

The Lyme Disease Vaccine: Conception, Development, and Implementation

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In the past 20 years, remarkable strides have been made toward understanding and preventing Lyme disease in humans. In December 1998, the U.S. Food and Drug Administration approved a recombinant outer surface protein A vaccine against Lyme disease (LYMERix, SmithKline Beecham, Philadelphia, Pennsylvania). The vaccine, which is derived from a lipidated outer surface protein of the causative spirochete *Borrelia burgdorferi*, is important because it may decrease the morbidity and financial costs associated with Lyme disease. Its mechanism is unique because it works inside the tick vector itself, preventing the human from becoming infected.

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A form of Lyme disease may have existed for centuries. The characteristic rash of Lyme disease, erythema migrans, was first described in Sweden in 1909 and was properly attributed to bites from the tick *Ixodes ricinus* (1). However, the syndrome now known as Lyme disease was not described until 1975, when a cluster of cases of juvenile oligoarthritis occurred in Lyme, Connecticut (2). The causative agent was isolated in 1981 and was subsequently identified as *Borrelia burgdorferi*, a previously unknown species of *Borrelia* (3). In 1996, *B. burgdorferi* was isolated from European ticks that were more than a century old, which suggests that Europeans may have been exposed to Lyme disease spirochetes as early as 1884 (4). Today, Lyme disease is the most common vector-borne illness in the United States and Europe and has been found in Russia, Japan, China, and Australia (5, 6). A 1998 pharmacoeconomic analysis sponsored by SmithKline Beecham (Philadelphia, Pennsylvania) estimated the direct and indirect costs of Lyme disease in the United States alone, including costs of therapeutic interventions, to be \$2.5 billion over 5 years (7).

Studies of nucleic acid hybridization have delineated eight *Borrelia* genospecies, four of which have been identified as causal agents of Lyme disease. *Borrelia burgdorferi sensu stricto* is dominant in North America. In Europe, where mixed infections have been reported, *B. burgdorferi sensu stricto*, *B. garinii*, and *B. afzelii* coexist (8). Newly described strains—*B. valaisiana*, *B. lusitaniae*, and *B. japonica*—have been isolated only in Europe and Japan. *Bor-*

relia garinii is thought to be the ancestor of the whole group and is believed to be largely responsible for the neurologic symptoms so common in Europe. In 1996, a syndrome similar to Lyme disease was described in Georgia and Missouri; an uncultivable spirochete, *B. lonestari*, is thought to be the etiologic agent (9). The approved vaccine is directed against *B. burgdorferi sensu stricto* only, and cross-protection against other strains is unlikely.

In North America, 90% of cases of Lyme disease occur in 10 states in the coastal northeastern and Great Lake regions. In some hyperendemic regions of New York and Connecticut, Lyme borreliosis is such a threat that it has depreciated real estate values (10). From 1980 through 1988, a total of 13 795 cases were reported in the United States (11). Official reports to the Centers for Disease Control and Prevention (CDC) place the number of cases in 1998 alone at 16 801; however, because underreporting has been a serious problem, the true number may have been six to nine times greater (Figure 1) (12).

The rationale for development of a safe, effective vaccine centered on the documented increase in the incidence of Lyme disease; the progressive geographic spread of the illness; the failure of infection to confer lasting immunity; and the association of Lyme disease with chronic rheumatologic and neurologic sequelae as well as transient, severe cardiac conduction disturbances.

Lyme Disease

Lyme disease is a multisystem inflammatory disease that occurs in similar frequencies in men and women and affects persons of all ages. Lyme disease occurs when the ixodid tick vector injects the causative spirochete *B. burgdorferi* into humans. The most characteristic clinical finding is a skin lesion, erythema migrans, which usually appears 3 to 30 days after inoculation; however, it appears in only 65% to 80% of infected persons. In early disseminated disease, erythema migrans may occur in isolation or may be associated with flu-like illness; multiple episodes of erythema migrans; or neuro-

logic (Bell palsy or meningitis), cardiac (arrhythmia), or rheumatologic (arthritic) features that suggest hematogenous dissemination of the spirochete. Dissemination from the site of the tick bite is now thought to occur in part through attachment of the causative agent to host plasmin and subsequent degradation of glycoproteins (13).

