B. burgdorferi sensu stricto and from each other, further subdivision into additional subbranches was done, dendrograms of genetic relatedness were constructed,* and some of the subbranches were designated as new genospecies—*Borrelia garinii* (formerly 20047),[53] *B. afzelii* (formerly VS461),[53, 67] *B. andersonii* (includes former groups 21038 and 21123),[56, 59, 66, 70, 100] *B. valaisiana* (formerly VS116 and M19),[61] *B. lusitaniae* (formerly PotiB2),[65] *B. japonica* (formerly HO14),[66, 69] *B. tanukii* (formerly Hk501),[58, 68] *B. turdae* (formerly Ya501),[58, 68] and *B. miyamotoi* (formerly HT31).[77] There is also a genomic group DN127, which includes strain CA55 and sometimes strain 25015, and which is distinct from the other genospecies.[55, 57, 64, 123, 884]

In 1998, isolation of an unusual strain of *B. burgdorferi sensu lato* was reported from *Ixodes dentatus* and *A. americanum* in southeastern Missouri, which is similar to strains isolated from *I. dentatus* in New York and Georgia, but different from *B. burgdorferi sensu stricto.*[153] Also, an uncultivable borrelia, *Borrelia lonestarii,* was found in *A. americanum* from New York, New Jersey, Missouri, and North Carolina,[73] which may be related to the Lyme-like disease in the southern states. A borrelia identified as *B. burgdorferi* has been found in *A. americanum* in New Jersey, Missouri, Texas, Oklahoma, Virginia, North Carolina, and Alabama.[156–158]

There is clustering of *B. burgdorferi* genospecies from different geographic areas, such as North America, Europe, Asia, and the circumpolar arctic and subantarctic regions, and from different tick vectors.[10, 13, 51–57, 59, 62, 71, 74] *B. garinii, afzelii, sensu stricto, valaisiana,* and *lusitaniae* accounted for 39.7, 37.1, 15.9, 6.7, and 0.6% of *B. burgdorferi sensu lato* genospecies isolated from arthropod vectors, animal hosts, and human patients in Europe.[54] *B. burgdorferi sensu stricto* is found in *I. scapularis* and *I. pacificus* in North America.[55, 56, 59, 63, 70, 100, 123, 159] *B. andersonii* is found in *I. dentatus,*[59, 100] and *I. scapularis* in North America.[64] *B. bissettii* is found in *I. pacificus*[55–57, 59, 123] and group CA55 in *Ixodes neotomae* in the western United States,[57, 59, 884] and group 25015 in *I. scapularis* from New York.[55–57, 123, 884] *B. bissettii* represents the only strain other than *sensu stricto* to be present in both Europe and North America.[83, 884] Four genospecies—*B. burgdorferi sensu stricto, B. afzelii, B. garinii,* and *B. valaisiana*—are found in *I. ricinus* in central Europe.[61, 74] Human co-infections[74, 84] and *I. ricinus* co-infections[83, 85, 160–162] with different genospecies have been reported. *B. afzelii* and *B. garinii* have been found in *Ixodes persulcatus* in eastern Europe and in Asia, including Japan, and *B. burgdorferi sensu stricto* has not been found.[67, 74, 163, 164] *B. japonica* is found in *Ixodes ovatus* in Japan[66, 69, 163]; *B. garinii,* and no other genospecies, is found in *Ixodes uriae* and *I. ricinus* in the far northern subarctic latitudes,[152, 165, 166] and in *I. uriae* in the far southern subantarctic latitudes; genetically heterogeneous *B. burgdorferi sensu stricto, B. garinii,* and *B. afzelii* occur in migratory passerine (perching) birds in Sweden.[13]

Hypotheses about the phylogenetic origins and historical patterns of global migration of the different *B. burgdorferi* genospecies have been developed, based on genetic analysis of the different strains. Initially, it was thought that there was greater diversity of genospecies in Europe,[53] with *B. garinii, afzelii,* and *sensu stricto,* and in Asia, with *B. garinii, afzelii,* and *japonica,* than in North America, where only *B. burgdorferi sensu stricto* was thought to occur; this led to hypotheses that *B. burgdorferi* was introduced into North America from Europe, possibly by migratory birds or small mammalian hosts of infected ticks.[13, 157, 165, 167–171] The initial genetic studies were done mainly on isolates from the restricted hyperendemic areas of the Northeast and Upper Midwest; later, when isolates from the South and West were studied, more genetic heterogeneity was found,[62, 63] raising the reverse hypothesis—that introduction was from North America into Europe. The similarity in Osp A phenotype of a few west central European strains and the North American strains raises the possibility that the *B. burgdorferi* originally introduced into the United States came from west central Europe,[92] or that North American strains were introduced into Europe. The differences in DNA sequences for outer surface proteins of North American and European strains of *B. burgdorferi* suggest that these strains may have diverged long ago and may be pathogenically different.

B. burgdorferi is clonal, and widespread genetic exchange between chromosomal genes is thought not to occur.[57, 60] The order of occurrence of genes is the same across different genospecies, and there is no evidence of chromosomal rearrangements since the evolutionary divergence of the different genospecies from a common ancestor.[57, 60] Genetic exchange between plasmid genes, such as the Osp A and Osp B linear plasmid genes, has been found[80] but is thought to be rare[57, 60]; there is evidence of exchange with other plasmid genes, such as the Osp D–encoding plasmid, which suggests that *B. afzelii* and *garinii* are closely related and that *B. burgdorferi sensu stricto* only recently acquired the Osp D gene.[117]

There are differences in vector competence of *I. ricinus* and *I. scapularis* for three genospecies of *B. burgdorferi sensu lato,* which correlates with the known geographic association of these vectors and genospecies: Acquisition of infection by *I. scapularis* was 83 to 90, 87, 10, and 5% for *B. burgdorferi sensu stricto, afzelii, garinii* VS286, and *garinii* VSBP, compared with acquisition of infection by *I. ricinus* of 3, 90, 5, and 3%, respectively.[878] Other genospecies are associated with some tick species and have not been found in others.[68, 69]

There is clustering of *B. burgdorferi* genospecies from different reservoir host species[74] and some host species, which may act as biologic filters.[172, 173]

Isolation and Cultivation

B. burgdorferi lives in hosts such as vertebrates or hematophagous arthropods and is not found living free in the environment. In 1981, it was first isolated by Burgdorfer and associates from the midgut and other tissues dissected from *Ixodes scapularis (dammini)* ticks from Shelter Island, a Lyme-endemic area of New York, and was cloned to become the B31 strain of *B. burgdorferi.*[1] In 1983, Burgdorfer and colleagues also first isolated a similar spirochete from *Ixodes ricinus* ticks from the Seo-

*See references 55–57, 59–63, 65, 66, 68, 76, 77, and 117.

wald Forest, a Lyme-endemic area of Switzerland, and showed it to be morphologically and antigenically similar to the *I. dammini* spirochete.[81] Since then, it has been isolated from several species of ticks, vertebrate hosts, and humans; this is described in the section Epidemiology and Transmission.

