Chapter 22

Tick-Borne Bacterial, Rickettsial, Spirochetal, and Protozoal Diseases

Approximately 900 tick species exist worldwide, parasitizing a broad array of mammals, including humans, and thereby playing a significant role in the transmission of infectious diseases *(1)*. In the United States, tick-borne diseases are generally seasonal and geographically distributed. They occur mostly during the spring and summer but can occur throughout the year.

These blood-feeding arthropods that parasitize all vertebrates can be classified into three families: (i) Ixodidae (hard ticks) comprising approximately 700 species and 13 genera; (ii) Argasidae (soft ticks), which consists of approximately 180 species and 5 genera; and (iii) Nuttaliellidae, which is composed of only one species and is found only in Africa *(1, 2)*.

Ticks are major vectors of arthropod-borne infections and not only can transmit a wide variety of pathogens, such as rickettsia and other bacteria, viruses, and protozoa, but also may carry more than one infectious agent and thus transmit one or more infections to humans at the same time *(1)*.

Infections transmitted by the Ixodidae family (hard ticks) include (i) Lyme disease (borreliosis); (ii) human ehrlichiosis; (iii) Rocky Mountain spotted fever; (iv) tularemia; (v) southern tick-associated rash illness; and (vi) babesiosis. Infections transmitted by the Argasidae family (soft ticks) include the tick-borne relapsing fever *(1)*.

Ticks of the Ixodidae family that transmit infections to humans are most often associated with the genera *Amblyomma, Ixodes, Dermacentor*, and *Rhicephalus*. They live in diverse but relatively humid habitats, and the infections they transmit are usually seasonal and geographically distributed. The hard ticks can attach securely to their hosts and feed slowly for prolonged periods, which will facilitate the transmission of infectious pathogens *(1)*. In contrast, the only important disease-transmitting soft ticks belonging to the Argasidae family are those of the genus *Ornithodoros*, and they transmit spirochetes throughout their life cycle. *Ornithodoros* feed rapidly, typically at night, and may transmit disease in as little as 30 seconds *(2)*.

In the United States, ticks have been found in all regions of the country, and the incidence rates of tick-borne diseases have increased steadily over the past decade or so (http://www3.niaid.nih.gov/research/topics/lyme/introduction/htm). Although Lyme disease and Rocky Mountain spotted fever are well known to the general public, recently emerging infections, such as ehrlichiosis and anaplasmosis (formerly known as human granulocytic ehrlichiosis), have now also been firmly established in the country. The increasing reports of tick-borne diseases likely reflect improved awareness, surveillance and diagnosis, but the growing U.S. population and the spread of human communities into previously undeveloped environments also increase the regions of tick-human contact.

Since the identification of *Borrelia burgdorferi* as the causative agent of Lyme disease in 1982, 11 tick-borne human bacterial pathogens have now been described throughout Europe. These include five spotted fever rickettsiae, the etiologic agent of human granulocytic anaplasmosis (HGA), four species of *B. burgdorferi* complex, and a new relapsing fever–causing *Borellia (3)*.

If left untreated, the tick-borne infections can be associated with significant morbidity and even mortality.

22.1 Lyme Disease (Lyme Borreliosis, Lyme Arthritis)

Lyme disease (borreliosis) is the most prevalent tick-borne infectious disease in the United States. The disease is caused by a spiral-shaped bacterium *Borrelia burgdorferi* and is spread by the deer tick *Ixodes scapularis*. The likelihood for a person to be bitten by a deer tick is greater during the times of the year when ticks are most active. Young deer ticks, called *nymphs*, are active from mid-May to mid-August and are approximately the size of poppy seeds. Adult ticks, which are approximately the size of sesame seeds, are most active from March to mid-May and from mid-August to November. Both nymphs and adult ticks can transmit Lyme disease at any time when the temperatures are above freezing.

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During 2003–2005, CDC received reports of 64,382 Lyme disease cases from 46 states and the District of Columbia *(4)*; 93% of cases occurred among residents of the 10 *Healthy People 2010* reference states (see Table and Fig. 1 in Ref. *4*). The average annual rate in these 10 reference states for the 3-year period was 29.2 cases per 100,000 population: 29.1 in 2003, 26.8 in 2004, and 31.6 in 2005 *(4)*.

Not all deer ticks are infected with Lyme disease. Ticks can become infected only if they feed on small animals that are infected. In most cases, the tick must stay attached to humans for 36 hours or more before the bacteria can be transmitted. There is no person-to-person spread of Lyme disease. In extremely rare cases reported, the bacteria may be transferred from an infected pregnant woman to the fetus. Even if successfully treated, a person may become re-infected if bitten later by another infected tick.

22.1.1 Pathophysiology of Lyme Borreliosis

The complex life cycle of *B. burgdorferi*, which passes through ticks and various intermediate hosts (mice and deer) before infecting humans, is still not completely understood *(5, 6)* (http://www3.niaid.nih.gov/research/topics/lyme). The outer surface protein A (OspA) of *B. burgdorferi* has been extensively studied, leading to a number of hypotheses regarding its role in conjunction with other cell surface proteins (OspB and OspC) in transmission of Lyme disease *(5)*.

Although *B. burgdorferi* depends on *Ixodes* ticks and mammalian hosts for its life cycle, the search for the borrelial genes responsible for its parasitic dependence on these diverse hosts has been hindered by the difficulties in genetically manipulating virulent strains of the pathogen. Nevertheless, there is strong evidence indicating that the inactivation and complementation of a linear plasmid-25-encoded gene, *bptA* (formerly known as *bbe16*), is essential for the persistence of *B. burgdorferi* in *I. scapularis* ticks, and therefore, it must be considered to be a major virulence factor that is critical for *B. burgdorferi*'s overall parasitic strategy *(7)*.

22.1.2 Symptoms of Lyme Disease

Early symptoms of Lyme disease usually appear within 3 to 30 days after the bite of an infected tick. In 60% to 80% of cases, a rash resembling a bull's eye or solid patch (about 2 inches in diameter) will appear and expand around or near the site of the bite. Occasionally, multiple rash sites may also appear. In the early stage of the Lyme disease, one or more of the following symptoms will appear: chills and fever, headache, fatigue, stiff neck, muscle and/or joint pain, and swollen glands. If not recognized or left untreated, symptoms become more severe, and include fatigue, a stiff aching neck, and tingling or numbness in the arms and legs, or facial paralysis.

The most severe symptoms can occur even weeks, months, or years after the tick bite and include severe headache, painful arthritis, swelling of the joints, and heart and central nervous system problems. Some evidence points to an autoimmune disease, perhaps triggered by the initial infection *(8, 9)* (http://www3.niaid.nih.gov/topics/lymeDisease/research/autoimmune.htm).

Early treatment of Lyme disease involves the use of antibiotics, which in nearly all cases results in a complete cure. However, the likelihood of a full cure will decrease if treatment is delayed. And whereas in most infected individuals Lyme disease can be easily treated with antibiotics, in a small percentage of patients it may lead to debilitating symptoms that may continue for years after treatment.

22.1.3 Neuropsychiatric Lyme Disease

As a result of dissemination through the bloodstream, *B. burgdorferi* can invade the central nervous system within days to a week after the initial skin infection. Once in the central nervous system, the spirochete may affect the brain, most commonly causing a disturbance in thinking (cognition), known as Lyme encephalopathy. Other symptoms may include headache, mood swings, irritability, depression, and a high degree of fatigue. These symptoms comprise the typical features of neuropsychiatric Lyme disease in adults (http://www.columbia-lyme.org/flatp/lymeoverview.html). Many of these symptoms are common manifestations in other disorders, such as mood or anxiety disorders, collagen vascular or autoimmune diseases, spinal cord compression, multiple sclerosis, metastatic diseases, endocrinologic disorders, fibromyalgia, chronic fatigue syndrome, and residual damage from past brain trauma or toxin exposure. Nevertheless, knowing the typical cluster of symptoms can be helpful in diagnosing this condition. The majority of patients with Lyme encephalopathy will not present with joint problems at the time that their cognitive symptoms have been recognized.

It is important to emphasize that bedside neurologic examination does not usually disclose neurologic findings, and standard office-based cognitive screening test may not detect cognitive impairment. To detect cognitive disturbance, a more comprehensive neuropsychological examination would be needed. In addition, lumbar puncture, even though important in the differential diagnosis, should not be used to exclude neurologic Lyme disease, as approximately 20% to 40% of patients with confirmed

neurologic Lyme disease may test negative in routine cerebrospinal fluid (CSF) assays (http://www.columbia-lyme.org/flatp/lymeoverview.html).

In general, the majority of patients who undergo early antibiotic therapy will not incur long-term central nervous system problems.

22.1.3.1 Time Course of Manifestations of Chronic Cognitive Disorders in Lyme Encephalopathy

The typical time course of the manifestations of Lyme encephalopathy (http://www.columbia-lyme.org/flatp/lymeoverview.html) is as follows:

(i) Very early: erythema migrans (a red, round, expanding rash).
(ii) One to 2 months after infection: cardiac or early neurologic involvement (meningitis, encephalitis, cranial neuropathies) with mild to marked neuropsychiatric symptoms.
(iii) Six to 10 months after infection: arthritis of multiple joints.
(iv) Two to 8 years after infection: chronic cognitive problems.

22.1.3.2 Symptoms of Neuropsychiatric Lyme Disease

Typical symptoms among adult patients with neuropsychiatric Lyme disease (http://www.columbia-lyme.org/flatp/lymeoverview.html) include:

(i) Mild to severe fatigue (a need for prolonged sleep), low-grade fevers, night sweats, migrating arthralgias (joint pains) or arthritis (joint inflammation or swelling), muscle pains, sleep disturbances, and frequent and severe headaches.
(ii) Cranial nerve disturbance. Though either facial nerve palsy or optic neuritis is not frequently manifested, patients may more commonly present with facial numbness and/or tingling.
(iii) Sharp, stabbing, deep/boring, burning, or lancinating (shooting) pains, as well as signs of peripheral neuropathy (multifocal numbness or tingling in hands or feet).
(iv) Cognitive problems may include problems of attention, memory, verbal fluency, and thinking speed. Some patients may experience what is otherwise a normal environmental stimulation to be excessive, resulting in a cognitive "short-circuiting" (cognitive overload) where patients may start to feel confused, lose focus, stutter, or panic.
(v) "Brain fog," a term frequently used by patients with Lyme disease to describe a syndrome characterized by lack of clarity in their cognitive processes similar to "depersonalization or derealization" in which the person's sense of self and place are altered.
(vi) Sensory hyperacuities characterized by heightened sensitivity to sound or to light, particularly in the early stages of neurologic Lyme disease.
(vii) Spatial or geographic orientation problems where a patient may bump into the door jambs; try to place an object on a table only to have it fall to the floor due to a misjudgment of spatial distance; or get lost in a familiar place.
(viii) Less common neurologic syndromes include partial or complex seizures, multiple sclerosis–like illness, dementia-like illness, Guillain-Barré syndrome, strokes, and Tullio phenomenon.

Symptoms of Neuropsychiatric Disorders in Children

Most common symptoms of neuropsychiatric disorders in children suffering from Lyme disease include headaches, disturbances of behavior or mood, fatigue (falling asleep in class), and problems with auditory and visual attention (*some children could be mistakenly diagnosed as having attention deficit disorder*).

They may have fluctuating symptoms: worse on some days, remarkably better on others, without a clear cause (http://www.columbia-lyme.org/flatp/lymeoverview.html).

As noted among adults, when Lyme disease is treated early, few children will develop long-term cognitive or neuropsychiatric problems.

22.1.4 Treatment of Lyme Borreliosis

Prior vaccination with the licensed recombinant outer surface protein A (OspA) vaccine reduces the risk of developing Lyme disease associated with tick bites *(10)*.

