http://www.expertreviews.org/

expert reviews in molecular medicine

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

Paul G. Auwaerter, John Aucott and J. Stephen Dumler

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

Paul G. Auwaerter
Associate Professor of Medicine, Divisions of General Internal Medicine and Infectious Diseases, The Johns Hopkins University School of Medicine, 10753 Falls Road, Suite 325, Lutherville, MD 21093, USA. Tel: +1 410 583 2774; Fax: +1 410 583 2883; E-mail: pauwaert@jhmi.edu

John Aucott
Assistant Professor of Medicine, Divisions of General Internal Medicine and Infectious Diseases, The Johns Hopkins University School of Medicine, Park Medical Associates, Suite 200, Greenspring Station, 10755 Falls Road, Baltimore, MD 21093, USA. Tel: +1 410 583 7124; Fax: +1 410 583 7128; E-mail: jaucott@jhmi.edu

J. Stephen Dumler (corresponding author)
Professor of Pathology, Division of Medical Microbiology, Department of Pathology, The Markey Graduate Program in Cellular and Molecular Medicine, The Johns Hopkins University School of Medicine, Department of Molecular Microbiology and Immunology, The Johns Hopkins University Bloomberg School of Public Health, Ross Research Building, Room 624, 720 Rutland Avenue, Baltimore, MD 21205, USA. Tel: +1 410 955 8654; Fax: +1 443 287 3665; E-mail: sdumler@jhmi.edu

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

to erythema migrans and ranging to disseminated infection with arthritis, carditis, cranial nerve palsy, peripheral neuropathy, meningitis, or other manifestations. Diagnostic tests have improved, but are unhelpful during certain stages of infection. Therapy varies depending on the degree of involvement, and recovery is usually rapid and complete. Post-treatment clinical manifestations in the absence of evidence for active infection are still poorly understood. The understanding of how *B. burgdorferi* survives in the environment and interacts with human and mammalian hosts has improved. However, further advances in prevention and therapy depend on continued investigation of the ecological risks and improved understanding of the pathobiology of this obligate bacterial parasite.

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 arboreous 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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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.

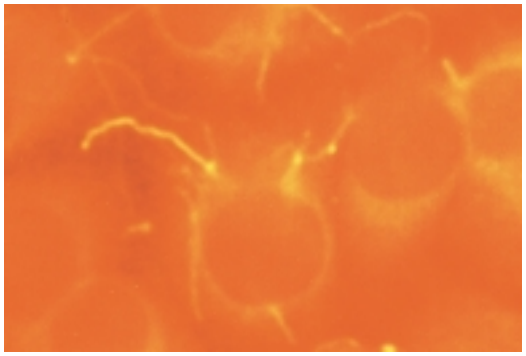
Microbiology, ecology and pathogenesis

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 bissettii*.

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



Immunofluorescent *Borrelia burgdorferi* in cell culture

Expert Reviews in Molecular Medicine
©2004 Cambridge University Press

Figure 1. Immunofluorescent *Borrelia burgdorferi* in cell culture. Note the long, spiral appearance of the bacterium. Immunofluorescent staining was carried out by reacting acetone-fixed *B. burgdorferi* strain 297 with diluted serum from a patient who was convalesing from Lyme disease; bound antibodies were detected with a fluorescein isothiocyanate-conjugated anti-human IgG. Evans blue was used to counterstain the culture. Magnification = ~400 X.

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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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 moults; 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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

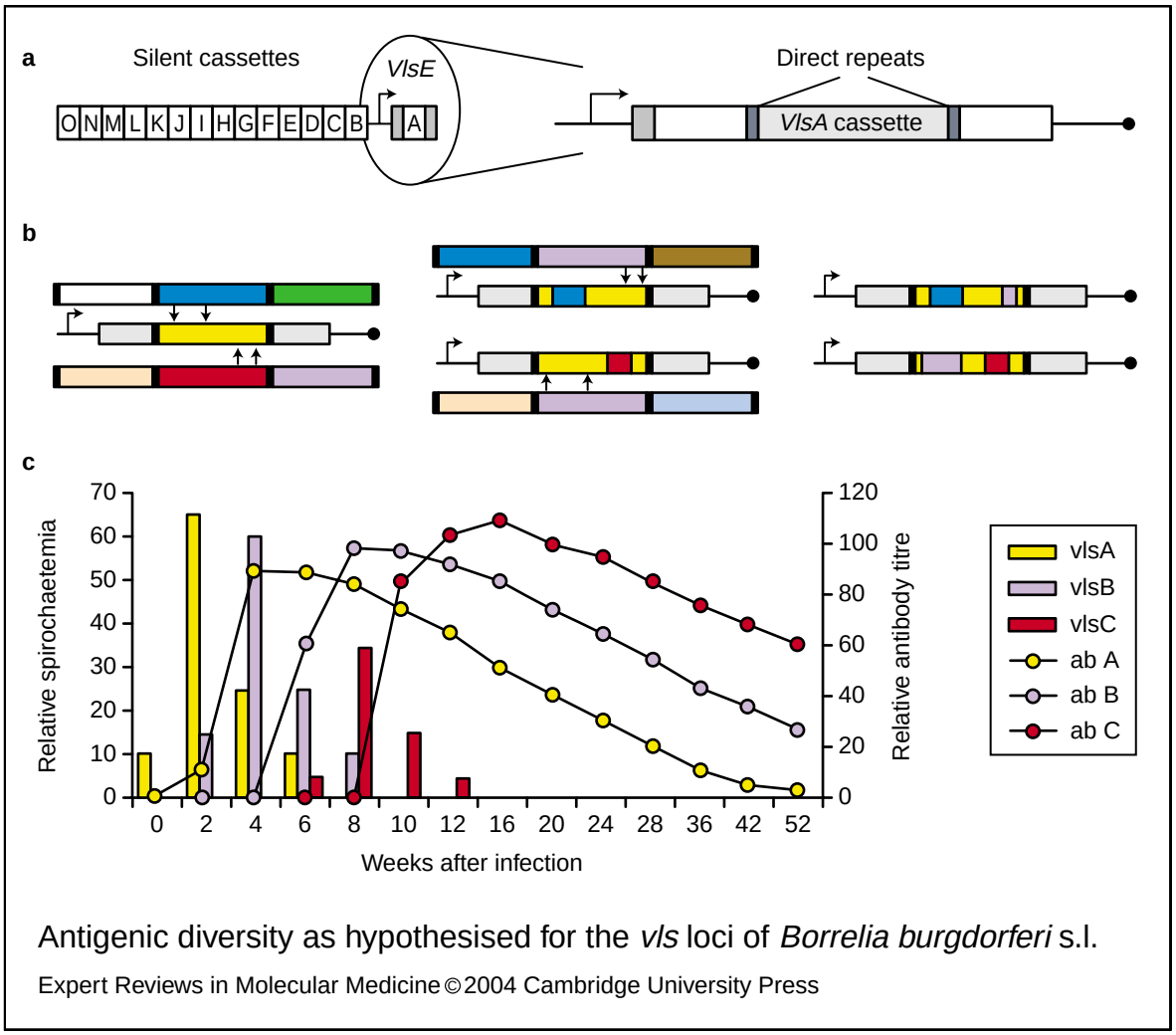


Figure 2. Antigenic diversity as hypothesised for the *vls* loci of *Borrelia burgdorferi* s.l. (a) The *vls* (variable membrane-like sequence) locus exists as a tandemly arranged series of paralog cassettes (left side, letters A–O), each flanked by direct repeats that mediate gene conversion into a site of active transcription preceded by a promoter sequence (horizontal arrow). (b) The specific cassette within the transcription site encodes the actively transcribed and expressed protein, or VlsE, here depicted by *vlsA* (right side). Because each paralog cassette contains regions of homology, inter-cassette recombination may occur, leading to a high degree of heterogeneity and increasing antigenic complexity with changes in VlsE. (c) As successive generations generate random recombinations in the *vls*E, new antigenic variants arise; antibodies that recognised predominant VlsE of one generation may successfully kill those bacteria, only permitting new antigenic variants to survive. The graph depicts a hypothetical progression of spirochaetemia characterised by expression of specific VlsE variants and the subsequent antibody or immune responses directed at those antigenic variants. The number of antigenic variants is exceedingly high and, thus, the length of persistence by this mechanism alone is very long. Immune responses against other invariant antigens probably influence bacterial survival with increasing persistence.

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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