B. burgdorferi is fastidious and microaerophilic and grows best in a liquid medium, modified Barbour-Stoenner-Kelly medium (BSK II), at 33° C to 35° C.[50, 81, 91] It has an 11- to 24-hour doubling time, which may be shortened to 11 to 12 hours under ideal conditions, but it still may take 3 weeks or longer to grow sufficiently in culture to become detectable by microscopy.[18, 50, 91, 174] However, the use of *B. burgdorferi*–specific PCR has shortened the time for detection in culture media.[175] It can also grow anaerobically, and has even been grown aerobically in the presence of 1 to 5% carbon dioxide.[99]

Unlike other spirochetes, *B. burgdorferi* can be grown in solid media.[97] It has been found to produce colonies of several types, including a compact 0.43-mm round colony at the agarose surface, and three types of colonies that penetrated into the agarose—a 1.43-mm colony with a raised center surrounded by a diffuse ring, a colony composed of many small aggregations, and a diffuse 1.8-mm colony. It was also found to cause intense hemolysis on solid BSK II medium with horse blood.[879] More recently, *B. burgdorferi* has been found to have shorter doubling times of even 7 hours, when grown in solid media under strict anaerobic conditions, and it may be considered an obligate anaerobe.[176]

B. burgdorferi can be seen in cultures by dark-field or phase-contrast microscopy. It stains with acridine orange, Giemsa, and silver stains such as Warthin-Starry or Dieterle's[25] or Bosma-Steiner stain,[82] and can be identified with immunofluorescence techniques using *B. burgdorferi*–specific polyclonal or monoclonal antibodies[177] or *B. burgdorferi*–specific PCR.[175]

Transformation of *B. burgdorferi* from typical motile spirochetes to immotile cystic spheroplast L-forms occurs when *B. burgdorferi* is grown in culture in the presence of antibiotics, *B. burgdorferi*–specific antibody, or normal CSF.[102] The conversion to spheroplast forms may be related to the ability of the spirochete to persist in tissues without elimination by the host immune response.

B. burgdorferi shows antigenic variation and loss of pathogenicity after 10 to 15 passages in culture, and becomes noninfectious; this correlates with loss of plasmids.[129, 133, 134, 178, 882] Loss of several outer surface proteins and their encoding plasmid genes, including Osp B, C, and D, with passage has been noted; there is a suggestion that linear plasmid of 24.7 kbp (lp24.7) is required for infectivity of *B. burgdorferi sensu stricto, garinii*, and *afzelii*, and that lp38 (which encodes Osp D) is not required. Loss of lp27.5 may increase infectivity, but correlation of individual plasmids with infectivity has been inconsistent.[117, 134, 178] High-passage strains of *B. burgdorferi* have also been found to decrease both invasiveness and cytopathic killing of B and T lymphocytes.[179]

B. burgdorferi is relatively easily isolated and grown from midgut and other tissues dissected from infected *Ixodes* ticks,[50, 74, 174, 180, 181] from which the isolation rate depends on the incidence of infection within the tick population (see section Epidemiology and Transmission: *B. burgdorferi* Tick Infection Rates); from blood and organ cultures of infected reservoir-competent host animals[167, 182] (see section Epidemiology and Transmission: *B. burgdorferi* Reservoir Animal Infection Rates); and from biopsy specimens of the leading edge of EM skin lesions, from which the isolation rate is usually 28 to 86% (it may be higher in disseminated infection).[183, 184] It has been isolated occasionally from blood, CSF, and ACA skin biopsy specimens, and rarely from borrelial lymphocytoma skin biopsies, synovium and synovial fluid, myocardium and heart valves, the iris, ligamentous tissue, placenta, fetal tissues, or other tissues because the organism density is low[50] (see section Diagnosis and Differential Diagnosis: Diagnostic Tests: Culture).

The *B. burgdorferi*–specific PCR[185–187] increases the sensitivity of detection of *B. burgdorferi* in body fluids and tissues by using DNA target sequences that are unique to *B. burgdorferi*, are not present in other closely related *Borrelia* species or other spirochetes, and are highly conserved among *B. burgdorferi* strains. PCR has been used to demonstrate the spirochetes in EM, ACA, and borrelial lymphocytoma skin biopsy specimens; serum, plasma, and bone marrow; CSF, brain biopsy, sural nerve biopsy, and vitreous fluid; synovial fluid and membrane; urine; breast milk; placental tissue; and various animal hosts and tick vectors (see section Diagnosis and Differential Diagnosis: Diagnostic Tests: Polymerase Chain Reaction).

Antibiotic Susceptibility

Isolates of *B. burgdorferi* from humans and ticks from different geographic areas, including the United States and Europe, generally have similar antimicrobial susceptibility patterns,[50, 174, 188–190, 192, 196–198] as is shown in Table 11–1. *B. burgdorferi* antibiotic susceptibility can be assessed in vitro by comparison of the minimal inhibitory concentrations (either mean MIC or MIC 50%) and the minimal bacteriocidal concentrations (either mean MBC or MBC 50%) for various antibiotics, and in vivo by comparison of the antibiotic dose required to cure 50% of infected animals of their infection (CD_{50}). However, there is one report[196] of lower doxycycline MIC values for cutaneous isolates than for CSF isolates.

B. burgdorferi was the most susceptible in vitro to the macrolides erythromycin, azithromycin, clarithromycin, and roxithromycin (MIC, 0.01 to 0.17 μg/ml); the penicillins penicillin, amoxicillin, ampicillin, amoxicillin–clavulanic acid, mezlocillin, azlocillin, and oxacillin (MIC, 0.02 to 1.1 μg/ml); the second- and third-generation cephalosporins ceftriaxone, cefotaxime, cefuroxime, ceftizoxime, and cefixime (MIC, 0.02 to 0.8 μg/ml); and the tetracyclines doxycycline, minocycline, and tetracycline (MIC, <0.13 to 0.79 μg/ml). Isolates were also susceptible to imipenem (MIC, 0.12 μg/ml) and chloramphenicol (MIC, 2 μg/ml). The mean MIC (or MIC 50%) value for penicillin was 0.02 to 1.1 μg/ml, but the range was wide (up to 8 μg/ml). According to MIC values, the aminoglycosides, sulfonamides, metronida-

TABLE 11–1
In Vitro and In Vivo Antimicrobial Susceptibilities of *Borrelia burgdorferi*

ANTIMICROBIAL AGENT	MEAN[a] (RANGE[b]) MIC (μg/ml)	MEAN[c] (RANGE[d]) MBC (μg/ml)	SUSCEPTIBILITY[e] IN VITRO	CD_{50}[f] (mg/kg/day)	SUSCEPTIBILITY IN VIVO
Penicillin	0.02–1.1(0.003–8)	1.08–8.7(0.1–50)	S-MS-R	>320–>1975	R
Amoxicillin	<0.03–0.25(<0.03–1)	0.06–1.9(<0.03–3.2)	S	50	S
Ampicillin	<0.25–0.47(<0.25–1)		S		
Amox/clav[g]	0.12(0.12–5)		S	25	S
Mezlocillin	0.5(0.25–1)		S		
Oxacillin	0.5(0.25–2)		S		
Cefaclor	(23–128)	(64–>256)	MS		
Cefadroxil	(11–128)	(32–>128)	MS		
Cefalexin	(16–32)	(32–>256)	MS		
Cefixime	0.8(0.8)	(0.8–1.6)	S		
Cefotaxime	<0.03–0.45(<0.03–1)	<0.03–0.17(<0.03–0.8)	S	50	S
Ceftizoxime	0.125(0.06–.5)	0.5(0.25–1)	S		
Ceftriaxone	0.02–0.06(0.006–1)	0.04–3.8(0.02–50)	S	50–240	S
Cefuroxime	(0.06–0.5)	(0.25–0.75)	S		
Doxycycline	0.125–1(0.1–2)	0.71–2(0.2–6.4)	S		
Minocycline	<0.13(<0.12–0.25)	2.3	S		
Tetracycline	0.14–0.79(0.01–2)	0.8–4(0.8–6)	S	50–287	S
Azithromycin	0.01–0.017(0.003–0.03)		S	8	S
Clarithromycin	0.01(0.003–0.06)	0.13(0.06–0.25)	S	>50	R
Erythromycin	0.03–0.15(0.007–1)	0.05–2.17(0.04–10)	S	400–2353	R
Roxithromycin	0.02–1.05(0.02–1.6)	1.1(0.02–1.6)	S	>50	R
Ciprofloxacin	1(0.25–4)	2–4(0.5–16)	MS		
Ofloxacin	2(0.5–8)	2(1–8)	MS		
Gentamicin	>16		R		
Amikacin	>32		R		
Chloramphenicol	2(1–3)		S		
Imipenem	0.12(0.06–1)		S		
Rifampin	>16		R		
Trimethoprim-sulfamethoxazole	>256		R		

