

Epidemiology of Lyme Neuroborreliosis



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KEYWORDS

• Lyme disease • *Borrelia burgdorferi* • Neurology • Public health • Epidemiology

KEY POINTS

- According to the Centers for Disease Control and Prevention, Lyme disease is the most commonly reported vector-borne illness and the fifth most common disease in the National Notifiable Diseases Surveillance System, making it an important public health concern.
- Lyme disease is caused by the bacterium *Borrelia burgdorferi* and is transmitted to humans through the bite of infected blacklegged *Ixodes* ticks.
- Typical symptoms include fever, headache, fatigue, and a characteristic skin rash called *erythema migrans*.
- Undiagnosed and therefore untreated, infection disseminates to the nervous system.
- The nonhuman primate model of Lyme neuroborreliosis accurately mimicked the microbiological, clinical, immunologic, and neuropathologic aspects of human Lyme neuroborreliosis.

INTRODUCTION

Lyme disease in humans is caused by the transmission of *Borrelia (B) burgdorferi* in the bite of infected blacklegged *Ixodes* ticks. Typical symptoms include fever, headache, fatigue, and a characteristic skin rash called *erythema migrans* (EM). If left undiagnosed and therefore untreated, infection disseminates to the nervous system causing Lyme neuroborreliosis (LNB). The clinical diagnosis is based on symptoms, physical findings, and the probability of exposure to infected ticks in endemic geographic areas and confirmed by serologic and cerebrospinal fluid (CSF) testing with the demonstration of intrathecal production of *Borrelia*-specific antibodies. There is general recognition for the potential of infectious-related autoimmune processes contributing to nervous system disease progression.

The author has nothing to disclose.

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HISTORY

Originally named for Lyme and Old Lyme, Connecticut, wherein a tight clustering of recurrent attacks of childhood and adult asymmetric oligoarticular arthralgia occurred beginning in 1972, Lyme disease showed a peak incidence of new cases in the summer and early fall.^{1,2} Epidemiologic analysis of the clustering suggested transmission of a causative agent by an arthropod vector to humans, in whom 25% describe an expanding annular EM rash before onset of the arthritis. Cultures of the synovium and synovial fluid did not suggest infection with agents known to cause other forms of arthritis. Those in whom arthritis developed seemed to have significantly elevated ESR, lower third and fourth components of complement (C3, C4), higher serum IgM levels, and serum cryoprecipitates at the time of the skin lesions, suggesting an active immunologic response. Five years later, Burgdorfer and colleagues³ isolated a spirochete from the tick *Ixodes (I) dammini* that bound immunoglobulins of patients convalescing from Lyme disease and recorded the development of lesions resembling EM in New Zealand white rabbits 10 to 12 weeks after being bitten by the ticks. One year later in the same volume of *The New England Journal of Medicine*, Steere and co-workers⁴ and Benach and colleagues⁵ described the spirochetal etiology of Lyme disease. Benach and colleagues⁴ isolated spirochetes from the blood of 2 of 36 patients in Long Island and Westchester County, New York with signs and symptoms suggestive of Lyme disease that were morphologically similar and serologically identical to organisms known to infect *I dammini* ticks, endemic to the area and epidemiologically implicated as vectors of Lyme disease.

CLINICAL INVOLVEMENT

By 1989 Steere⁶ summarized the causation, vector and animal hosts, clinical manifestations, pathogenesis, and treatment of human Lyme disease. Three stages of infection were recognized, each with different clinical manifestations. Stage 1 followed the bite by the tick with spread of bacteria locally in the skin in 60% to 80% of patients, resulting in EM rash that faded in 3 to 4 weeks but often accompanied by fever, minor constitutional symptoms, or regional adenopathy. At this time, the patient's mononuclear cells responded minimally to spirochete antigens, and even specific antibody might be lacking. Stage 2 of early infection followed days or weeks after the bite with bloodstream or lymphatic spread to many organ sites. More common in the United States than in Europe, widespread dissemination resulted in recovery of spirochete from tissue specimens of meninges, brain, myocardium, retina, muscle, bone, synovium, spleen, and liver.⁷