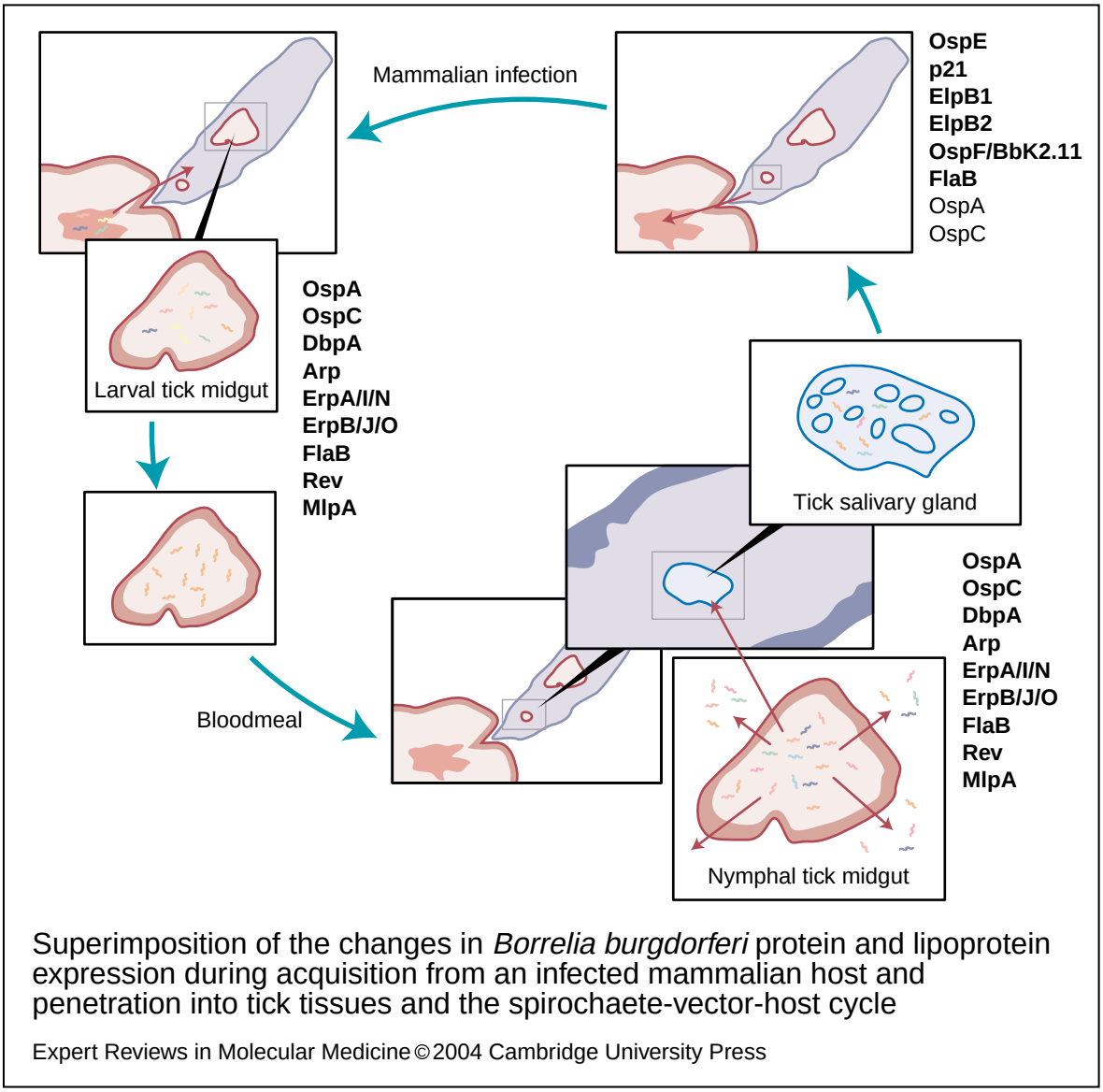


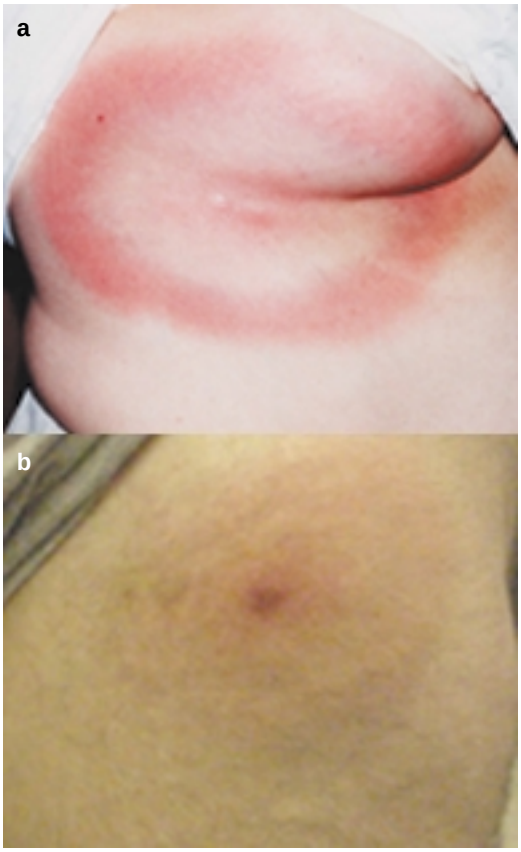
Figure 3. Superimposition of the changes in *Borrelia burgdorferi* protein and lipoprotein expression during acquisition from an infected mammalian host and penetration into tick tissues and the spirochaete-vector-host cycle. The patterns and changes in protein and lipoprotein expression are listed in 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.

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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.



Photographs showing erythema migrans

Expert Reviews in Molecular Medicine

Figure 4. Photographs showing erythema migrans. (a) This photograph shows a classical erythema migrans lesion following a tick bite on the side of the torso, and is characterised by a bright-red border surrounding a central target lesion, with a partial clearing of the redness of the skin between the border and lesion. (b) This photograph shows a lesion on the arm that is more homogeneous in appearance. The features of these lesions 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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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[a]

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[b].

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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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

Assay	Specimen type	Clinical manifestation or phase	Sensitivity (%)	Specificity (%)
PCR or other molecular diagnostic test	Skin	Erythema migrans	71	100
		Acrodermatitis chronica atrophicans[b]	84	
	Plasma/serum	Early localised or disseminated	29	100
	Synovial fluid	Arthritis	65	99
	Cerebrospinal fluid	Meningitis/encephalopathy	24	100
Whole-cell ELISA	Serum	Early[c]	40–60	72–94
		Late[d]	89–100	
C6 peptide VlsE ELISA	Serum	Early	75	90–99
		Late	100	
IgM western blot	Serum	Early	32–59	92–100
		Late	20–40	
IgG western blot	Serum	Early	36–83	94–96
		Late	80–100	

Abbreviations: ELISA, enzyme-linked immunoabsorbent assay; PCR, polymerase chain reaction; VlsE, variable membrane-like sequence E protein.
[a] See Refs 144, 145, 146, 147, 148, 149, 150 for further details.
[b] A gradually progressive late skin manifestation comprising erythematous plaques appearing first on feet, hands, elbows or knees.
[c] Early indicates <30 days after erythema migrans or onset.
[d] Late indicates >30 days after erythema migrans or onset.

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 β-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).

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

Early disseminated disease

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 afflicting 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, mononeuritis 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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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 B 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, ocassionally 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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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 arthritis 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 (CRASPs) 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 haemocele 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 CRASPs 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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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 spirochaetaemia 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). 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

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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.

Acknowledgements and funding

We gratefully acknowledge the National Institutes of Health for their generous grant support for tick-borne infectious diseases (NIAID RO1-AI44102 to JSD). We also thank Barbara Detrick, Clara Lema, Amy Lindhart and Peggy Coulter, and the Departments of Pathology and Medicine of the Johns Hopkins University School of Medicine. We also thank our peer reviewers: Gary P. Wormser (Division of Infectious Diseases, Department of Medicine, New York Medical College, Valhalla, NY, USA) and Thomas G. Schwan (Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA).

References

1 Steere, A.C. et al. (1977) Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. Arthritis Rheum 20, 7-17, PubMed: 836338
2 Burgdorfer, W. et al. (1982) Lyme disease-a tick-borne spirochetosis? Science 216, 1317-1319, PubMed: 7043737
3 Randolph, S.E. (2001) The shifting landscape of tick-borne zoonoses: tick-borne encephalitis and Lyme borreliosis in Europe. Philos Trans R Soc Lond B Biol Sci 356, 1045-1056, PubMed: 11516382
4 Anderson, J.F. (1989) Epizootiology of *Borrelia* in *Ixodes* tick vectors and reservoir hosts. Rev Infect Dis 11 Suppl 6, S1451-1459, PubMed: 2682957
5 Bosler, E.M. and Schulze, T.L. (1986) The prevalence and significance of *Borrelia burgdorferi* in the urine of feral reservoir hosts. Zentralbl Bakteriol Mikrobiol Hyg [A] 263, 40-44, PubMed: 3577491
6 Humair, P. and Gern, L. (2000) The wild hidden face of Lyme borreliosis in Europe. Microbes Infect 2, 915-922, PubMed: 10962275
7 Fish, D. (1995) Environmental risk and prevention of Lyme disease. Am J Med 98, 2S-8S; discussion 8S-9S, PubMed: 7726188

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

8 Fix, A.D., Strickland, G.T. and Grant, J. (1998) Tick bites and Lyme disease in an endemic setting: problematic use of serologic testing and prophylactic antibiotic therapy. Jama 279, 206-210, PubMed: 9438740
9 Costello, C.M. et al. (1989) A prospective study of tick bites in an endemic area for Lyme disease. J Infect Dis 159, 136-139, PubMed: 2642519
10 Robertson, J.N., Gray, J.S. and Stewart, P. (2000) Tick bite and Lyme borreliosis risk at a recreational site in England. Eur J Epidemiol 16, 647-652, PubMed: 11078122
11 Orloski, K.A. et al. (2000) Surveillance for Lyme disease—United States, 1992-1998. MMWR CDC Surveill Summ 49, 1-11, PubMed: 10817483
12 Wang, G. et al. (1999) Molecular typing of *Borrelia burgdorferi* sensu lato: taxonomic, epidemiological, and clinical implications. Clin Microbiol Rev 12, 633-653, PubMed: 10515907
13 Fraser, C.M. et al. (1997) Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature 390, 580-586, PubMed: 9403685
14 Casjens, S. et al. (2000) 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 35, 490-516, PubMed: 10672174
15 Schwan, T.G. and Piesman, J. (2002) Vector interactions and molecular adaptations of Lyme disease and relapsing fever spirochetes associated with transmission by ticks. Emerg Infect Dis 8, 115-121, PubMed: 11897061
16 Pal, U. et al. (2000) Attachment of *Borrelia burgdorferi* within *Ixodes scapularis* mediated by outer surface protein A. J Clin Invest 106, 561-569, PubMed: 10953031
17 Pal, U. et al. (2001) Inhibition of *Borrelia burgdorferi*-tick interactions in vivo by outer surface protein A antibody. J Immunol 166, 7398-7403, PubMed: 11390491
18 Ohnishi, J., Piesman, J. and de Silva, A.M. (2001) Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. Proc Natl Acad Sci U S A 98, 670-675, PubMed: 11209063
19 Seinost, G. et al. (1999) Four clones of *Borrelia burgdorferi* sensu stricto cause invasive infection in humans. Infect Immun 67, 3518-3524, PubMed: 10377134
20 Zuckert, W.R. et al. (2001) Structural conservation of neurotropism-associated VspA within the variable *Borrelia* Vsp-OspC lipoprotein family. J Biol Chem 276, 457-463, PubMed: 11018048
21 Wormser, G.P. et al. (1999) Association of specific subtypes of *Borrelia burgdorferi* with hematogenous dissemination in early Lyme disease. J Infect Dis 180, 720-725, PubMed: 10438360
22 Stewart, P.E. et al. (2001) Isolation of a circular plasmid region sufficient for autonomous replication and transformation of infectious *Borrelia burgdorferi*. Mol Microbiol 39, 714-721, PubMed: 11169111
23 Sartakova, M.L. et al. (2001) Complementation of a nonmotile *flaB* mutant of *Borrelia burgdorferi* by chromosomal integration of a plasmid containing a wild-type flaB allele. J Bacteriol 183, 6558-6564, PubMed: 11673425
24 Jones, C.G. et al. (1998) Chain reactions linking acorns to gypsy moth outbreaks and Lyme disease risk. Science 279, 1023-1026, PubMed: 9461433
25 Gern, L. et al. (1998) European reservoir hosts of *Borrelia burgdorferi* sensu lato. Zentralbl Bakteriol 287, 196-204, PubMed: 9580423
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
27 Hofmeister, E.K. et al. (1999) Population dynamics of a naturally occurring heterogeneous mixture of *Borrelia burgdorferi* clones. Infect Immun 67, 5709-5716, PubMed: 10531219
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
29 Anguita, J. et al. (2001) *Borrelia burgdorferi*-induced inflammation facilitates spirochete adaptation and variable major protein-like sequence locus recombination. J Immunol 167, 3383-3390, PubMed: 11544329
30 Liang, F.T., Nelson, F.K. and Fikrig, E. (2002) Molecular adaptation of *Borrelia burgdorferi* in the murine host. J Exp Med 196, 275-280, PubMed: 12119353
31 Revel, A.T., Talaat, A.M. and Norgard, M.V. (2002) DNA microarray analysis of differential

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

gene expression in *Borrelia burgdorferi*, the Lyme disease spirochete. Proc Natl Acad Sci U S A 99, 1562-1567, PubMed: 11830671
32 Gilmore, R.D., Jr., Mbow, M.L. and Stevenson, B. (2001) Analysis of *Borrelia burgdorferi* gene expression during life cycle phases of the tick vector *Ixodes scapularis*. Microbes Infect 3, 799-808, PubMed: 11580974
33 Zhang, J.R. and Norris, S.J. (1998) Kinetics and in vivo induction of genetic variation of *vlsE* in *Borrelia burgdorferi*. Infect Immun 66, 3689-3697, PubMed: 9673250
34 Purser, J.E. and Norris, S.J. (2000) Correlation between plasmid content and infectivity in *Borrelia burgdorferi*. Proc Natl Acad Sci U S A 97, 13865-13870, PubMed: 11106398
35 Labandeira-Rey, M. and Skare, J.T. (2001) Decreased infectivity in *Borrelia burgdorferi* strain B31 is associated with loss of linear plasmid 25 or 28-1. Infect Immun 69, 446-455, PubMed: 11119536
36 Zhang, J.R. and Norris, S.J. (1998) Genetic variation of the *Borrelia burgdorferi* gene *vlsE* involves cassette-specific, segmental gene conversion. Infect Immun 66, 3698-3704, PubMed: 9673251
37 Sung, S.Y. et al. (2000) Mutation and recombination in the upstream homology box-flanked *ospE*-related genes of the Lyme disease spirochetes result in the development of new antigenic variants during infection. Infect Immun 68, 1319-1327, PubMed: 10678944
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
39 Hagman, K.E. et al. (1998) Decorin-binding protein of *Borrelia burgdorferi* is encoded within a two-gene operon and is protective in the murine model of Lyme borreliosis. Infect Immun 66, 2674-2683, PubMed: 9596733
40 Probert, W.S. and Johnson, B.J. (1998) Identification of a 47 kDa fibronectin-binding protein expressed by *Borrelia burgdorferi* isolate B31. Mol Microbiol 30, 1003-1015, PubMed: 9988477
41 Kraiczy, P. et al. (2001) Further characterization of complement regulator-acquiring surface proteins of *Borrelia burgdorferi*. Infect Immun 69, 7800-7809, PubMed: 11705962
42 Stevenson, B. et al. (2002) 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 70, 491-497, PubMed: 11796574
43 Piesman, J., Schneider, B.S. and Zeidner, N.S. (2001) Use of quantitative PCR to measure density of *Borrelia burgdorferi* in the midgut and salivary glands of feeding tick vectors. J Clin Microbiol 39, 4145-4148, PubMed: 11682544
44 Ramamoorthy, R. and Scholl-Meeker, D. (2001) *Borrelia burgdorferi* proteins whose expression is similarly affected by culture temperature and pH. Infect Immun 69, 2739-2742, PubMed: 11254645
45 Yang, X. et al. (2000) Interdependence of environmental factors influencing reciprocal patterns of gene expression in virulent *Borrelia burgdorferi*. Mol Microbiol 37, 1470-1479, PubMed: 10998177
46 Piesman, J. (1993) Dynamics of *Borrelia burgdorferi* transmission by nymphal *Ixodes dammini* ticks. J Infect Dis 167, 1082-1085, PubMed: 8486940
47 Hefty, P.S. et al. (2002) Changes in temporal and spatial patterns of outer surface lipoprotein expression generate population heterogeneity and antigenic diversity in the Lyme disease spirochete, *Borrelia burgdorferi*. Infect Immun 70, 3468-3478, PubMed: 12065486
48 Liveris, D. et al. (1999) Genetic diversity of *Borrelia burgdorferi* in Lyme disease patients as determined by culture versus direct PCR with clinical specimens. J Clin Microbiol 37, 565-569, PubMed: 9986813
49 Schoeler, G.B. and Wikel, S.K. (2001) Modulation of host immunity by haematophagous arthropods. Ann Trop Med Parasitol 95, 755-771, PubMed: 11784430
50 Valenzuela, J.G. et al. (2002) Exploring the sialome of the tick *Ixodes scapularis*. J Exp Biol 205, 2843-2864, PubMed: 12177149
51 Hodzic, E. et al. (2002) *Borrelia burgdorferi* population kinetics and selected gene expression at the host-vector interface. Infect Immun 70, 3382-3388, PubMed: 12065476
52 Wang, G. et al. (2002) Disease severity in a murine model of Lyme borreliosis is associated with the genotype of the infecting *Borrelia burgdorferi* sensu stricto strain. J Infect Dis 186, 782-791, PubMed: 12198612
53 Guo, B.P. et al. (1998) Decorin-binding adhesins from *Borrelia burgdorferi*. Mol Microbiol 30, 711-723, PubMed: 10094620

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

54 Brown, E.L. et al. (2001) Resistance to Lyme disease in decorin-deficient mice. J Clin Invest 107, 845-852, PubMed: 11285303
55 Grab, D.J., Givens, C. and Kennedy, R. (1998) Fibronectin-binding activity in *Borrelia burgdorferi*1. Biochim Biophys Acta 1407, 135-145, PubMed: 9685613
56 Parveen, N. and Leong, J.M. (2000) Identification of a candidate glycosaminoglycan-binding adhesin of the Lyme disease spirochete *Borrelia burgdorferi*. Mol Microbiol 35, 1220-1234, PubMed: 10712702
57 Coburn, J. et al. (1998) Integrins alpha(v)beta3 and alpha5beta1 mediate attachment of Lyme disease spirochetes to human cells. Infect Immun 66, 1946-1952, PubMed: 9573074
58 Klempner, M.S. et al. (1995) Binding of human plasminogen and urokinase-type plasminogen activator to the Lyme disease spirochete, *Borrelia burgdorferi*. J Infect Dis 171, 1258-1265, PubMed: 7751701
59 Coleman, J.L. et al. (1995) *Borrelia burgdorferi* binds plasminogen, resulting in enhanced penetration of endothelial monolayers. Infect Immun 63, 2478-2484, PubMed: 7790059
60 Hu, L.T. et al. (1995) Binding of human plasminogen to *Borrelia burgdorferi*. Infect Immun 63, 3491-3496, PubMed: 7642282
61 Klempner, M.S. et al. (1996) Binding of human urokinase type plasminogen activator and plasminogen to *Borrelia* species. J Infect Dis 174, 97-104, PubMed: 8656020
62 Coleman, J.L., Roemer, E.J. and Benach, J.L. (1999) Plasmin-coated *Borrelia burgdorferi* degrades soluble and insoluble components of the mammalian extracellular matrix. Infect Immun 67, 3929-3936, PubMed: 10417158
63 Perides, G., Noring, R. and Klempner, M.S. (1996) Inhibition of *Borrelia burgdorferi*-bound fibrinolytic enzymes by alpha2-antiplasmin, PAI-1 and PAI-2. Biochem Biophys Res Commun 219, 690-695, PubMed: 8645243
64 Hu, L.T. et al. (2001) Host metalloproteinases in Lyme arthritis. Arthritis Rheum 44, 1401-1410, PubMed: 11407701
65 Gebbia, J.A., Coleman, J.L. and Benach, J.L. (2001) Borrelia spirochetes upregulate release and activation of matrix metalloproteinase gelatinase B (MMP-9) and collagenase 1 (MMP-1) in human cells. Infect Immun 69, 456-462, PubMed: 11119537
66 Coleman, J.L., Gebbia, J.A. and Benach, J.L. (2001) *Borrelia burgdorferi* and other bacterial products induce expression and release of the urokinase receptor (CD87). J Immunol 166, 473-480, PubMed: 11123326
67 Coleman, J.L. et al. (1997) Plasminogen is required for efficient dissemination of B. burgdorferi in ticks and for enhancement of spirochetemia in mice. Cell 89, 1111-1119, PubMed: 9215633
68 Piesman, J. et al. (1987) Duration of tick attachment and *Borrelia burgdorferi* transmission. J Clin Microbiol 25, 557-558, PubMed: 3571459
69 Gerber, M.A. et al. (1996) Lyme disease in children in southeastern Connecticut. Pediatric Lyme Disease Study Group. N Engl J Med 335, 1270-1274, PubMed: 8857006
70 Steere, A.C. (2001) Lyme disease. N Engl J Med 345, 115-125, PubMed: 11450660
71 Smith, R.P. et al. (2002) Clinical characteristics and treatment outcome of early Lyme disease in patients with microbiologically confirmed erythema migrans. Ann Intern Med 136, 421-428, PubMed: 11900494
72 Reed, K.D. (2002) Laboratory testing for Lyme disease: possibilities and practicalities. J Clin Microbiol 40, 319-324, PubMed: 11825936
73 Nowakowski, J. et al. (2001) Laboratory diagnostic techniques for patients with early Lyme disease associated with erythema migrans: a comparison of different techniques. Clin Infect Dis 33, 2023-2027, PubMed: 11700579
74 Liveris, D. et al. (2002) Quantitative detection of *Borrelia burgdorferi* in 2-millimeter skin samples of erythema migrans lesions: correlation of results with clinical and laboratory findings. J Clin Microbiol 40, 1249-1253, PubMed: 11923340
75 Dattwyler, R.J. et al. (1990) Amoxicillin plus probenecid versus doxycycline for treatment of erythema migrans borreliosis. Lancet 336, 1404-1406, PubMed: 1978873
76 Dattwyler, R.J. et al. (1997) Ceftriaxone compared with doxycycline for the treatment of acute disseminated Lyme disease. N Engl J Med 337, 289-294, PubMed: 9233865
77 Reik, L. et al. (1979) Neurologic abnormalities of Lyme disease. Medicine (Baltimore) 58, 281-294, PubMed: 449663
78 Oschmann, P. et al. (1998) Stages and syndromes of neuroborreliosis. J Neurol 245, 262-272, PubMed: 9617706
79 McAlister, H.F. et al. (1989) Lyme carditis: an