22.1.4.1 Early Lyme Disease

Administration of doxycycline (100 mg, twice daily) or amoxicillin (500 mg, 3 times daily) for 14 to 21 days is recommended for the treatment of early localized or early disseminated Lyme disease associated with erythema migrans, in the absence of neurologic involvement or third-degree atrioventricular heart block *(9)*. In prospective studies, these two drugs have been shown to be effective in treating erythema migrans and associated symptoms. Doxycycline has the advantage of being effective also for treating human granulocytic anaplasmosis (HGA), another tick-borne infection

that may occur simultaneously with Lyme disease. However, doxycycline may be relatively contraindicated for pregnant women, during lactation, and for children aged 8 years or younger (see also Section 22.2.2.2).

Because of its higher cost, cefuroxime axetil (500 mg orally, twice daily), which is as effective as doxycycline in the treatment of erythema migrans, should be reserved as an alternative agent for those patients who can take neither doxycycline nor amoxicillin *(9)*.

For children, the recommended dose of amoxicillin is 50 mg kg^{-1} day^{-1}, divided into 3 doses per day (maximum, 500 mg/dose). Cefuroxime axetil is an acceptable alternative given at a dose of 30 mg kg^{-1} day^{-1}, divided into 2 doses daily (maximum, 500 mg/dose).

Macrolide antibiotics (azithromycin, erythromycin, and clarithromycin) are not recommended as first-line therapy for early Lyme disease; when used they should be reserved for patients who are intolerant of amoxicillin, doxycycline, and cefuroxime axetil *(9)*.

Intravenous ceftriaxone (2.0 g daily), although effective, is not superior to oral agents and is not recommended as a first-line agent for treatment of Lyme disease in the absence of neurologic involvement or third-degree atrioventricular heart block. However, ceftriaxone is recommended for acute neurologic disease manifested by meningitis or radiculopathy *(9)*. For children, the recommended dose of ceftriaxone is 75 to 100 mg kg^{-1} day^{-1}, in a single daily intravenous dose (maximum, 2.0 g), or cefotaxime (150 to 200 mg kg^{-1} day^{-1}) divided into 3 or 4 doses (maximum, 6.0 g/daily) for 14 to 28 days *(9)*.

Patients with first- or second-degree atrioventricular heart block associated with early Lyme disease should be treated with the same antimicrobial regimens as patients with erythema migrans without carditis. The recommended treatment for patients with a third-degree atrioventricular heart block is parenteral antibiotics such as ceftriaxone in a hospital setting *(9)*.

Although antibiotic treatment does not hasten resolution of seventh cranial nerve palsy associated with *B. burgdorferi* infection, antibiotics should be used to prevent further sequelae *(9)*.

22.1.4.2 Lyme Arthritis

Lyme arthritis usually can be treated successfully with antimicrobial agents administered orally or intravenously. Thus, administration of oral doxycycline (100 mg, twice daily) or amoxicillin (500 mg, 3 times daily), in each instance for 28 days, is recommended for patients without clinically evident neurologic disease *(9)*. For children, the recommended dose of doxycycline (1.0 to 2.0 mg/kg, twice daily; maximum, 100 mg/dose) could be given to children age 8 and older, or amoxicillin (50 mg kg^{-1} day^{-1}, divided into 3 doses per day; maximum, 500 mg/dose) for 28 days *(9)*.

Whereas oral therapy is easier to administer than intravenous antibiotics, is associated with fewer adverse effects, and is significantly less expensive, its disadvantage is that some patients treated with oral antimicrobials have subsequently developed overt neuroborreliosis, which may require intravenous therapy for successful resolution *(9)*.

22.1.4.3 Late Neuroborreliosis

The recommended therapy for patients with late neuroborreliosis affecting the central or peripheral nervous system is treatment with intravenous ceftriaxone (2.0 g, once daily for 2 to 4 weeks) *(9)*. Response to treatment is usually slow and may be incomplete. However, unless relapse is shown by reliable objective means, repeat treatment is not recommended *(9)*. For children, the recommended treatment is a 14- to 28-day course of ceftriaxone (75 to 100 mg kg^{-1} day^{-1}, in a single daily intravenous dose; maximum, 2.0 g) *(9)*.

22.1.4.4 Chronic Lyme Disease or Post–Lyme Disease Syndrome

After an episode of Lyme disease that is treated appropriately, some patients have a variety of subjective complaints, such as myalgia, arthralgia, or fatigue. Such patients may then be classified as having either *chronic Lyme disease* or *post–Lyme disease syndrome*. However, both conditions are poorly defined because these patients represent a heterogenous group. Because there have not been any randomized, controlled studies of patients who remain unwell after standard courses of antibiotic therapy for Lyme disease, there are no convincing published data demonstrating that repeated or prolonged courses of either oral or intravenous antimicrobial therapy are effective for such patients *(9)*.

22.1.5 NIAID Research Agenda in Lyme Disease

22.1.5.1 Background and Goals

The NIAID has had a long-standing commitment to conduct research on Lyme borreliosis, or Lyme disease, beginning more than 20 years ago when the cause of the disease was not yet known (http://www3.niaid.nih.gov/research/topics/lyme/research/). In 1981, NIAID-funded research efforts resulted in identifying *Borrelia burgdorferi*, a spiral-shaped bacterium, or spirochete, as the causative

agent of Lyme disease *(11)*. Since then, basic and clinical research efforts have been expanded in scope to address many different aspects of this infectious disease (http://www3.niaid.nih.gov/research/topics/lyme/research/). They include systematic studies of:

- Animal models of disease.
- Microbial physiology.
- Molecular, genetic, and cellular mechanisms of pathogenesis.
- Mechanisms of protective immunity.
- Vectors, as well as vector competency and their influence on transmission of the disease.
- Efficacy of different modes of antibiotic therapy.
- Development of more sensitive and reliable diagnostic tests for both early (acute) and late (chronic) Lyme disease.
- Diagnosis, including the development and application of new technologies for rapid and sensitive diagnostic assays, as well as assessment, refinement, and standardization of improved diagnostic procedures.
- Treatment and prevention, including the development, application, and evaluation of novel and safe therapeutic approaches, as well as identification and characterization of candidate vaccines.
- Immune mechanisms, including understanding the development of protective immunity, characterizing the immunomodulatory properties of microbial antigens and evaluating their role in the pathogenesis, and characterizing the response of the host's immune system both during infection and after deliberate immunization.
- Pathogenesis, including the identification and characterization of virulence factors and the molecular basis for damage to host tissues during infection, and defining the role of cytokines and other immunomodulatory agents in the expression of disease.
- Epizootiology/ecology, including defining potential and established vectors and reservoirs, assessing the role of ticks and other vectors in transmitting the disease and maintaining virulence, relating the role of genetic variation in the incidence of disease in endemic areas, and defining effective measures for significantly reducing or eliminating populations of infected ticks in endemic areas.

Other developments of NIAID-supported Lyme borreliosis and tick-borne rickettsial disease research include:

(i) The sequencing of the *B. burgdorferi* genome, including the DNA within plasmids—small segments of DNA that reside outside the *B. burgdorferi* chromosome and can be exchanged among bacteria. Microarray technology is now being applied to identify genes involved in pathogenesis and adaptation to various host environments, as well as to evaluate the utility of this approach for the early diagnosis of infection.

(ii) The development of tick salivary protein-based, transmission blocking vaccines to interfere with the ability of infected ticks to feed on intermediary mammalian hosts.

(iii) Research is continuing to sequence multiple *Rickettsia* species, including the spotted fever group bacteria *R. rickettsii* and *R. akari*, and the typhus-group pathogen *R. canada*. In addition, the genome sequence of *R. bellii*, which represents a third group of *Rickettsia* species, is also under way.

22.1.5.2 Ongoing Research

NIAID's current Lyme disease research portfolio is extensive and diverse (http://www3.niaid.nih.gov/research/topics/lyme/research/). It encompasses basic and clinical research studies conducted by extramural and intramural investigators, including intramural scientists at NIAID's Rocky Mountain Laboratories (RML) in Hamilton, Montana, as well as at NIAID intramural laboratories in Bethesda, Maryland.

Specific NIAID-supported activities on Lyme disease include extramural research on:

- The transmission of Lyme disease
- Diagnostic procedures
- Co-infection
- Antibiotic therapy
- The role of autoimmune reactivity
- Vaccine production

Lack of Evidence of Borrelia Involvement in Alzheimer's Disease. Because various published reports have suggested the possibility that *B. burgdorferi* may play a role in the etiology of Alzheimer's disease, NIAID intramural scientists have examined this issue in greater detail. The results of these studies, using a very sensitive polymerase chain reaction (PCR) assay capable of amplifying a *Borrelia*-specific DNA target sequence from all strains of *B. burgdorferi sensu lato* species known to cause disease in humans, have provided *no evidence* to indicate the presence of *B. burgdorferi* in the brains of patients with Alzheimer's disease *(12)*.

22.1.5.3 Antibiotic Therapy and Animal Models

Whereas early acute Lyme borreliosis is easily cured by conventional antibiotic therapy, some patients who have been correctly diagnosed initially as having Lyme disease may experience serious neurologic and musculoskeletal symptoms several months after receiving what appeared to have been successful antibiotic therapy. Because it is unclear

whether such symptoms are due to long-term persistent infections or other causes, the term *posttreatment chronic Lyme disease (PTCLD)* is often used to describe this condition, so as not to impose any judgment on the actual mechanism(s) that might be involved (see also Section 22.1.4.4).

Over the years, NIAID has supported research regarding PTCLD as well as other clinical issues (http://www3.niaid.nih.gov/research/topics/lyme), including:

- *New England Medical Center (NEMC) Clinical Study*. This study, which was carried out in NEMC in Boston and completed in 2000, was aimed at studying the clinical efficacy of antibiotic therapy for treating PTCLD. It involved randomized, double-blind, placebo-controlled, multicenter trials to examine the safety and efficacy of ceftriaxone and doxycycline in patients with either seropositive or seronegative chronic Lyme disease. The trials compared treatment with 30 days of intravenous ceftriaxone followed by 60 days of oral doxycycline to treatment with intravenous placebo followed by oral placebo for the same duration in patients who were either seropositive or seronegative at the time of enrollment. Preliminary results from the trials showed that after 90 days of continuous antibiotic therapy, there were no significant differences in the percentage of patients who felt that their symptoms had improved, gotten worse, or stayed the same between the antibiotic treatment and placebo groups in either trial *(13)*. Other results from the trials indicated that patients with PTCLD did not show objective evidence of cognitive impairment and that 90 days of continuous antibiotic therapy was not more beneficial for these patients than was administering a placebo *(14)*.
- *State University of New York (SUNY) Clinical Study*. In another placebo-controlled study conducted at SUNY at Stony Brook, patients with PCTLD were treated with either intravenous ceftriaxone or a placebo for 28 days. They were then evaluated to determine whether there was significant improvement with respect to fatigue, cognitive function, and the clearance of OspA antigen that was present in the spinal fluid of only 16% of all enrolled patients. The results of the trial have shown that ceftriaxone therapy was associated with improvement in fatigue but not with the other primary outcome markers considered *(15)*. Because fatigue, which is a nonspecific symptom, was the only primary outcome measure affected and because the treatment examined was associated with adverse events, the results of the SUNY study do not support the use of additional antibiotic therapy with parenteral ceftriaxone in posttreatment, persistently fatigued PTCLD patients (http://www3.niaid.nih.gov/research/topics/lyme).
- *Animal Models*. Appropriate animal models also have provided considerable information on the transmission and pathogenesis of Lyme borreliosis, as well as on the mechanisms involved in the development of protective immunity. NIAID, in collaboration with the National Institute of Neurological Disorders and Stroke (NINDS), has broadened these efforts to include comprehensive studies on non-human primate animal models for experimental research on the neuropathology associated with chronic Lyme borreliosis *(16)*. A major goal of these studies is to optimize the rhesus model of Lyme borreliosis as well as to determine the pathogenesis of the disease with a focus on the neurologic manifestations. It is anticipated that these studies will expand the knowledge of those factors that contribute to the pathology associated with persistent infection of the central nervous system by *B. burgdorferi* and ultimately will enable scientists to devise more effective clinical approaches for treating chronic Lyme borreliosis in humans. These studies will also supplement and enhance the results of current clinical research on the efficacy of antibiotic therapies for treating chronic Lyme disease and provide precedents for use in designing future clinical studies and will ultimately enhance the results of current clinical studies on chronic Lyme disease.

Inflammation of skeletal muscle is a consistent feature of Lyme borreliosis, both in humans and in experimental animal models of infection. Although several cytokines are expressed in muscle tissue, proinflammatory cytokines commonly associated with inflammation are not upregulated in *Borrelia*-infected muscle. However, the expression of *B-lymphocyte chemoattractant (BLC)*, a chemokine implicated in the trafficking of B cells to tissues, is increased in *Borrelia*-infected muscles of non-human primates *(17)*. Using protein expression profiling, it has been shown that BLC is upregulated in the spinal fluid of patients with neuroborreliosis but not in patients with noninflammatory and various other inflammatory neurologic diseases *(18)*. Because the upregulation of BLC was found in every neuroborreliosis patient examined, it may be a valuable diagnostic marker for neuroborreliosis.

Other studies have shown that *B. burgdorferi* can be detected in mice for at least 3 months after treatment with therapeutic doses of various antibiotics (ceftriaxone, doxycycline, or azithromycin). These surviving spirochetes could not be transmitted to healthy mice and some lacked plasmid genes associated with infectivity. By 6 months, antibiotic-treated mice no longer tested positive for the presence of *B. burgdorferi*, and even cortisone immunosuppression failed to alter this result; that is, it failed to activate infection. Nine months after antibiotic treatment, low levels of *Borrelia* DNA still could be detected in some, but not all of the mice. These findings *(19)* have indicated that noninfectious *B. burgdorferi* can persist for a limited time after

antibiotic therapy. The implications of these findings to persistent infection and the nature of chronic Lyme disease in humans remain to be assessed.

22.1.5.4 The Role of Autoimmune Reactivity in Lyme Disease

Results from recent studies have indicated that T cells from patients with chronic Lyme disease were reactive not only against *B. burgdorferi*–specific antigens but also against various host (self) antigens *(20)*. Such antigenic mimicry might generate autoimmune inflammatory reactions that could be responsible for arthritic as well as neurologic symptoms associated with chronic Lyme disease (http://www3.niaid.nih.gov/research/topics/lyme/research/autoimmune/).

In other studies, antibodies against the OspA epitopes of *B. burgdorferi* have also been shown to cross-react with neural tissue *(21)* as well as myocin *(22)*. Such antigenic mimicry may have the potential to generate autoimmune inflammatory reactions that could be responsible for the neurologic symptoms associated with chronic Lyme disease. In this context, it is interesting to note that homologies between proteins of *B. burgdorferi* and thyroid antigens have also been reported *(23)*.

In NIAID-supported clinical studies, case subject patients with PTCLD were compared with control subjects without such symptoms for the presence of several human leukocyte antigen (HLA) class II (DRB1 and DQB1) genetic markers, some of which are known to be associated with the expression of autoimmune reactivity. The results obtained did not support the involvement of an autoimmune mechanism in PTCLD *(24)*. However, because not all autoimmune diseases are associated with specific HLA haplotypes, these findings do not necessarily exclude that possibility. Definitive proof would clearly involve demonstrating the presence of significant levels of relevant autoimmune antibodies and/or autoreactive T cells in patients with PTCLD but not in treated control subjects without such symptoms. A greater frequency of DRB1*0401, which has been reported to be associated with antibiotic-treatment-resistant arthritis, was noted in the case subject patients; although this finding appeared to be nominally significant ($p < 0.05$), its biological significance is ambiguous because none of the case subjects considered had symptoms of inflammatory arthritis (http://www3.niaid.nih.gov/research/topics/lyme/research/autoimmune/).

22.1.5.5 Co-infections

Co-infection could represent a major potential problem, mainly because the *Ixodes* ticks that transmit *B. burgdorferi* often carry—and simultaneously transmit—other emerging pathogens, such as *Anaplasma (Ehrlichia)* species, the causative agent of human granulocytic ehrlichiosis (HGE), and *Babesia microti*, which causes babesiosis (http://www3.niaid.nih.gov/research/topics/lyme/research/co-infection/). In Europe and Asia, *Ixodes* ticks also are known to transmit tick-borne encephalitis viruses. Fortunately, this tick-borne viral infection has not yet been reported in the United States, although co-infections with Powasan virus and deer tick virus have been reported.

Co-infection by some or all of these other infectious agents may interfere with the clinical diagnosis of Lyme borreliosis and/or adversely influence host defense mechanisms, thereby altering landmark characteristics of the disease and the severity of infection *(25)*. NIAID-supported studies have indicated that co-infection with HGE increases the severity of Lyme borreliosis *(26)*. By contrast, when mice were co-infected with *B. microti* and *B. burgdorferi*, neither agent influenced the course of infection induced by the other as evidenced by the percentage of parasitemia, spleen weights, and hematologic and clinical chemistry parameters *(27)*.

In NIAID-supported clinical studies on chronic Lyme disease, patients with persisting symptoms were examined to determine if they might have been co-infected with other tick-borne infectious diseases at the time of their acute episode of Lyme disease. Among the tick-borne infectious diseases considered were babesiosis (*Babesia microti*), granulocytic ehrlichiosis (*Anaplasma phagocytophilum*), and tick-borne encephalitis virus infection. The seroprevalence rates for *B. microti* and *A. phagocytophilum* were found to be 2.5% and 8.6%, respectively, and no patient examined was found to be positive for tick-borne encephalitis viruses *(27)*. Thus, the persistence of symptoms in patients with "post–Lyme syndrome" could not be attributed to co-infection with one of these pathogens.

An examination of pathogen distributions in the tissues of mice infected with both *B. burgdorferi* and *A. phagocytophilum*, the bacterium that causes HGE in humans, showed an increase in the numbers of *B. burgdorferi* in the ears, heart base, and skin of co-infected mice; however, the numbers of *A. phagocytophilum* remained relatively constant. The serum antibody response to *A. phagocytophilum* (but not to *B. burgdorferi*) decreased as a result of co-infection. These findings suggest that co-infection can influence not only pathogen burden but also host antibody responses *(28)*. NIAID intramural and extramural research programs have initiated clinical studies on chronic Lyme disease. The intramural research program is conducting a comprehensive clinical, microbiologic, and immunologic assessment of patients with Lyme disease. This involves multiple lines of investigation with emphasis on (i) defining various biological markers of infection; (ii) assessing clinical course and outcomes of patients with Lyme borre-

liosis; and (iii) characterizing the immune response generated in response to *B. burgdorferi* (http://www3.niaid.nih.gov/research/topics/lyme/research/co-infection/).

22.1.5.6 Diagnostic Procedures

NIAID is supporting various efforts to evaluate and improve existing diagnostic procedures. Approximately 20% of its extramural Lyme disease research portfolio is devoted to developing novel and more sensitive diagnostic procedures (http://www3.niaid.nih.gov/research/topics/lyme/research/diagnostics/). In 1998, the FDA granted approval to Chembio Diagnostic Systems to market the Wampole *PreVue Borrelia burgdorferi* Antibody Detection Assay. The assay is a single-use, unitized immunochromatographic test that uses recombinant *B. burgdorferi* antigens for the qualitative presumptive (first step) detection of IgG and IgM antibodies to *B. burgdorferi* in human serum or whole blood. This test is to be used only in patients with history, signs, and symptoms that are consistent with Lyme disease. It is intended for use in clinical and physicians' office laboratories.

In collaboration with CDC, NIAID is also playing a major role in encouraging the development of novel approaches to improve the diagnosis of Lyme borreliosis in humans with various co-infections (e.g., ehrlichiosis or babesiosis), as well as in immunized people (http://www3.niaid.nih.gov/research/topics/lyme/research/diagnostics/). For example, it has been shown in NIAID-supported research that a synthetic peptide composed of 26 amino acid residues (C6) derived from a variable surface antigen (VlsE) of *B. burgdorferi* can be used in a new, rapid, and extremely sensitive ELISA test (the C6 ELISA) for diagnosing Lyme disease. Because this diagnostic test for Lyme disease, which has been approved by FDA, does not detect antibodies specific for recombinant OspA, it can be used even for those who have been immunized with the licensed OspA-based LYMErix vaccine *(29)*.

Although the *Lyme Urinary Antigen Test (LUAT)* is one of several diagnostic tests used routinely in NIAID's clinical studies on chronic Lyme disease, the results of independent quality control assessments of tests conducted by extramural and intramural scientists showed the LUAT to be unreliable because it yields an unacceptably high percentage of false-positive reactions *(30)*. A critical evaluation of urine-based PCR assays for the diagnosis of Lyme borreliosis likewise affirmed that urine is not a suitable material for the diagnosis of Lyme borreliosis *(31)*. By contrast, the similar assessments confirmed a high degree of reproducibility and concordance (virtually 100%) for the results obtained using ELISA and Western blot assays *(30)*.

Of great importance is the fact that decreases in the titer of antibodies against C6 can be used as an indicator of the efficacy of antibiotic therapy for patients with localized or disseminated Lyme disease, but not for chronic Lyme disease *(32)*. This is indeed a major advancement, because no other laboratory test enables one to obtain such information *(29)*. The results obtained with the C6 ELISA assay are consistent with those obtained with other diagnostic tests and may eliminate the time and expense of conducting additional laboratory tests to confirm the diagnosis of Lyme disease *(33)*. NIAID-supported investigators are now working closely with the CDC to determine if the C6 ELISA can eventually replace the traditional two-tiered conventional ELISA and Western blot assays. The results of other studies confirmed that a decline in the anti-C6 antibody titer coincides with the efficacy of antimicrobial therapy in patients with early localized or early disseminated Lyme borreliosis *(34)* (see also *22.1.6*).

The *B. burgdorferi*–specific immune complex (IC) test in which polyethylene glycol (PEG) is used to isolate ICs from serum has been advocated by some investigators as an approach for the early diagnosis of active borreliosis. However, recent findings indicate that it may not be more effective in detecting early and active infections than other conventional tests in which unprocessed serum specimens are used *(35)*.

There is a great need to develop additional simple, sensitive, and rapid procedures to distinguish those persons who are actively infected with *B. burgdorferi* from those who have either recovered from a previous infection or have been immunized previously. Because the genome of *B. burgdorferi* has now been completely sequenced, greater advances toward this goal are anticipated as this information is used in conjunction with microarray technology and proteomics to improve diagnosis, as well as to provide new insights on the pathogenesis of this disease and pathogen-specific host response mechanisms (http://www3.niaid.nih.gov/research/topics/lyme/research/diagnostics/).

22.1.5.7 Transmission of Lyme Disease

There is no clear understanding about the molecular basis of how *B. burgdorferi* maintains itself in nature via a complex life cycle that involves passage through ticks and various intermediate hosts, such as mice and deer, before infecting humans. The outer surface protein A (OspA) of *B. burgdorferi* has been well studied, and there is much speculation about its role—in conjunction with other cell surface proteins (OspB and OspC)—in transmitting Lyme disease *(5)*.

Although *B. burgdorferi* depends on *Ixodes* ticks and mammalian (rodent) hosts for its persistence in nature *(6)*, the search for borrelial genes responsible for its parasitic dependence on these types of diverse hosts has been

hampered by limitations in the ability to genetically manipulate virulent strains of *Borrelia*. Despite this constraint, there is evidence to indicate that the inactivation and complementation of a gene (*bbe16*) encoded by a linear plasmid (lp25) plays a major role in the virulence, pathogenesis, and survival of *B. burgdorferi* during its natural life cycle *(36)*. This gene, which has been renamed BptA (for borrelial persistence in ticks-gene A), potentiates virulence in mice and is essential for the persistence of *B. burgdorferi* in *Ixodes scapularis* ticks. Although BptA appears to be a lipoprotein expressed on the outer surface membrane of *B. burgdorferi*, the molecular mechanism(s) by which BptA promotes persistence within its tick vector remains to be elucidated. Because BptA appears to be highly conserved (>88% similarity and >74% identity in amino acid sequence) in all *B. burgdorferi sensu lato* strains examined, it may be widely used to promote persistence in nature. Given the absolute dependence on—and intimate association with—its tick and rodent hosts, BptA must be considered to be a major virulence factor that is critical for *B. burgdorferi's* overall infectious strategy *(7)*. Strategies designed to block the synthesis or expression of BptA could be of great value in preventing the transmission of Lyme disease.

The potential role that differentially upregulated surface proteins play in the transmission of borreliosis and Lyme disease pathogenesis have prompted investigators to conduct a comprehensive gene expression profiling analysis of temperature-shifted and mammalian host–adapted *B. burgdorferi*. The combined microarray analyses revealed that many genes encoding known and putative outer surface proteins are downregulated in mammalian host–adapted *B. burgdorferi*. However, at the same time, several different genes encoding at least seven putative outer surface proteins were found to be upregulated during the transmission and infection process. All seven proteins are immunogenic and generate the production of bactericidal antibodies in infected baboons *(37)*. This suggests that these outer surface proteins might be excellent second-generation vaccine candidates.

The above findings have been consistent with the results of published studies *(19)* in which a novel experimental technique (*xenodiagnosis by ticks*) was used to determine whether *B. burgdorferi* can persist in mice long after antibiotic therapy. In these studies, an immunofluorescence assay and the PCR assay were used to demonstrate that *B. burgdorferi* could be detected in doxycycline- and ceftriaxone-treated mice for at least 3 months (if not longer) after antibiotic therapy. However, the resulting surviving spirochetes were unable to infect other naïve mice because they lacked those linear plasmids (lp25 and lp28) that are essential for their ability to transmit infection *(19)*. It is noteworthy that lp25 also encodes for a gene product (PncA or BBE22) that is essential for the survival of *B. burgdorferi* in a mammalian host *(36)*.

NIAID-supported investigators have now been able to create various mutant strains of *B. burgdorferi* and have shown that although OspA and OspB are not required for infection of mice, they were essential for the colonization and survival of *B. burgdorferi* in ticks (http://www3.niaid.nih.gov/research/topics/lyme/research/transmission/). *Ixodes scapularis* ticks have a receptor on the inner wall of their intestines to which *B. burgdorferi* is able to bind tenaciously by means of OspA, a cell surface protein. This receptor is called the *tick receptor for OspA (TROSPA)*. Attachment to TROSPA will enable *B. burgdorferi* to persist in the gut from the time they were ingested by ticks through a subsequent molt, thereby avoiding elimination; this would allow *Borrelia* to be injected into a new host when the ticks take their next blood meal *(38)*. When ticks take a blood meal, the production of OspA is downregulated in favor of the increased production of OspC. This results in gut-bound spirochetes becoming detached, which enables them to migrate to the salivary glands, where they can be injected into mammalian hosts. Thus, TROSPA, in addition to other bacterial cell surface components, such as OspA, appears to play a key role in the transmission of Lyme disease to humans. Other studies have shown that if ticks are permitted to feed on mice that have been immunized previously with OspA, or have been treated with the antibody specific for OspA, the attachment and subsequent colonization of ticks by *B. burgdorferi* would be significantly impaired, if not prevented. This suggests the feasibility of developing oral- or vector-expressed transmission-blocking vaccines that involve the immunization of the intermediate hosts upon which ticks feed *(39)*. Several NIAID-supported investigators are now examining and testing this approach under controlled laboratory conditions (http://www3.niaid.nih.gov/research/topics/lyme/research/transmission/).

Results from other studies conducted by NIAID-supported investigators *(40)* have demonstrated that *B. burgdorferi* uses an immunosuppressive *tick salivary protein (Salp 15)* to facilitate the transmission of infection to mammalian hosts. This finding is based on observations that (i) the level of Salp 15 expression is enhanced by the presence of *B. burgdorferi* in infected ticks; (ii) Salp 15 adheres specifically to spirochete surface OspC both *in vivo* and *in vitro*, thereby increasing the ability of *B. burgdorferi* to infect mice; and (iii) the binding of Salp 15 protects *B. burgdorferi* from antibody-mediated killing *in vitro*, a factor that confers marked survival advantage. All of these observations suggest that Salp 15 and/or other tick salivary proteins might be excellent candidates for vaccines to block the transmission of Lyme disease *(41)*. In this context, prior and repeated exposure of experimental animals to uninfected ticks—and presumably their salivary proteins—has been shown to limit the capacity of infected ticks to transmit Lyme disease *(42)*.

22.1.5.8 Vaccine Production

Two large pharmaceutical companies [GlaxoSmithKline (SKB) and Pasteur Merieux Connaught (PMC)] have devoted considerable effort to developing a vaccine for Lyme disease. Double-blind, randomized, placebo-controlled clinical trials, involving more than 10,000 volunteers from areas of the United States where Lyme disease is highly endemic, have been completed for each of two *B. burgdorferi* recombinant OspA vaccines manufactured by SKB and PMC. These vaccines were found to be 49% to 68% effective in preventing Lyme disease after two injections and 68% to 92% effective in preventing Lyme disease after three injections. The duration of the protective immunity generated in response to the SKB vaccine (LYMErix), which was licensed by the FDA in December 1998, is not known. Consequently, the need for yearly booster injections remains to be established. Researchers and health experts anticipate that the use of these vaccines in endemic areas would likely result in a significant reduction in the incidence of Lyme disease in the future.

NIAID was not directly involved in the design and implementation of these particular vaccine trials; however, patents for cloning the genes used for the expression of recombinant OspA, as well as knowledge of the role of antibodies against OspA in the development of protective immunity, were derived from basic research funded by NIAID (http://www3.niaid.nih.gov/research/topics/lyme/research/vaccine/).

In April 2002, GlaxoSmithKline announced that even with the incidence of Lyme disease continuing to increase, sales for LYMErix declined from about 1.5 million doses in 1999 to a projected 10,000 doses in 2002. Although studies conducted by FDA failed to reveal that any reported adverse events were vaccine-associated, GlaxoSmithKline has discontinued manufacturing the vaccine for economic reasons *(43)*.

NIAID-funded investigators have developed an experimental bait delivery system for an OspA-based vaccine against *B. burgdorferi* in which mice were immunized orally (via gavage or bait feeding) with a strain of *Escherichia coli* expressing the gene for OspA, which resulted in the appearance of serum antibody specific for OspA. When mice were exposed to *Ixodes* nymphs carrying multiple strains of *B. burgdorferi*, oral vaccination was found to protect 89% of the mice from infection, and the resultant serum antibody response confirmed the presence of IgG2a/2b antibody specific for OspA (http://www3.niaid.nih.gov/topics/lymeDisease/research/vaccine.htm). This vaccination approach is able to generate a significant protective immune response against a variety of infectious strains of *B. burgdorferi*, thereby indicating that it can eliminate *B. burgdorferi* from a major host reservoir. It suggests that the broad delivery of an oral vaccine to wildlife reservoirs in an endemic area is likely to disrupt the transmission of Lyme disease *(44)*. These findings are consistent with the results reported by other investigators *(39)*, thus affirming the utility of this approach.

In other NIAID-supported studies, scientists have developed a murine-targeted OspA vaccine using the vaccinia virus to interrupt the transmission of disease in reservoir hosts, thereby having the potential to reduce the incidence of human disease. Oral vaccination of mice with a single dose of vaccinia virus expressing OspA resulted in high antibody titers to OspA, 100% protection of vaccinated mice from infection by *B. burgdorferi*, and a significant clearance of *B. burgdorferi* from infected ticks fed on vaccinated animals *(44)*. These findings indicate that such a vaccine may effectively reduce the incidence of Lyme disease in endemic areas.

NIAID is also funding preclinical studies to develop and test other candidate vaccines (e.g., decorin-binding protein A, or DbpA) for Lyme disease. Thus, MedImmune, Inc., and Sanofi-Aventis Pharmaceuticals have reported that a combination vaccine composed of the DbpA and OspA of *B. burgdorferi* was more effective than either one given alone in preventing the development of borreliosis in experimental animals. On the basis of these encouraging findings, both companies have entered into an agreement to develop a new, more effective second-generation vaccine to prevent Lyme disease in humans. Although the results of previous studies indicate that DbpA induces the development of protective immunity in a murine model of Lyme borreliosis when mice have been challenged (needle-inoculated) intradermally with *in vitro*–cultivated *B. burgdorferi*, such mice were not protected from infection transmitted by ticks carrying virulent *B. burgdorferi*.

22.1.5.9 Other NIH Institutes and Centers Working on Lyme Disease

The principal mission of NIAID is to study infectious diseases and host immune defense mechanisms; therefore, the institute conducts and supports most of the basic and clinical research on Lyme disease funded by the National Institutes of Health (NIH). However, because Lyme disease affects different tissue and organ systems of the body, it is also a matter of great concern to other NIH institutes and centers (http://www3.niaid.nih.gov/research/topics/lyme/centers/).

The *National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)* is funding research on chronic Lyme-induced arthritis, including the role of the immune system and genetic factors in contributing to its development.

The *National Institute of Neurological Disorders and Stroke (NINDS)* is funding research to characterize the neurologic, neuropsychological, and psychosocial manifestations

of early and late Lyme disease in both adults and children, as well as to characterize pathogenic mechanisms associated with the neurologic symptoms of chronic Lyme disease.

The *National Center for Research Resources (NCRR)* provides resource support (non-human primates) for basic and clinical studies on both acute and chronic infection, as well as support for testing and developing candidate vaccines for Lyme disease.

In addition, the *Fogarty International Center (FIC)* is funding research on Lyme disease abroad, and the *National Institute on Aging (NIA)* and the *National Institute of Mental Health (NIMH)* have focused on those aspects of Lyme disease that relate to their specific missions.

To facilitate cooperative interactions as well as to ensure that the research activities of all NIH components are complementary, an *NIH Lyme Disease Coordinating Committee (LDCC)* was established in 1992. LDCC meets annually to review the results of current studies and recent advances in research on Lyme disease. Because the FDA is responsible for evaluating the efficacy and safety of vaccines against Lyme disease (e.g., the LYMErix vaccine) and the CDC is especially interested in developing new and improved diagnostic procedures, representatives from FDA and CDC have been invited to serve on the LDCC and to provide updates on their activities related to Lyme disease.

22.1.6 Recent Scientific Advances

- *C6 ELISA Diagnostic Procedure*. It has been shown that a synthetic peptide comprising 26 amino acid residues (C6) derived from a variable surface antigen (V1sE) of *B. burgdorferi* can be used in a new, rapid, and extremely sensitive ELISA test (the C6 ELISA) for diagnosing Lyme disease *(29)*. The C6 ELISA test is sensitive only to antibodies generated during an active infection (both early and late stages of Lyme disease). Another advantage of the test is its ability to detect antibodies specific for both North American and European strains of *Borrelia*. Of great importance is the fact that decreases in the titer of antibodies against C6 can be used as an indicator of the efficacy of antibiotic therapy for patients with localized or disseminated Lyme disease, but not for chronic Lyme disease. Because the C6 ELISA test would not detect antibodies specific for recombinant OspA, it can be used even for those patients who have been immunized with the licensed OspA-based LYMErix vaccine *(29)*. This is a major advance, because except for the C6 ELISA no other laboratory test is capable of obtaining such information *(32)*.
- *An Ecologic Approach to Preventing Lyme Disease*. In a recently developed, ecologic approach to Lyme disease prevention, researchers have intervened in the natural life cycle of *B. burgdorferi* by immunizing the wild white-footed mouse (*Peromyscus leucopus*), a reservoir host species, with either a recombinant antigen (OspA) of the spirochete or a negative control antigen in a repeated field experiment with paired experimental and control grids stratified by site *(39)*. OspA vaccination significantly reduced the prevalence of *B. burgdorferi* in nymphal black-legged ticks (*I. scapularis*) collected at the sites the following year in both experiments. The magnitude of the vaccine's effect at a given site correlated with the prevalence of tick infection found on the control grid, which in turn correlated with mouse density. These data, as well as differences in the population structure of *B. burgdorferi* in sympatric ticks and mice, indicated that non-mouse hosts contributed more toward infecting ticks than previously expected. Thus, where non-mouse hosts play a large role in the infection dynamics, vaccination should be directed at additional species *(39)*.
- *Variable Nature of Antibodies Specific for OspC Influence Virulence*. The outer surface protein C (OspC) of *B. burgdorferi*, the spirochete that causes Lyme disease, has been studied for its potential in the development of a vaccine *(45)*. Of the 21 OspC types currently identified, a surprisingly large number (types A, B, C, D, K, N, and 1) are associated with invasive disease. Because a detailed knowledge of the antigenic structure of OspC would be essential for vaccine development, the antibody response against several different recombinant OspC proteins was examined in detail. The results have revealed a high degree of specificity, indicating that the immunodominant epitopes of OspC reside in the variable regions of the protein. To localize these epitopes, OspC fragments were generated and screened against serum collected from infected mice, thus allowing the identification of previously uncharacterized epitopes that define the type specificity of the OspC antibody response. The reported findings have provided valuable insights into the antigenic structure of OspC, as well as a basis for understanding the variable nature of the antibody response to this important virulence factor.
- *B. burgdorferi Uses an Immunosuppressive I. scapularis Salivary Protein to Infect the Mammalian Host*. The Lyme disease spirochete, *B. burgdorferi*, is maintained in a tick-mouse cycle. Evidence has demonstrated that the spirochete usurps a tick (*I. scapularis*) salivary protein, Salp15, to facilitate the infection of mice *(40)*. The level of *salp15* expression was selectively elevated by the presence of *B. burgdorferi* in *I. scapularis*. The salivary protein was shown to adhere to the spirochete and to specifically interact with *B. burgdorferi*'s outer surface protein C. The binding of Salp15 protected *B. burgdorferi* from antibody-mediated killing, thereby providing the spirochetes with a marked advantage when

they were inoculated into naïve mice or mammals previously infected with *B. burgdorferi*.

- *Development of a New Transmission Blocking Vaccine for Lyme Disease*. It has long been known that immunization of mice with outer surface protein A (OspA) will protect against transmission of *B. burgdorferi* infection and will reduce the carriage of this pathogen in feeding ticks. In a recent study, the development of a murine-targeted OspA vaccine using vaccinia virus to interrupt the transmission of disease in reservoir hosts has been reported *(44)*. Thus, oral vaccination with a single dose of the OspA-expressing vaccinia virus construct resulted in high antibody titers against OspA, 100% protection against infection by *B. burgdorferi*, and a significant clearance of *B. burgdorferi* from infected ticks that fed on immunized mice. The reported findings indicated that such a vaccine was effective and may provide a means to lower the incidence of human disease in endemic areas.
- *Identification of a Genetic Deficiency That Enhances Acquisition and Transmission of Lyme Disease by Ticks.* *B. burgdorferi* strains exhibit various degrees of infectivity and pathogenicity in mammals, which may be due to their relative ability to evade initial host immunity. Innate immune cells recognize *B. burgdorferi* by Toll-like receptors (TLRs) that use the intracellular molecule myeloid differentiation factor-88 (MyD88) to mediate effector functions *(46)*. In a mouse model of Lyme disease using mutant strains of mice, the absence of MyD88 was found to facilitate tick-transmission of strains of *B. burgdorferi* of both low and high infectivity *(47)*. The reported data will broaden the understanding of factors that contribute the degree of pathogenicity observed between different clinical isolates of *B. burgdorferi*, as well as the genetic basis for host resistance or susceptibility to infection.

22.2 Tick-Borne Rickettsial Diseases

Tick-borne rickettsial diseases (TBRDs) are caused by pathogens of the second main group (the *spotted fever group*) of the genus *Rickettsia* (the other being the *typhus group*; see Chapter 21). TBRDs continue to cause severe illness and death in otherwise healthy adults and children despite the availability of low-cost, effective antimicrobial therapy, and the reported incidence of TBRDs has increased during the previous decade. The greatest challenge to clinicians is the difficulty of diagnosing these illnesses early in their clinical course when antibiotic therapy is most effective—early signs and symptoms are often nonspecific or mimic benign viral diseases, making diagnosis difficult *(48)*.

Although clinically similar, the TBRDs are epidemiologically and etiologically distinct diseases. In the United States, they include (i) human monocytotropic (or monocytic) ehrlichiosis (HME); (ii) human granulocytotropic (or granulocytic) anaplasmosis (HGA; formerly known as human granulocytotropic ehrlichiosis or HE); (iii) Rocky Mountain spotted fever (RMSF); (iv) *Ehrlichia ewingii* infection; and (v) other emerging TBRDs *(48)*.

Additional diseases caused by the pathogenic members of the spotted fever group of *Rickettsia* are the African tick typhus and rickettsial pox. It is interesting to note that the pathogenic tick-borne *R. rickettsii, R. parkeri*, and *R. sibirica* are phylogenetically distinct from the nonpathogenic species *R. rhipicephali* and *R. montana*. Other species, such as *R. felis* and *R. helvetica*, are early diverging within the spotted fever group. The difference at the molecular level between the pathogenic and nonpathogenic species has not yet been completely elucidated.

Genome Sequence of Rickettsia conorii. Complete genome sequence data has been generated for only one species of the spotted fever group, *R. conorii (49)*. The genome of *R. conorii* is very small, only 1.29 Mb, and similar to that of *R. prowazekii* (see Chapter 21). The overall architecture of these two rickettsial genomes is essentially the same, with the exception of a few rearrangements near the terminus of replication. Symmetric DNA inversions at the regions surrounding the origins of replication and termination have been observed also in *Chlamydia (50)*. The symmetric nature of these rearrangements is thought to be the outcome of recombination events at the open replication forks. Such translocation and inversion events have since been identified in a variety of genomes, suggesting that the replicating DNA at the open replication fork is particularly vulnerable to recombination events *(49)*.

22.2.1 Treatment and Management of TBRDs: General Comments

Appropriate antibiotic treatment should be initiated immediately after diagnosis is made based on clinical, laboratory, or epidemiologic findings *(48)*. Any delay in treatment may lead to severe disease and even a fatal outcome.

Because any of the TBRD pathogens is susceptible to tetracycline antibiotics (especially doxycycline), these drugs are considered the therapy of choice in nearly all clinical situations. Fever typically subsides within 24 to 48 hours after doxycycline treatment is initiated during the first 4 to 5 days of illness *(48)*.

Doxycycline is bacteriostatic against rickettsiae and is active in both children and adults. The recommended dose for adults is 100 mg, twice daily (orally or intravenously). For children weighing less than 100 lb (45.5 kg), the recommended dose is 2.2 mg/kg body weight, twice daily (orally

or intravenously). Intravenous administration is frequently indicated for hospitalized patients. The length of antibiotic therapy would be at least 3 days after the fever subsides and until evidence of clinical improvement is noted (typically 5 to 7 days) *(48)*.

The tetracycline antibiotics are generally *contraindicated for use in pregnant women* because of risks associated with malformation of teeth and bones in the fetus and hepatotoxicity and pancreatitis in the mother *(48, 51)*. However, tetracycline has been used successfully to treat HME in pregnant women *(52)*, and its use may be warranted during pregnancy in life-threatening situations where clinical suspicion of TBRD is high. Nevertheless, therapeutic choices for pregnant women with ehrlichiosis should be weighed cautiously, even when the benefits of doxycycline therapy generally outweigh its risks *(1)*.

Chloramphenicol (no longer available as an oral formulation) is an alternative drug that has been used to treat TBRDs such as Rocky Mountain spotted fever (RMSF) *(53)*. However, the drug is associated with various side effects and may need monitoring of the patient's blood indices. Moreover, epidemiologic studies using CDC case report data have suggested that patients with RMSF treated with chloramphenicol have a higher risk of dying than do patients who receive tetracycline *(48, 54)*. Whereas chloramphenicol is typically the preferred treatment for RMSF during pregnancy, care *must be used* especially when administering the drug late in the third trimester of pregnancy because of the risk of gray baby syndrome *(51)*.

22.2.1.1 Drug Resistance

Only the tetracycline antibiotics have demonstrated *in vitro* susceptibility and *in vivo* activity toward *Ehrlichia* species. In spite of *in vitro* susceptibility against *Ehrlichia*, the clinical effectiveness of rifampin is unknown *(55)*.

Ehrlichia chaffeensis has demonstrated resistance to gentamicin, ciprofloxacin, penicillin, macrolides, and sulfa-containing drugs *(56)*.

22.2.1.2 Severe Manifestations of TBRDs

A substantial number of patients with TBRDs may require hospitalization because of severe manifestations, including prolonged fever, renal failure, disseminated intravascular coagulopathy (DIC), hemophagocytic syndrome, meningoencephalitis, and acute respiratory distress syndrome (ARDS) *(48)*. A notable exception is anaplasmosis (HGA), which has not been associated with meningoencephalitis.

Rocky Mountain spotted fever frequently presents as a severe illness, during which patients commonly require hospitalization. Up to 20% of untreated cases and 5% of treated cases have a fatal outcome, *making RMSF the most often fatal rickettsial disease in the United States (57)*. Host factors associated with severe or fatal RMSF include advanced age, male gender, black race, chronic alcohol abuse, and *glucose-6-phosphate dehydrogenase (G6PD)* deficiency *(53)*. Deficiency of G6PD is a sex-linked genetic condition affecting approximately 12% of the U.S. black male population *(48)*. Deficiency of G6PD is associated with a high proportion of fulminant cases of RMSF *(53)*. Fulminant cases follow a clinical course that is fatal within 5 days of the onset of infection. Long-term health effects of severe, life-threatening RMSF that may persist for more than 1 year include partial paralysis of the lower extremities, gangrene requiring amputation (fingers, toes, arms, or legs), hearing loss, blindness, loss of bowel or bladder control, movement disorders, and speech disorders *(58)*.

Similarly to RMSF, HME and HGA can also cause serious or fatal illness, although at a lower frequency than that observed with RMSF. Clinical conditions that may require hospitalization may include immunocompromised state, pain (headache, myalgia), mental confusion, cough, infiltrate in chest radiograph, abnormal spinal fluid findings, or specific acute organ failure *(48)*.

22.2.1.3 TBRDs Overlapping with Invasive Meningococcal Infection

It must be emphasized that during diagnosis, clinicians should be aware of the overlap of early symptoms of invasive meningococcal infection and TBRDs. These conditions are difficult to distinguish early in the course of the illness. In patients for whom both conditions are included in the initial differential diagnoses, after performing blood cultures and lumbar puncture, empirical treatment for both diseases *would be appropriate*. Such treatment could be accomplished by adding an appropriate parenteral penicillin (or cephalosporin) that has activity against *Neisseria meningitides* to doxycycline therapy *(48)*.

22.2.1.4 Pathogen Tropism

R. rickettsii, E. chaffeensis, E. ewingii, and *A. phagocytophilum* have specific and distinct cell tropism *(48)*.

R. rickettsii infects endothelial cells and, more rarely, underlying smooth muscle cells, where it multiplies freely in the cytoplasm. The rickettsiae cause a small-vessel vasculitis resulting in a maculopapular or petechial rash in the majority of patients. Vasculitis, when occurring in organs (brain or lungs), could cause life-threatening complications *(48)*.

Rickettsiae are not evident in blood smears and do not stain with the majority of conventional stains.

Ehrlichiosis and anaplasmosis are characterized by infection of leukocytes where the causative pathogens multiply in cytoplasmic membrane-bound vacuoles to form microcolonies known as *morulae. E. chaffeensis* most frequently infect monocytes, whereas *A. phagocytophilum* and *E. ewingii* demonstrate a predilection for granulocytes *(48)*. Morulae can be stained with conventional Wright or Giemsa stains and are occasionally observed in leukocytes in smears of peripheral blood, buffy coat preparations, or cerebrospinal fluid. Although a routine blood smear can provide a presumptive clue *for early diagnosis* because of the visualization of morulae, still a confirmatory testing for *Ehrlichia* or *Anaplasma* species *is required* by serology, PCR, or immunostaining methods. Also important to note is that the available methodology to demonstrate morulae in blood smears is not very sensitive, and a case of ehrlichiosis or anaplasmosis might be missed if the diagnosis relies solely on detecting morulae on blood smears. Although the diagnostic sensitivity of blood smears is greater for HGA than for HME, blood smears might only be positive in up to 60% of patients with HGA *(59)*.

22.2.2 Human Ehrlichiosis

Since 2000, the CDC has been tracking reported cases of several diseases collectively called human ehrlichiosis. However, the term "ehrlichiosis" is somewhat misleading, because when studied in detail it became clear that the etiologic agents of these emerging tick-borne infections are two different bacterial genera, *Ehrlichia* and *Anaplasma*. Geographically, they have occurred primarily east of the Rocky Mountains *(8, 48)*.

In the United States, infections caused by *Ehrlichia* spp. are typically transmitted by tick species of the genera *Amblyomma* (*A. americanum*) and *Ixodes* (*I. scapularis* and *I. pacificus)*. Both genera use small mammals and birds as their primary reservoirs *(1)*. Morphologically, *Ehrlichia* spp. are small intracellular Gram-negative cocci that infect different hematopoietic cells, causing two etiologically and epidemiologically distinct forms of ehrlichiosis: *human monocytic ehrlichiosis (HME)* and *human granulocytic anaplasmosis (HGA)*. In the United States, most cases of both HME and HGA occur in the spring and summer (April to September for HME, and May to August for HGA), when ticks are at their peak *(1)*.

Recently, the CDC has described a new group of diseases called "other and unspecified" human ehrlichiosis. These infections include diseases caused by a second *Ehrlichia* species as well cases of previously mentioned illnesses that could not be definitely diagnosed as either HME or anaplasmosis *(8, 48)*.

22.2.2.1 Human Monocytic Ehrlichiosis

The etiologic agent of human monocytic ehrlichiosis (HME) is *Ehrlichia chaffeensis* (Lone Star tick), which infects the macrophages and monocytes. The pathogen is transmitted primarily by *Amblyomma americanum*, but *Dermacentor variabilis* (American dog tick) can also transmit the disease. The major reservoir for *E. chaffeensis* is the white-tailed deer, with most cases being reported in the south central and southeastern regions of the United States. HME has been mainly associated with males (3 times more often than females), the elderly (over 65 years of age), and immunocompromised hosts (HIV/AIDS patients, and those with asplenia or Down's syndrome, and patients receiving immunosuppressive therapy) *(48, 60, 61)*.

Clinical manifestations of HME include fever, headache, and rash presented as part of a prodrome consisting of abrupt, high-grade fever (>95% of patients) often with an associated headache (60% to 80%), malaise (30% to 80%), nausea (40% to 50%), myalgia (40% to 60%), arthralgia (30% to 35%), lower back pain (30% to 40%), and gastrointestinal disorders (20% to 25%). The rash (on the trunk, extremities, and face, but rarely on the sole and palms) may be petechial, macular, maculopapular, or erythematous *(60)*. The prodrome typically manifests itself 7 to 14 days (median 9 days) after exposure to a tick. Neurologic manifestations (symptoms of meningitis and encephalopathy) have been observed in approximately 20% of patients.

Laboratory findings of HME are characterized by reduction in the multiple hematopoietic cell lines (occurring early in the course of the disease), thrombocytopenia, and leukopenia. A large decline in the total lymphocyte count is often seen in the early stage of the disease, whereas lymphocytosis occurs later, during the recovery phase of HME. Elevated liver enzyme levels (aspartate aminotransferase and alanine aminotransferase) are another characteristic laboratory finding of the disease and occur in 80% to 90% of patients *(1)*.

The manifestations of HME are typically moderate to severe and would require hospitalization of at least 50% of infected patients. If left untreated, HME may be fatal within the first 2 weeks, especially in men, the immunocompromised, and the elderly *(1)*.

22.2.2.2 Human Granulocytic Anaplasmosis

The black-legged tick (*Ixodes scapularis*) is the vector for *Anaplasma phagocytophilum* in the New England and north central regions of the United States, whereas the western

black-legged tick (*Ixodes pacificus*) is the principal vector in northern California. Because these *Ixodes* species also transmit *Borrelia burgdorferi* (the causative agent of Lyme disease) and various *Babesia* species, the preponderance of cases of HGA occur in the same states that usually report high incidence of Lyme disease and human babesiosis *(48)*. Simultaneous infection with *A. phagocytophilum* and *B. burgdorferi* has been reported *(55, 62)*, and discerning such a mixed infection is vital because it *might affect the choice of antimicrobial medication*; whereas amoxicillin can be used to treat early stage of Lyme disease, it is not effective against HGA *(48)*.

In the absence of tick exposure, other modes of HGA transmission have also been reported—butchers cutting fresh deer carcasses had contracted the disease *(63)*. This suggests blood as a potential source of transmission and represents a risk of occupational exposure.

At-risk populations include the elderly, patients with chronic diseases (e.g., diabetes, collagen-vascular diseases), and patients on immunosuppressive therapy.

HGA is manifested as a constellation of nonspecific symptoms that occur after an incubation period of 7 to 10 days after tick exposure; generally 4 to 8 days elapse before a patient will seek medical care *(55)*.

The disease is commonly characterized by high-grade fever (over 39°C), rigors, nonspecific myalgia, severe headache, and malaise *(1)***.** Other symptoms may include nausea, nonproductive cough, arthralgia, and anorexia. Although less common, 11% of patients with HGA will present with rash *(55)*, which is thought to be due to co-infection with Lyme borreliosis *(62)*. Though associated with less morbidity and mortality than is HME, 50% of patients with HGA will require hospitalization *(1)*.

Unlike patients with HME, those with HGA may have normal blood cell counts *(1)*. Nevertheless, approximately 70% of patients will have leukopenia and thrombocytopenia. Increased levels of liver enzymes, in particular, hepatic transaminases and C-reactive protein, are also commonly observed. In general, laboratory abnormalities will reach their peaks within 1 week after the onset of symptoms.

During acute HGA, morulae can be visualized (with microscopy) in the cytoplasm of leukocytes. This finding, if present, is diagnostic of HGA. However, the absence of morulae does not exclude the diagnosis of HGA *(55)*.

Typically, the nonspecific disease presentation, lack of morulae, and the transient nature of the blood cell counts *would make the diagnosis of HGA difficult*. As a result, the Consensus Approach for Ehrlichiosis (CAFE) Society has developed a set of definitions to help clinicians with the diagnosis of HGA *(1, 64)*.

Although HME and HGA are two distinct forms of human ehrlichiosis, the treatment is the same for both infections *(1)*. Doxycycline is the primary agent recommended for treatment of HGA. However, similarly to HME, doxycycline is *contraindicated for pregnant women and children younger than age 9*, posing a dilemma for clinicians treating these patients. Data demonstrating the efficacy of rifampin in the treatment of ehrlichiosis in pregnant women are limited to just case reports *(56)*. Therapeutic choices for pregnant women with ehrlichiosis should be weighed cautiously, but the benefit of doxycycline therapy generally outweigh its risks *(1)*, and according to recommendations by the American Academy of Pediatrics and the CDC, doxycycline should be used in the treatment of children *(65)* and neonates *(66)*. It is recommended for children to start with oral doxycycline (4.4 mg/kg in 2 divided doses) on day 1, followed by a single dose of 2.2 mg kg^{-1} day^{-1}; the CDC is recommending the use of doxycycline 4.0 mg/kg in divided doses for children weighing less than 45 kg, and 100 mg twice daily (adult dose) for children weighing 45 kg or more (http://www.cdc.gov/ncidod/dvrd/rmsf/treatment).

22.2.2.3 Rocky Mountain Spotted Fever

In 1916, *Rickettsia rickettsii* was described in the blood vessels of infected patients and later identified as the etiologic agent of the Rocky Mountain spotted fever (RMSF).

The Rocky Mountain spotted fever has long been established in the United States. In spite of its common name, it is relatively rare in the Rocky Mountain region but far more prevalent in the southeastern regions of the United States.

Most often the infection is transmitted by ticks of the genus *Dermacentor*, which include the American dog tick (*D. variabilis)* in the eastern, central, and Pacific coastal United States, and by the Rocky Mountain wood tick (*D. andersoni*) in the western United States. In 2005, the common brown dog tick (*Rhipicephalus sanguineus*), a vector in Mexico, was also implicated in an Arizona outbreak *(8)*. The cayenne tick (*Amblyomma cajennense*) is a common vector of RMSF in Central and South America, and its range has extended into the United States in Texas *(48)*.

A case report of *Rickettsia parkeri* infection was recently published *(67)*. The organism was first discovered in Texas in *Amblyomma maculatum* (Gulf Coast tick); before that, the disease had not been reported in humans.

The main reservoirs for *D. variabilis* are small animals, such as mice and voles, and dogs and other large animals; and for *D. andersoni*, both small and large animals, typically wild rodents *(1)*. Ticks become infected by feeding on infected animals, by transtadial and transovarian passage. Humans are not a primary reservoir for *R. rickettsii* but are merely secondary hosts that enter the organism's life cycle tangentially through contact with arthropods. For humans to become infected with rickettsiosis, a tick may need to be attached for as little as 6 to 8 hours. However, attachment

of 24 hours or more is generally needed for transfer of the disease *(1)*. Human infection may also result from contact with contaminated tick fluid and tissues during tick removal or from laboratory contact during culture and isolation.

In cases reported to the CDC, approximately 90% occurred from April to September and 40% during the May to June period, although infections have occurred in every month *(57)*.

Today, if left untreated, RMSF is the most fatal tick-borne infection in the United States, with an overall mortality of 25% *(68)*. However, treatment with antibiotics has reduced the mortality rates to 3% to 4%, and this, in most cases, may be due to delay in the diagnosis of the disease. The causative agent of RMSF, *R. rickettsii*, has been included as a NIAID Category C biodefense priority pathogen.

Dogs are susceptible to RMSF, and they frequently develop the disease concurrently with other household members in an endemic area *(48)*.

The clinical and laboratory manifestations of RMSF are similar to those of HME and HGA and generally appear within 2 to 14 days after a tick bite *(1, 48)*. In RMSF, a rash typically appears 2 to 4 days after the onset of fever and will occur earlier in children than in adults; it is eventually observed in approximately 90% of children. The exanthema typically will begin with the appearance of small, blanching, pink macules on the ankles, wrists, or forearms that evolve to maculopapules. The classic centripetal spread of rash is typically not noticed by the patient and might be difficult to elicit from the clinical history *(48)*. Although the rash may expand to involve the entire body, its presence on the face is usually limited. Patients with petechial rash are often severely ill, and although fever and organ dysfunction may resolve quickly with treatment, complete recovery can take longer.

The rash progression of RMSF includes several critical exceptions and considerations as follows *(48)*:

(i) A rash on the palms and soles is not pathognomonic and may occur in illnesses caused by drug hypersensitivity reactions, infective endocarditis, and a diverse group of other pathogens, including *Treponema pallidum, Streptobacillus moniliformis, E. chaffeensis*, and especially *Neisseria meningitides*, as well as certain enteroviruses.
(ii) The rash might be evanescent or localized to a particular region of the body.
(iii) A rash might be completely absent or atypical in up to 20% of patients with RMSF.

In certain cases, patients with RMSF (or ehrlichiosis) may seek medical attention for a febrile illness that mimics viral meningoencephalitis. Focal neurologic deficits, including cranial or peripheral motor nerve paralysis or sudden transient deafness, may also be observed *(48)*.

Laboratory findings, especially the complete blood cell count (CBC), are essential for the diagnosis of RMSF *(48)*. The total white blood cell (WBC) count is typically normal in patients with RMSF, but increased numbers of immature bands are generally observed. Thrombocytopenia, mild elevation in hepatic transaminases, and hyponatremia may be observed with RMSF. By comparison, leukopenia (up to 53% of patients), thrombocytopenia (up to 94% of patients), and modest elevation of liver transaminase levels are particularly suggestive for HME and HGA *(48)*.

Patients with RMSF may have various signs and symptoms that differ in degree of severity *(1, 48)*. Orally given antibiotics are adequate in cases of mild illness, whereas severely ill patients should be hospitalized and treated with intravenous antibiotics. In a retrospective study, information based on multivariate analysis has shown that only increased serum creatinine levels and neurologic symptoms were associated with mortality *(69)*. The clinical outcome of RMSF is apparently strongly dependent on the time span between the patient's first visit and the start of therapy; if therapy had begun more than 5 days after the first visit, the outcome is significantly poorer than if treatment had been initiated earlier.

The tetracyclines are the cornerstone of therapy for RMSF, with doxycycline being the drug of choice *(1, 48)*. However, as with ehrlichiosis, the use of doxycycline in pediatric and pregnant patients again poses a problem. Generally, short courses of doxycycline may be administered in children younger than 9 years of age. However, according to the guidelines of the American Academy of Pediatrics and the CDC, the *empiric use* of doxycycline in children and pregnant women, although possible, should be applied with caution and with careful consideration for maternal hepatotoxicity and permanent tooth discoloration. Chloramphenicol has long been recommended as an alternative therapy for RMSF and is considered a suitable choice for patients who are pregnant or allergic to tetracyclines *(1)*. However, the adverse effects of chloramphenicol are well known: aplastic anemia, reversible bone marrow suppression, and gray baby syndrome *(70)*. Moreover, the chloramphenicol concentrations and reticulocyte counts should be monitored when the treatment exceeds 3 days.

Clearly, the administration of either doxycycline or chloramphenicol in pregnant women is not without risks.

22.2.2.4 Ehrlichia ewinglii Infection

Amblyomma americanum is also the principal vector of the ehrlichial pathogen *Ehrlichia ewinglii (48)*. The ecologic features of *E. ewinglii* are not completely known. However, dogs and deer have been naturally infected. Cases of granulocytotropic ehrlichiosis caused by *E. ewinglii* have been reported primarily in immunocompromised hosts. Human

infections with this pathogen have been reported throughout the range of the Lone Star tick *(48)*.

Early clinical presentations in patients with *E. ewinglii* include fever, headache, myalgia, and malaise, and they are difficult to distinguish from other TBRDs and noninfectious diseases *(48)*.

As in patients with HGA, rash is rare in patients with *E. ewinglii* infection, and blood smears are useful for identifying patients with *E. ewinglii*. Furthermore, evaluation of CSF in patients with *E. ewinglii* has shown neutrophilic pleocytosis *(71)*.

Appropriate antibiotic treatment should be initiated immediately when a diagnosis of *E. ewinglii* is made. Doxycycline is the drug of choice for both children and adults, and as with the treatment of other TBRDs, caution must be applied when doxycycline is used for the treatment of *E. ewinglii (48)*.

22.3 Tularemia

The first report of tularemia in the United States occurred in 1911, in Tulare County, California *(72)*. One year later, the pathogen responsible for this outbreak was isolated and named *Bacterium tularense (72)*. The first report of tularemia in humans occurred in 1914 in two patients bitten by deerfly *(72)*. The infection is transmitted by ticks and is passed transovarially among ticks. In 1959, the organism was renamed *Francisella tularensis*.

F. tularensis is a highly contagious organism, which, in the context of biological weapons defense, is considered to be a potential threat. In fact, a tularemia outbreak in 1942 before the Battle of Stalingrad was the result of weaponized *F. tularensis*. There is no person-to-person transmission, but tularemia delivered as an aerosol could infect a large number of people.

Ecologically, tularemia is a disease of the northern hemisphere (North America, Northern Asia, Scandinavia, Europe, Japan, and Russia).

In addition to transmission by ticks and other arthropods, *F. tularensis* can be transmitted by inhalation, ingestion of contaminated food or drinking water supplies, and animal bites *(1)*. More than 250 animal species are implicated as carriers of *F. tularensis*. Consequently, in different regions of the world the disease is known by different names (rabbit fever, hare fever, deerfly fever, and lemming fever).

In the United States, when transmitted by ticks, *F. tularensis* is primarily transmitted by *A. americanum, D. andersoni*, and *D. variabilis (72)*. Except for Hawaii, all states have reported cases of tularemia, with the highest rates coming from Arkansas, Missouri, South Dakota, and Oklahoma.

The disease has a predilection for males, especially Native Americans and Alaskan Natives, and children age 5 to 9 and adults age 75 or older. Most human outbreaks occur in spring and summer, which correlates with arthropod transmission *(1, 72)*.

22.3.1 Bacteriology and Taxonomy of F. tularensis

The bacteriology and taxonomy of *F. tularensis* is complex *(1)*. It is a small, pleomorphic, aerobic, Gram-negative coccobacillus that can be found both inside and outside of cells.

The genus *Francisella* is divided into three major biovars. Biovar A (biogroup tularensis) predominates in North America and is the most virulent. Biovar B (biogroup holarica) is found primarily in Europe and Asia, but also exists in North America. Biovar C (biogroup novicida; formerly known as *F. novicida*) is found in parts of North America and has very low virulence *(73)*.

22.3.2 Clinical Manifestations and Laboratory Findings

The clinical course of tularemia is quite diverse, ranging from asymptomatic disease to septic shock and death *(1)*. Typically, tularemia is divided into six forms, reflecting the mode of transmission: (i) ulceroglandular; (ii) glandular; (iii) oculoglandular; (iv) oropharyngeal; (v) pneumonic (pleuritic); and (vi) typhoidal.

Tularemia is characterized by abrupt but nonspecific symptoms, such as fever, chills, headache, vomiting, fatigue, and anorexia, which make the disease difficult to diagnose. Pulse-temperature disparity in which the patients may exhibit a high temperature without reflexive increase in pulse is a *hallmark manifestation of the disease (73)*.

Ulceroglandular tularemia is the most common syndrome, accounting for 70% to 80% of *F. tularensis* infections. The pathogen enters through a scratch, abrasion, or tick and spreads via the proximal lymphatic system. As few as 10 organisms can cause disease. This syndrome usually appears as a papule at the tick-bite site and progresses to a pustular, ulcerated lesion called an inoculation eschar *(73)*.

Glandular tularemia is a relatively rare syndrome (15% of patients) with no ulcer present. The organism causes regional lymphadenopathy and is presumed to have gained access to the host through clinically unapparent abrasion *(74)*. Diagnosis may be difficult because the patient presents one or several enlarged lymph nodes with no skin lesion.

Oculoglandular tularemia occurs in approximately 1% of patients and results from inoculation of the eye by tularemia-contaminated fluids or fingers, perhaps after removal of the tick *(1)*. The clinical manifestations of oculoglandular tularemia are conjunctivitis with adjacent lymph node involvement, periorbital edema, and erythema.

Oropharyngeal tularemia accounts for less than 5% of all cases. This syndrome is not acquired by contact with ticks but results from ingestion of infected raw meats or contaminated water supplies *(1)*. Symptoms include fever, exudative pharyngitis, or oropharyngeal ulcerations. Because the manifestations mimic those of other upper respiratory infections, the diagnosis of oropharyngeal tularemia is based on exclusion from lack of response to antibiotics given for bacterial pharyngitis.

Pneumonic tularemia, the most severe form of the infection, may not be directly associated with tick exposure, but rather can develop through inhalation or secondarily by hematogenous spread *(1)*. Disease mortality is estimated to be around 7% *(72)*.

Typhoidal tularemia is a rare syndrome *(72)* manifested by fever, chills, and local findings to culture-negative septic shock. The syndrome may also be accompanied by pneumonia, elevated transaminase levels, and rhabdomyolysis, which leads to renal failure *(75)*.

The diagnosis of tularemia is confirmed when an antibody response occurs approximately 2 weeks after the onset of disease. The preferred serologic methods are agglutination (latex or tube agglutination tests) or PCR. The latter is highly sensitive and safer, but its specificity is dependent on the DNA sample and its purity *(1)*.

22.3.3 Antibiotic Therapy of Tularemia

Treatment of tularemia is based solely on case reports and anecdotal experience. Based on the latter, aminoglycosides, especially streptomycin and gentamicin, are regarded as the cornerstone of therapy *(1)*. A meta-analysis found that streptomycin was successful in 97% of patients, whereas gentamicin was successful in 86% *(76)*. In addition, gentamicin was associated with a 6% relapse rate and an 8% failure rate. However, despite these drawbacks of gentamicin, its cure rate was equal to or greater than that of other classes of antimicrobials, thus making it an acceptable alternative to streptomycin.

Results from tetracycline therapy of tularemia have shown 88% success and no treatment failures; however, it was associated with 12% relapse rate *(76)*. The high relapse rate of tetracycline may be the result of its bacteriostatic mode of action. In addition, tetracycline can be given only orally, which limits its use in patients with severe tularemia—the drug levels achieved with oral administration only minimally exceeded the minimum inhibitory concentration (MIC) for *F. tularensis*.

In patients treated with chloramphenicol, the success rate was 77% with a 21% relapse rate *(76)*. Like tetracycline, chloramphenicol is bacteriostatic. However, when compared with aminoglycosides and tetracycline, one advantage of chloramphenicol is its enhanced penetration into the central nervous system. This feature makes chloramphenicol a *therapeutic option for treatment of meningeal tularemia*.

The fluoroquinolones are another therapeutic option for treating tularemia. Ciprofloxacin and levofloxacin have been used in the treatment of pneumonic tularemia, with the former showing a low failure rate and fewer adverse effects *(77)*, and no relapses (levofloxacin) 1 year later. Although data describing the efficacy of fluoroquinolones in the treatment of tularemia are still evolving, these agents have been as successful as other treatments of the disease *(1)*.

In vitro data demonstrated that *F. tularensis* isolates have shown resistance to β-lactams and therefore they should not be recommended for treatment of tularemia *(1)*.

22.4 Southern Tick-Associated Rash Illness

Erythema migrans, the characteristic rash associated with Lyme disease, has been reported in patients living in the south central and southeastern United States. Typically, it is associated with the bite of *Amblyomma americanum*. However, the spirochete that causes Lyme disease in North America, *Borrelia burgdorferi* sensu stricto, has not been confirmed in these regions of the United States by culture from clinical specimens, and serum antibodies rarely indicate exposure *(1)*. Although *Amblyomma americanum* is apparently not a vector for *B. burgdorferi sensu stricto*, the same ticks carry another spirochete, *Borrelia lonestari*. In a case report *(78)*, a patient was described with erythema migrans and *Amblyomma americanum* attachment. Importantly, *Borrelia lonestari* was identified both in the patient and the tick, and serology for *B. burgdorferi sensu stricto was negative*. Therefore, this observation strongly suggested that *Amblyomma americanum* can transmit the spirochete to humans, and the resulting rash, which resembled that seen with Lyme disease, has become known as southern tick-associated rash illness (STARI).