NONHUMAN PRIMATE STUDIES

Between 1998 and 1993 two animal models, a murine⁸ and nonhuman primate (NHP)^{9,10} accurately mimicked the microbiological, clinical, immunologic, and neuropathologic aspects of LNB. Two methods of spirochete inoculation, by needle injection of 1 million N40Br strain spirochetes and feeding of infected ticks were found to be comparable in establishing infection. Transient immunosuppression maximized the yield of infection in some of the NHPs. The central nervous system (CNS) was a major reservoir of spirochetal infection and showed that a strong, well-developed anti-*Borrelia* humoral immune response did not clear spirochetes from NHP during the months of infection. Accordingly, spirochetal presence was a necessary but not sufficient condition for inflammation.

PUBLIC HEALTH SURVEILLANCE

The public health surveillance of Lyme disease is reviewed elsewhere.¹¹ Lyme disease is the most commonly reported vector-borne illness and the fifth most common disease in the National Notifiable Diseases Surveillance System. The Centers for Disease Control and Prevention (CDC) reported 22,014 confirmed and 8817 probable incident US cases of Lyme disease reported during 2012. These data were similar to those of 2010 and 2011 but substantially lower than the number reported in 2008 and 2009. One important development, however, was an increase in the geographic distribution. In 2012, a total of 356 counties had a reported incidence of ≥ 10 confirmed cases per 100,000 persons compared with 324 counties in 2008. In 2013, 95% of confirmed incident cases were reported from 14 northeast and midwestern states including Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and Wisconsin. The seasonal occurrence of Lyme disease follows the life cycle of *Ixodes* ticks. Children age 5 to 14 years and adults age greater than 65 years are most susceptible when they engage in activities that heighten exposure to tick bites infected with *B burgdorferi*. Most cases occur from late spring to early fall when larval ticks mature into nymphs. Nymphs are infected by primary mammalian hosts transmitting the disease to secondary human hosts. Biodiverse habitats have reduced risk of Lyme disease. Forest clearings favor more efficient mammalian hosts such as mice spreading infection to people.

Surveillance methods to ascertain cases of Lyme disease use rigorous clinical and laboratory criteria to verify the diagnosis for reporting purposes, the results of which are verified and tabulated in final numbers in the *Morbidity and Mortality Weekly Report* in early August of the following year and summarized in the annual *Morbidity and Mortality Weekly Report Summary of Notifiable Diseases* (www.cdc.gov/mmwr/mmwr_nd/). A CDC public use dataset provides the number of confirmed cases by county in 5-year intervals, enabling investigators to access and download the information into compatible research-driven computer software for epidemiologic analysis. It should be emphasized that the methodology and specific criteria used in case ascertainment for epidemiologic and public health activities are not intended to be applicable to routine clinical diagnosis or the selection of antibiotic regimens, as a sizable population would be excluded from consideration of the diagnosis and treatment, specifically those with less-compelling, incomplete, or atypical presentations.

For surveillance purposes, the clinical description of Lyme disease is a systemic tick-borne disease with protean manifestations including dermatologic, rheumatologic, neurologic, and cardiac manifestations. The most common clinical marker for the disease is the EM rash, the initial skin lesion so noted in up to three-quarters of confirmed cases. Late manifestations include musculoskeletal (joint swelling, monoarthritis and oligoarthritis), nervous system (lymphocytic meningitis, cranial neuritis, radiculoneuropathy, and encephalomyelitis), and cardiovascular (high-grade heart block and atrioventricular conduction defects). Arthralgia, myalgia, and fibromyalgia; headache, fatigue, paresthesia, stiff neck; and palpitation, bradycardia, bundle branch heart block, and myocarditis, which may be highly suggestive of an index case of Lyme disease-related musculoskeletal, neurologic, and cardiac disease, are not specific criteria for case designation.