[a]MIC = minimal inhibitory concentration (either mean MIC or MIC 50%).
[b]MIC Range, minimum and maximum MIC values reported.
[c]MBC = minimal bactericidal concentration (either mean MBC or MBC 50%).
[d]MBC Range, minimum and maximum MBC values reported.
[e]S = susceptible to antimicrobial agent; MS = moderately susceptible to antimicrobial agent; R = resistant to antimicrobial agent.
[f]CD_{50} = dose of antimicrobial agent required to cure 50% of infected animals in animal model.
[g]Amox/clav = amoxicillin-clavulanic acid.
Data obtained from references 50, 79, 174, 188, 189, 191, 192, 194–200, and 621.

zole, rifampin, and quinolones were not useful for *B. burgdorferi*. Although *B. burgdorferi* is resistant to co-trimoxazole in vitro, a minor synergistic decrease in the roxithromycin MIC from 0.031 to 0.015 μg/ml and a significant decrease in spirochetal motility were reported to occur in combination with co-trimoxazole.[199]

For the various antibiotics, the in vitro MIC efficacy and the in vivo CD_{50} efficacy were in agreement except for penicillin, erythromycin, clarithromycin, and roxithromycin. For erythromycin, clarithromycin, and roxithromycin, evaluation of the CD_{50} showed that despite excellent MIC values, they were poorly active in vivo in the animal models. For penicillin, the poor in vivo efficacy may be due to strains of *B. burgdorferi* with high MIC values.

B. burgdorferi is killed slowly even by antibiotics to which it is sensitive, and prolonged exposure of the spirochetes to the antibiotics is necessary to achieve adequate killing.[188, 192, 200] In one study,[188] the length of time required to kill 99% of *B. burgdorferi* exposed to twice the MIC of antibiotic ranged from 72 hours for ceftriaxone and cefuroxime to 96 hours for cefixime. In another study,[192] the length of time needed to kill 99% of *B. burgdorferi* was 72 hours for 0.1 μg/ml and 48 hours for 1.0 μg/ml of both penicillin and ceftriaxone, and 72 hours for 1.0 μg/ml of tetracycline. Low concentrations of tetracycline (0.1 and 1.0 μg/ml) allowed regrowth of organisms after prolonged incubation for 96 hours or longer, but no such regrowth occurred with low concentrations of penicillin or ceftriaxone, or higher concentrations of tetracycline (above 10 μg/ml). In one study,[200] some differences in the kinetics of killing of different *B. burgdorferi* strains by different antibiotics were found after 48 hours, but all strains were effectively killed by antibiotics to which they were susceptible after 72 hours.

Results of the animal model efficacy studies show better correlation for some antibiotics than others with

clinical human patient results. For example, Steere and colleagues reported[201] that, of the oral antibiotics, tetracycline was most effective, penicillin was next most effective, and erythromycin was least effective for treatment of early Lyme disease. Clarithromycin[202] and azithromycin[193, 203] have been found to be equally or almost equally as efficacious as amoxicillin and doxycycline in the treatment of EM. Several factors, in addition to the MIC of the antibiotic, play a role in determining whether an antibiotic will be clinically effective in the elimination of *B. burgdorferi* infection; these include the duration of adequate serum, spinal fluid, intraocular, intrasynovial, and tissue antibiotic concentrations; the efficacy of the host immune response; and the potential sequestration of organisms in protected sites.

Interactions with the Immune System

B. burgdorferi infection triggers a sequence of immunologic and other cellular events that are involved in the local and systemic dissemination of the infection, the immunopathogenesis of the various manifestations of the infection, and the host elimination of the infection, as well as in the ability of the spirochete to evade host defenses.[148, 149, 151, 204–207] A discussion of the immunopathogenesis of Lyme borreliosis is provided in the section Pathology and Pathogenesis.

T LYMPHOCYTE REACTIVITY

B. burgdorferi antigen–triggered T cell activation occurs within a few days of the tick bite, develops before the B cell antibody response, rises during infection, is directed initially against the 41-kd flagellar and the 31-kd Osp A antigens, and is directed later against additional outer surface membrane proteins.[208, 209, 211, 212] *B. burgdorferi* spirochetes, Osp A, and Osp B have been reported to induce specific proliferation in T lymphocytes from Lyme disease patients[213, 214]; the response is predominantly due to $CD4^+$ and $CD8^+$ T lymphocytes,[214] and there is also a response due to $CD56^+$ NK (natural killer) cells.[213] *B. burgdorferi*, Osp A, Osp B, and even Osp-containing membrane blebs have been found to possess nonspecific B lymphocyte proliferative activity.[215, 216] However, *B. burgdorferi*–induced nonspecific T lymphocyte or mononuclear cell proliferation has been found by some groups[217] and not by others.[213]

B. burgdorferi antigen–specific T lymphocyte reactivity, measured by the *B. burgdorferi*–specific lymphocyte proliferative assay, is long lasting, and may persist even in seronegative patients with Lyme borreliosis.[208, 214, 218, 219] The lymphoproliferative response may be greater in spinal fluid and synovial fluid than in peripheral blood in some patients with neurologic or arthritic manifestations of Lyme borreliosis.[214, 220, 221] There is *B. burgdorferi*–specific synovial fluid T lymphocyte production of Th1-type cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).[393] There is peripheral blood and intrathecal *B. burgdorferi*–specific T lymphocyte production of the Th1-type cytokine IFN-γ, as well as specific B lymphocyte production of IgG antibody, all of which persist for several months after clinical recovery from treated neuroborreliosis.[214] After successful antibiotic therapy of Lyme disease, the reactivity may decrease somewhat but is usually still detectable if the most sensitive assay methods are used.[208–210, 212, 213, 231]

DEVELOPMENT OF SERUM ANTIBODY

The antibody response to *B. burgdorferi* infection begins to develop a few days after the tick bite, after the development of the T lymphocyte response,[211] and there are several studies of the temporal evolution of serum IgG and IgM antibody responses to the infection in North American[222, 226, 232] and European[227, 228] patients. *B. burgdorferi sensu stricto* is the only major genospecies causing Lyme disease in North America; all three of the major genospecies, *B. burgdorferi sensu stricto*, *B. garinii*, and *B. afzelii*, cause Lyme borreliosis in Europe, resulting in some differences between the antibody responses of North American and European patients. Because of these differences, distinct criteria for Western blot positivity for each of the three genospecies in European patient sera, and for *B. burgdorferi sensu stricto* in North American patient sera, have been recommended.[233, 234, 237, 238] In both North American and European patients, the initial polyvalent antibody response to *B. burgdorferi* infection is directed primarily against the 24-kd Osp C[223, 235, 239, 240] and the 41-kd flagellar antigen. The early response to the 39-kd antigen is more common in North American than European patients,[233, 237] and the late antibody response is more often directed primarily against the outer surface membrane proteins, that is, 31-kd Osp A and 34-kd Osp B, in North American than in European patients.[234, 237, 241, 242]