Late-stage Lyme disease usually affects the joints (Lyme arthritis). Approximately 70% of untreated persons develop arthralgia, arthritis, or synovitis. Chronic Lyme arthritis typically occurs in the knees (14).

In the United States, an estimated 10% of patients with Lyme arthritis have persistent arthritis for months or even years after antibiotic therapy (14, 15). This chronic arthritis, which is usually monoarticular, can be severe and disabling. Kalish and colleagues (16) report that in persons with HLA-DR4 haplotype, antibody reactivity to two outer surface proteins of *Borrelia* species (OspA and OspB) is seen in association with chronic arthritis and a lack of response to antibiotic therapy in some patients (16). It is theorized that this relative antibiotic resistance could be caused by a persistent immunologic response, which might continue even in the absence of the antigen (15, 17).

Extra-articular manifestations of Lyme disease include cardiac disease, which typically develops weeks to months after infection and is usually manifested by a fluctuating degree of atrioventricular block. Pericarditis, left ventricular dysfunction, and cardiomegaly are rare. Neurologic Lyme borreliosis accounts for no more than 10% of cases, according to 1990–1995 data from the State of Connecticut (18). Early in the illness, Bell palsy is a common manifestation of disseminated disease and may take weeks to months to resolve (19). Other peripheral

neuropathies and Lyme meningitis are also seen at this stage. In late-stage disease, the central nervous system may be involved. A new diagnostic test measuring glial fibrillary acidic protein in cerebrospinal fluid may prove to be a useful tool for measuring such involvement (20). In Europe, untreated Lyme disease can manifest as acrodermatitis chronica atrophicans, a condition in which the skin becomes reddened, wrinkled, and paper-thin. One third of patients have an associated polyneuropathy. This is extremely uncommon in the United States because of the absence of *B. afzelii*.

Antibiotic treatment is at least 90% curative in active early Lyme disease (21–24) but is less successful in late-stage disease. The antibiotic therapy of choice for early Lyme disease is a 21- to 28-day course of doxycycline, 100 mg twice per day, or amoxicillin, 500 mg three times per day. For arthritis, a 30-day course is preferable. Optimal treatment for Lyme carditis or neurologic disease is ceftriaxone, 2 g intravenously for 14 to 28 days.

Environment of the Tick

The ixodid tick is virtually restricted to forested areas in which its hosts are plentiful. Human disease is thought to be caused largely by nymphal ticks, whose peak activity is in spring and summer. The adult female transmits disease in the fall. In endemic areas, 25% to 35% of nymphal ticks and 50% to 70% of adult ticks are infected with *B. burgdorferi*. Other species of pathogens, such as *Babesia* and *Ehrlichia*, are perpetuated in the same vector ticks and the same reservoir mice as *B. burgdorferi* (25). Mixed infections in humans are not uncommon.

In the western United States, the low rate of *B. burgdorferi* infection seems to have two causes. The

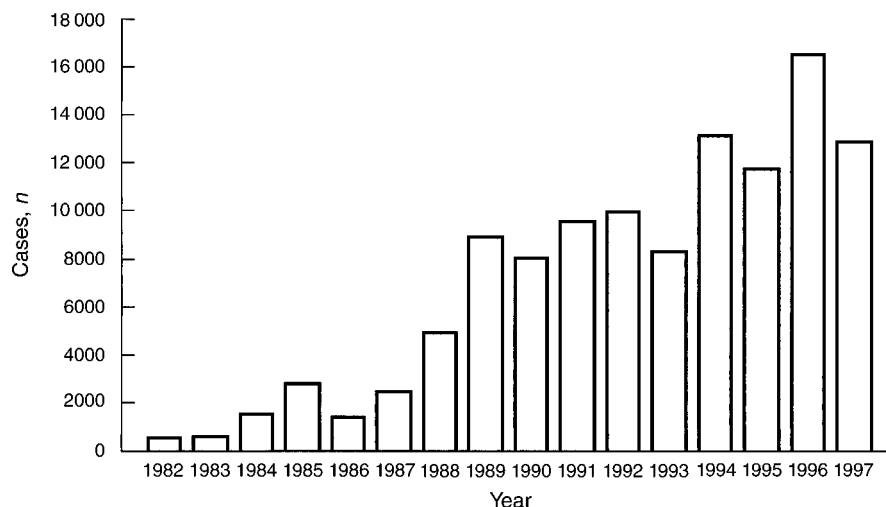


Figure 1. Number of reported cases of Lyme disease in the United States, 1982–1997. Adapted from the Centers for Disease Control and Prevention.