Borrelia lonestari (family Treponemataceae) is a spirochete that has been detected in *Amblyomma americanum* by DNA analysis. Unlike *Ixodes scapularis*, a vector for Lyme disease, *Amblyomma americanum* is less likely to be infected with a spirochete, with only 1% to 3% of *Amblyomma americanum* infected *(79)*. In contrast, 10% to 20% of nymph stage

ticks and 30% to 40% of the adult-stage ticks of *I. scapularis* are infected with a spirochete.

The natural reservoir for *B. lonestari* has not been identified even though it was detected in white-tailed deer.

22.4.1 Clinical Manifestations, Laboratory Findings, and Treatment

In the only published case report of STARI, the patient showed only mild symptoms, such as fatigue, cough, and right shoulder discomfort *(78)*. Fever and headache were absent, and results of musculoskeletal, neurologic, pulmonary, and cardiac examinations were normal. Two erythematous lesions were also noted. The only abnormal laboratory finding was a slightly elevated serum alkaline phosphatase level.

There is no specific serologic test for exposure to *B. lonestari (1)*.

In the only case reported, the patient underwent antibiotic therapy with doxycycline for 2 weeks *(78)*; the skin lesions resolved in 11 days, and the patient returned to health about 24 days after therapy was initiated.

22.5 Babesiosis

Babesiosis, a malaria-like disease caused by intraerythrocyte protozoa named *Babesia bigemina*, was first described in 1888 *(80)*. The parasite is also the cause of the Texas cattle fever *(1)*. The first case of babesiosis in humans was reported in the former Republic of Yugoslavia in 1957 and in the United States in the late 1960s *(1)*.

The *Babesia* protozoa may vary in size (1 to 5 μm) and can be oval, round, or pear shaped *(81)*. More than 100 different species have been identified, but only four have been reported to be pathogenic in humans. In the United States, infection is caused primarily by *B. microti*, and two new strains of *Babesia* that can cause infection are designated as WA-1 and MO-1. Although human infections in the United States caused by *B. divergens* have not been reported, this protozoa is the primary cause of babesiosis in Europe *(1)*.

In the northeastern United States, the primary vector for *B. microti* is *Ixodes scapularis*, and the primary reservoir is the white-footed mouse. Although all stages of *I. scapularis* feed on humans, the nymph-stage tick is typically responsible for transmission of *B. microti* in humans. Vectors and reservoirs for WA-1 and MO-1 have not been identified *(1)*.

While babesiosis infections have been observed in patients of all ages, it appears that the occurrence is higher in men, and persons older than 40 years are prone to more severe infection. The infection is contracted most commonly during the summer (June to August) *(1)*.

22.5.1 Clinical Manifestations and Laboratory Findings of Babesiosis

Like malaria, the *Babesia* species reproduce within the red blood cells and produce hemolysis, which is responsible for the clinical presentation of babesiosis *(82)*. Manifestations of the disease are diverse and may range from asymptomatic to fulminant, leading to prolonged illness and even death. Although in the United States most cases of babesiosis are subclinical, when patients become symptomatic, manifestations usually appear after an incubation period of 1 to 6 weeks. The most common symptoms include fever (85% of patients), fatigue (79%), chills (63%), and headache (39%). Less often, myalgia, anorexia, cough, nausea, vomiting, arthralgia, emotional liability, depression, sore throat, abdominal pain, conjunctival injection, photophobia, and weight loss have been reported *(82)*. Physical findings are generally nonspecific (high fever, mild splenomegaly, and hepatomegaly). Unlike other tick-borne infections, rash is not common in babesiosis.

The most common complications in patients with severe babesiosis are acute respiratory failure (21% of patients), disseminated intravascular coagulation (18%), heart failure (12%), coma (9%), and renal failure (6%). Babesiosis is fatal in 5% to 9% of cases *(83)*.

Laboratory findings may include a decreased hematocrit value, thrombocytopenia, and a normal or decreased white blood cell count. Elevated levels of hepatic transaminases, bilirubin, and lactate dehydrogenase have also been observed. Urinalysis may reveal proteinuria and hemoglobinuria *(81)*.

22.5.2 Treatment of Babesiosis

Most cases of human babesiosis in the United States are mild and may resolve without treatment *(1)*. However, therapy is required in those patients who have undergone splenectomy, are immunosuppressed, are elderly, or have significant symptoms.

Historically, the cornerstone of babesiosis therapy is a combination of clindamycin and quinine given for period of 7 to 10 days *(82)*. However, even though effective, the combination clindamycin-quinine has been associated with significant drug-related toxicities, such as hearing loss, tinnitus, vertigo, and diarrhea.

Atovaquone, an antiprotozoal drug, has been studied in combination with azithromycin for the treatment of *B. microti* infections *(83)*. The atovaquone-azithromycin combination was compared with a 7-day oral treatment with clindamycin-quinine in immunocompetent adults with non–life-threatening babesiosis *(84)*. Resolution of parasitemia and symptoms was similar in both groups; however, the adverse reactions were significantly less in patients receiving atovaquone-azithromycin (15%) than in those receiving clindamycin-quinine (72%).

Another drug combination found effective in the treatment of babesiosis was clindamycin-doxycycline-azithromycin in an AIDS patient who developed an allergy to quinine *(81)*.

The combination of sulfamethoxazole-trimethoprim-pentamidine has been used successfully in the treatment of *B. divergens (84)*.

Exchange transfusions, administered concurrently with antibiotic therapy, may be necessary for patients with severe babesiosis showing significant parasitemia (more than 5%), coma, hypotension, heart failure, pulmonary edema, or renal failure *(82)*. Exchange transfusions reduce parasitemia and will facilitate the removal of *Babesia*-, erythrocyte-, and macrophage-produced by-products *(85)*.

22.6 Tick-Borne Relapsing Fever

In 1905, tick-borne relapsing fever (TBRF) was first described in West Africa where it was transmitted by *Ornithodoros moubata* soft ticks *(86)*. Tick-borne relapsing fever (TBRF) is a systemic *Borrelia* infection caused by a group of closely related species of spirochetes: *B. hermsii, B. turicatae*, and *B. parkeri*.

22.6.1 Pathophysiology of TBRF

The *Ornithodoros* species are soft ticks belonging to the Argasidae family. In the United States, two of these species, *O. hermsii* and *O. turicata*, cause most cases of TBRF *(1)*. *O. hermsii* transmits *Borrelia hermsii, O. turicata* transmits *B. turicatae*, and *O. parkeri* transmits *B. parkeri (86)*.

TBRF is endemic in the western United States. It occurs sporadically, but several common source epidemics have been reported. As with other tick-borne diseases, TBRF is a seasonal illness; 71% of reported cases have occurred during the June to September period. However, in Texas most episodes occur during the late autumn and early winter, with 50% reported from November to January *(1)*. This difference in seasonality may be related to differences in both organisms and habitats; in Texas, cases typically represent *B. turicatae* infections acquired in caves, whereas in the northwestern United States, cases are generally *B. hermsii* infections acquired in mountainous regions *(1)*.

These spirochetes possess the unique ability to change outer surface proteins under pressure from the host immune system in a process known as *antigenic variation*, a phenomenon responsible for the recurring nature of the disease *(87)*. Thus, borreliae will sequester themselves in internal organs during afebrile periods and then will reappear with modified surface antigens to evade eradication.

As *Borrelia* organisms invade the endothelium, this can produce a low-grade, disseminated intravascular coagulation and thrombocytopenia. The relapse phenomenon occurs because of the antigenic variation—a genetically programmed shifting of outer surface proteins of *Borrelia* that allows a new clone to avoid destruction by antibodies directed initially against the majority of the original infecting organisms. As a result, the patient will improve clinically until the new clone multiplies sufficiently to cause another relapse. The tick-borne illness tends to have more relapses (average of 3) than does the louse-borne variety (often just one relapse).

22.6.1.1 Louse-Borne Relapsing Fever

Relapsing fever (RF) is an infectious disease transmitted to humans by two vectors, ticks and lice. The human body louse, *Pediculus humanus*, is the specific vector for borreliae. *Pediculus pubis* is not a vector. Louse-borne relapsing fever is a more severe form than the tick-borne variety. Regardless of the mode of transmission, a spirochetemia will develop.

The louse-borne relapsing fever is caused by *Borrelia recurrentis*. No animal reservoir exists. The lice that feed on infected humans acquire the *Borrelia* organisms, which then multiply in the gut of the louse. When an infected louse feeds on an uninfected human, the organism gains access when the victim crushes the louse or scratches the area where the louse is feeding. *B. recurrentis* infects the person through either abraded or intact skin (or mucous membranes) and then invades the bloodstream.

22.6.2 Clinical Manifestations and Laboratory Findings of TBRF

Because *Ornithodoros* ticks feed so rapidly, patients with TBRF are often unaware of the tick bite. A pruritic eschar may develop at the soft tick attachment site *(1)*.

Symptoms will appear abruptly on average 7 days after tick exposure *(88)*. Common manifestations include fever, headache, myalgia, arthralgia, nausea, and vomiting. The

primary febrile period when the temperature can rise as high as 104°F (or even higher) lasts about 3 days (range, 12 hours to 17 days). Patients then experience an afebrile period lasting about 1 week, during which time they may experience malaise before symptoms suddenly recur. Without treatment, several (three to five) relapses can be expected. However, the length and severity of the illness will typically decrease with each relapse *(88)*.

Less common symptoms of TBRF include abdominal pain, confusion, dry cough, eye pain, diarrhea, dizziness, photophobia, and neck pain. Rash (petechial, macular, or popular) occurs in about 18% of patients and develops as the fever subsides. Other physical findings can be splenomegaly and hepatomegaly *(1)*.

Neurologic complications (neuroborreliosis) will occur predominately in patients infected with *B. turicatae* (27% to 80%), but much less in patients infected with *B. hermsii* (5%). The most common manifestations of neuroborreliosis are cranial nerve palsies and meningisms *(89)*. Rare complications of TBRF are ocular disorders, myocarditis, and ruptured spleen *(86)*.

The most common hematologic abnormalities are thrombocytopenia (32% of patients), proteinuria (46%), and hematuria (30%). Most patients with TBRF have a normal white blood cell count.

22.6.3 Treatment of TBRF

No controlled studies have been published regarding treatment of TBRF. Based on clinical experience, the tetracycline antibiotics have been the treatment of choice *((90, 91)*. Oral doxycycline, 100 mg every 12 hours for 7 to 10 days, is the typical treatment. In addition, penicillin, chloramphenicol, and erythromycin have all been used successfully to treat TBRF. Tetracycline, erythromycin, and chloramphenicol are usually administered at dosages of 500 mg every 6 hours *(90)*. Patients with meningitis should receive intravenous therapy with penicillin G, cefotaxime, or ceftriaxone for 14 days or more *(91)*.

22.6.3.1 Jarisch-Herxheimer Reaction

The Jarisch-Herxheimer reaction is a serious consequence of TBRF treatment. It is manifested as an acute exacerbation of the patient's symptoms that can occur with the start of the antibiotic treatment. It has been reported in more than 50% of patients treated for TBRF *(89)*.

The pathophysiology of the Jarisch-Herxheimer reaction has been studied most extensively in patients with louse-borne relapsing fever. The reaction is associated with transient increases in plasma concentrations of tumor necrosis factor-α (TFN-α), interleukin-6, and interleukin-8 *(92)*. Anti-TNF-α antibodies prevented this reaction in patients with louse-borne relapsing fever *(93)*. Furthermore, meptazinol, an opioid partial agonist, reduced the severity of symptoms, whereas naloxone was ineffective *(94)*.

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