The *B. burgdorferi*–specific IgM response develops in 1 to 2 weeks, peaks at 2 to 8 weeks, and usually disappears after several months in uncomplicated treated patients but may persist in patients with disseminated rather than localized infection, patients with persistent infection, some with late chronic infection,[18, 222, 226, 240, 243] patients with initially delayed antibiotic therapy (even after clinical recovery),[226, 244] and some patients with promptly and successfully treated EM and neuroborreliosis.[226, 227, 233] Although comparisons of the temporal evolution of antibodies detectable by Western blots to individual *B. burgdorferi* antigens are often difficult because of lack of standardization of band and molecular weight nomenclature, a general pattern of progressive expansion of the antibody repertoire after infection emerges. There is general agreement that the initial specific IgM response is made to the 24-kd Osp C antigen and to the 41-kd flagellar antigen. Several investigators describe early development of IgM antibody and other antigens as well. After recovery, Western blot IgM antibody reactivity to several antigens declines after 1 month and usually disappears after several months. IgM reactivity to the 24-kd Osp C and 41-kd flagellin may persist,[226, 240, 247, 248] and is even still detectable in 38% of patients with successfully treated EM 1 year later[226]; IgM antibody to Osp C is detectable in 45% of patients with chronic arthritis for months to years, and in 20% of those with chronic neuroborreliosis.[240] However, in a follow-up study of resolved pediatric Lyme arthritis,

only 5% had any IgM Western blot reactivity at a mean of 10 months after treatment, and this was only to the 41-kd flagellar antigen.[229] In very early infection, in both North American and European patients, IgM antibody to Osp A may be bound in immune complexes, and may be detectable only when these are dissociated.[246] The acute IgM response during EM in North American patients who progress to severe persistent Lyme disease includes the 83-kd and 34-kd antigens, and these responses persist into chronic disease.[222] In North American patients, the IgM Osp C antibody response is greatest in patients with EM and meningitis, early in the course of infection, and decreases to low levels in those who develop chronic neuroborreliosis.[240]

In North America, the CDC criteria for a positive IgM Western immunoblot are the presence of two of the following three bands in early disease: 24-kd Osp C, 39-kd Bmp A, and 41-kd Fla.[238] In Europe, proposed criteria for IgM Western blot positivity include the following: for *B. burgdorferi sensu stricto* IgM, at least one of 39, Osp C, and 17a or a strong 41; for *B. afzelii* IgM, at least one of 39, Osp C, and 17 or a strong 41; and for *B. garinii* IgM, at least one of 39 and Osp C or a strong 41.[237]

A delay in initial antibiotic therapy appears to be associated with increased dissemination, with development of higher polyvalent enzyme-linked immunosorbent assay (ELISA) titers and greater numbers of Western blot IgM bands, and with persistence of IgM positivity even after clinically successful treatment[226, 232, 244]; however, prompt antibiotic treatment of early Lyme disease appears to be associated with disappearance of IgM positivity within several months.[226, 229, 248] Longer disease duration is associated with a higher incidence of IgM seropositivity.[124] The IgM ELISA antibody is higher in neuritis and arthritis patients with early Lyme disease than in patients with only EM.[224] In late chronic Lyme borreliosis, such as arthritis, neuroborreliosis, and sometimes even in acrodermatitis chronica atrophicans, the specific IgM is often persistently positive by immunofluorescent assay (IFA), ELISA, or Western blot assays.[18, 209, 222, 223, 241, 242, 244, 249–252]

The *B. burgdorferi*–specific IgG response develops at 2 to 8 weeks, peaks at 4 to 6 months, and in uncomplicated treated patients, usually gradually declines and sometimes eventually disappears after several months, but it may persist for years in persistent infection, sometimes even after successful antibiotic therapy.[18, 222, 226–228] This response may be aborted by early antibiotic therapy.[226, 253] Delays in initial antibiotic therapy are associated with a higher incidence of dissemination, progression to later stages of infection, strongly positive IgG responses, higher polyvalent ELISA antibody titers, and increased numbers of IgG Western blot bands.[222, 226, 234, 235, 254] In one study, the number of Western blot bands reacting with serum IgG antibody decreased after successful treatment of pediatric Lyme arthritis, and no new ones appeared.[229] As is the case for IgM Western blot bands, direct comparison of IgG bands from different studies is not always possible. However, general patterns of the temporal evolution of the IgG antibody response to infection emerge. The initial IgG response is made to the 41-kd flagellar and the 24-kd Osp C antigens; it progressively expands to include additional antigens, such as the 26-kd Osp F,[245] and eventually, within the first month after successful treatment, it includes many additional antigens.[211, 222, 223, 226, 235, 240, 245] In very early infection, in both European and North American patients, IgG antibody to Osp A is often present in immune complexes, but may be detectable only if these are dissociated.[246] In persistent infection, the IgG response expands over months to years.[211, 222, 255, 256] In late European Lyme borreliosis, IgG antibody is almost always directed toward the 41-kd flagellin, and the 58-kd and 83/100-kd antigens.[107, 108, 237, 247, 257] The progressive expansion of the IgG antibody response develops regardless of whether the late manifestations are arthritic, neurologic, or cardiac,[279] although the Western blot antibody patterns may differ with various late manifestations.[234, 237, 247, 257]

In North America, the CDC criteria for a positive IgG Western immunoblot are the presence of five of the ten most common bands after the first few weeks of disease.[238] The proposed criteria for IgG Western blot positivity in Europe can be seen in reference 237.

The development of IgM and IgG antibody to new antigens months to years after onset of infection suggests either the persistence of viable *B. burgdorferi* throughout the illness, or reinfection.[222, 226, 228] There are varying opinions regarding the significance of positive IgM antibody in Lyme disease of more than 1 month's duration, but there is agreement that a diagnosis of active Lyme disease should not be made on this basis alone.[24, 226–228, 233, 244, 254]

Patients with neuroborreliosis usually have higher polyvalent *B. burgdorferi* antibody in spinal fluid than in serum,[258, 259] and some may have spinal fluid antibody in the absence of serum antibody.[253, 260, 261] Patients with arthritis usually have higher polyvalent specific antibody in synovial fluid than in serum.[262]

Highly specific antibody capable of killing *B. burgdorferi* in culture and of passively protecting mice against experimental *B. burgdorferi* challenge develops during infection and quantitatively increases with increasing severity and duration of the infection.[230] In one study, the seroprotective and borreliacidal activity occurring in patient sera from late but not early Lyme borreliosis correlated with the presence of reactivity to Osp A and Osp B.[230] Borreliacidal antibody is seroprotective against the homologous strain, and sometimes against heterologous strains.[263]

INDUCTION OF OTHER ANTIBODIES

The *B. burgdorferi*–specific IgM antibody rise during infection is also associated with polyclonal B lymphocyte activation that peaks 3 to 6 weeks after onset of infection and corresponds to the time of maximal total and *B. burgdorferi*–specific IgM antibody.[243, 264] This B cell hyperactivity leads to the development of several antibodies that are not specific for *B. burgdorferi* and are directed against host tissues, such as rheumatoid factor,[211, 243, 264] antinuclear antibody,[211, 243] anti-cardiolipin antibody,[211, 243] antibody to fibronectin-binding protein,[255]

antibody to neuronal axons,[151, 265] antibodies to myelin basic proteins,[266] and antibody to neurofilament proteins[266] and oligoclonal bands.[258, 267, 268] False-positive Venereal Disease Research Laboratory (VDRL) antibody,[211] cryoglobulins,[211, 243] and circulating immune complexes[211, 243, 269] are also found during this time. In patients with Lyme arthritis, the circulating immune complexes disappear from serum in 3 months but increase in synovial fluid; in patients with cardiac or neurologic involvement, the immune complexes persist in the serum.[262, 269]

Induced low levels of rheumatoid factor are detectable in 32% of Lyme patients by ELISA IgM and in 4% by latex agglutination assay.[264] Serum IgM antibodies to neuronal axons were found in all patients with neuroborreliosis in one study[151]; autoantibodies were found in the spinal fluid of 20% of patients with neuroborreliosis in another study.[266] *B. burgdorferi*–specific oligoclonal bands were found in the spinal fluid of 40 to 100% of patients with neuroborreliosis.[258, 267]

Anti–tick saliva antibody (ATSA) develops after a tick bite in response to the bolus of tick saliva injected, peaks at 3 to 5 weeks, persists for weeks to months, and subsequently decreases.[270] This antibody is a good biologic marker for tick exposure and may be useful in confirming tick exposure in seronegative patients with suspected Lyme borreliosis.

FAILURE TO DEVELOP SERUM ANTIBODY

Early antibiotic therapy may attenuate or eliminate the *B. burgdorferi*–specific antibody response.[18, 208, 209, 218, 225, 253, 273] Normally, *B. burgdorferi* antigen triggers B lymphocyte as well as T lymphocyte responses, but if antigen is removed by early antibiotic therapy, the antigen-dependent T cell stimulation of B cell maturation does not occur, and the mature antibody response does not develop.[253] Thus, if antibiotic therapy is given before the development of the mature IgG antibody response, this response may be aborted even though the infection may not be fully eradicated, and the patient may be seronegative. If antibiotic therapy is given after the development of the mature IgG response, the antibody response may eventually decrease, disappear, or persist, even after successful eradication of the infection.[274–277] The longer the Lyme disease persists before antibiotic therapy is begun, the more *B. burgdorferi*–specific antibody bands develop by Western blot assay.[208, 226, 244] Persistent *B. burgdorferi* infection may also occur in sequestered sites such as the central nervous system, inducing local CSF but not systemic antibody responses. Seronegative patients usually still have detectable T lymphocyte proliferative responses.[208, 218, 219, 234, 269, 277, 278] Seropositivity or seronegativity alone is not always a reliable indicator of cure.

Steere and colleagues[279] reported the incidence of true seronegative Lyme disease to be 4% in a large study of 180 patients with confirmed North American Lyme disease; they noted that all were EM history–positive, 75% had *B. burgdorferi*–specific T lymphocyte reactivity, and manifestations were usually neurologic or musculoskeletal. In seronegative patients, clinical manifestations were attenuated compared with those in seropositive patients; in seronegative patients with symptoms of significant arthritis, the term *seronegative Lyme arthritis* is contradictory, as the *B. burgdorferi* antibody response is considered to be involved in the pathogenesis of the arthritis, and these patients are unlikely to have Lyme disease.

In some patients, apparent seronegativity is due to testing by standard ELISA and Western blot assays, which detect free antibody, and specific antibody may be detected by using methods that dissociate immune complexed antibody.[278]

Failure to develop *B. burgdorferi* serum antibody in patients with confirmed Lyme borreliosis may be due to serologic testing done very early after onset of infection, during the spirochetemic phase, before the development of even a very early antibody response. Thirty-five to 100% of early Lyme borreliosis patients with *B. burgdorferi* detectable in plasma or serum by PCR were seronegative,[280, 281] and 53% of seronegative Lyme borreliosis patients had *B. burgdorferi* DNA detectable in serum by PCR, compared with none of the seropositive patients.[281]

DEVELOPMENT OF CEREBROSPINAL FLUID ANTIBODY

B. burgdorferi invasion of the central nervous system (CNS) occurs early in two thirds of patients with disseminated infection even in the absence of neurologic symptoms; this has been reported from both North America[282, 283] and Europe.[284] Patients who develop either acute or chronic neurologic involvement may have intrathecal production of specific IgG, IgM, or IgA antibodies to *B. burgdorferi* demonstrable by IFA, ELISA (standard, antibody capture, or immune-complex ELISA), or Western blot assay.*

Intrathecal production of *B. burgdorferi*–specific antibody confirms neuroborreliosis. Patients with late neuroborreliosis may be seronegative and still have intrathecal specific antibody production, presumably because oral antibiotic therapy eradicates the majority of organisms systemically, but it may fail to achieve adequate MICs in the CSF, thus allowing persistence of the organism in this privileged site.[253] Some patients with early neuroborreliosis may also have specific intrathecal antibody production, as has been observed with *B. burgdorferi* IgM antibody, without seropositivity.[253, 260, 261] Early in CNS invasion, *B. burgdorferi*–specific CSF antibody may be located in immune complexes, which are not detected by free antibody assays.[283]

There are some differences in intrathecal *B. burgdorferi* antibody between North American and European patients.[259, 290, 293] Polyclonal intrathecal *B. burgdorferi*–specific antibody was found in almost all North American patients with early Lyme meningitis, and in almost half of those with late central nervous system borreliosis, but not in those with late peripheral nervous system borreliosis. Polyclonal intrathecal *B. burgdorferi*–specific antibody was found in almost all European patients with either early or late neuroborreliosis. In one study of North American Lyme disease,[292] there was intrathecal

*See references 211, 253, 259, 260, 265, 267, 268, 283, and 285–292.

B. burgdorferi–specific ELISA IgM in 100% and IgG in 40% of patients with meningitis, as well as ELISA IgM and IgG in 26 to 30% of patients with encephalitis; in another study of North American early and late neuroborreliosis, intrathecal free antibody detectable by ELISA was found in 48% and specific immune complex–associated IgG and IgM antibody in 43%.[283]

B. burgdorferi–specific CSF antibody was directed primarily against the 41-kd flagellar antigen, and also against the 33-kd Osp A and 17-kd antigens.[258, 260, 283, 293] CSF ELISA antibody levels were higher than serum antibody levels,[259] but IFA antibody levels were higher in serum than in CSF.

INTERACTIONS WITH COMPLEMENT

B. burgdorferi activates the alternate and classic complement pathways but is resistant to the nonspecific bactericidal activity of normal human serum. However, in the presence of *B. burgdorferi* immune serum, it is sensitive to serum and is killed via the classic pathway.[96] Host-specific differential transmission of different *B. burgdorferi sensu lato* genospecies by ticks has been found to correlate with the differential susceptibility of the genospecies to bacteriolysis by serum complement, including via the alternate pathway, of the different host species.[294]

INTERACTIONS WITH PHAGOCYTES

Peripheral blood polymorphonuclear and mononuclear phagocytes and macrophages are able to phagocytose opsonized and nonopsonized *B. burgdorferi*.[295, 296] *B. burgdorferi* binds to polymorphonuclear phagocytes via integrin $\alpha_m\beta_2$, the CR3 complement receptor, during nonimmune phagocytosis.

B. burgdorferi stimulates human endothelial cells to express the neutrophil adhesion molecule, E-selectin, and the neutrophil chemotactic agent, interleukin-8 (IL-8), both of which are probably involved in recruitment of neutrophils to sites of *B. burgdorferi*–induced inflammation, and in transmigration of neutrophils across the endothelium.[150, 297] Whole *B. burgdorferi* spirochetes were demonstrated to be strong inducers, equivalent to or more potent than lipopolysaccharide (LPS), of chemoattractant cytokine production by human monocytes, including MIP-1α (macrophage inflammatory protein-1α), MCP-1 (monocyte chemotactic protein-1), and RANTES (regulated upon activation, normal T cell expressed and secreted), which attract monocytes and lymphocytes, and IL-8 (interleukin-8) and GRO-α (melanoma growth–stimulatory activity), which attract neutrophils and contribute to tissue inflammation and damage.[298]

Recombinant lipidated, but not unlipidated, *B. burgdorferi* Osp A, even in minute amounts, is a potent human neutrophil activator that induces neutrophil responses similar to those induced by bacterial LPS. Neutrophils are the main cell type in Lyme arthritic joints; they are involved both in maintaining an inflammatory response and in the destruction of opsonized *B. burgdorferi*, presumably via a combination of reactive oxygen intermediates and lysosomal products, including the proteolytic enzyme elastase.[299] Elastase has been demonstrated to be the main borreliacidal factor in human neutrophils.[300]

A possible additional mechanism by which the spirochete might evade borreliacidal antibody and temporarily persist in a protected niche is by invasion and killing of both B and T lymphocytes.[179]

EVASION OF HOST DEFENSES AND PERSISTENCE IN TISSUE

B. burgdorferi has the unusual, but fortunately uncommon, ability to evade the host immune response and persist in tissues for months to years, sometimes even after antibiotic therapy, and sometimes even after intravenous antibiotic therapy.* When it occurs, this persistence is usually either in immunologically privileged sites inaccessible to host defenses, after local or systemic steroid therapy, after initially delayed or inadequate antibiotic therapy, or in patients with risk factors such as HLA-DR4 specificity, and may occur in the presence or absence of seropositivity.[306, 307]

B. burgdorferi has been isolated, months to several years after oral or intravenous (IV) antibiotic therapy, from CSF[284, 302]; synovial fluid[200]; EM skin lesions[83, 200, 302, 303]; mitral valve tissue[200]; ligamentous tissue[304]; and iris biopsy tissue.[284] It has also been isolated, 1 month to 10 years after onset, without preceding antibiotic therapy, from CSF[302]; synovial fluid[301]; EM skin biopsy[305–307]; ACA skin lesions[19, 20]; and myocardium.[308] Its presence has been demonstrated, months to 27 years after antibiotic therapy, by *B. burgdorferi*–specific PCR or antigen capture ELISA in CSF[269, 283, 287, 309–311]; brain[311]; synovial fluid and membrane[312–314]; ACA skin biopsy[315]; and serum, blood, plasma, and bone marrow.[281, 311] Persistence for 1 month to 10 years without antibiotic therapy has also been demonstrated by PCR or antigen-detection methods in CSF and ACA skin biopsy.[143, 316, 317] The development of *B. burgdorferi*–specific IgM antibody responses to new spirochetal antigens late in the course of Lyme disease also indicates long-term persistence of live organisms in these patients.[222]

Differential gene expression of *B. burgdorferi* antigens, which results in variation in antigenicity of the spirochete during different stages of infection, is thought to be involved in evasion of the immune response.[120, 137, 142]

It has been proposed that the spirochete may be able to evade the host immune response while still inducing the inflammatory pathology characteristic of the various manifestations of Lyme disease. Differential expression of surface lipoproteins during various stages of infection allows the spirochete to vary its antigenicity[120, 130, 131, 135–137, 141, 142] while maintaining its ability to activate cells because the lipid moiety of the lipoproteins is responsible for cell activation.[147]

The use of the host's own fibrinolytic enzymes for invasion, while eliciting minimal immunologic response by the host, is an immunologically silent method of invasion called "stealth pathogenesis,"[144] which may ex-

*See references 19, 20, 206, 269, 281, 283, 284, 287, 302–308, and 311–318.

plain the long-term persistence of *B. burgdorferi* in host tissues with only minimal mononuclear cell infiltration. *B. burgdorferi* invasion of epidermal dendritic Langerhans' cells induces downregulation of major histocompatibility class II (MHC II) molecules on this major antigen-presenting cell, and may result in inability of Langerhans' cells to eliminate the spirochete and long-term *B. burgdorferi* persistence in the skin.[148]

The immunosuppressive and immunomodulatory properties of *B. burgdorferi* may also be involved in its ability to evade the host immune response. The addition of *B. burgdorferi* to lymphocyte proliferative assays reduces the proliferative responses of human peripheral blood lymphocytes to concanavalin A and phytohemagglutinin. It has been proposed that this immunosuppressive effect may allow the spirochete to rapidly disseminate from the skin inoculation site and persist in the host; it could also explain the better efficacy of prompt antibiotic therapy in elimination of the spirochete.[217]

Another mechanism by which the spirochete might evade borreliacidal antibody is by entering a protected niche such as an intracellular or other environment that is inaccessible to either a borreliacidal immune response or antibiotic therapy. Proposed potential sites for such persistence include the central nervous system, the eye, and the joints.[179, 320, 321] Temporarily, persistence in a protected niche occurs by invasion of B and T lymphocytes. *B. burgdorferi* persistence in ligamentous tissue, the iris, synovium, and the central nervous system may also represent the use of a protected niche.

Several antigens of *B. burgdorferi* have portions that share amino acid homology with human cellular proteins; molecular mimicry may also be involved in immune evasion.[113, 126, 151, 204, 205, 256, 265, 323]

CORRELATION OF CLINICAL MANIFESTATIONS WITH HLA TYPE

Differences in HLA specificities may determine *B. burgdorferi* antigen binding and presentation to T cells and the composition of the T cell response, and may be related to susceptibility to infection.[256]

Several studies by Steere and colleagues and others reported that HLA-DR4 specificity and Osp A or Osp B IgG seropositivity are associated with chronic antibiotic-resistant Lyme arthritis but not with EM or acute or chronic neuroborreliosis.[256, 312, 324, 325] Long-duration chronic Lyme arthritis patients had high frequencies of HLA-DR4 or -DR2 positivity (89%) compared with those with short-duration Lyme arthritis (27%), and HLA-DR4 positivity but not -DR2 positivity correlated with lack of response to antibiotic therapy.

Correlation of HLA specificity with outcome of antibiotic therapy of Lyme arthritis is discussed in the section Therapy: Predictors of Antibiotic Therapy Cure.

EPIDEMIOLOGY AND TRANSMISSION

World Wide Web sites for the Centers for Disease Control and Prevention (CDC), http://www.cdc.gov/ncidod/dvbid/lymegen.htm, and the European Union Concerted Action of Risk Assessment in Lyme Borreliosis (EUCALB), http://www.dis.strath.ac.uk/vie/LymeEU,[326] have updated Lyme borreliosis epidemiologic and clinical information.

Historical Review

In 1909, Afzelius described a migrating annular skin lesion in a Swedish woman at the site of an *Ixodes ricinus* sheep tick bite, called it erythema chronicum migrans (ECM), and proposed that it was a zoonosis transmitted by a tick from an animal reservoir to humans.[14, 17] ECM became a well-recognized European disease thought initially to be caused by either a tick-associated toxin or an infectious agent.[17]

Another European disease, acrodermatitis chronica atrophicans (ACA), which had first been described by Buchwald in 1883 in Germany,[327] was noted to be preceded frequently by ECM and was named ACA Herxheimer by Herxheimer and Hartman in 1902. In 1922, Garin and Boujadoux described cutaneous lesions and paralysis after a tick bite and suspected a spirochetal etiology,[328] and in 1944, Bannwarth described chronic lymphocytic meningitis after European ECM; this became known as Garin-Boujadoux-Bannwarth syndrome, or simply Bannwarth's syndrome.[17, 329]

In 1948, Lennhoff reported spirochetes in ECM skin biopsy specimens,[17, 330] but this finding could not be confirmed by others and was essentially forgotten. By 1949, there were suggestions in Europe that penicillin therapy was beneficial in ECM,[17, 331] and between 1948 and 1957, Hollstrom found that most European ECM cleared within 2 weeks after intramuscular penicillin therapy.[79, 331] In 1949, Thyresson successfully treated patients with ACA with penicillin, and in 1952, Gruneberg considered spirochetes as possible etiologic agents.[20]

In 1955, Binder and associates, in Europe, transplanted skin biopsy specimens from the rim of an ECM lesion from a patient to three scientist-volunteers who then developed ECM lesions within 3 weeks. They established that ECM was caused by a penicillin-susceptible infectious agent transmitted by the *Ixodes ricinus* tick.[17] In 1955, Gotz transmitted ACA from patient to patient by transplantation of ACA skin biopsy specimens[20] and thus confirmed ACA as an infectious disease. Both ECM and ACA became well-known European skin diseases.

The first report in the medical literature of North American erythema migrans (EM), as ECM was eventually called, was from Wisconsin in 1970 by Scrimenti,[333] although retrospective studies have found that it existed in small foci in New England as early as 1962 and 1965.[334, 335]

The recognition of Lyme arthritis as a distinct disease came in 1975, when two mothers from the small village of Old Lyme, Connecticut, brought the existence of an epidemic of children diagnosed as having juvenile rheumatoid arthritis to the attention of the state health department and the Yale Rheumatology Clinic. Steere and colleagues investigated and recognized an outbreak

of infectious arthritis, noted that many patients had an unusual rash similar to European EM, proposed that transmission occurred via an arthropod vector, and named the disease Lyme arthritis.[15] By 1980, it became known as Lyme disease because meningoencephalitis and myocarditis were also recognized as part of the disease.

In 1980, Steere and co-workers[339] found that penicillin or tetracycline therapy shortened the duration of EM and reduced the severity and frequency of subsequent arthritis. They concluded that antibiotic therapy was useful and that the disease was caused by a penicillin-sensitive bacterium such as a spirochete.

In 1981, a new spirochete was accidentally discovered by Burgdorfer in *I. dammini* ticks (now renamed *I. scapularis*) collected for a rickettsial study from Shelter Island, New York, a highly Lyme-endemic focus.[1, 17] It induced EM lesions in rabbits, and convalescent sera from Lyme patients reacted with it.[1, 17] In 1983, two groups of investigators, Steere and associates[18] and Benach and colleagues,[80] isolated the same spirochete from patients with Lyme disease, found specific antibody titers against this spirochete in convalescent sera of Lyme disease patients, and concluded that the *I. dammini* spirochete was the etiologic agent of Lyme disease. In 1984, it was named *B. burgdorferi* when it was confirmed to be a new species.[16]

In 1983, Barbour, Burgdorfer, and co-workers isolated a spirochete similar to the *I. dammini* spirochete from *Ixodes ricinus* ticks[81]; it was indistinguishable from *B. burgdorferi* and was also confirmed to be the etiologic agent of European ECM,[19] European ACA,[19, 20] European Bannwarth's syndrome,[21, 302] and European borrelial lymphocytoma.[22]

The recent application of new molecular biologic techniques such as the polymerase chain reaction (PCR) to the historical study of *B. burgdorferi* in museum specimens of ticks and animals has made it possible to retrospectively document its presence in Europe in museum tick specimens as early as 1882 to 1897,[340] and in North America in museum mouse specimens as early as 1894. This dates the presence of the spirochete in Europe to the times of the earliest clinical descriptions of Lyme borreliosis.

The first case of congenitally transmitted Lyme borreliosis was described by Schlesinger and associates in 1985 after gestational Lyme disease acquired in Wisconsin.[25] Since then, several additional cases have been reported, and it has become clear that gestational Lyme borreliosis carries a low but serious risk of congenital infection.

Tick (and Other Arthropod) Vectors

Epidemiologic studies have indicated that Lyme borreliosis is caused by *B. burgdorferi sensu lato* transmitted from animals to humans by ixodid ticks that are members of the *Ixodes ricinus* complex,[167, 336] and that this transmission occurs during tick feeding because of either tick salivation or regurgitation of organisms.[341, 342] Ticks that are members of the *I. ricinus* complex and have been associated with human Lyme borreliosis transmission are the deer tick *Ixodes dammini/scapularis* in the northeastern and upper midwestern United States,[335, 337] the black-legged tick *Ixodes pacificus* in the western United States,[2, 180, 335, 337, 343] the sheep tick *Ixodes ricinus* in Europe,[11, 81] and the *Ixodes persulcatus* tick in Asia.[163, 344] Other ticks that are not members of the *I. ricinus* complex are also associated with enzootic *B. burgdorferi* cycles, but either are not or are rarely involved in human Lyme borreliosis transmission and may be involved in bridging between separate enzootic cycles.[13] (In this chapter, the name *I. scapularis* is used to indicate both northern and southern ticks.[346, 348])

B. burgdorferi is often found in nymphal and adult stages of *Ixodes scapularis, pacificus, ricinus,* and *persulcatus,* but rarely in unfed larvae, because infection is acquired by larvae feeding on *B. burgdorferi*–infected animal reservoirs, is passed transstadially (between stages) from larvae to nymphs to adults, and is rarely passed transovarially from infected female ticks to less than 1% of eggs and larvae.[18, 154, 167, 182, 352–356] However, because occasional female ticks may produce progeny with high infection rates, rare transovarial transmission may be important for establishment of new endemic foci of Lyme disease in instances in which an infected tick is transported by birds or other methods into a new, previously nonendemic area. Partially fed larval ticks (in which feeding on infected hosts was interrupted) are able to transmit *B. burgdorferi* during refeeding, which may explain some larval positivity.[357]

In North America, most Lyme disease transmission is due to northern *I. scapularis*[348] and *I. pacificus* tick vectors,[180, 348] which frequently bite humans, but several other species of ticks have been thought to be vectors in some geographic areas, particularly in areas where northern *I. scapularis* and *I. pacificus* are not prevalent[348]; the southern *I. scapularis* has been considered a Lyme disease vector in parts of the southern United States.[358–361] In the western United States, *I. neotomae* and *Ixodes spinipalpus* are involved in *B. burgdorferi* enzootic cycles, and *I. pacificus* serves as a bridge vector to man[361]; in the eastern United States, *I. dentatus* and *Ixodes minor* are also involved in *B. burgdorferi* enzootic cycles, and *I. scapularis* may be involved as a bridge vector.[361] Although infrequent, human bites have been documented for *I. spinipalpus*[362] and *I. dentatus*,[70, 363] and for two other ticks that are not members of the subgenus *Ixodes*—*Ixodes angustus*[364] and *Ixodes cookei*[363]; rare cases of possible EM have been reported after human bites by *I. angustus* in Washington state[364] and *I. cookei* in West Virginia.[363]

Other ticks in North America commonly biting humans are the dog tick *Dermacentor variabilis,* and the Pacific Coast tick *Dermacentor occidentalis*[348, 365]; *D. variabilis* in Kentucky[366] has been considered a possible secondary human Lyme disease vector. The Lone Star tick *Amblyomma americanum,* which is the most common tick biting humans in the southeastern and south central United States,[367] has been considered a potentially important alternate human vector in New Jersey,[155] southeastern Missouri,[153, 367, 368] North and South Carolina,[359, 360] Kentucky,[366] Alabama,[156] and Texas.[369] *B. burgdorferi sensu lato*[153] and *Borrelia lonestari,*[73] a noncultivable *Borrelia* possibly related to Lyme-like disease in

the South, have been found in *A. americanum*. *H. leporispalustri* and *Dermacentor parumapertus* rarely bite humans.[336] There have been occasional reports of suspected Lyme borreliosis transmission by other hematophagous arthropods such as mosquitoes[370] and tabanid flies (deer and horseflies) in North America and Europe.[371, 372] Figure 11–4 shows different stages of three common North American ticks: *I. scapularis*, *A. americanum*, and *D. variabilis*.

In South America, the *Ixodes affinis* and *Ixodes pararicinus* ticks from Peru are also members of the *Ixodes ricinus* complex and are considered potential vectors of *B. burgdorferi*.[373] In Asia, although the *Ixodes ovatus* tick in Japan frequently bites humans and has been found to harbor *B. japonica*, this has not been found to be associated with human Lyme borreliosis.[163, 374] The *Ixodes holocyclus* tick in Australia is the tick most often biting humans, but it has not been found to harbor *B. burgdorferi*.[375]

In addition to *Ixodes scapularis*, *pacificus*, *ricinus*, and *persulcatus* ticks,[336] *B. burgdorferi* has been isolated from ticks of other *Ixodes* species and of four additional genera (Table 11–2).

For a tick to be vector-competent for *B. burgdorferi*, it must be able to become and remain infected, pass the infection transstadially, and transmit the infection to a host. *Ixodes scapularis*, *pacificus*, *ricinus*, *persulcatus*, *dentatus*, *neotomae*, and *hexagonus* are efficient and competent *B. burgdorferi* vectors,[170, 336, 355, 357, 365, 378, 390] and *I. uriae*,[165, 166] and *I. spinipalpus*[362] are probably efficient and competent vectors.

It has been recognized that there are significant differ-

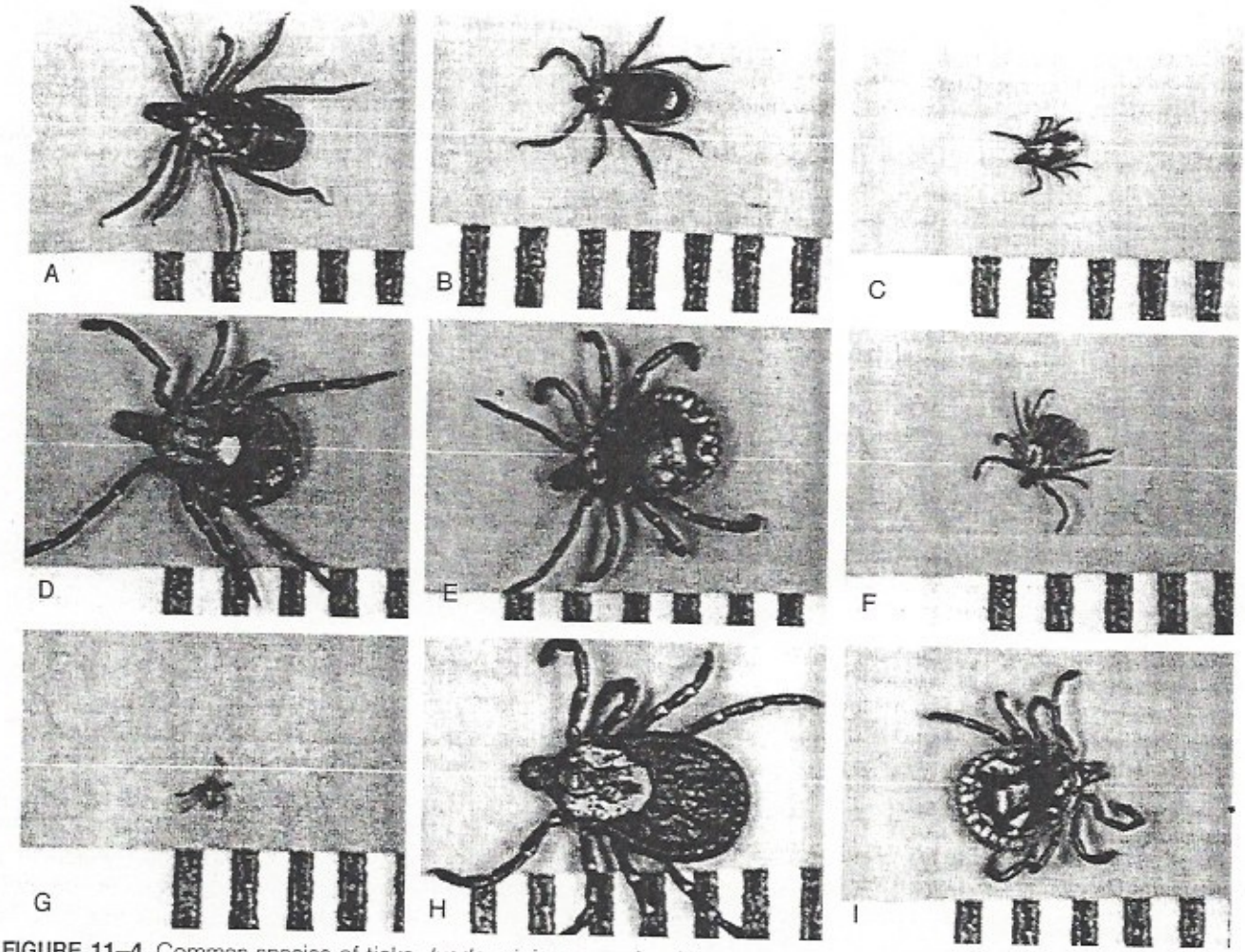

FIGURE 11–4 Common species of ticks. *Ixodes ricinus* complex ticks are vectors of transmission of the Lyme disease spirochete, *Borrelia burgdorferi*, to humans. *A*, *Ixodes dammini/scapularis* (northern species) adult female. *B*, Adult male. *C*, Nymph. The North American deer tick *Ixodes dammini* (the same species as the black-legged tick *Ixodes scapularis*) is the vector in the northeastern and north central, and possibly in southeastern and south central, United States. Other *Ixodes* ticks are similar in appearance, such as the western black-legged tick *Ixodes pacificus* (the vector in the northwestern United States), the European wood or sheep tick *Ixodes ricinus* (the vector in Europe), and the taiga tick *Ixodes persulcatus* (the vector in Eurasia). Some non-*Ixodes* ticks have been suspected but not proved to be associated with transmission of the Lyme disease spirochete to humans. *(D) Amblyomma americanum* adult female, *(E)* adult male, *(F)* nymph, and *(G)* larva. The Lone Star tick *A. americanum* may be a vector in the southeastern and south central United States. *H, Dermacentor variabilis* adult female. *I*, Adult male. The American dog tick *Dermacentor variabilis* and the Rocky Mountain wood tick *Dermacentor andersoni* may be occasional vectors and are similar in appearance.