western fence lizard, a principal blood source for the nymphal tick, contains a thermolabile borreliacidal factor (isolated but not yet identified) that has been shown to destroy almost all spirochetes in vitro in less than 1 hour (26). This factor travels to the tick's mid-gut and kills the spirochetes in a fashion similar to that of the newly developed recombinant vaccine. In addition, the *I. neotomae* tick, which is common in the western United States, does not feed on humans.

Fortunately, it takes 24 to 72 hours for spirochetes to be transmitted into humans after the tick's initial attachment. This allows time for persons to safely remove the tiny (less than 3 mm) ixodid ticks. After much review, experts have decided that post-exposure prophylactic antibiotics should not be used, even in high-risk areas (27, 28).

Prevention on a regional level has proven to be cumbersome and ineffective. Communities have attempted to reduce the prevalence of ixodid ticks by burning vegetation, spraying acaricide, locally distributing acaricide, and reducing the deer population. These measures, however, are expensive, inefficient, and harmful to the environment. Avoidance of tick bites remains the mainstay of prevention.

Early Vaccine Research: Whole-Cell Studies

Research for a vaccine against Lyme disease progressed in earnest when, in 1986, Johnson and coworkers (29) reported passive immunization studies in which Syrian hamsters were successfully protected against live spirochete challenge. In addition, hamsters actively immunized with a single dose of a formalin-inactivated whole-cell lysate vaccine were protected after intraperitoneal inoculation of *B. burgdorferi* (30). These studies indicated the feasibility of vaccination as a method for the prevention of borreliosis in other species.

Canine borreliosis was first reported in 1984 (31). The recognized seroprevalence in dogs increased rapidly. The canine form of the disease produced lameness, fever, and anorexia. In 1992 and 1994, two inactivated whole-cell vaccines were developed and licensed for use in dogs. An inactivated whole-cell vaccine with adjuvant (*Borrelia burgdorferi* bacterin, Fort Dodge Laboratories, Fort Dodge, Iowa) and a bivalent whole-cell killed vaccine (Solvay Animal Health, Mendota Heights, Minnesota). Studies done under experimental conditions showed that the bacterin was immunogenic and efficacious in protecting vaccinated dogs from developing spirochetemia (32).

Protection seemed to be antibody-mediated because the inoculation of serum from immunized animals protected recipient animals (30). Concern

was later raised that this immune response may mediate some of the adverse effects of Lyme disease, particularly large-joint arthritis. Furthermore, in vitro experiments showed that certain antibodies to *B. burgdorferi* cross-reacted with nerve cell axons, synovial cells, hepatocytes, and heart and skeletal muscle protein (33, 34). The *B. burgdorferi* antigens thought to be primarily responsible for this molecular mimicry are the 41-kD flagellin subunit and heat-shock proteins. Because a whole-cell vaccine posed a potential for cross-reactivity to various antigens, research then focused on identifying recombinant proteins for the development of a human vaccine.

Outer Surface Protein A

After the causative Lyme disease agent was identified, it became possible to culture *B. burgdorferi* in a complex liquid medium called Barbour-Stoenner-Kelly medium (35). In culture medium, *B. burgdorferi* expresses abundant OspA (molecular mass, 31 kD), a species-specific lipoprotein that with flagellin makes up approximately one third of the total spirochete protein. The crystal structure of OspA has recently been clarified as containing a single-layer beta-sheet connecting N-terminal and C-terminal globular domains. The central beta-sheet consists largely of polar amino acids that are solvent-exposed on both faces; this seems to be unique among protein structures (36). More than 100 other proteins have also been identified in the spirochete's genome, including OspB, OspC, OspD, OspE, and OspF (37, 38).

Two weeks after cultured spirochetes are injected into animals, antibodies against flagellin, P39, and OspC developed. Soon thereafter, reactivity against OspA and OspB was demonstrated (39). However, when naturally infected through the tick vector, humans (40), rhesus monkeys (41), dogs (42), mice (43), and hamsters (44) did not seroconvert to OspA, converted with minimal titers, or did not respond until several months after the onset of infection (45).

This was unexpected. Explanations for the weak antibody response to OspA after natural infection were found only after examination of the spirochete's life cycle. It was shown that the *B. burgdorferi* spirochete changes its expression of OspA during attachment and feeding by ticks. During feeding, the spirochete, which resides in the mid-gut of the tick, is warmed to 37 °C and migrates to the tick's salivary gland. The spirochete downregulates the expression of OspA and begins rapid synthesis of OspC (46). Although some patients may have an early, transient (IgM) antibody response to OspA

and some patients with arthritis have a marked IgG response to this antibody, it is this downregulation of OspA before inoculation into the host that explains the general lack of OspA antibody response in naturally occurring infection. Downregulation also demonstrates that a vaccine directed against OspA must necessarily be arthropod-specific, acting solely in the mid-gut of an unengorged tick (47).

Animal Trials of Outer Surface Protein A Vaccine

In 1990, a mouse model was discovered in which C3H/HeJ mice develop cardiac and rheumatologic manifestations of Lyme disease (48). Fikrig and colleagues (49) reported that these mice, when immunized with recombinant OspA, were protected from natural infection. In fact, serologic evaluation of the mice after spirochete challenge showed no detectable antibody response to nonvaccine *B. burgdorferi* antigens, suggesting that the infection was entirely aborted (50). Schaible and coworkers (51) demonstrated that the transfer of monoclonal antibodies to OspA and the transfer of serum containing upregulation polyclonal antibodies against OspA also protected against disease in mice. In addition, successful immunization of hamsters, dogs, and monkeys was reported (52–54).

The mechanism of protection is thought to result from the development of high-titered antibodies to a conformational epitope in the C-terminal end of OspA that is identified by the human monoclonal antibody LA-2 (51). Direct antiborrelial activity of antibodies can be measured by assays of spirochetal immobilization, growth inhibition, or killing. Antibody-induced inhibition or killing seem to be complement-dependent and -independent; the complement-mediated effects seem more efficient (55). Antibodies may also facilitate phagocytosis by macrophage/monocytes through binding the Fc receptors (56).

The use of a fully lipidated OspA or OspA adsorbed to adjuvant increases antibody titers (57). The finding that the nonlipoprotein OspA is less immunogenic than the lipoprotein is consistent with observations of the syphilis spirochete *Treponema pallidum*, in which the lipoproteins are the most immunogenic antigens (58).

Human Trials and Approval of the First Lyme Disease Vaccine

The U.S. Food and Drug Administration approved the first human vaccine against Lyme disease (LYMERix, SmithKline Beecham) in December

1998. Two vaccine preparations, LYMERix and another recombinant OspA vaccine (Pasteur Merieux Connaught, Swiftwater, Pennsylvania), have been found safe in phase I and phase II studies. The most common adverse reactions have been local pain or tenderness at the injection site. Systemic reactions were infrequent and included self-limited headache, fatigue, fever, and arthralgia (59–61). The now-approved formulation was also found to be safe and immunogenic in 30 patients with previous Lyme disease (62).

In July 1998, Steere and colleagues (63) reported an extensive multicenter, double-blind, placebo-controlled phase III human trial of the now approved vaccine involving 10 936 participants (63). Ninety-two percent of participants completed the trial. LYMERix, the vaccine studied by Steere and colleagues, contains 30 μg of lipidated recombinant OspA of *B. burgdorferi* adsorbed to 0.5 mg of aluminum hydroxide adjuvant. Alum adsorption increased the immunogenicity of the vaccine 14- to 128-fold (64). The immunization was given at 0, 1, and 12 months. Participants were 15 to 70 years of age ($n = 10\,936$) and included those who had had Lyme disease more than 3 months earlier. Persons with active or recent Lyme disease, chronic arthritis, second- or third-degree atrioventricular block, immunodeficiency, and history of alcohol or drug abuse were excluded, as were pregnant women.

To detect asymptomatic infection, all participants were tested for IgG seroconversion by Western blot analysis at 0, 12, and 20 months. Additional serologic tests, cultures, and polymerase chain reaction were done on all persons with clinically suspect illness. Definite Lyme disease was defined as characteristic clinical illness and confirmation of *B. burgdorferi* infection by one or more of these laboratory tests. After three injections, the overall efficacy of LYMERix was 76% in definite cases of disease, increasing to 88% when persons older than 65 years of age were retrospectively excluded. In addition, the vaccine was 100% protective against asymptomatic infection after three injections; therefore, it is unlikely to induce partial protection.

The formulation was safe and well-tolerated. The main adverse reaction to the vaccine was mild tenderness at the injection site (81% of participants). Severe reactions, defined as those limiting daily activities, included tenderness at the injection site (3.5% of participants), fatigue (<1% of participants), headache (<1% of participants), and rash or arthralgia (<0.5% of participants) (63).

In a subset of 938 patients, Steere and colleagues (63) measured human monoclonal LA-2–equivalent antibody levels in an effort to determine the role of LA-2 antibody levels in vaccine failure (**Figures 2 and 3**). The LA-2–equivalent antibody titers proved

to be substantially lower in the vaccinated patients who had breakthrough cases of Lyme disease ($P < 0.01$). This suggests that LA-2 antibody titers may predict the vaccine's efficacy.

Another phase III trial, reported by Sigal and coworkers, initially involved 10 305 participants, of which 7453 (72%) completed testing. The formulation consisted of full-length OspA containing 30 μg of the purified recombinant protein without adjuvant. After three injections on a 0-, 1-, and 12-month schedule, the trial reported an efficacy rate of 92% (65). The end point was the number of clinically significant cases of serologically proven Lyme disease. In this study, Western blot analysis was performed only if participants were clinically suspected of having Lyme disease. The trial by Steere and colleagues (63), in contrast, conducted IgG antibody studies at three intervals on all participants regardless of reported symptoms. This vaccine was found to be safe without any pattern of unusual adverse experiences. Both phase III trials were conducted during two seasons of Lyme disease transmission.

Since the U.S. Food and Drug Administration approved LYMERix, several alternate dosing schedules have been reported. Each schedule is based on the finding that antibody levels and seroprotection are related and that a cutoff titer of 1200 enzyme-

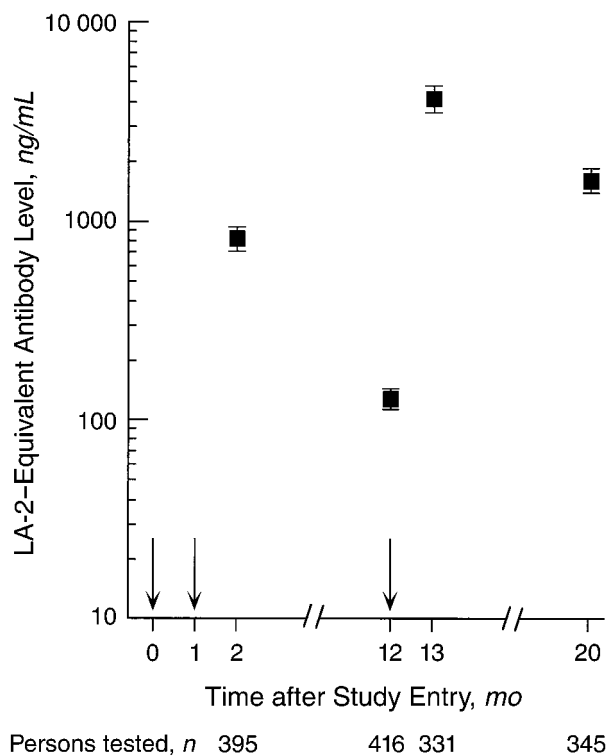


Figure 2. Levels of antibody to the protective epitope of outer surface protein A (LA-2-equivalent antibody in vaccine recipients) over time. Arrows indicate month of primary immunization given. Error bars represent 95% CIs. Adapted with permission from Steere et al. (63).

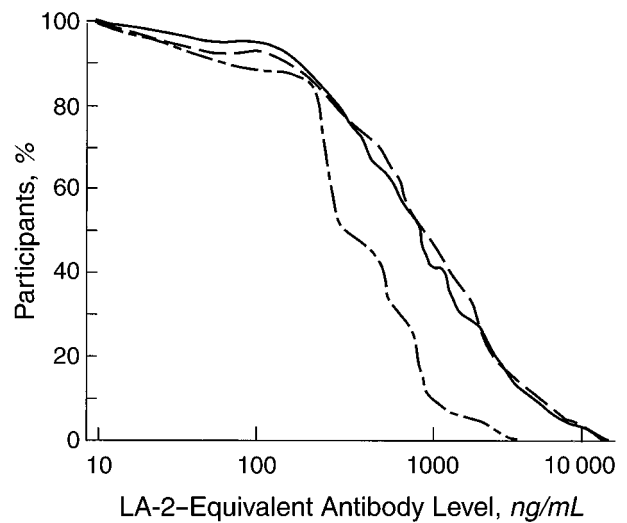


Figure 3. Reverse cumulative curve of LA-2-equivalent antibody levels at month 2. Dashed-and-dotted lines indicate 20 vaccinated persons with breakthrough cases of definite Lyme disease; dashed lines indicate 512 vaccinated persons in whom Lyme disease was not confirmed at year 1; solid lines indicate 395 vaccinated controls. Adapted with permission from Steere et al. (63).

linked immunosorbent assay (ELISA) units per milliliter can be used as a surrogate marker of protection. Depending on the methods and sensitivity selected, antibody titers between 700 and 1400 ELISA units per milliliter provided 70% to 95% sensitivity in correctly discriminating vaccine failure and are highly predictive of protection against Lyme disease (66). A schedule of immunizations given at 0, 1, and 2 months (instead of at the 12th month) proffered titers of more than 4500 ELISA units per milliliter, implying that this schedule will provide protection approximately equal to that of the pivotal trial within a much shorter time frame (67). Adverse effects did not increase. In 800 participants, an alternate dosing schedule of 0, 1, and 6 months showed similar results (68).

After the initial three-dose immunization series, recommendations for a “booster” strategy are needed. Because of the decrease in protective antibody titers seen in the phase III trial, it is anticipated that a booster dose given 12 months after the initial series (in the following spring) will be necessary to maintain immunity. The finding of a correlate of protection will allow determination of subsequent intervals for booster doses. These studies are in progress.

It is important to note that both phase III trials were conducted in the eastern United States, where *B. burgdorferi sensu lato* is the predominant species. With the recognition of worldwide (and even domestic) subspecies heterogeneity (69), the problem of cross-protection arises. It is unclear whether and to what extent the vaccine will prove protective against other *Borrelia* subspecies.

Table. Prevention of Lyme Disease through Active Immunization: Recommendations of the Advisory Committee for Immunization Practices

High risk
Persons who reside, work, or recreate in areas of high or moderate risk during seasons of Lyme disease transmission
Persons who engage in activities (recreation, property maintenance, occupation, or leisure) that result in frequent or prolonged exposure to tick-infested habitats
Moderate risk
Persons who reside, work, or recreate in areas of high or moderate risk during seasons of Lyme disease transmission
Persons who are exposed to tick-infested habitats, but whose exposure is not frequent or prolonged
Low risk
Persons who reside, work, or recreate in areas of low or no risk during seasons of Lyme disease transmission

Even in the subspecies *B. burgdorferi sensu lato*, substantial genetic diversity has been demonstrated in the outer surface proteins, including OspA (70). In early studies of syringe challenge, vaccination with OspA-N40 did not protect mice against heterologous spirochetes in which OspA differs by 40 amino acids (71). The studies suggested that protective immunity might be strain- and site-specific. However, it must be noted that information on subspecies was not well known and that not all of the *B. burgdorferi* subspecies used in this experiment may have been *sensu lato*. In the phase III trials, OspA typing was not performed. Some variation in sequences is possible, and the efficacy of the vaccine in varieties of OspA is unknown.

Indications for Vaccine Use

LYMERix is approved in persons 15 to 70 years of age. Its use should target persons at risk for exposure to infected ticks. This risk can be assessed by considering the person's likelihood of being outdoors in nonurban areas in endemic regions. Vaccination of persons who experience frequent or prolonged exposure is likely to be an important method of decreasing the incidence and associated morbidity of Lyme disease. It is important to note that the vaccine is not effective against other tick-borne diseases.

The Advisory Committee for Immunization Practices recommends that the vaccine be "considered" for persons at high risk and that it "may be considered" for persons at moderate risk. It is "not recommended" for persons who are at no or low risk for *B. burgdorferi* infection (Table) (72). The same guidelines apply to persons traveling to endemic areas during the high season. The vaccine is not recommended for pregnant women or persons older than 70 years of age. Vaccinated persons should continue to practice personal protective measures

against ticks and should seek early medical attention when infection is suspected.

Pregnant women, immunocompromised persons, and persons with treatment-resistant Lyme arthritis were not included in the phase III trials. Therefore, the safety and efficacy of the vaccine in these persons are unknown, and its use is not recommended. The safety and efficacy of the simultaneous administration of the recombinant OspA vaccine with other immunizations have also not been delineated.

Twenty-three percent of all cases of Lyme disease in the United States occur in children, and clinical trials of the vaccine in children are under way. In a 1998 European dose-ranging trial, LYMERix was well-tolerated without adverse safety results in patients 5 to 15 years of age (73). Other trials are under way in the United States.

Limitations of the Vaccine

The vaccine's usefulness is limited because it has been evaluated and approved only in persons older than 15 years of age. In addition, the vaccine's cross-protection overseas and over time is unclear because *Borrelia* strains differ outside the United States. The heterogeneity of OspA among isolates of *B. burgdorferi* strains in Europe may limit the utility of a monovalent OspA vaccine and may necessitate development of multivalent vaccines. The stability of the OspA sequence in nature is also unknown, and alterations in the structure or sequence over time could change the vaccine's effectiveness.

The safety and cost-effectiveness of the vaccine have been questioned (74). As mentioned, unvaccinated persons with the HLA-DR4 haplotype may be at increased risk for chronic Lyme arthritis. In addition, chronic Lyme arthritis may be associated with increased OspA reactivity in the synovial fluid (16). The possibility of some type of vaccine-induced molecular mimicry causing reactivity or exacerbation of a current illness has been raised as a theoretical concern; however, during the 20 months of the pivotal trial, vaccine recipients had no greater incidence of unexplained arthritis or neurologic disease than persons in the placebo group. In addition, no unexpected pattern of arthritis-related circumstances have been reported after widespread vaccine use. Persons with a history of immune-mediated neurologic disease or arthritis, immunocompromised persons, and pregnant women were excluded from the phase III trials.

Because Lyme disease is difficult to diagnose, it has proven extremely expensive. As previously mentioned, a recent analysis estimated the costs of Lyme disease in the United States to be \$2.5 billion

over 5 years (7). However, the morbidity associated with Lyme disease is often minor, antibiotic treatment is effective, the disease is not communicable from human to human, and up to 80% of patients develop the characteristic rash of erythema migrans, which allows early diagnosis and treatment if recognized.

It is also important to note that persons who receive the recombinant OspA vaccine may have positive results on ELISA and on the newly approved rapid detection assay (PreVue *B. burgdorferi* Antibody Detection Assay, Chembio Diagnostic Systems, Medford, New York) for antibodies to whole-cell *B. burgdorferi*. It will therefore be important to rely on immunoblotting (evaluating antibody reactivity to non-OspA borrelial antigens) when Lyme disease is suspected in previously vaccinated persons.

Summary

Just 16 years after its isolation from humans, the ability of the spirochete *B. burgdorferi* to cause Lyme disease has been severely limited. Two inactivated whole-cell vaccines were developed for canines, and a monovalent recombinant OspA vaccine is now approved for use in humans. Phase III trials report substantial efficacy in preventing asymptomatic and clinical disease. Extensive safety data gathered from almost 11 000 volunteers over 20 months showed no evidence that the vaccine induces auto-immune arthritis or other lasting adverse reactions. In addition, no unexpected safety or efficacy patterns have arisen after widespread public use of the vaccine. However, questions on the duration of the immunity conferred by the vaccine and the vaccine's safety and efficacy in children must be addressed.

Perhaps the most interesting aspect of the OspA vaccine is that the vaccine-induced immune response takes effect within the tick vector itself before the causative spirochete ever enters the human host. This intravector mode of action is unique and opens the door to a new method of preventing insect-borne illnesses in humans.

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