The specific laboratory criteria for case ascertainment according to the CDC^{12–15} include a positive *B burgdorferi* culture or one of the following: (1) a positive result of 2-tier testing interpreted using established criteria in which a positive IgM titer is used for symptom onset ≤ 30 days and a positive IgG titer for any point during the

infectious illness; (2) single-tier IgG immunoblot or Western blot seropositivity; and (3) CSF positivity for *B burgdorferi* by enzyme-linked immunoassay or immunofluorescence assay notably when the titer is higher in CSF than in serum referred to an intrathecal production of *Borrelia*-specific antibody. The terminology of Lyme exposure often used in clinical notes is defined as having been in wooded, brushy, or grassy areas, all potential tick habitats, in a county in which Lyme disease is endemic. The term *endemic* refers to a county in which at least 2 confirmed cases have been acquired or a county with a population of known tick vectors infected with *B burgdorferi*. A confirmed case of Lyme disease for surveillance meets the criteria of an EM rash with known exposure, or an EM rash with laboratory evidence of infection without known exposure, or one with at least a late clinical manifestation with laboratory evidence of infection. Suspected or probable cases are those with an EM rash or laboratory evidence of infection, respectively. A history of tick bite is not required for case ascertainment. Tokarz and colleagues¹⁶ determined the prevalence of polymicrobial co-infection with *B burgdorferi*, *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia miyamotoi*, and Powassan virus in 286 adult tick-endemic areas of Lyme disease in New York State using a MassTag multiplex polymerase chain reaction assay.¹⁷ The investigators noted 2 or more co-infections in 30% of ticks and some having up to 3 and 4 organisms.

To improve public health, the CDC has been conducting 3 complementary projects. The first project is to achieve an estimate of the number of people with Lyme disease diagnosed based on medical claims information from a large insurance database. The second study is to estimate the number of people who test positive for Lyme disease based on data obtained from a survey of clinical laboratories. A third study aims to estimate the number of people who report that they have had Lyme disease diagnosed in the previous year.

INFECTION VERSUS IMMUNITY

Inherent in the public health debate is the contribution of infectious versus immune-mediated mechanisms to clinical manifestations and disease progression.

Treatment-Resistant Arthritis

Steere and colleagues¹⁸ first called attention to concomitant immune processes of infectious Lyme disease in the investigation of treatment-resistant Lyme arthritis, a complication rarely noted in Europe. With only about 10% of patients presenting with persistent joint inflammation for months to years after standard courses of antibiotic treatment, Steere and colleagues¹⁹ studied the binding of outer surface protein A and human lymphocyte function-associated antigen 1 peptides to 5 major histocompatibility complex molecules noting the outer surface protein A identified the critical epitope in triggering antibiotic treatment-resistant Lyme arthritis. The hypothesis of infection-induced autoimmunity²⁰ was based on T-cell epitope mimicry between a spirochetal and host protein of bystander activation of a T-cell response to a self-epitope unrelated to spirochetal proteins. Either way, the T-cell response or linked antibody response to the self-protein could stimulate persistent synovial inflammation. Only some major histocompatibility complex molecules bound particular autoantigens, accounting for the human leukocyte antigen (HLA) association with autoimmune diseases, which made more important that most patients with treatment-resistant Lyme arthritis had the HLA-DRB1*0401 or HLA-DRB1*001 alleles, and to a lesser degree, the HLA-DRB1*0404 alleles. These 3 alleles, which have a similar sequence in the third hypervariable region of the HLA-DRB1 chain were also associated with

the severity of adult rheumatoid arthritis. However, in a study of European Lyme disease,²¹ there was no association among 283 patients between HLA determinants and any of the various early or late infectious manifestations. There is limited information on postinfectious autoimmune syndromes of the nervous system caused or mediated by the Lyme spirochete. However, early susceptibility and protracted involvement, combined with the presence of serologic markers of altered immunity in affected patients with neurologic involvement²² should lead to a consideration of concomitant autoimmune processes and treatment with immune modulatory therapy.

