Review
Lyme disease and current aspects of immunization
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Abstract

Lyme disease is a tick-borne multisystem disease that affects primarily the skin, nervous system, heart and joints. At least three species of *Borrelia burgdorferi* sensu lato, namely *Borrelia burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii*, can cause the disease. This review will focus mainly on the pathophysiology of Lyme arthritis, the long-term outcome of Lyme disease, and the recently licensed vaccine against Lyme disease.

Keywords: arthritis, autoimmunity, infection, Lyme disease, T lymphocytes

Etiology and epidemiology

Lyme arthritis was recognized as a clinical entity in 1976 when researchers at Yale University investigated a cluster of arthritis in children from the three contiguous communities of Old Lyme, Lyme, and East Haddam, Connecticut [1]. It was soon discovered that Lyme arthritis was only one aspect of the clinical spectrum of Lyme disease and that some manifestations of the disease had been described in Europe early in the 20th century [2–4]. Rational treatment for Lyme disease became possible after a tick-borne spirochete, *Borrelia burgdorferi*, was identified as the cause of Lyme disease [5,6].

Lyme disease is the most common vector-borne disease in the United States, where approximately 15,000 cases are reported every year [7]. The disease has a highly focal distribution pattern, with over 90% of the cases reported from eight states along the Atlantic Coast and from Wisconsin. The annual reported incidence in these areas ranges from 20 to slightly more than 100 cases per 100,000 inhabitants. In some areas the incidence may be as high as 1000 cases per 100,000 inhabitants. The incidence in children aged 5–10 years is approximately twice as high as that in adults [7].

In Europe most cases occur in Scandinavia and Central Europe. A prospective, population-based survey in southern Sweden revealed an annual incidence of 69 cases per 100,000 population. As in the United States, there were areas of endemicity in which the annual incidence reached 160 per 100,000 inhabitants [8]. A similar study in southern Germany found an annual incidence of 111 cases per 100,000 inhabitants. The incidence in children was higher than that in adults in both these European studies [9].

B. burgdorferi is maintained in enzootic cycles between ixoid ticks (*Ixodes ricinus* in Europe, and *Ixodes scapularis* and *Ixodes pacificus* in the United States) and small mammal reservoirs. In addition *to B. burgdorferi*, the ixoid ticks may carry other pathogens such as viruses, *Babesia*, or the agent of human granulocytic ehrlichiosis [10].

ACA = acrodermatitis chronica atrophicans; CDC = Centers for Disease Control; ELISA = enzyme-linked immunosorbent assay; EM = erythema migrans; FDA = