Lyme borreliosis

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Abstract | Lyme borreliosis is a tick-borne disease that predominantly occurs in temperate regions of the northern hemisphere and is primarily caused by the bacterium *Borrelia burgdorferi* in North America and *Borrelia afzelii* or *Borrelia garinii* in Europe and Asia. Infection usually begins with an expanding skin lesion, known as erythema migrans (referred to as stage 1), which, if untreated, can be followed by early disseminated infection, particularly neurological abnormalities (stage 2), and by late infection, especially arthritis in North America or acrodermatitis chronica atrophicans in Europe (stage 3). However, the disease can present with any of these manifestations. During infection, the bacteria migrate through the host tissues, adhere to certain cells and can evade immune clearance. Yet, these organisms are eventually killed by both innate and adaptive immune responses and most inflammatory manifestations of the infection resolve. Except for patients with erythema migrans, Lyme borreliosis is diagnosed based on a characteristic clinical constellation of signs and symptoms with serological confirmation of infection. All manifestations of the infection can usually be treated with appropriate antibiotic regimens, but the disease can be followed by post-infectious sequelae in some patients. Prevention of Lyme borreliosis primarily involves the avoidance of tick bites by personal protective measures.

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Lyme borreliosis is an important emerging infectious disease[1,2] that is commonly reported in North America and Europe, but is also found in parts of Asia[3]. The US Centers for Disease Control and Prevention estimates that ~300,000 new cases of the disease occur annually in the United States, primarily in northeastern states[4].

Lyme arthritis was recognized in 1976 because of geographical clustering of children with arthritis in Lyme, Connecticut in the United States[5]. Following this report, Lyme arthritis was revealed to be part of a complicated multisystem illness, which included erythema migrans (a slowly expanding skin lesion), Bannwarth syndrome and acrodermatitis chronica atrophicans, which had been described in Europe[1]. These syndromes were brought together following the isolation of *Borrelia burgdorferi*, a spirochaete, from *Ixodes scapularis* (also known as *Ixodes dammini* ticks)[6]. In addition, *B. burgdorferi* was recovered from patients with these clinical manifestations of the infection[1], and the disease, commonly affecting the skin, joints, heart or nervous system, is now referred to as Lyme borreliosis.

B. burgdorferi sensu lato (*B. burgdorferi* s.l.; that is, *B. burgdorferi* in the general sense) now comprises 20 different genospecies[3]. Three genospecies of *B. burgdorferi* s.l. are primarily responsible for human Lyme borreliosis: *B. burgdorferi*, *Borrelia afzelii* and *Borrelia garinii*[7]. These genospecies are transmitted by different species of ticks and are responsible for Lyme borreliosis in different geographical regions, for example, *B. burgdorferi* sensu stricto (that is, *B. burgdorferi* in the strict sense) is the primary cause of Lyme borreliosis in the United States, whereas *B. afzelii* and *B. garini* (and less often *B. burgdorferi*) cause Lyme borreliosis in Europe. The heterogeneity among *B. burgdorferi* s.l. strains seems to be the main factor causing the regional differences in the clinical expression of human Lyme borreliosis[8]. For example, *B. burgdorferi* in the northeastern United States is particularly arthritogenic, *B. afzelii* primarily causes skin infections and *B. garinii* is especially neurotropic[2].

In this Primer, we detail the epidemiology, pathophysiology, clinical manifestations, prevention, management and post-treatment sequelae of the infection. Moreover, we provide an outlook for improved diagnostics, treatment studies, tick control and vaccination for this complex, tick-transmitted illness.

Epidemiology

In the United States, the age distribution of Lyme borreliosis is typically bimodal, with peaks among children 5–15 years of age and adults 45–55 years of age[3,9] (FIG. 1a). The incidence of Lyme borreliosis is higher among men than among women in those <60 years of age, but the sex ratio is nearly equal or slightly higher in women in older

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age groups. In some European countries, such as Slovenia and Germany, the incidence of Lyme borreliosis is higher among adult women (55%) than among men (45%)[9–11]. In the northeastern United States and in most of Europe, the peak months of disease onset are June and July, which is owing to the feeding habits of nymphal ticks (FIG. 1b).

Tick vectors and animal hosts

Worldwide, four tick species belonging to the *Ixodes ricinus* complex are major vectors for the transmission of *B. burgdorferi* s.l. to humans[12] (FIG. 2). The natural enzootic cycles of *B. burgdorferi* s.l. are complex and vary by geographical location. In the northeastern United States, transmission of *B. burgdorferi* occurs from some rodents, particularly white-footed mice and chipmunks, to larval and nymphal *I. scapularis*[13]. The fact that the larval and nymphal stages of the tick feed on the same animal is essential, as the life cycle of the spirochaete depends on horizontal transmission from infected nymphs to rodents in early summer and from infected rodents to larvae in late summer, which then moult to infected nymphs to begin the cycle the following year[14]. White-tailed deer, which are not involved in the life cycle of the spirochaete, are the primary host of adult *I. scapularis* and, as such, are the primary site of tick mating, and, therefore, are important for the maintenance of tick populations[15]. In Europe, *I. ricinus* feed on >300 animal species, including large and small mammals, birds and reptiles[16]. Birds are an important reservoir for *B. garinii*, whereas rodents are an important reservoir for *B. afzelii*. Migratory birds might have a role in expanding the range of ticks to new areas[12], as has been observed in central and eastern Canada[17].

Distribution and incidence

Lyme borreliosis has been reported in countries throughout the northern hemisphere. In Europe and Asia, the reported country-wide incidence ranges from low to negligible in the United Kingdom, Turkey and Japan, to >80 cases per 100,000 individuals in the Netherlands, Belgium, Austria, Slovenia, Lithuania and Estonia[10,18]. Data on the incidence of Lyme borreliosis are scarce from Asia, although cases have been reported in, for example, China and Mongolia[19]. In North America, >90% of cases are reported from two regions in the United States: the northeast and mid-Atlantic region and the north-central region[9,12,20]. Both regions have expanded substantially over the past 20 years[20] and have reached the southern parts of Canada[21]. Pockets of intense transmission of *B. burgdorferi* can exist within smaller, well-defined areas and in individuals who are at high risk for tick bites, such as hikers or forestery workers[9,11]. In a trial investigating a Lyme disease vaccine that was conducted in such areas of the northeastern United States, the yearly incidence of *B. burgdorferi* was >1 per 100 participants in the placebo group[22].

Risk factors

Within an endemic area, the risk of human infection by *B. burgdorferi* s.l. is determined by the local abundance and infection rate of vector ticks and by human behaviours that affect the likelihood of being bitten. Occupations and hobbies that increase tick exposure (for example, forestry workers, hunters and hikers) are associated with an increased risk of infection[20,23,24]. Where homes are situated in tick-infested areas, exposure occurs primarily in the peridomestic environment that is influenced by the amount of suitable tick habitat, the density of ticks and deer, landscaping practices that promote tick survival and the undertaking of outdoor activities, such as gardening[25]. Infection through alternate modes of transmission, including blood transfusion, sexual contact, semen, urine or breast milk, has not been demonstrated.

Mechanisms/pathophysiology

Most mechanistic studies of *B. burgdorferi* s.l. have been carried out in mouse models using strains of *B. burgdorferi* that are found in the United States. However, several caveats are associated with the translation of findings in mouse models to human Lyme borreliosis. First, although *B. burgdorferi* infection shows a reproducible temporal progression in mice as the organism disseminates from the inoculation site, disease in inbred strains of mice does not closely mimic human Lyme borreliosis. Second, the pathogenic characteristics of *B. burgdorferi* might not be identical for all of the genospecies that cause Lyme borreliosis. Thus, except for instances in which a given characteristic is known to apply to all *B. burgdorferi* s.l. genospecies, we use the term *B. burgdorferi* throughout this section and we do not equate pathological characteristics with a stage of the human disease, unless we are reporting features of the human infection.

B. burgdorferi *cellular architecture*

Like all spirochaetes, members of the *B. burgdorferi* s.l. complex are bound by an inner cytoplasmic membrane and an outer membrane. The defining characteristic of *B. burgdorferi* s.l. is periplasmic flagella, which are

responsible for the flat-wave morphology of the bacteria. The flagella are attached to each cell pole and wind around the cell cylinder in the periplasmic space between the peptidoglycan layer and the outer membrane[26] (FIG. 3a,b). Flagellar motors are located at the cell poles and are situated next to the methyl-accepting chemotaxis proteins that direct movement of the bacteria towards chemoattractants (for example, nutrients such as rabbit serum and *N*-acetylglucosamine) and away from repellants (for example, ethanol and butanol)[27–29].

B. burgdorferi differs considerably from typical Gram-negative bacteria. Notably, the outer membrane lacks lipopolysaccharide and consists of a lipid bilayer that is composed of phospholipids and glycolipids[30] (FIG. 3c). Cholesterol glycolipids in the outer membrane form lipid-raft-like microdomains that change in order and size in response to temperature, which is an important environmental cue for *B. burgdorferi* during transmission between the tick vector and the mammalian host[31]. The *B. burgdorferi* outer membrane also contains surface lipoproteins, which can change depending on the environment. For example, outer-surface protein A (OspA) is expressed during tick colonization and OspC is expressed during early infection of the mammal[32–34].

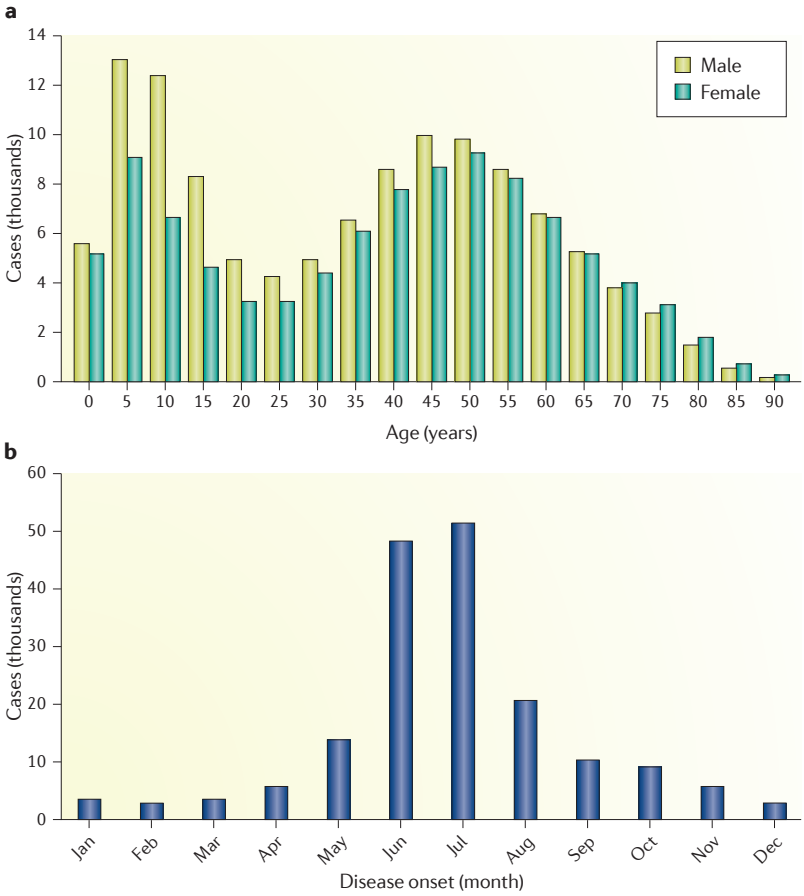

Figure 1 | **Age and sex distribution and seasonality of Lyme borreliosis. a** | Among cases of Lyme borreliosis reported to the US Centers for Disease Control and Prevention in 2010–2013, the peak of ages of disease onset were 5–15 and 45–55 years of age. In individuals <60 years of age, Lyme borreliosis was more prevalent among males than females, but for those >60 years of age, the sex ratio was nearly equal or was higher in women. **b** | Nymphal ticks are a key source of human infection with *Borrelia burgdorferi* and typically feed in the late spring or early summer, which produces a June–July peak in human illness in the United States. In most of Europe, the peak months of onset are also June and July, but a later peak in August has been reported in Estonia and Sweden, perhaps owing to the northern latitudes of these countries[11,217]. In milder climates, such as California, the onset might be more spread out over spring and summer months[3]. Smaller regional and year-to-year variations in human illness have been correlated with meteorological conditions that influence tick feeding and human behaviour, such as temperature, humidity and rainfall[218]. These figures were obtained from the Centers for Disease Control and Prevention: http://www.cdc.gov/lyme/stats/graphs.html.

B. burgdorferi *genome and metabolism*

All genospecies of the *B. burgdorferi* s.l. complex have small, highly segmented genomes. The 1.5 million-base genome of *B. burgdorferi* strain B31 consists of a linear chromosome of 910 kilobases, 9 linear plasmids and 12 circular plasmids of various sizes[35,36]. The linear chromosome is highly conserved among *B. burgdorferi* s.l. genospecies, whereas the plasmids show a high degree of variation. Although sequence data on plasmids are often incomplete, available data indicate that some plasmids in *B. burgdorferi* are missing in *B. afzelii* and *B. garinii*. Differences in plasmids among *B. burgdorferi* s.l. are thought to contribute to the clinical variability in Lyme borreliosis in different geographical regions.

Intraspecies diversity has also been noted for several *B. burgdorferi* s.l. genospecies[8]. For example, in the United States, *B. burgdorferi* strains have been divided into several subtypes[8] based on variations in the genes that encode OspC, 16S–23S rRNA intergenic spacer region or eight selected housekeeping genes. This subtyping has been used to correlate the strain variation with the risk for haematogenous dissemination and clinical outcome[37–39].

B. burgdorferi has a very limited metabolic capacity and is highly dependent on its tick vector and vertebrate host for many essential factors. For example, *B. burgdorferi* lacks genes encoding proteins that have a role in the tricarboxylic acid cycle and oxidative phosphorylation, and relies exclusively on glycolysis for energy production. For this purpose, *B. burgdorferi* uses several host or vector-derived carbohydrates, including glucose, glycerol, maltose, *N*-acetylglucosamine, trehalose and chitobiose[40]. The *B. burgdorferi* genome also lacks genes that are required for the synthesis of amino acids, lipids, nucleotides and cofactors; to obtain these factors, the *B. burgdorferi* genome encodes 16 distinct membrane transporters, many of which have broad substrate specificity[35]. Owing to the inability of *B. burgdorferi* to synthesize fatty acids, its lipid composition reflects that of the host tissues[41]; *B. burgdorferi* exchanges lipids with the plasma membrane of eukaryotic cells, either through direct contact or via outer membrane vesicles[42].

Invasion, dissemination and immune evasion

B. burgdorferi uses differential gene expression for survival in the different environments of the tick vector and the mammalian host. After tick attachment to the host, cues from tick engorgement (that is, the process of a tick taking a blood meal), such as increases in temperature, the availability of nutrients, changes in oxygen tension and decreased pH, stimulate *B. burgdorferi* in the tick

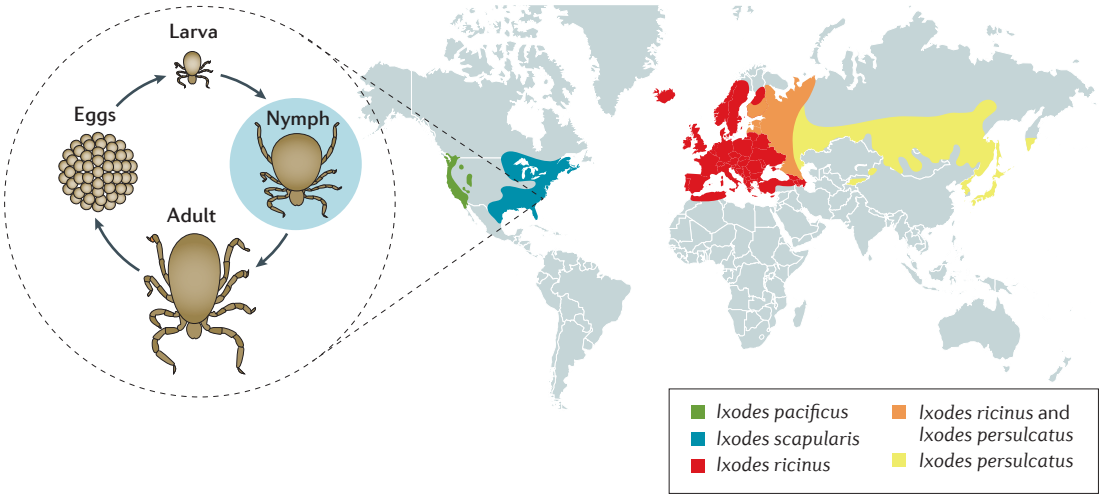

Figure 2 | **Distribution of *Ixodes* ticks that transmit *Borrelia burgdorferi* s.l. to humans.** *Borrelia burgdorferi* s.l. are transmitted by ticks of the *Ixodes ricinus* complex. In Europe, the principal tick vector is *I. ricinus* (red), which transmits all three major pathogenic genospecies of *B. burgdorferi* s.l. The tick *Ixodes persulcatus* (yellow), is found in western Russia, the Baltic countries, parts of Finland, central regions of eastern Russia, northern Mongolia, China and Japan. *I. persulcatus* transmits *Borrelia afzelii* and *Borrelia garinii*, but is not known to transmit *B. burgdorferi*[219,220]. In eastern Europe, *I. ricinus* and *I. persulcatus* overlap (orange). In North America, the main tick vectors are *Ixodes scapularis* in the eastern and mid-western United States (blue) and some areas in middle southern and southeastern Canada[12,221,222] and *Ixodes pacificus* in the western United States (green)[9,12]; both of these ticks transmit *B. burgdorferi*. Within these broad areas, tick abundance and infection prevalence vary widely and are influenced by microclimate, vegetation and the abundance of reservoir vertebrate hosts. The regions of most intense transmission of *B. burgdorferi* s.l. are in the northeastern United States and in central Europe[3], where the prevalence of *B. burgdorferi* s.l. infection among ticks can be as high as 40–50%. By contrast, infection prevalence in the southern United States is <1%. The life cycle of ticks is illustrated in the inset. Larvae, nymph and adult are the main life stages (illustrated as would be seen with magnification), but each stage of each of these species is nearly identical in appearance. The tiny nymphal stage, which feeds in the late spring and early summer, is primarily responsible for transmission of the disease.

midgut to undergo transformation from a state that is adapted for tick colonization to one that is primed for infection of the mammal. This transformation requires a complex regulatory network that includes but is not limited to the expression of the RNA polymerase alternative σ-factor RpoS and *Borrelia* oxidative stress regulator (BosR)[32,33] (FIG. 3c). A large number of genes that are targeted by RpoS and BosR encode surface lipoproteins, and RpoS and BosR act in concert to promote the adaptation of *B. burgdorferi* to the mammalian host through transcriptional activation of mammalian-phase-specific genes (for example, *ospC*) by RpoS[43] and transcriptional repression of the tick-phase-specific genes (for example, *ospA*) by BosR[44]. Replacing OspA (a tick midgut adhesin[45]) with OspC is crucial for establishing early infection in the mammal[33,46].

The establishment of *B. burgdorferi* infection in the host is substantially aided by the tick in both mechanical and biochemical ways. Mechanically, the penetration of the host's skin by the tick enables the delivery of *B. burgdorferi* deep into the dermis, near the blood vessels. Biochemically, tick salivary proteins help *B. burgdorferi* establish an infection by modulating host activities, such as coagulation, fibrinolysis and the immune response[47]. One of the mechanisms by which OspC enhances bacterial colonization of a mammalian host is through direct recruitment of the tick salivary protein Salp15, a molecule with immunosuppressive properties, to the bacterial surface[48]. After being deposited in the skin, *B. burgdorferi* usually multiplies locally before spreading through tissues and into the blood or lymphatic system, which facilitates migration to distant sites. Motility (generated by the flagella) and adherence to host molecules (mediated by the surface lipoproteins) are key to *B. burgdorferi* moving through the host's blood and tissues and evading immune responses. Mutant bacteria that have defects in motility or chemotaxis fail to disseminate and are quickly cleared from the inoculation site[26].

Many of the mammalian-phase-specific surface lipoproteins can directly interact with several host macromolecules, including plasminogen, complement regulatory proteins and components of the extracellular matrix, such as fibronectin, collagen, laminin and glycosaminoglycans (GAGs)[49,50]. These interactions are thought to have distinct functions, such as plasminogen for proteolysis of tissues, extracellular matrix molecules for adhesion and complement regulatory proteins for immune evasion. However, there is a high degree of functional redundancy among these *B. burgdorferi* lipoproteins, which makes it difficult to ascertain the *in vivo* significance of each protein. An exception is the fibronectin-binding protein BBK32, in which unique and sequential roles are assigned to the fibronectin-binding and GAG-binding domains in the interaction of *B. burgdorferi* with the host vasculature[51]. BBK32–fibronectin binding initiates the 'tethering' of circulating

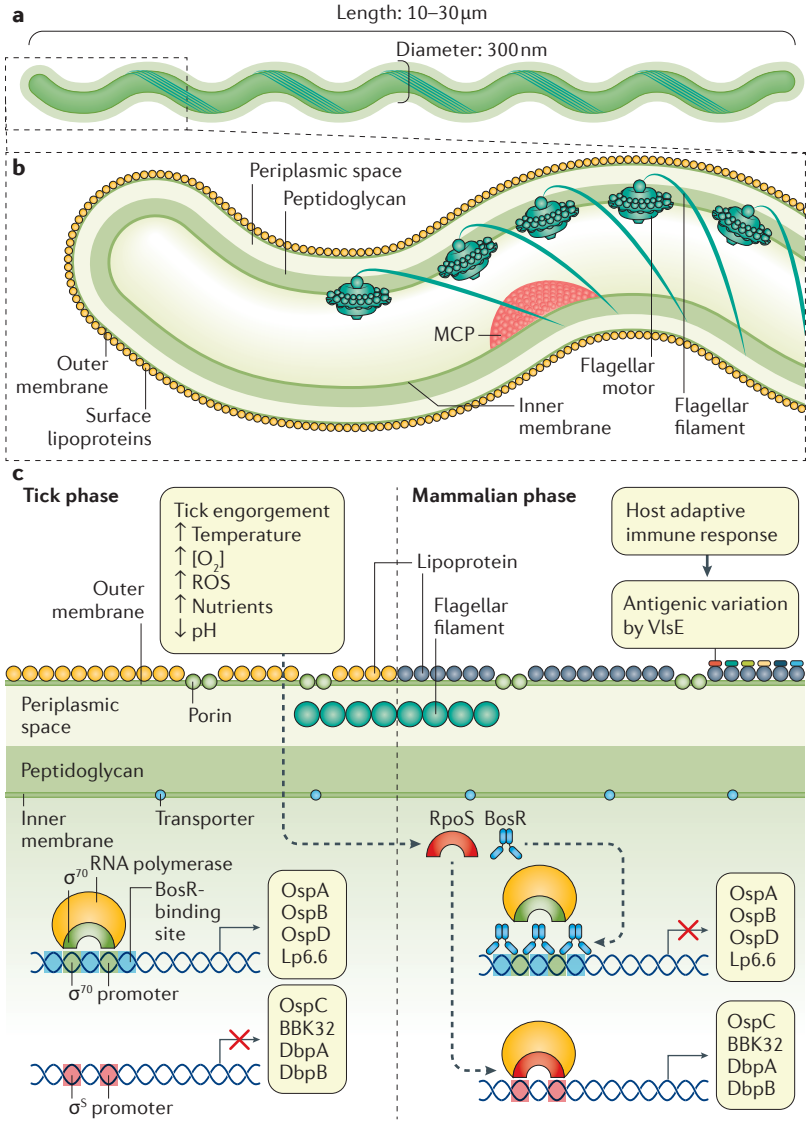

Figure 3 | **Morphology and cellular architecture of *Borrelia burgdorferi*.** **a** | *Borrelia burgdorferi* has a flat-wave morphology, is ~300 nm in diameter and 10–30 µm in length. **b** | A coloured 3D model of one end of *B. burgdorferi* generated by cryo-electron tomography[27]. The flagellar filaments are confined to the periplasmic space and are anchored to each cell pole by the flagellar motors, which are located next to methyl-accepting chemotaxis proteins (MCPs) that direct movement. **c** | The outer membrane of *B. burgdorferi* consists of a lipid bilayer that is heavily decorated with lipoproteins. Different sets of lipoproteins are expressed on the surface in the tick or mammalian environments. When a larval tick acquires *B. burgdorferi* from an infected mammalian host, the bacteria express tick-phase lipoproteins in the tick midgut. After larval ticks moult to nymphal-stage ticks and when these ticks feed, cues from tick engorgement block the expression of tick-phase lipoproteins and activate the expression of mammalian-phase lipoproteins through a complex regulatory network, including but not limited to *Borrelia* oxidative stress regulator (BosR) and the RNA polymerase alternative σ-factor RpoS. As the host mounts an adaptive immune response to *B. burgdorferi*, outer-surface protein C (OspC) is downregulated and VlsE, a lipoprotein that undergoes antigenic variation, is upregulated and expressed on the bacterial surface. The regulatory network for the adaptation of *B. burgdorferi* to different environments is much more complex than depicted in this figure; for example, the pathways leading to the activation of BosR and RpoS are still being determined, RpoS-dependent expression of lipoproteins could involve mechanisms other than the recruitment of RNA polymerase, the expression of some mammalian-phase-specific lipoproteins, such as VlsE and complement regulator-acquiring surface proteins (CRASPs), is not regulated through RpoS and not all tick-phase-specific lipoproteins are directly repressed by BosR. Lp6.6, 6.6-kDa lipoprotein; ROS, reactive oxygen species.

B. burgdorferi to the vascular surface and BBK32–GAG binding contributes to a more-stable vascular interaction, after which *B. burgdorferi* transmigrates across the endothelium and disseminates into tissues.

B. burgdorferi uses several strategies for evading the host innate and adaptive immune systems. Several *B. burgdorferi* lipoproteins, which are known collectively as complement regulator-acquiring surface proteins (CRASPs), can bind to host factor H, factor H-like protein and factor H-related proteins[52,53], which prevents complement-mediated killing of the bacteria *in vitro*[52,53]. After the establishment of infection, the evasion of bactericidal antibodies becomes crucial for the survival of the bacteria. For this purpose, *B. burgdorferi* again alters the lipoproteins that are expressed on its outer surface, by replacing OspC with VlsE. On the basis of structural similarities between OspC and VlsE, these two proteins might serve a similar physiological function. However, unlike OspC, VlsE undergoes extensive antigenic variation to evade the host immune response[54]. Millions of *vlsE* alleles can be generated through gene conversion, which occurs when segments of the 15 silent *vls* loci are assembled into the *vlsE* locus in random combinations. The highly variable segments of VlsE are located in a 'dome' region that is distal to the lipid anchor and exposed on the cell surface. *B. burgdorferi* can evade host antibodies to one VlsE variant by expressing a different one on the surface (FIG. 3c). Mutants that express non-variable VlsE are unable to reinfect animals that have been previously infected with *B. burgdorferi*, whereas bacteria that express variable VlsE can[55].

The host immune response

Despite the expression of CRASPs and the antigenic variation of *B. burgdorferi* surface lipoproteins that enable the bacteria to evade host immune defences, *B. burgdorferi* is still recognized and killed by both innate and adaptive immune responses. As *B. burgdorferi* does not produce toxins or extracellular matrix-degrading proteases, most of the manifestations of human Lyme borreliosis at each of the three stages of disease result from inflammation generated by these immune responses.

In stage 1 (in humans, this is typically an expanding skin lesion known as erythema migrans), biopsies that are taken during the first days of infection show papillary dermal oedema and a mixed infiltrate that consists predominantly of T cells, neutrophils, dendritic cells and monocytes or macrophages[56]. Cytokine expression during this stage is predominantly pro-inflammatory and includes increased levels of tumour necrosis factor (TNF), IL-2, IL-6 and type I interferons (IFNs)[57–59]. The levels of chemokines that attract neutrophils (such as CXC-chemokine ligand 1 (CXCL1; also known as growth-regulated-α protein)), macrophages (such as CC-chemokine ligand 3 (CCL3) and CCL4) and T cells (such as CXCL9, CXCL10 and CXCL11) are also increased in erythema migrans lesions[60]. In both animal and human studies, neutrophils, which are highly effective at killing *B. burgdorferi*, are notably absent as the erythema migrans rash progresses past 24 hours, whereas T cells, dendritic cells and monocytes

remain[59,61]. In animals that artificially express increased levels of the neutrophil chemoattractant KC, *B. burgdorferi* is rapidly cleared following incoculation to the dermis, which suggests that the disappearance of neutrophils is important for permitting the establishment of the infection[62]. Complement also plays an early part in controlling the infection, probably by augmenting phagocytosis and opsonization through the classical pathway. As erythema migrans lesions evolve over days, they progress to dense perivascular and interstitial infiltrates that consist of lymphocytes, plasma cells and occasionally mast cells[56]. The anti-inflammatory cytokine IL-10 can be found in these lesions and higher levels of IL-10 have been associated with fewer systemic symptoms of Lyme borreliosis[57,63]. In animals, IL-10 deficiency is associated with increased inflammation and decreased numbers of *B. burgdorferi*[64], suggesting that, although IL-10 induction might lead to less inflammation and tissue damage, this might enable *B. burgdorferi* to escape the immune system.

Much of the initial host inflammatory response seems to be mediated by pathogen-associated molecular patterns (PAMPs) that are detected by pattern recognition receptors, such as Toll-like receptors (TLRs) and the cytosolic nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors (FIG. 4). Different TLRs can recognize different PAMPs of *B. burgdorferi*, including but not limited to lipoproteins (detected by TLR1 and TLR2)[65–67], flagellin (detected by TLR5)[68], RNA (detected by TLR7 and TLR8)[69,70] and CpG sites in DNA (detected by TLR9)[68,69]. However, responses to *B. burgdorferi* lipoproteins seem to be the main stimulus causing induction of host enzymes that result in the digestion of extracellular matrix proteins, allowing bacteria to move within tissues and the release of inflammatory cytokines, which leads to the symptoms of Lyme borreliosis[71]. Studies of knockout mice have shown a role for some TLRs and adaptors (including TLR2 (REF. 72) and the myeloid differentiation primary response protein MYD88 (REFS 73–75)), but not all[76–78], in control of the infection.

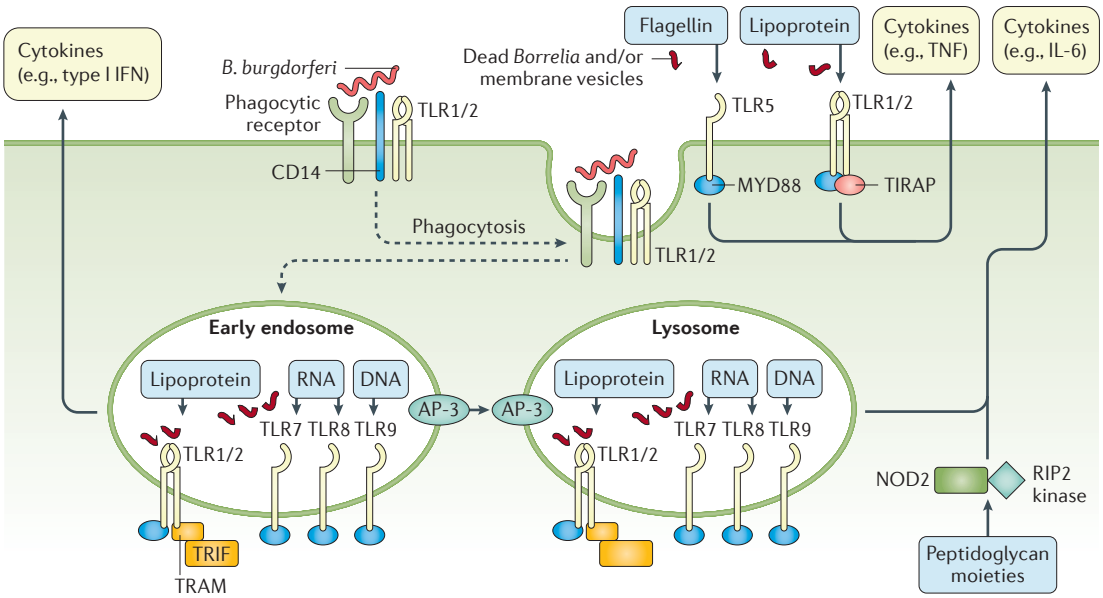

Figure 4 | **Mechanisms of innate immune recognition of *Borrelia burgdorferi*.** The initial innate immune response is triggered by recognition of *Borrelia burgdorferi* or its pathogen-associated molecular patterns (PAMPs) by host immune cells, for example, dendritic cells and macrophages or monocytes. These cells express pattern recognition receptors, particularly Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors (NLRs). Some TLRs, including TLR1/2 heterodimers and TLR5, bind to their ligand on the cell surface, which results in the activation of a regulatory cascade that leads to the production of specific cytokines and chemokines. TLR1/2, along with other TLRs (such as TLR7, TLR8 and TLR9), can also be activated after phagocytosis of the organism and signal from within endosomes. Several different molecules that are involved in phagocytosis have been identified, including complement receptor 3, CD14 and the macrophage receptor MARCO. The exact cytokines produced as a result of these interactions are probably dependent on the specific endosome and/or adaptors and effectors that are recruited to that site. NLRs (for example, NOD2) recognize *B. burgdorferi* or its PAMPs in the cytosol and can increase cytokine induction by activation of TLRs. The balance of pro-inflammatory and anti-inflammatory cytokine production evolves over time and shifts towards an increased production of anti-inflammatory cytokines, such as IL-10, which limits tissue pathology. Signalling is mediated through the recruitment of adaptor molecules, such as the myeloid differentiation primary response protein MYD88, TIR domain-containing adaptor molecule 1 (TRIF), Toll/IL-1 receptor domain-containing adaptor protein (TIRAP) and receptor-interacting serine/threonine-protein kinase 2 (RIP2), which help to transduce signals from receptors to effector molecules, such as interferon (IFN) regulatory factor 3 (IRF3), IRF7 and nuclear factor-κB — each of which can result in the production of different subsets of cytokines. The dashed arrows represent predictions for which there are currently no experimental evidence. AP-3, adaptor protein complex 3; TRAM, TRIF-related adapter molecule (also known as TICAM2); TNF, tumour necrosis factor.

Although deficiencies in TLRs or their adaptors can result in reduced induction of inflammatory cytokines upon exposure to *B. burgdorferi in vitro*, a deficiency in one TLR or adaptor does not diminish inflammation during infection in animals, and might even result in increased inflammation, as observed in mice deficient in the TLR components TLR2, MYD88, TIR domain-containing adapter molecule 1 (TRIF) or CD14 (REFS 66,67,73,76). This finding suggests that there is redundancy in the ability of the innate immune system to recognize *B. burgdorferi* and/or that these components can activate pathways that produce anti-inflammatory cytokines, such as IL-10. During later stages of infection — namely, stage 2 (in humans known as early disseminated infection that is manifested by inflammation at multiple sites) and stage 3 (in humans known as late infection, typically involving arthritis in the United States) — the anti-inflammatory effects might be the more important function of TLR signalling[79,80]. In human Lyme arthritis, a polymorphism in *TLR1* (which results in decreased expression of TLR1) is associated with increased levels of pro-inflammatory cytokines and persistent arthritis after antibiotic therapy[39]. This high level of cytokines parallels the phenotype of untreated innate immune-deficient mice, but mice do not develop persistent arthritis after antibiotic therapy. This phenotype might result from persistent stimulation from residual TLR ligands, either pathogen or host-derived, leading to continued receptor activation.

As the organism disseminates from the original inoculation site, the pattern of recruitment of inflammatory cells, the release of cytokines and inflammation continue at the sites where the bacteria localize, including the heart, joints and nervous system. All affected tissues show mononuclear cell infiltrates, particularly $CD4^+$ and $CD8^+$ T cells, and vascular abnormalities, suggesting that spirochaetes were located in or around blood vessels[81]. In animal models, differences in the inflammatory infiltrates at different sites of infection, as well as at different stages of the disease, have been observed[82]. For example, macrophages are abundant in cardiac lesions, but B cells and plasma cells are plentiful in synovial lesions. In stage 3, synovial lesions in patients with antibiotic-refractory Lyme arthritis show synovial cell hypertrophy, vascular proliferation and sometimes obliterative microvascular lesions, in addition to mononuclear cell infiltrates, which primarily consist of $CD4^+$ and $CD8^+$ T cells and macrophages, often with large numbers of plasma cells[83].

In *in vitro* studies, phagocytic cells, including macrophages, monocytes, neutrophils, dendritic cells and microglia, can effectively kill *B. burgdorferi*. Both γδ T cells and natural killer T cells (NKT cells) are recruited quickly to sites where bacteria are present and seem to be involved in modulating antibody responses to the bacteria. The diacylglycerol glycolipid in the outer membrane of the spirochaete can directly activate invariant NKT cells, which have an important role in controlling infection and augmenting phagocytosis[84].

B cells are crucial for the control of infection. In the spleen, marginal zone B cells produce antibodies to T cell-independent antigens and are a source of *B. burgdorferi*-specific IgM antibodies during stage 1 of the disease[85]. Subsequent development of *B. burgdorferi*-specific IgG antibodies is correlated with a reduction in spirochaetal numbers in mice, and passively administered IgG antibodies can prevent the establishment of infection in animal models[33]. Probably owing to variability of proteins among strains of *B. burgdorferi*, many antibodies against specific proteins are only able to prevent infection in isogenic strains.

Within several weeks to months, innate and adaptive immune mechanisms can reduce bacterial numbers such that, even without antibiotic treatment, the systemic symptoms of Lyme borreliosis wane. Spirochaetes might survive in localized niches in untreated patients for several more years, which can cause persistent symptoms in some cases. However, in humans, all of the inflammatory manifestations of disease, with the possible exception of acrodermatitis chronica atrophicans, resolve eventually, even without antibiotic therapy. Wild-life reservoirs of *B. burgdorferi*, such as mice, do not develop any pathology related to the infection, despite lifelong persistence of the bacteria. This finding suggests that their immune systems have evolved to 'ignore' the presence of the organism, which, because the bacteria do not produce toxins or degradative factors, poses less of a threat than continued activation of the immune system. In humans, who are not reservoir hosts, the bacteria, although able to escape killing in localized niches for months or years, are more-efficiently cleared.

Diagnosis, screening and prevention

Except for erythema migrans, which is usually diagnosed clinically, the other manifestations of Lyme borreliosis are typically diagnosed by recognition of characteristic clinical signs and symptoms along with serological testing[2]. Without antibiotic therapy, the clinical manifestations of the disease typically occur in three stages, beginning with early localized infection of the skin and ending with late infection, most commonly Lyme arthritis in the United States or acrodermatitis chronica atrophicans in Europe.

Clinical manifestations

In all parts of the world with endemic Lyme borreliosis, the infection typically begins during summer with erythema migrans (stage 1), which occurs at the site of the tick bite (FIG. 5). In the United States, where the infection is caused by *B. burgdorferi*, erythema migrans is often accompanied by malaise, fatigue, headache, arthralgias, myalgias, fever and regional lymphadenopathy[86]. In addition, erythema migrans is the presenting manifestation of Lyme borreliosis in ~80% of patients in the United States; ~18% of patients have nonspecific symptoms during summer without recognition of erythema migrans and the remaining 2–3% present with a manifestation of early or late disseminated infection, such as facial palsy, trigeminal neuropathy or Lyme arthritis[87]. Even without antibiotic therapy, erythema migrans typically improves or resolves within weeks.

In Europe, erythema migrans caused by any of the *B. burgdorferi* s.l. genospecies expands slower than that evident in cases in the United States and is not usually accompanied by other symptoms. Interestingly, the clinical features of *B. burgdorferi* infection in Europe more closely resemble that of infection with *B. afzelii* or *B. garinii*, which share the same ecological niche, than the clinical features of *B. burgdorferi* infection in the United States[88]. Although *B. garinii* infection also begins in most cases as a solitary skin lesion, itching and burning symptoms within the lesion are more common and local spreading is faster than with *B. afzelii* or *B. burgdorferi* infection in Europe[89,90]. A rare skin manifestation of early Lyme borreliosis in Europe is borrelial lymphocytoma, which is typically located on the earlobe in children or on the nipple in adults (FIG. 5b) and is most often caused by *B. afzelii* infection[91].

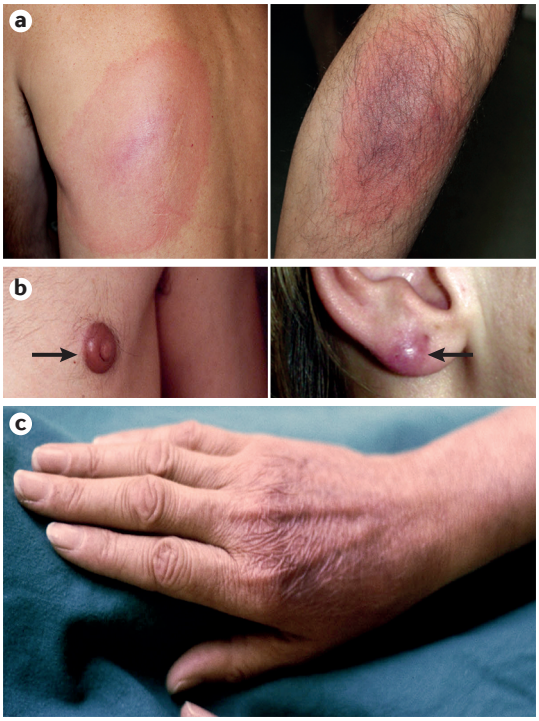

Figure 5 | **Dermatological manifestations of Lyme borreliosis.** All pathogenic *Borrelia burgdorferi* s.l. genospecies typically cause an expanding skin lesion known as erythema migrans, which occurs at the site of the tick bite. **a** | Classic erythema migrans lesions, with a brighter red outer border, partial central clearing and a bull's eye centre. Other erythema migrans lesions can have a more-intense inflammation and purplish discolouration in the centre. **b** | Borrelial lymphocytoma (arrows) is a subacute lesion that typically occurs on the nipple in adults or on the earlobe in children. **c** | Acrodermatitis chronica atrophicans is the most common late manifestation of Lyme borreliosis in Europe. These lesions have an inflammatory phase with a reddish or blue colour followed by an atrophic phase, in which the skin thins considerably, sometimes with fibrotic features. Borrelial lymphocytoma and acrodermatitis chronica atrophicans have been noted in Europe and Asia, but not in North America.

Early disseminated infection. Within days to weeks, the strains of *B. burgdorferi* in the United States commonly disseminate from the site of the tick bite to other regions of the body. *B. burgdorferi* can spread to other skin sites, causing multiple erythema migrans lesions, or to other organs, particularly to the peripheral and/or central nervous system (CNS), heart or joints[2] (FIG. 6). *B. afzelii* does not disseminate as often as *B. burgdorferi*, but can persist at skin sites, for months or years, either at the site of the previous tick bite or at other sites, whereas *B. garinii* is particularly neurotropic and may cause both peripheral nervous system (radiculoneuritis, that is, inflammation of the spinal nerve root) and CNS (meningitis) abnormalities.

During early disseminated infection (stage 2), patients might develop acute Lyme neuroborreliosis[1]. In the United States, the most common clinical features of Lyme neuroborreliosis are lymphocytic meningitis with episodic headaches and mild neck stiffness, cranial neuropathy (particularly facial palsy), or motor or sensory radiculoneuritis[92]. Rarely, patients might have cerebellar ataxia or encephalomyelitis[92]. Electrophysiological studies of peripheral nerve lesions in affected extremities (that is, the extremities that are innervated by branches of the spinal nerve with radiculoneuritis) suggest primarily axonal nerve involvement[92,93]. Histologically, these lesions show axonal injury with perivascular infiltration of lymphocytes and plasma cells around epineural blood vessels[93].

In Europe, *B. garinii* infection causes a type of neurological involvement, called Bannwarth syndrome or tick-borne meningopolyneuritis[94]. Bannwarth syndrome begins with painful radiculoneuritis that is associated with lymphocytic meningitis, often without headache, and can be followed by cranial neuropathy or pareses of the extremities[95]. *B. afzelii* can also cause neurological involvement, but the clinical manifestations are not as clear as with *B. garinii*[96]. Of 10 patients in whom *B. afzelii* was isolated from cerebrospinal fluid (CSF), only one fulfilled criteria for confirmed Lyme neuroborreliosis, which is defined as an objective neurological deficit accompanied by CSF pleocytosis and intrathecal antibody production to *B. burgdorferi* s.l. (REF. 96). In the other nine patients, clinical symptoms of CNS involvement were often vague, primarily consisting of headache, dizziness, concentration and memory disturbances and paresthesias; only two of these patients had CSF pleocytosis. Even without antibiotic therapy, acute Lyme neuroborreliosis usually improves or resolves within months[97].

Acute cardiac involvement can also occur during early disseminated infection and mostly manifests as fluctuating degrees of atrioventricular nodal block, in which electrical impulses from the atria are interrupted before conduction to the ventricles. Other, less common, manifestations include acute myopericarditis or mild left ventricular dysfunction and, rarely, cardiomegaly or pancarditis[1]. Acute carditis usually resolves within weeks, even without antibiotic therapy. However, fatal cases have been reported[98]. In Europe, the spirochaete was isolated from endomyocardial biopsy samples of a patient with chronic dilated cardiomyopathy[99].

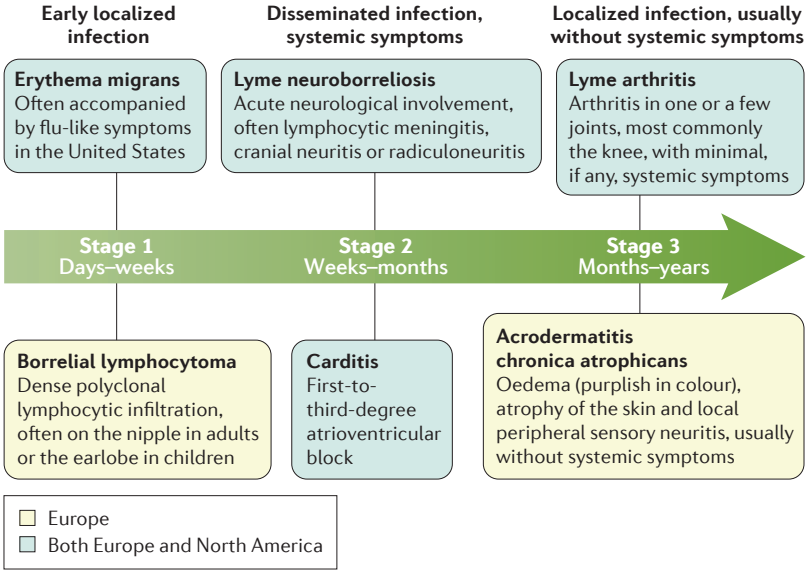

Figure 6 | **The stages and most common clinical features of Lyme borreliosis.** The natural history of the infection without antibiotic therapy begins with localized infection in the skin, then dissemination of the bacteria to numerous sites, but long-term survival in only one or a few localized niches. In patients who have not been treated with antibiotics, Lyme borreliosis typically occurs in stages, with different clinical manifestations at each stage. However, the stages can overlap and late manifestations can be the presenting feature. The infection typically begins as a localized infection of the skin, but, particularly in the United States, *Borrelia burgdorferi* often disseminates, which is commonly associated with systemic symptoms. However, as the disease progresses and as the immune response matures, the infection typically becomes more localized, such as to the knee joint, and is accompanied by minimal, if any, systemic symptoms.

Late infection. The late, stage 3 manifestations of Lyme borreliosis in the United States diverge from those in Europe even more than the early-stage manifestations (FIG. 6). In an initial study, carried out before the cause of the disease was known, ~60% of patients with erythema migrans who were not treated with antibiotics in the northeastern United States developed arthritis an average of 6 months (range: 4 days to 2 years) later[100]. Joint swelling and pain typically occurred in intermittent attacks primarily in large joints, especially the knee, over a period of several years, but some patients had persistent synovitis for 4–5 years. Lyme arthritis usually resolves following appropriate oral or intravenous antibiotic therapy[101]. However, some patients have persistent, proliferative synovitis for months to several years, which is termed post-infectious, antibiotic-refractory Lyme arthritis[102]. In Europe, Lyme borreliosis can also cause arthritis, usually in one or a few large joints, but this occurs less frequently and earlier in the disease course than in the United States. In one study from Germany, the mean period from erythema migrans to the onset of arthritis was 3 months (range: 10 days to 16 months)[103]. In addition, chronic arthritis occurs rarely in Europe.

In Europe, acrodermatitis chronica atrophicans — a slowly progressive lesion that is located primarily on the extensor (acral) surfaces of the extremities — is the most frequent late manifestation of Lyme borreliosis and is more common in older women, for unclear reasons, and is rare in children[91,104] (FIGS 5,6). About 20% of patients with acrodermatitis chronica atrophicans have a history that is consistent with a preceding spontaneously healed erythema migrans lesion, usually on an extremity where the acrodermatitis chronica atrophicans lesion developed 6 months to 8 years later[105]. Acrodermatitis chronica atrophicans results primarily from *B. afzelii* infection, although it can also be caused by *B. garinii* or European *B. burgdorferi* infections[106] and starts with an inflammatory phase that is followed by an atrophic phase, sometimes with features. The bacteria have been isolated from acrodermatitis chronica atrophicans lesions >20 years after its first appearance. Peripheral neuropathy and joint involvement can occur within the skin sites affected with acrodermatitis chronica atrophicans lesions.

Several rare neurological manifestations have also been associated with *B. burgdorferi* s.l. infection. In Europe, a severe neurological syndrome of chronic encephalomyelitis that is characterized by spastic paraparesis, cranial neuropathy or cognitive impairment has been described[94]. Stroke-like signs and symptoms in patients with Lyme borreliosis have been reported in both the United States and Europe[107]. In the United States, a mild, late encephalopathy that manifests primarily as subtle cognitive disturbances[108] and/or a mild sensory polyneuropathy have been reported to occur[109]. However, the existence of these neurological syndromes is controversial and how much of the disease is due to active infection, post-infectious immune phenomena or other factors is not always clear.

Diagnostic tests

Demonstration of borrelial infection by laboratory testing is required for reliable diagnosis of Lyme borreliosis, with the exception of erythema migrans[1,2,97,110]. The culture of *Borrelia* spp. from patient specimens enables definitive diagnosis, but is generally restricted almost exclusively to research studies because of the need for special expertise and equipment. Moreover, except for patients with acrodermatitis chronica atrophicans[104], positive cultures have been obtained only during the first weeks of infection, primarily from skin biopsy samples of erythema migrans lesions[2]. Later in the infection, PCR-based testing for *B. burgdorferi* DNA in synovial fluid is often positive in patients with Lyme arthritis before antibiotic therapy[111], but is only positive in the CSF of a small number of patients with late Lyme neuroborreliosis. Because *Borrelia* DNA can persist after spirochaetal killing, PCR is not an accurate test for active infection[111].

Thus, in both the United States and Europe, serological testing is the only practical and readily available method to support a diagnosis of Lyme borreliosis. In the United States, diagnosis is usually based on recognition of characteristic clinical features along with the detection of antibodies to *B. burgdorferi*, determined by enzyme-linked immunosorbent assay (ELISA) and western blotting. BOX 1 summarizes the recommended approaches for serological testing for *B. burgdorferi* s.l. and the pitfalls to be avoided. In the United States, western blots should be interpreted according to the criteria of the Centers for Disease Control and Prevention[112,113] (BOX 1).

Box 1 | **Serological testing for Lyme borreliosis**

Recommended approach to support diagnosis

- Recognition of characteristic clinical manifestations
- Positive antibody response against *Borrelia burgdorferi* determined by a two-tiered approach of enzyme-linked immunosorbent assay (ELISA) and western blot, except in patients with erythema migrans
- In the United States, the recommended criteria from the US Centers for Disease Control and Prevention to interpret a positive western blot are:
  - During the first 30 days of symptoms in a patient with early disease, at least two IgM bands of 23, 39 or 41 kD[214]
  - During early or late infection, at least five IgG bands of 18, 23, 28, 30, 39, 41, 45, 58, 66 or 93 kD[215]
- In Europe, there is no single set of criteria to interpret western blot results with high levels of sensitivity and specificity
- In European and North American countries, testing for antibody in cerebrospinal fluid (CSF) must be corrected for passive diffusion of anti-*Borrelia* antibodies from blood
  - Total IgG levels can be measured at both sites, adjusting the dilutions accordingly for ELISA testing[110,216], or antibody capture enzyme immunoassay can be used to compare the ratio of total to specific immunoglobulin at both sites[117]

Pitfalls to be avoided

- Omission of first-tier ELISA and using only a second-tier western blot to support the diagnosis
- Using an IgM western blot to support a diagnosis of late Lyme borreliosis
- Using unvalidated western blot criteria
- Testing for antibody in CSF without correcting for passive diffusion of anti-*Borrelia* antibodies in blood
- Attributing a positive antibody response against *Borrelia* as an indication of persistent infection after recommended courses of antibiotic therapy, given that the anti-*Borrelia* antibody response (even IgM) declines slowly after antibiotic treatment and can remain positive for months or years

However, these criteria cannot be used in Europe, as no single set of interpretive criteria provides results with high sensitivity and specificity in all countries, owing to the presence of different genospecies of bacteria[114]. In a multicentre study to develop criteria for Europe, each laboratory identified eight bands for use in diagnosis, but no single set of criteria could be formulated[114].

Serodiagnostic tests are insensitive during the first several weeks of infection when most patients with Lyme borreliosis have erythema migrans. In the United States, 20–50% of patients have positive responses, usually of the IgM isotype, during acute, early infection[115,116]. During the convalescent period at the end of 2–3 weeks of antibiotic treatment, 70–80% of patients have seroreactivity, still usually of the IgM isotype. However, after 4–8 weeks of untreated infection, virtually 100% of patients have IgG antibody responses.

In patients with acute Lyme neuroborreliosis, especially those with meningitis, intrathecal antibody production of IgG and IgM antibodies against *Borrelia* spp. can often be observed, particularly in European patients with *B. garinii* infection[117]. To demonstrate intrathecal antibody production, the testing must be corrected for the diffusion of *Borrelia* antibodies from the blood (BOX 1).

The major limitation of serological testing is that IgG and even IgM antibodies against *B. burgdorferi* can remain for months or years after near or complete spirochaetal elimination with antibiotics[118]. The amount of antibody declines slowly after treatment, but findings on western blot, which is a non-quantitative test, do not change appreciably in the post-antibiotic period. Thus, serological testing cannot be used to determine active infection or the adequacy of antibiotic therapy. Moreover, in the United States, *B. burgdorferi* can cause asymptomatic infections in ~10% of patients[119] and, in a study of seroprevalence in Sweden, >50% of individuals who were seropositive for *B. burgdorferi* detected by ELISA did not remember having symptoms of Lyme borreliosis[120]. Thus, if patients with past or asymptomatic *Borrelia* infection develop another illness, particularly one with neurological or joint symptoms, the symptoms might be attributed incorrectly to Lyme borreliosis. For this reason, serological testing becomes somewhat less useful in supporting the diagnosis of current symptoms in patients with a past history of *Borrelia* infection.

Co-infection

In the United States, *I. scapularis* has been implicated in the transmission of at least five other infectious agents, most prominently *Anaplasma phagocytophilum*[101] (previously called the agent of human granulocytic ehrlichiosis, which infects granulocytes), *Babesia microti*[101] (a red blood cell parasite), *Borrelia miyamotoi* (a relapsing fever *Borrelia*)[121] and *Borrelia mayonii* (a newly recognized species in the northern midwestern United States)[122]. Each of these agents (except that very little is currently known about *B. mayonii* infection) causes nonspecific symptoms, such as headache, myalgia, arthralgia and fatigue, with the fever being generally more prominent than in patients with Lyme borreliosis. However, chronic illness is not generally associated with these agents, although *B. miyamotoi* infection caused chronic meningoencephalitis in one reported immunocompromised individual[123] and *B. mayonii* infection apparently caused arthritis in one of the six reported cases[122,124].

A. phagocytophilum and *B. microti*, as well as *Babesia divergens* and *Babesia venatorum*, have been found in *I. ricinus* in Europe[125,126]. In addition, in Europe, *I. ricinus* can transmit tick-borne viral encephalitis[127]. In the United States, ~0.2% of *I. scapularis* are infected with a related virus, called the Powassan virus (also known as the deer tick virus), but only sporadic cases of the infection have been recognized. In a small percentage of patients, particularly in older or immunocompromised patients, these neurotropic viruses can cause fatal infection[128].

Prevention

Interventions for the prevention of Lyme borreliosis focus primarily on personal protective measures. Protective measures can include the avoidance of areas with high numbers of ticks, wearing protective clothing, the use of tick repellents and acaricides, checking for the presence of ticks and changing the landscape in, or nearby, residential areas to make it less habitable for ticks[129]. These measures have been associated with a decreased risk of infection in some studies[130],

but not in others[131]. Landscaping practices and the application of synthetic acaricides can substantially reduce the abundance of ticks, but might not reduce the incidence of Lyme borreliosis, which shows that the remaining ticks can still transmit the bacteria[129]. For individuals who are reluctant to use synthetic acaricides, entomopathogenic fungi and several compounds extracted from herbs or coniferous trees have been shown to kill or repel *Ixodes* spp. in field trials[132,133]. The frequency of the infection after an *I. scapularis* bite has been shown to be 1–4% in the United States[134], presumably because the tick must usually be attached for >36 hours before transmission occurs[135]. However, shorter periods of tick attachment that are necessary to cause transmission have been reported for *B. afzeli*[12]. Thus, if the tick is removed quickly, then other treatments are not generally required. However, on the basis of a study conducted in the United States, one dose of doxycycline, if administered within 72 hours following a tick bite, can prevent the development of Lyme borreliosis in most individuals[134].

In Europe, the frequency of clinically symptomatic infection after *I. ricinus* bites is 1–5%, as in the United States. On the basis of animal studies, the transmission of *Borrelia* might occur in <36 hours with *I. ricinus* than with *I. scapularis*[12]. However, as the frequency of infected ticks tends to be less in Europe than in the United States and as the efficacy of prophylactic antibiotic therapy has not been determined there, only observation is recommended for individuals with tick bites in Europe. Vaccination for human Lyme borreliosis is not currently available in either North America or Europe.

Table 1 | **Recommended treatment for adults with Lyme borreliosis**

Manifestation	Antibiotic	Treatment duration (days)
Erythema migrans, borrelial lymphocytoma or acrodermatitis chronica atrophicans*	Doxycycline	10
	Amoxicillin	14
	Cefuroxime axetil	14
	Phenoxymethylpenicillin	14
	Azithromycin‡	5–10
Lyme meningitis, cranial neuropathy or radiculopathy	Doxycycline§	14
	Ceftriaxone‖	14
Lyme encephalomyelitis	Ceftriaxone	14–28
Cardiac Lyme disease	Doxycycline§	14–21
	Amoxicillin§	14–21
	Cefuroxime axetil§	14–21
	Ceftriaxone‖	14–21
Lyme arthritis	Doxycycline	28
	Amoxicillin	28
	Cefuroxime axetil	28
	Ceftriaxone¶	14–28

*Treatment duration for borrelial lymphocytoma is 14 days for β-lactam and tetracycline antibiotics; the duration for acrodermatitis chronica atrophicans is 21–28 days. ‡For patients who are unable to take β-lactams or tetracyclines. §For ambulatory patients. ‖For hospitalized patients. ¶Used when there is only a minimal response to oral antibiotics. Adapted from the Infectious Diseases Society of America (IDSA) treatment guidelines[101] and from Sanchez *et al.*[140].

Management

Despite differences in the *B. burgdorferi* s.l. genospecies that cause Lyme borreliosis in Europe and North America and the absence of studies that directly compare treatment outcomes, the overall results of antibiotic treatment seem to be remarkably similar on both continents[97,136–138]. Several groups have reported treatment guidelines for Lyme borreliosis, but the most comprehensive are the 2006 Infectious Diseases Society of America (IDSA) guidelines that were authored by both US and European authorities[101]. In 2010, the IDSA Lyme borreliosis guidelines were reviewed by an independent panel and found to be current and valid[139]. A recent review includes slight modifications of the IDSA guidelines[140].

Early localized infection

Erythema migrans. All *B. burgdorferi* s.l. genospecies tested seem to be susceptible, *in vitro*, to tetracyclines and many β-lactam antibiotics[101]. No differences in the level of susceptibility among active members of these drug classes for the different genospecies that are thought to be clinically relevant have been observed and no evidence suggests the emergence of drug resistance. More variability exists among strains of Lyme borrelia with regard to the *in vitro* activity of certain macrolides, particularly erythromycin[141]. In a single study conducted in the United States, azithromycin, which is quite active *in vitro*, was less effective than amoxicillin in patients with erythema migrans[142].

In the United States, patients with erythema migrans can usually be treated successfully with a 10–14-day course of doxycycline, amoxicillin or cefuroxime axetil[101,137,138,143] (TABLE 1). In Europe, patients can also be treated with phenoxymethylpenicillin. Patients with erythema migrans who cannot take doxycycline or β-lactam antibiotics can be treated with a 5–10-day course of azithromycin[97,101]. One advantage of doxycycline treatment is that it has efficacy against the rickettsial agent *A. phagocytophilum*, which causes human granulocytic anaplasmosis, a possible co-infection with *B. burgdorferi* s.l. However, doxycycline is intended for use in adults and adolescents and should generally be avoided in children <8 years of age and in women who are pregnant or breastfeeding. Although erythema migrans will resolve eventually without antibiotic therapy, treatment shortens the duration and prevents later manifestations of Lyme borreliosis[97,101] (FIG. 6).

Borrelial lymphocytoma. The antibiotic regimens used to treat erythema migrans are also highly effective for the treatment of borrelial lymphocytoma[144] (TABLE 1). If untreated, borrelial lymphocytoma can last for months.

Early extracutaneous disseminated infection

Depending on the disease severity, patients with neurological or cardiac involvement of Lyme borreliosis can usually be treated successfully with the same oral antibiotics that are used for the treatment of erythema migrans (TABLE 1). However, with neurological or cardiac manifestations, a parenterally administered antibiotic might be used in certain cases. The most commonly

prescribed parenterally administered antibiotic is ceftriaxone, as this is administered once daily, but cefotaxime and intravenous penicillin are also highly effective[145,146].

Lyme neuroborreliosis. Patients who are hospitalized for Lyme-associated meningitis are typically treated with parenteral antibiotics (TABLE 1) for either a 14-day course or until they can be discharged from the hospital, at which time oral doxycycline is often substituted to complete a 14-day course of treatment. In a prospective, double-blind study in Europe, 200 mg once-daily treatment with oral doxycycline was found to be as effective as once-daily intravenous ceftriaxone (2 g) for adults with early neurological Lyme borreliosis, with no treatment failures in either group[147]. Whether twice-daily administration of 100 mg of oral doxycycline, which is perhaps better tolerated, would be equally as effective has not been studied systematically; anecdotally, twice-daily administration seems to be effective (G.P.W., unpublished observations). In patients with seventh nerve palsy, antibiotics do not lead to faster resolution of the facial weakness, but are effective in preventing later clinical sequelae, such as Lyme arthritis[148]. Patients with cranial neuropathy have been treated successfully with oral antibiotics other than doxycycline, including amoxicillin or cefuroxime axetil, but published data documenting the efficacy of these therapies are limited[101]. Encephalomyelitis, a rare neurological manifestation of Lyme borreliosis, is treated with a full course of parenteral antibiotics, although oral doxycycline has shown promising results[149]. No comparative trial that included an oral antibiotic has been performed in patients with Lyme encephalomyelitis.

Carditis. Cardiac manifestations commonly include varying degrees of atrioventricular block. In those with a complete (third-degree) block, hospitalization is recommended for monitoring and administration of parenteral antibiotics and patients might also need to have a temporary pacemaker implanted. Patients with advanced forms of partial heart block, including those with second-degree block or first-degree block with a PR interval of >0.3 seconds, should also be hospitalized for monitoring, as these patients are at risk for progression to complete heart block[150]. If substantial improvement allows discharge before day 14, a 2-week course of antibiotics can be completed with oral administration of any of the β-lactam or tetracycline antibiotics that are used for the treatment of erythema migrans[140].

Late infection

Lyme arthritis. Lyme arthritis is typically treated with a 28-day course of an oral antibiotic[101,151]. Parenteral antibiotic therapy of 14–28 days in duration is reserved for patients who still have synovial inflammation following oral therapy[101,152]. No clinical trials have been performed comparing a 28-day course of oral antibiotic with a 14-day course of oral treatment and no clinical trials have compared the outcome of Lyme arthritis in patients treated with oral antibiotics versus parenteral therapy.

NSAIDs can be given with antibiotic therapy, but intra-articular steroids are not recommended before or during antibiotic treatment as this has the potential to result in worse outcomes, such as prolonged joint inflammation[145,153]. However, prospective studies on the use of NSAIDs or intra-articular steroids are lacking. After resolution of arthritis of the knee, physical therapy might be needed if atrophy of the quadriceps has developed.

The persistence of at least mild joint inflammation immediately following antibiotic therapy is found in at least 25% of patients with Lyme arthritis, irrespective of whether intravenous ceftriaxone[153] or an oral antibiotic[154] was the initial treatment. Data from observational studies of children with Lyme arthritis indicate that the residual joint inflammation will resolve with NSAIDs and/or intra-articular injections of corticosteroids in most patients[153,155]. Disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate, are usually effective in those who do not respond to NSAIDs or intra-articular corticosteroids and in adults with post-antibiotic proliferative synovitis[156]. In responding patients, DMARDs can usually be discontinued after 6–12 months of treatment. In patients with incomplete responses, arthroscopic synovectomy is an option, but the debridement of synovial tissue down to the cartilage interface is necessary for a successful result[156].

Acrodermatitis chronica atrophicans. Acrodermatitis chronica atrophicans is usually treated with a 21–28-day course of oral antibiotic therapy (TABLE 1), which can halt progression and improve or resolve the skin lesion, but not reverse the neuropathy[106]. If untreated, the skin lesion does not typically resolve spontaneously, and fibrosis and atrophy can develop[104].

Post-treatment symptoms

In ~10% of patients with erythema migrans and perhaps a higher percentage of patients with Lyme neuroborreliosis[157–159], subjective symptoms such as fatigue, cognitive complaints and musculoskeletal pain can persist for ≥6 months after antibiotic therapy. These symptoms have been shown to continue, at least intermittently, for >10 years in some cases[157]. In an undefined minority of patients with residual subjective symptoms following treatment of Lyme borreliosis, these symptoms are functionally disabling and are referred to as post-treatment Lyme disease syndrome (PTLDS). A proposed definition for PTLDS is the presence of subjective symptoms that begin within 6 months after the diagnosis and treatment of an objective clinical manifestation of Lyme borreliosis and persists for at least 6 months[101]. Whether post-treatment symptoms are more common with Lyme borreliosis than other infections, or even in healthy controls, is unknown owing to a lack of studies. In one prospective European study of patients with erythema migrans and healthy controls, the rate of new or increased symptoms at 12 months was greater in the healthy control population[158].

In the United States and Europe, placebo-controlled, randomized retreatment trials using either parenteral antibiotic therapy alone or followed by a course of oral antibiotics in patients with PTLDS did not show any

Box 2 | **Therapies not recommended for Lyme borreliosis or PTLDS**

- Long-term antibiotic therapy
- Multiple repeated courses of antibiotics for the same episode of Lyme borreliosis
- Combinations of antibiotics
- Pulsed dosing in which antibiotics are given on some days but not on other days
- First-generation cephalosporins, such as cephalexin, benzathine penicillin G, fluoroquinolones, carbapenems, vancomycin, metronidazole, tinidazole, trimethoprim-sulfamethoxazole, amantadine, ketolides, isoniazid, rifampin or fluconazole
- Drug therapy for babesiosis (that is, a malaria-like parasitic disease) or *Bartonella* spp. infection in the absence of evidence for active infection
- Hyperbaric oxygen, intravenous hydrogen peroxide or ozone therapy
- Energy-based or radiation-based therapies, such as 'rife therapy'
- Nutritional therapies, such as burnt mugwort or glutathione
- Chelation and heavy-metal therapies
- Fever therapy
- Intravenous immunoglobulin
- Urotherapy (that is, the ingestion of one's own urine)
- Apheresis
- Stem cell transplantation
- Drugs (such as cholestyramine), enemas, bee venom, various hormonal therapies (such as thyroid hormone), lithium orotate, olmesartan, naltrexone or bleach

PTLDS, post-treatment Lyme disease syndrome

clinical benefit, or showed a benefit that was so modest it was outweighed by the risk of adverse effects from the treatment (for example, intravenous-line sepsis)[160,161]. Evidence for the persistence of infection was not found in any of these studies and a recent editorial suggests that a different approach than prolonged treatment with antibiotics for PTLDS is required[162]. Additional studies are needed to better understand the pathogenesis of persistent symptoms and to determine the best approaches for symptomatic relief.

Depression in patients with PTLDS should be managed as per standard practice[163]. Symptomatic therapy is recommended for other types of symptoms, such as joint pain. However, optimal approaches for symptomatic management have not been defined as systematic studies — except those evaluating retreatment with antibiotics — are uncommon or completely lacking in these patients. Some authorities, borrowing from treatment studies of fibromyalgia, have advocated multidisciplinary approaches that combine medications, such as gabapentin or amitriptyline, with non-pharmacological therapies, for example, cognitive–behavioural therapy, acupuncture or massage[164].

Many unsubstantiated therapies have been proposed for patients with Lyme borreliosis and for those patients with residual pain, neurocognitive symptoms or fatigue following treatment[101,165] (BOX 2). These therapies lack proven benefit and, in some cases, pose a real risk of adverse outcomes.

Quality of life

Regardless of the disease manifestation, most patients with Lyme borreliosis respond well to antibiotic therapy and experience a complete recovery. However, some patients do not recover completely and post-infectious sequelae can affect quality of life.

In the northeastern United States, a small percentage of patients have marked proliferative synovitis that lasts for months to several years after treatment of the infection[102]. Rather than persistent infection, post-infectious immune responses resulting from excessive inflammation[39], immune dysregulation[166,167], infection-induced autoimmunity[168–170] or retained bacterial antigens[171] are thought to contribute to this. Severe and prolonged Lyme arthritis can result in premature degenerative arthritis, particularly if associated with tendon rupture or marked atrophy of the quadriceps. In a 10–20-year follow-up study in the United States, 10 of 42 patients with previous Lyme arthritis had findings that were suggestive of degenerative arthritis in previously affected knees on physical examination compared with 0 of 42 patients with previous Lyme borreliosis who did not develop Lyme arthritis ($P=0.001$)[172].

Long-term motor deficits can occur after Lyme neuroborreliosis. In a 10–20-year follow-up study of patients with Lyme neuroborreliosis in the United States, most of whom were seen before knowledge of antibiotic treatment for the disease, 23% of patients with facial palsy had mild-to-moderate residual deficits of facial nerve function and 26% had subtle motor or sensory peripheral nerve abnormalities[172]. By contrast, in a study of antibiotic-treated European patients with Bannwarth syndrome, 7% with facial palsy and 50% with motor pareses of extremities had incomplete recoveries, although only one patient had motor weakness that was functionally limiting[95]. Similarly, in patients with late, stage 3 Lyme neuroborreliosis, antibiotic therapy halts disease progression and patients experience substantial improvement. However, depending on the degree of parenchymal CNS involvement, recovery might be incomplete[173]. Risk factors for unfavourable outcomes (such as poor recovery of sensory and motor function) include female sex, pretreatment duration of symptoms of >6 weeks, a high CSF white blood cell count before treatment[174,175] and the presence of symptoms at the end of a 14-day treatment with ceftriaxone[95]. Similarly, in patients with acrodermatitis chronica atrophicans who were treated with antibiotics, the manifestations associated with long-term skin infection, such as skin atrophy, peripheral nerve abnormalities or subluxation of small joints, might improve little if at all[104]. Rare cases have been reported of the development of autoimmune diseases in the weeks to months after Lyme neuroborreliosis, for example, chronic idiopathic demyelinating polyneuropathy[176] and cerebral vasculitis[177].

Finally, patients with post-treatment symptoms often experience improvement of their symptoms within months[158], although some patients can have severe joint and muscle pain, neurocognitive difficulties and/or incapacitating fatigue that persist for years[178]. The pathogenesis of these symptoms is unclear and might not be the same in all patients. These symptoms might be a part of a central sensitization syndrome[179] or be attributable to immune system abnormalities, for example, increased levels of IL-23 (REF. 180) or heightened levels of antineuronal antibodies[181]. This area is further confused as PTLDS, or what has been referred to as 'chronic Lyme

disease', has become a diagnosis for medically unexplained symptoms, even when patients have no evidence of past or present Lyme borreliosis[182,183]. Importantly, the various post-infectious complications of Lyme borreliosis should not be confused with signs of persistent infection with *B. burgdorferi*, and prolonged antibiotic therapy has not been shown to be beneficial[182].

Outlook

Diagnostic tests

In the United States, a two-tiered approach with whole-cell sonicate ELISA and western blotting has worked well for serological diagnosis of Lyme borreliosis, but has limitations. Second-generation screening immunoassays have been designed using recombinant *B. burgdorferi* proteins or synthetic peptides as antigen targets[184], which show an improvement in specificity compared with conventional whole-cell sonicate ELISAs. Similarly, line immunoblots or other multiplexed antibody detection platforms prepared from purified or recombinant *B. burgdoferi* proteins have been developed as a substitute for western blots[185]. These assays are easier to interpret and their adoption might reduce the misreading of weak bands and improve reproducibility, particularly of western blotting for IgM antibodies[186]. In addition, western blotting might be unnecessary in routine cases of Lyme borreliosis if two different ELISAs are used instead[187]. This strategy has improved sensitivity during the first weeks of infection[187,188] and reduced the costs and complexity compared with conventional two-tiered testing[189]. Efforts are also underway to develop rapid serological assays that can be performed in doctors' offices, so-called point-of-care tests[190], which would reduce turnaround times.

Another avenue of investigation involves the measurement of biomarkers. For example, increased concentrations of CXCL13 (a chemoattractant for B cells) in CSF have been proposed as a biomarker for Lyme neuroborreliosis. Although European patients with Bannwarth syndrome typically have high levels of CXCL13 in CSF, which correlates with intrathecal antibody production against Lyme borrelia[191,192], numerous other conditions can also cause increased levels of CXCL13, including neurosyphilis, HIV infection, CNS lymphoma and multiple sclerosis[192–194]. Thus, for use as a diagnostic test for Lyme neuroborreliosis, the cut-off concentration of CXCL13 becomes crucially important and needs definition.

Another investigative approach involves the development of proteomic, inflammatory, nucleic acid, cellular or metabolomic biosignatures of Lyme borreliosis. In one proof-of-concept study, a metabolic biosignature of 44 molecular features, such as polyunsaturated fatty acids or products of prostaglandin metabolism, correctly classified patients with early Lyme borreliosis (the majority of whom were seronegative by conventional two-tiered testing) and healthy controls with a sensitivity of 88% and a specificity of 95%[195]. By contrast, in the United States, standard two-tiered testing in patients with acute erythema migrans has a sensitivity of 30% and a specificity of 99%, although sensitivity rises to 60–70% with post-treatment convalescent testing[115].

Antibiotic treatment

Studies on antibiotic treatment for Lyme borreliosis are relatively limited. A double-blind, placebo-controlled trial of intramuscular benzathine penicillin that started before the identification of *B. burgdorferi* showed the efficacy of antibiotic therapy in Lyme arthritis, but did not lead to the resolution of arthritis in all patients[196]. Conversely, several double-blind, placebo-controlled trials have demonstrated no benefit from additional courses of oral or intravenous antibiotic therapy in patients with PTLDS[160]. In addition, several randomized trials of different antibiotic regimens have been conducted on both continents in patients with erythema migrans, neuroborreliosis or Lyme arthritis. Treatment of unusual manifestations of the disease is based on case series or expert opinion. These trials are detailed in the IDSA guidelines[101].

Numerous questions remain about the best approaches to treat Lyme borreliosis and well-designed studies are to be encouraged. For example, a recent retrospective analysis of patients with Lyme borreliosis and facial palsy reported a worse long-term outcome in those who received antibiotics and prednisone than in those who received antibiotics alone[197]. However, these regimens have not been compared in a randomized clinical trial. Another important example is the treatment of Lyme arthritis in the United States, which is currently based on an algorithm[156]. In formulating this step-wise approach, randomized studies of initial antibiotic therapy are available, but only case series have been published regarding patients who did not respond to oral antibiotic therapy or those who developed antibiotic-refractory Lyme arthritis. Thus, any definitive study would need to compare multiple therapies and include long-term follow-up.

Control of ticks or animal hosts

Considerable research has been devoted to interventions that decrease tick abundance or interrupt the transmission of *B. burgdorferi* in the environment[129]. In areas where deer are a primary host for adult *Ixodes* spp., deer exclusion or population reduction has been proposed as an indirect means of tick control. Although studies on islands have suggested that deer elimination can be successful, interventions that are short of complete deer elimination have yielded mixed results[198]. As a possible alternative, a '4-poster' device has been developed that applies topical acaricide to deer that are drawn to a feeding station[199]. Several interventions designed to reduce or prevent infection among reservoir rodents are also being tested. These tools include bait boxes that treat rodents with a tick-control agent (such as fipronil), oral vaccines to immunize wild rodents against infection with *B. burgdorferi* and doxycycline hyclate-laden bait to treat and eliminate infection[129,200].

Despite the theoretical promise of environmental controls, numerous obstacles exist to widespread implementation, including cost, regulatory issues and concerns over pesticide use and deer control[201]. Perhaps most importantly, empirical evidence demonstrating public health efficacy is lacking for most environmental interventions. Nearly all studies have focused on

entomological outcomes, such as tick abundance, rather than human illness and most have been conducted on a small scale under optimized conditions. The importance of documenting health outcomes is highlighted by a placebo-controlled trial in which yard pesticide treatments were associated with a 60% reduction in the number of ticks, but no reduction in tick bites or tick-borne illness among household members[202].

Vaccination

Although several vaccines for Lyme borreliosis are available for use in animals[203], human vaccines are not available. A recombinant OspA-based vaccine was marketed in the United States from 1998 to 2002 (REF. 22), but, although safe and efficacious, this was withdrawn from the market. One important reason for this was that Lyme borreliosis advocacy groups, which initially supported the vaccine, became crucial opponents[204], leading to the threat of class action lawsuits. Other reasons included vaccine cost, the need for booster vaccinations and the theoretical concern that the vaccine might trigger autoimmune arthritis[205]. Despite the demand for a protective vaccine for Lyme borreliosis and that vaccination is cost-effective in high-risk endemic areas[206], pharmaceutical companies have subsequently been reluctant to test and market human vaccines.

Nonetheless, modified OspA-based vaccines, other spirochaetal protein-based vaccines (including one that targets BB0405 (REF. 207)) and novel delivery strategies have been the subject of investigation[208]. Importantly, phase I and phase II trials with a modified multivalent OspA-based vaccine that lacked a T cell epitope initially thought to have autoreactive potential, confirmed that vaccination is safe and results in robust antibody responses[209]. However, the involved pharmaceutical company is not currently moving ahead with further testing of this vaccine.

An alternative vaccination strategy to prevent Lyme borreliosis and potentially other diseases that are transmitted by the same tick would be to vaccinate against tick proteins[208]. This approach is based on the observation that some laboratory animals, such as guinea pigs and rabbits, when repetitively infested with ticks, develop immune responses against tick proteins, which results in impaired tick feeding and partial protection against *B. burgdorferi*[210]. An anti-tick vaccine against another tick species is available for the veterinary market, which underscores the feasibility of this approach[208]. Finally, multiple tick saliva proteins are involved in the transmission of *B. burgdorferi* from the tick to the host, and targeting such proteins by vaccination has yielded partial success in experimental settings[48,211,212]. A new vaccine would need to meet high standards of safety, efficacy, cost and public acceptance[213]. Nevertheless, the feasibility of human vaccination for Lyme borreliosis has been demonstrated and vaccination could be beneficial in preventing this and other tick-borne diseases.

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Competing interests
A.C.S. declares grant support from Immunetics, Inc. and Viramed. F.S. has served as a consultant to Baxter Biosciences regarding Lyme vaccine development and has been a member of the steering committee of the European Society of Clinical Microbiology and Infectious Diseases Study Group on Lyme Borreliosis (ESGBOR). G.P.W. has received research grants from Immunetics Inc., Institute for Systems Biology, Rarecyte, Inc. and Quidel Corporation. G.P.W. also owns equity in Abbott, has been an expert witness in malpractice cases involving Lyme borreliosis and is an unpaid board member of the American Lyme Disease Foundation. J.A.B. declares grant support from Immunetics, Inc. and Diasorin. J.W.R.H. is a member of the steering committee of ESGBOR. L.T.H., X.L. and P.S.M. declare no competing interests.