (Refs 28, 33, 36, 122). However, early infection with a cloned strain of B. burgdorferi does allow the generation of diverse populations that differ in vlsE sequence and VlsE protein. The generation of this diversity is stimulated by immunological responses directed against the spirochaete and dominated by the production of IFN-γ (Ref. 29). Because at least 15 distinct vls loci may recombine with each other in various ways, the total potential antigenic variation is enormous and, thus, the potential for long-term infections exists. Paradoxically, the immune response itself drives the generation of a diverse population among which some spirochaetes are fit for survival (Ref. 29). When IFN-γ-receptor deficient mice passively immunised with B. burgdorferi immune serum are infected, B. burgdorferi are killed. However, B. burgdorferi survive and demonstrate recombination at the vls locus when immunocompetent mice are administered the same immune serum, suggesting that the murine immune response promotes VlsE antigenic variation and in vivo adaptation.

**Prevention strategies for Lyme disease**

Prevention strategies for Lyme disease include reducing tick abundance, minimising human exposure to ticks and the development of effective vaccines or post-exposure antibiotic regimens (Refs 123, 124, 125, 126, 127). Risk in tick habitats directly surrounding residential areas can be reduced by the removal of leaf litter and brush. Tick abundance can be further reduced by applying acaricides directly to tick habitats or by new approaches developed to apply acaricides to deer and rodents harbouring ticks (Refs 123, 124, 125, 126, 127). Deer control is an impractical approach to reducing tick numbers because it would require either extensive fencing or near eradication of deer from the environment (Ref. 128). Future areas of research might include the development of rodent vaccines to decrease the reservoir for B. burgdorferi (Ref. 129).

Personal protective options include the avoidance of tick habitats, use of insect repellents such as DEET (N,N-diethyl-meta-toluamide) or IR3535 (3-[N-Butyl-N-acetyl]-aminopropionic acid ethyl ester) and regular tick checks with prompt tick removal (Refs 130, 131, 132). Of these options, tick checks remain the most acceptable to the general population; however, many Lyme disease patients do not recall a tick bite preceding their illness. Ticks should be removed directly and promptly using tweezers to grasp them and pull them off at the point where their mouthparts meet the skin.

An effective vaccine against B. burgdorferi OspA (Ref. 133) was available between 1999 and 2002, but is no longer being marketed. Protection with the vaccine is not long lived and would have required booster vaccinations every 1–3 years. Post-exposure prophylaxis with a single 200-mg dose of doxycycline, a broad-spectrum antibiotic, is effective when used within 72 hours of tick attachment (Ref. 126). When this antibiotic is used in Lyme-endemic areas after engorged tick bites, it is calculated that 40 patients with tick bites will be treated for each patient in whom Lyme disease is prevented. For some practitioners this is an unacceptable use of antibiotics.

**Research in progress and outstanding research questions**

Three important areas of clinical concern have remained present since the early descriptions of Lyme disease. First, although EM is often sufficient to diagnose Lyme disease, atypical rashes and summertime febrile syndromes that are not associated with a rash can represent a diagnostic challenge because serological testing is often negative in the early stages of the disease. An extremely sensitive PCR method or other rapid testing technique is needed for routine laboratory confirmation. New serological assays that appear sensitive and specific for Lyme
disease, such as those based on the VlsE C6 region recombinant peptides, are demonstrating promise in streamlining and reducing the cost of testing (Ref 134). Second, although Lyme disease is typically an infection that responds easily to appropriate antimicrobial therapy, some patients despite treatment are left with debilitating symptoms that do not appear to represent active infection (Refs 86, 89, 114). Understanding the molecular mechanisms that are important in this subset of patients might help to develop new therapies for treating or preventing these sequelae. Last, because the commercially available Lyme vaccine has been withdrawn by the manufacturer from the US market, work is ongoing on developing an alternative or multivalent vaccine that could offer effective worldwide protection against exposure to different genospecies (Ref. 135).