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

important cause of reversible heart block. Ann Intern Med 110, 339-345, PubMed: 2644885
80 Asbrink, E. (1991) Cutaneous manifestations of Lyme borreliosis. Clinical definitions and differential diagnoses. Scand J Infect Dis Suppl 77, 44-50, PubMed: 1947811
81 Kindstrand, E. et al. (2000) Polyneuropathy in late Lyme borreliosis - a clinical, neurophysiological and morphological description. Acta Neurol Scand 101, 47-52, PubMed: 10660152
82 Asbrink, E. and Hovmark, A. (1985) Successful cultivation of spirochetes from skin lesions of patients with erythema chronicum migrans Afzelius and acrodermatitis chronica atrophicans. Acta Pathol Microbiol Immunol Scand [B] 93, 161-163, PubMed: 4013743
83 Steere, A.C., Schoen, R.T. and Taylor, E. (1987) The clinical evolution of Lyme arthritis. Ann Intern Med 107, 725-731, PubMed: 3662285
84 Herzer, P. (1991) Joint manifestations of Lyme borreliosis in Europe. Scand J Infect Dis Suppl 77, 55-63, PubMed: 1947813
85 Steere, A.C. et al. (1994) Treatment of Lyme arthritis. Arthritis Rheum 37, 878-888, PubMed: 8003060
86 Carlson, D. et al. (1999) Lack of *Borrelia burgdorferi* DNA in synovial samples from patients with antibiotic treatment-resistant Lyme arthritis. Arthritis Rheum 42, 2705-2709, PubMed: 10616021
87 Logigian, E.L. and Steere, A.C. (1992) Clinical and electrophysiologic findings in chronic neuropathy of Lyme disease. Neurology 42, 303-311, PubMed: 1310529
88 Kalish, R.A. et al. (2001) Evaluation of study patients with Lyme disease, 10-20-year follow-up. J Infect Dis 183, 453-460, PubMed: 11133377
89 Klempner, M.S. et al. (2001) Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 345, 85-92, PubMed: 11450676
90 France, D. (2000) A war over Lyme disease. Newsweek 136, 72, PubMed: 11186832
91 Seltzer, E.G. et al. (2000) Long-term outcomes of persons with Lyme disease. Jama 283, 609-616, PubMed: 10665700
92 Ma, Y. and Weis, J.J. (1993) *Borrelia burgdorferi* outer surface lipoproteins OspA and OspB possess B-cell mitogenic and cytokine-stimulatory properties. Infect Immun 61, 3843-3853, PubMed: 8359905
93 Radolf, J.D. et al. (1995) *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins and synthetic lipopeptides activate monocytes/macrophages. J Immunol 154, 2866-2877, PubMed: 7876555
94 Ebnet, K. et al. (1997) *Borrelia burgdorferi* activates nuclear factor-kappa B and is a potent inducer of chemokine and adhesion molecule gene expression in endothelial cells and fibroblasts. J Immunol 158, 3285-3292, PubMed: 9120285
95 Hirschfeld, M. et al. (1999) Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. J Immunol 163, 2382-2386, PubMed: 10452971
96 Lien, E. et al. (1999) Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. J Biol Chem 274, 33419-33425, PubMed: 10559223
97 Wooten, R.M. et al. (2002) Toll-like receptor 2 is required for innate, but not acquired, host defense to *Borrelia burgdorferi*. J Immunol 168, 348-355, PubMed: 11751980
98 Bockenstedt, L.K. et al. (2001) CD4+ T helper 1 cells facilitate regression of murine Lyme carditis. Infect Immun 69, 5264-5269, PubMed: 11500394
99 de Silva, A.M. et al. (1996) *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. J Exp Med 183, 271-275, PubMed: 8551231
100 Barthold, S.W. et al. (1995) Circumvention of outer surface protein A immunity by host-adapted *Borrelia burgdorferi*. Infect Immun 63, 2255-2261, PubMed: 7768606
101 Rathinavelu, S., Broadwater, A. and de Silva, A.M. (2003) Does host complement kill *Borrelia burgdorferi* within ticks? Infect Immun 71, 822-829, PubMed: 12540562
102 Schulze, J. et al. (1996) High- and low-level cytokine induction in human peripheral blood mononuclear cells by different *Borrelia burgdorferi* strains. Med Microbiol Immunol (Berl) 185, 31-37, PubMed: 8803951
103 Oksi, J. et al. (1996) Decreased interleukin-4 and increased gamma interferon production by peripheral blood mononuclear cells of patients with Lyme borreliosis. Infect Immun 64, 3620-3623, PubMed: 8751908
104 Miller, L.C. et al. (1993) Balance of synovial fluid IL-1 beta and IL-1 receptor antagonist and recovery from Lyme arthritis. Lancet 341, 146-148, PubMed: 8093746

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

105 Gross, D.M., Steere, A.C. and Huber, B.T. (1998) T helper 1 response is dominant and localized to the synovial fluid in patients with Lyme arthritis. J Immunol 160, 1022-1028, PubMed: 9551943
106 Kang, I. et al. (1997) T-helper-cell cytokines in the early evolution of murine Lyme arthritis. Infect Immun 65, 3107-3111, PubMed: 9234761
107 Keane-Myers, A. and Nickell, S.P. (1995) Role of IL-4 and IFN-gamma in modulation of immunity to *Borrelia burgdorferi* in mice. J Immunol 155, 2020-2028, PubMed: 7636253
108 McKisic, M.D., Redmond, W.L. and Barthold, S.W. (2000) Cutting edge: T cell-mediated pathology in murine Lyme borreliosis. J Immunol 164, 6096-6099, PubMed: 10843657
109 McKisic, M.D. and Barthold, S.W. (2000) T-cell-independent responses to *Borrelia burgdorferi* are critical for protective immunity and resolution of Lyme disease. Infect Immun 68, 5190-5197, PubMed: 10948143
110 Sigal, L.H. et al. (2001) H9724, a monoclonal antibody to *Borrelia burgdorferi*'s flagellin, binds to heat shock protein 60 (HSP60) within live neuroblastoma cells: a potential role for HSP60 in peptide hormone signaling and in an autoimmune pathogenesis of the neuropathy of Lyme disease. Cell Mol Neurobiol 21, 477-495, PubMed: 11860186
111 Steere, A.C. et al. (2001) Autoimmune mechanisms in antibiotic treatment-resistant Lyme arthritis. J Autoimmun 16, 263-268, PubMed: 11334491
112 Trollmo, C. et al. (2001) Molecular mimicry in Lyme arthritis demonstrated at the single cell level: LFA-1 alpha L is a partial agonist for outer surface protein A-reactive T cells. J Immunol 166, 5286-5291, PubMed: 11290815
113 Sigal, L.H. (1999) Lyme arthritis: lessons learned and to be learned. Arthritis Rheum 42, 1809-1812, PubMed: 10513793
114 Weinstein, A. and Britchkov, M. (2002) Lyme arthritis and post-Lyme disease syndrome. Curr Opin Rheumatol 14, 383-387, PubMed: 12118171
115 Benoist, C. and Mathis, D. (2001) Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? Nat Immunol 2, 797-801, PubMed: 11526389
116 Nocton, J.J. et al. (1994) Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Engl J Med 330, 229-234, PubMed: 8272083
117 Keller, T.L., Halperin, J.J. and Whitman, M. (1992) PCR detection of *Borrelia burgdorferi* DNA in cerebrospinal fluid of Lyme neuroborreliosis patients. Neurology 42, 32-42, PubMed: 1734321
118 Hellwage, J. et al. (2001) The complement regulator factor H binds to the surface protein OspE of *Borrelia burgdorferi*. J Biol Chem 276, 8427-8435, PubMed: 11113124
119 Kraiczy, P. et al. (2002) Complement regulator-acquiring surface proteins of *Borrelia burgdorferi*: a new protein family involved in complement resistance. Wien Klin Wochenschr 114, 568-573, PubMed: 12422603
120 Nowling, J.M. and Philipp, M.T. (1999) Killing of *Borrelia burgdorferi* by antibody elicited by OspA vaccine is inefficient in the absence of complement. Infect Immun 67, 443-445, PubMed: 9864253
121 Kitten, T. and Barbour, A.G. (1990) Juxtaposition of expressed variable antigen genes with a conserved telomere in the bacterium *Borrelia hermsii*. Proc Natl Acad Sci U S A 87, 6077-6081, PubMed: 2385585
122 Zhang, J.R. et al. (1997) Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. Cell 89, 275-285, PubMed: 9108482
123 Deblinger, R.D. and Rimmer, D.W. (1991) Efficacy of a permethrin-based acaricide to reduce the abundance of *Ixodes dammini* (Acari: Ixodidae). J Med Entomol 28, 708-711, PubMed: 1941940
124 Stafford, K.C., 3rd, Ward, J.S. and Magnarelli, L.A. (1998) Impact of controlled burns on the abundance of *Ixodes scapularis* (Acari: Ixodidae). J Med Entomol 35, 510-513, PubMed: 9701937
125 Poland, G.A. (2001) Prevention of Lyme disease: a review of the evidence. Mayo Clin Proc 76, 713-724, PubMed: 11444404
126 Nadelman, R.B. et al. (2001) Prophylaxis with single-dose doxycycline for the prevention of Lyme disease after an *Ixodes scapularis* tick bite. N Engl J Med 345, 79-84, PubMed: 11450675
127 Sonenshine, D.E. et al. (1996) A self-medicating applicator for control of ticks on deer. Med Vet Entomol 10, 149-154, PubMed: 8744707
128 Wilson, M.L., Levine, J.F. and Spielman, A. (1984) Effect of deer reduction on abundance of the deer tick (*Ixodes dammini*). Yale J Biol Med 57, 697-705, PubMed: 6516462
129 Kurtenbach, K. et al. (1997) Vaccination of natural reservoir hosts with recombinant

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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
130 Staub, D. et al. (2002) Effectiveness of a repellent containing DEET and EBAAP for preventing tick bites. Wilderness Environ Med 13, 12-20, PubMed: 11929056
131 Fradin, M.S. and Day, J.F. (2002) Comparative efficacy of insect repellents against mosquito bites. N Engl J Med 347, 13-18, PubMed: 12097535
132 Piesman, J. and Dolan, M.C. (2002) Protection against Lyme disease spirochete transmission provided by prompt removal of nymphal *Ixodes scapularis* (Acari: Ixodidae). J Med Entomol 39, 509-512, PubMed: 12061448
133 Steere, A.C. et al. (1998) Vaccination against Lyme disease with recombinant *Borrelia burgdorferi* outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. N Engl J Med 339, 209-215, PubMed: 9673298
134 Bacon, R.M. et al. (2003) Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with 2-tiered testing using whole-cell lysates. J Infect Dis 187, 1187-1199, PubMed: 12695997
135 Luft, B.J., Dunn, J.J. and Lawson, C.L. (2002) Approaches toward the directed design of a vaccine against *Borrelia burgdorferi*. J Infect Dis 185 Suppl 1, S46-51, PubMed: 11865439
136 Weis, J.J. (2002) Host-pathogen interactions and the pathogenesis of murine Lyme disease. Curr Opin Rheumatol 14, 399-403, PubMed: 12118174
137 Coburn, J., Medrano, M. and Cugini, C. (2002) *Borrelia burgdorferi* and its tropisms for adhesion molecules in the joint. Curr Opin Rheumatol 14, 394-398, PubMed: 12118173
138 Guerau-de-Arellano, M. and Huber, B.T. (2002) Development of autoimmunity in Lyme arthritis. Curr Opin Rheumatol 14, 388-393, PubMed: 12118172
139 Bunikis, J. and Barbour, A.G. (2002) Laboratory testing for suspected Lyme disease. Med Clin North Am 86, 311-340, PubMed: 11982304
140 Piacentino, J.D. and Schwartz, B.S. (2002) Occupational risk of Lyme disease: an epidemiological review. Occup Environ Med 59, 75-84, PubMed: 11850549
141 Wormser, G.P. et al. (2000) Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. Clin Infect Dis 31 Suppl 1, 1-14, PubMed: 10982743
142 Wharton, M. et al. (1990) Case definitions for public health surveillance. MMWR Recomm Rep 39, 1-43, PubMed: 2122225
143 (1995) Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep 44, 590-591, PubMed: 7623762
144 Dressler, F. et al. (1993) Western blotting in the serodiagnosis of Lyme disease. J Infect Dis 167, 392-400, PubMed: 8380611
145 Tugwell, P. et al. (1997) Laboratory evaluation in the diagnosis of Lyme disease. Ann Intern Med 127, 1109-1123, PubMed: 9412316
146 Engstrom, S.M., Shoop, E. and Johnson, R.C. (1995) Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J Clin Microbiol 33, 419-427, PubMed: 7714202
147 Aguero-Rosenfeld, M.E. et al. (1996) Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture-confirmed erythema migrans. J Clin Microbiol 34, 1-9, PubMed: 8748261
148 Hauser, U., Lehnert, G. and Wilske, B. (1999) Validity of interpretation criteria for standardized Western blots (immunoblots) for serodiagnosis of Lyme borreliosis based on sera collected throughout Europe. J Clin Microbiol 37, 2241-2247, PubMed: 10364592
149 Liang, F.T. et al. (1999) Sensitive and specific serodiagnosis of Lyme disease by enzyme-linked immunosorbent assay with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi* vlsE. J Clin Microbiol 37, 3990-3996, PubMed: 10565920
150 Dumler, J.S. (2001) Molecular diagnosis of Lyme disease: review and meta-analysis. Mol Diagn 6, 1-11, PubMed: 11257206

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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.

Schwan, T.G. and Piesman, J. (2002) Vector interactions and molecular adaptations of Lyme disease and relapsing fever spirochetes associated with transmission by ticks. Emerg Infect Dis 8, 115-121, PubMed: 11897061

Humair, P. and Gern, L. (2000) The wild hidden face of Lyme borreliosis in Europe. Microbes Infect 2, 915-922, PubMed: 10962275

Weis, J.J. (2002) Host-pathogen interactions and the pathogenesis of murine Lyme disease. Curr Opin Rheumatol 14, 399-403, PubMed: 12118174

Coburn, J., Medrano, M. and Cugini, C. (2002) *Borrelia burgdorferi* and its tropisms for adhesion molecules in the joint. Curr Opin Rheumatol 14, 394-398, PubMed: 12118173

Guerau-de-Arellano, M. and Huber, B.T. (2002) Development of autoimmunity in Lyme arthritis. Curr Opin Rheumatol 14, 388-393, PubMed: 12118172

Weinstein, A. and Britchkov, M. (2002) Lyme arthritis and post-Lyme disease syndrome. Curr Opin Rheumatol 14, 383-387, PubMed: 12118171

Bunikis, J. and Barbour, A.G. (2002) Laboratory testing for suspected Lyme disease. Med Clin North Am 86, 311-340, PubMed: 11982304

Luft, B.J., Dunn, J.J. and Lawson, C.L. (2002) Approaches toward the directed design of a vaccine against *Borrelia burgdorferi*. J Infect Dis 185 Suppl 1, S46-51, PubMed: 11865439

Piacentino, J.D. and Schwartz, B.S. (2002) Occupational risk of Lyme disease: an epidemiological review. Occup Environ Med 59, 75-84, PubMed: 11850549

Reed, K.D. (2002) Laboratory testing for Lyme disease: possibilities and practicalities. J Clin Microbiol 40, 319-324, PubMed: 11825936

Wormser, G.P. et al. (2000) Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. Clin Infect Dis 31 Suppl 1, 1-14, PubMed: 10982743

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

http://www.niaid.nih.gov/publications/lyme/default.htm

The National Institute of Neurological Disorders and Stroke

http://www.ninds.nih.gov/health_and_medical/disorders/lyme_doc.htm

(Continued on next page)

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004

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/

http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gbb

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

Paul G. Auwaerter, John Aucott and J. Stephen Dumler (2004) Lyme borreliosis (Lyme disease): molecular and cellular pathobiology and prospects for prevention, diagnosis and treatment. Expert Rev. Mol. Med. Vol. 6, Issue 2, 19 January, DOI: 10.1017/S1462399404007276

Accession information: DOI: 10.1017/S1462399404007276; Vol. 6; Issue 2; 19 January 2004