Inflammation in the Pathogenesis of Lyme Neuroborreliosis

With the aim of evaluating whether inflammation induced by *B burgdorferi* was causal in mediating the pathogenesis of acute LNB, hypothesizing that *B burgdorferi*-induced production of inflammatory mediators in glial and neuronal cells and that this response had a role in potentiating glial and neuronal apoptosis, investigators²³ recently studied the inflammatory changes induced in CNS, spinal nerves, and dorsal root ganglia (DRG) of rhesus macaques inoculated with live *B burgdorferi* into the cisterna magna. Some animals were left untreated or given the anti-inflammatory drug, dexamethasone, a corticosteroid that inhibited the expression of several immune mediators and studied for either 8 or 14 weeks postinoculation. Enzyme-linked immunosorbant assay (ELISA) of CSF showed significantly elevated levels of interleukin (IL)-6, IL-8, chemokine ligand 2, and CXCL 13 and pleocytosis in all infected animals except dexamethasone-treated animals; however, CSF and CNS tissues of infected animals were culture positive for *B burgdorferi* regardless of treatment. *B burgdorferi* antigen was present in DRG and dorsal roots by immunofluorescence staining and confocal microscopy. Histopathology findings showed leptomeningitis, vasculitis, and focal inflammation in the CNS; necrotizing focal myelitis in the cervical spine cord; radiculitis; neuritis and demyelination in spinal roots; and inflammation with neurodegeneration in the DRG that was concomitant with significant neuronal and satellite glial cell apoptosis. These changes were absent in the dexamethasone-treated animals. In accordance with their hypothesis, the investigators¹⁰ noted that the effective suppression of inflammation by dexamethasone treatment resulted in inhibition of glial and neuronal damage, suggesting that host immunity to infection by *B burgdorferi* with subsequent inflammation, and not infection alone, had a causal role in the pathogenesis of LNB.

Blood-Brain Barrier

General considerations

Central to the question of active or chronic CNS infection is whether *B burgdorferi* has disseminated to the CNS across the blood-brain barrier (BBB) and if there is intrathecal production of *Borrelia*-specific antibodies. The BBB is a neurovascular unit comprising capillary vascular endothelial and neural cells, extracellular matrix components, and a variety of immune cells and has been intensely investigated in health and disease.²⁴⁻²⁷

A schematized and electron microscopic appearance of cerebral capillaries in the BBB (Figs. 1 and 2) shows layers of pericytes adherent to the abluminal or parenchymal surface of endothelial cells, together surrounded by a layer of basal lamina comprising extracellular matrix protein molecules. The end feet of neighboring astrocyte processes ensheath the blood vessels. Monolayers of adjacent endothelial cells that form tight junction (TJ) strands connect adjacent endothelial cells by adhesions of transmembrane (occluding-, claudin-, and junctional-associated molecules) across the intercellular space. Cytoplasmic scaffolding and regulatory proteins such as

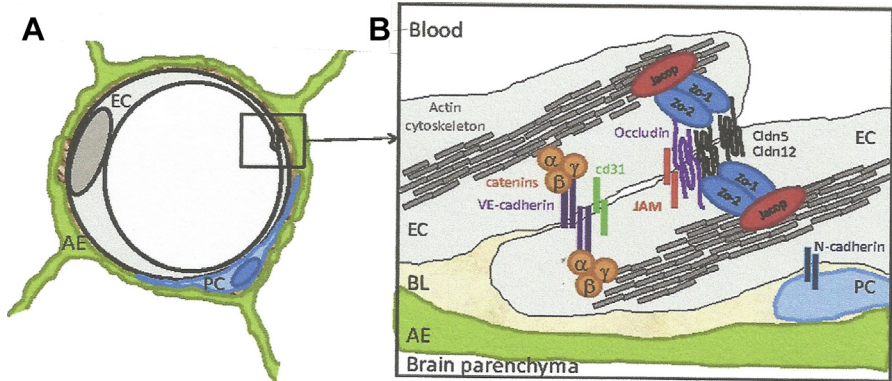


Fig. 1. (A) Cross-section schematic representation of a capillary in the human BBB over an endothelial tight junction. (B) The insert shows the molecular composition of tight and adherens junctions. (From Daneman R. The blood-brain barrier in health and disease. *Ann Neurol* 2012;72:648–72; with permission.)

zona occludens type 1 and 2 provide linkage to the actin cytoskeleton and initiate several signaling mechanisms via protein-protein interactions. Endothelia BBB cells are also linked by adherens junctions comprising vascular endothelial cadherin, which mediates cell-cell adhesion interactions, linking adherens junctions to the actin

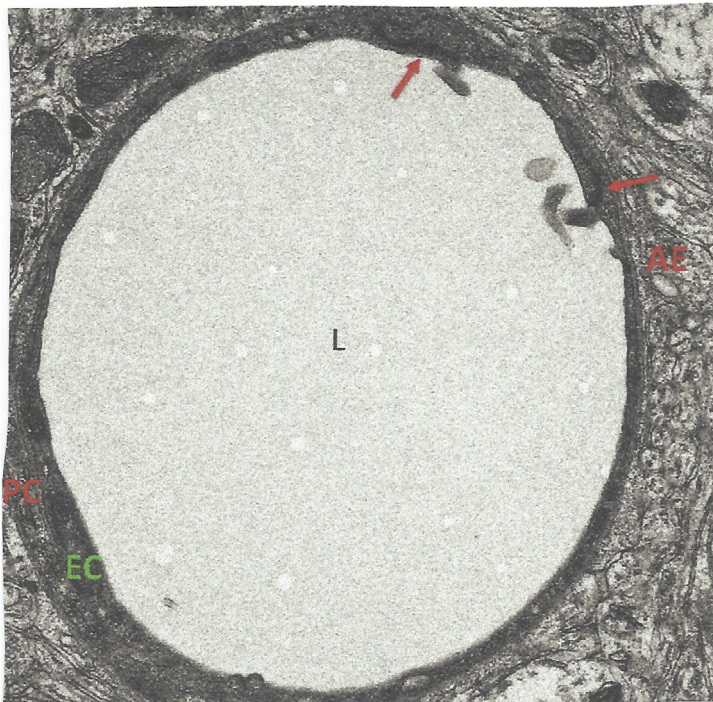


Fig. 2. Electron micrograph of a capillary in the adult murine BBB. Endothelial cells are held together by tight junctions (red arrow). (From Daneman R. The blood-brain barrier in health and disease. *Ann Neurol* 2012;72:648–72; with permission.)

cytoskeleton via catenins. The extended neurovascular unit comprises perivascular macrophages that reside between astrocyte end feet and the vessel wall, mast cells associated with specific regions of the CNS, resident microglia that act as antigen-presenting cells, circulating leukocytes that can penetrate the intact BBB via interactions with endothelial adhesion molecules to mediate bidirectional crosstalk between immune cells, and endothelium for normal surveillance. The abnormal entry of plasma components, immune molecules, and cellular elements across the BBB leads to further neural dysfunction and varying degrees of irreversible neural degeneration.

Implications for Lyme neuroborreliosis

Interest in concepts of BBB disruption in LNB started with experimental and clinical observations beginning in the early 1990s focusing on the etiopathogenesis of CNS manifestations, notably encephalopathy in acute and chronic Lyme infection in humans and NHP. The mechanisms by which bacteria breach the BBB have generally been incompletely understood; however, it was proposed that during other forms of bacteremia, microbial factors acted directly or indirectly to trigger production of endogenous inflammatory mediators that altered endothelial TJ to facilitate bacterial entry. There are increasing experimental data in the understanding of the human illness.