Currently, Lyme disease can be controlled by several methods, but none are satisfactory. Because the US Centers for Disease Control and Prevention have established an ambitious goal of reducing the incidence of Lyme disease by 50% in the year 2010, significant progress is urgently required. The most frequent method of managing the infection currently is clinical detection and antibiotic treatment, which does little to address the increasing incidence of infection. The withdrawal of a vaccine for Lyme disease has removed an effective although costly tool for the prevention of human infections. Environmental control, championed by a few, has a real possibility of success if further investigation is permitted. Thus, a fundamental understanding of the ecological and biological processes that underlie transmission and disease is critical to advance the clinical needs. Such goals include: (1) investigating the specific mechanisms that *B. burgdorferi* uses to cause cellular injury and inflammation in a genetically heterogeneous human population; (2) understanding how infection persists with ongoing inflammatory injury or induction of immunopathology in the absence of propagating bacteria; (3) determining whether the availability of the complete *B. burgdorferi* genome sequence can facilitate novel studies that could lead to insights into pathogenesis and potentially important therapeutic targets; and (4) predicting models for human risk based on studying the ecology of *B. burgdorferi*. Zoonoses are best understood by a full understanding of the complex natural ecology of the organism; thus, more fundamental studies of the ecology of *B. burgdorferi*, including the range and distribution of reservoir hosts, and the events that influence changes in populations of hosts and parasites alike on global and molecular scales are crucially important to understanding and controlling the disease.

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**References**


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Review

Lyme borreliosis (Lyme disease): molecular and cellular pathobiology and prospects for prevention, diagnosis and treatment

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26 Oliver, J.H., Jr. et al. (1999) Ticks and antibodies to Borrelia burgdorferi from mammals at Cape Hatteras, NC and Assateague Island, MD and VA. J Med Entomol 36, 578-587, PubMed: 10534951
28 McDowell, J.V. et al. (2002) Evidence that the variable regions of the central domain of VlsE are antigenic during infection with Lyme disease spirochetes. Infect Immun 70, 4196-4203, PubMed: 12117928
38 Katona, L.I. et al. (2000) A bactericidal monoclonal antibody elicits a change in its antigen, OspB of *Borrelia burgdorferi*, that can be detected by limited proteolysis. J Immunol 164, 1425-1431, PubMed: 10640758
79 McAlister, H.F. et al. (1989) Lyme carditis: an


129 Kurtenbach, K. et al. (1997) Vaccination of natural reservoir hosts with recombinant
Lipidated OspA induces a transmission-blocking immunity against Lyme disease spirochaetes associated with high levels of LA-2 equivalent antibodies. Vaccine 15, 1670-1674, PubMed: 9364698


Further reading, resources and contacts

The following recent review articles on Lyme disease and *Borrelia burgdorferi* summarise existing knowledge of the interactions of ecology and biology that lead to disease manifestations. Some reviews also provide contrasts and comparisons of these features with other infectious agents and diseases that might facilitate understanding of general and specific mechanisms of disease.


The websites listed below are for organisations and societies that provide funding or help to disseminate evidence-based information regarding aspects of Lyme disease. Many other sites and much other information exist, but care must be exercised when searching to be certain about the validity of the data provided.

The Centers for Disease Control and Prevention – Division of Vector-Borne Infectious Diseases
  
  http://www.cdc.gov/ncidod/dvbid/lyme/

The National Institutes of Allergy and Infectious Diseases
  
  http://www.niaid.nih.gov/research/lyme.htm
  

The National Institute of Neurological Disorders and Stroke
  

(Continued on next page)
American College of Physicians/American Society of Internal Medicine
http://www.acponline.org/lyme/

European Union Concerted Action On Lyme Borreliosis (EUCALB)
http://vie.dis.strath.ac.uk/vie/LymeEU/

The American Lyme Disease Foundation, Inc.
http://www.aldf.com/

Arthritis Foundation
http://www.arthritis.org/conditions/DiseaseCenter/lyme_disease.asp

The B. burgdorferi genome has been sequenced by the Brookhaven National Laboratory and by the Institute for Genomic Research. Further details can be found at the following websites.
http://www.genome.bnl.gov/Sequencing/Bburgdorferi/

Features associated with this article

Figures
Figure 1. Immunofluorescent Borrelia burgdorferi in cell culture.
Figure 2. Antigenic diversity as hypothesised for the vls loci of Borrelia burgdorferi s.l.
Figure 3. Superimposition of the changes in Borrelia burgdorferi protein and lipoprotein expression during acquisition from an infected mammalian host and penetration into tick tissues and the spirochaete-vector-host cycle.
Figure 4. Photographs showing erythema migrans.

Table
Table 1. Sensitivity and specificity of assays for the diagnosis of Lyme disease.

Box
Box 1. Lyme disease case definition for reporting in the USA as recommended by the Centers for Disease Control and Prevention.

Citation details for this article