In 1990, Szczepanski and colleagues²⁸ studied the emigration of *B burgdorferi* across cultured human umbilical vein endothelial cells (HUVEC). Low-passage human clinical isolates (HSA1 and HBD1) cultured from skin and blood, respectively, of patients with EM and a tick isolate (T11) from *I dammini* collected in Montauk, NY, adhered 22- to 30-fold greater than the continuously passaged strain B31 to the subendothelial matrix. Spirochete binding and adherence to the subendothelial matrix were inhibited 48% to 63% by pretreatment of the matrix with antiserum to fibronectin, a major component of the matrix produced by cultured endothelial cells and a constituent of the basement membrane of blood vessels in vivo. The inhibition of spirochete adherence to the matrix by antifibronectin indicated that the spirochetes recognized the insoluble matrix form of this glycoprotein. Spirochete migration across endothelial monolayers cultured on amniotic membrane was increased when the monolayers were damaged by chemical or physical means. Electron microscopic examination of spirochete–endothelial interactions demonstrated the presence of spirochetes in the intercellular junctions between endothelial cells and beneath the monolayers. Scanning electron microscopy identified a mechanism of transendothelial migration whereby spirochetes passed between cells into the amniotic membrane at areas where subendothelium was exposed. The adherence of *B burgdorferi* to subendothelial matrix is an important finding, as spirochetes must penetrate the subendothelial basement membrane of the BBB to enter the CNS compartment. Spirochete recognition of endothelial cells or subendothelial matrix seems to be mediated by separate mechanisms, as pretreatment of endothelial cells with antifibronectin antiserum reduced spirochete adherence to the cells slightly, whereas matrix binding was greatly diminished. Moreover, little fibronectin is expressed on the surface of endothelial cells in culture or in vivo. Spirochete transendothelial migration was facilitated by prior damage of the endothelial cell monolayer by physical or chemical injury. Spirochete migration at regions where a small gap in the monolayer exposed the underlying connective tissue on scanning electron microscopy would likewise be expected to occur in vivo in areas where endothelial cell contraction or damage occurred. A similar sequence of events of attachment to the apical surfaces of cultured cells, between cells, and beneath endothelial cell monolayers, and migration via an intercellular route and not by a transcytotic process, was described for the transendothelial migration of *Treponema pallidum* spirochete.²⁹

Grab and colleagues³⁰ studied the traversal of human brain microvascular endothelial cells (BMEC) and HUVEC by *B burgdorferi* noting facilitation in the former by proteases. The spirochete organism seemed to bind human BMEC by their tips near or at cell borders, inducing the expression of plasminogen activators, plasminogen activator receptors, and matrix metalloproteinases. Grab and colleagues³⁰ noted that about a 21-fold more low-passage *Borrelia* crossed HUVEC than BMEC, underscoring the importance of extrapolating data concerning *B burgdorferi* penetration of the BBB from experimental data using nonbrain vascular endothelial cell models. The authors hypothesized that *B burgdorferi* induces the expression of plasminogen activators and matrix metalloproteinases and that these enzymes, linked by an activation cascade, could lead to the focal and transient degradation of TJ proteins. This mechanism allows the spirochete organism to invade the CNS, binding via their tips before crossing the in vitro human BBB model without evidence of loss of BBB integrity. Unlike purulent bacterial meningitis, *B burgdorferi* causes aseptic meningitis in which the permeability of the BBB may not be substantially altered.³¹ In a later investigation of the traversal of *B burgdorferi* across the human BBB using in vitro model systems constructed of HBMEC, Grab and coinvestigators³² used cell monolayers pretreated with the intracellular calcium chelator BAPTA-AM (1,2-Bis [2-aminophenoxy]ethane-N,N,N',N'-tetra-acetic acid tetrakis [acetoxymethyl ester]) and the phospholipase C (PLC) inhibitor U-73122 (1-(6-[[[17b]-3Methoxyestra-1,3,5[10]-trien-17-y]amino]heyl)1H-pyrrole-2,5-dione). The results were significant to total inhibition of transmigration of *B burgdorferi* as a result of barrier tightening based on electric cell-substrate impedance sensing. These data suggest a role for calcium in CNS spirochete invasion through the endothelial cell barrier. Nyarko and colleagues³³ noted that *B burgdorferi* and *Anaplasma phagocytophilum*-infected neutrophils co-incubated with HUVEC and HBMEC were associated with increased blood and tissue spirochete loads and heightened traversal through endothelial cell barriers.

Garcia-Monco and coworkers³⁴ found early invasion of the CNS in experimental Lewis rats by *B burgdorferi* accompanied by increased BBB permeability measured as the ratio of Iodine-125-labeled albumin CSF to that in blood. Dose-dependent BBB permeability changes were noted 12 hours after inoculation and reversed within a week. Only live, intravenously inoculated organisms produced disruption of the BBB. More marked BBB changes were noted with inoculation of the more recent low-passage strain termed *J31* acquired from Long Island than with the original isolate of the B31 strain in long-term in vitro culture from Shelter Island, both of which were grown in serum-free media to log phase. Mild pleocytosis and retrievable spirochetes were noted in the CSF of rats with increased BBB permeability. Specific *B burgdorferi* antigens were detectable in the CSF of human patients with early Lyme disease with use of murine monoclonal antibodies as probes providing evidence for early CNS invasion.

Garcia-Monco and coworkers^{35,36} described the affinity of the Lyme spirochete for cells of primary neonatal rat brain cultures, providing evidence of spirochete binding to cell surfaces and processes of glial fibrillary acidic protein-bearing cells and to the surfaces and processes of myelin basic protein (MBP) and galactocerebroside-bearing cells and their extracellular visible by microscopy. Given that most of the cells in primary rat brain culture were astrocytes and oligodendrocytes, the investigators³⁵ suggested that affinity and adherence to these cells and their known proximity to brain capillary endothelial cells in the BBB were likely determinants of the initiation of CNS injury and might contribute to the secondary persistence of *B burgdorferi* in the CNS and the development of cross-reactivity between microbial antigens and neural components. Using chromium-51 assays for the detection of damage to cells

of neural origin, Garcia-Monco and coworkers³⁶ showed a higher degree of injury in the primary brain than in astroglial cultures on scanning electron microscopy, revealing marked contraction of the membrane sheets and bleb production of oligodendroglia in neonatal rat brain culture after incubation with *B burgdorferi*, whereas the astroglial layers appeared unharmed. The damage to oligodendroglia was evident on the surface of the cells without detection of intracellular *B burgdorferi*, suggesting that the ensuing morphologic changes were not the result of internalization of spirochetes.

The presence of CNS white matter injury and *B burgdorferi*-specific and autoreactive T-cell lines from the CSF have been described in affected patients with Lyme Meningo-radiculo-myelitis,^{37,38} as have antibodies to MBP in CSF specimens from patients with chronic meningo-encephalopathy,³⁹ suggesting a role for antibodies to MBP in the pathogenesis of the disease manifestations. However, a quarter century later, it is still not known with absolute certainty whether autoreactivity causes tissue damage or is a secondary epiphenomenon.⁶

Moriarty and coworkers⁴⁰ engineered a fluorescent strain of *B burgdorferi* that expressed green fluorescent protein. Using real-time 3-dimensional and 4-dimensional intravital microscopy with quantitative analysis, the investigators studied fluorescent spirochete dissemination noting it to be a multistage process that included transient tethering-type associations, short-term dragging interactions, and stationary adhesion. The latter in association with extravasating *Borrelia* spirochetes were most commonly observed at endothelial junctions, whereas translational motility of spirochetes seemed to play an integral role in transendothelial migration. Stationary adhesions that projected deep into and sometimes through platelet endothelial cell adhesion molecule 1 (PECAM-1)-stained regions of vessels, a phenomenon termed *embedding*, and that occurred along the length of the spirochete or at one end only, found spirochetes protruding through both sides of the PECAM-1 signal, suggesting migration more deeply into junctions or endothelial cells than partially embedded adhesions. This observation seemed to be consistent with early electron microscopic studies that found that *B burgdorferi* invaded or was taken up by endothelial cells in monolayer cultures.⁴¹ However, the investigators did not study aspects of the BBB.

More recently, Brissette and coworkers⁴² analyzed the transcriptional responses to the incubation of *B burgdorferi* in primary cultures with primary human astrocytes and HBMEC over a 72-hour period noting a robust increase in IL-8, CXCL-1, and CXCL-10 chemokines in response to virulent spirochetes. The results were confirmed by ELISA and individual sets of polymerase chain reaction primers. The up-regulation of chemokines receptors from brain microvascular endothelial cells and astrocytes has the potential to facilitate entry of neurotoxic neutrophils into the CNS. NHP astrocytes that expressed the neutrophil chemoattractant IL-8 in response to *B burgdorferi* seemed to contribute to the inflammatory response both in vivo and in vitro in a macaque model of CNS Lyme disease.⁴³⁻⁴⁵

Future progress in studies of the BBB in humans may yet lead to improved outcome in early and late manifestations of the disease, taking advantage of the selective expression of membrane-bound proteins expressed by brain endothelial cells or circulating leukocytes to target new drugs and improving the effectiveness of conventional oral and parenteral antibiotics.

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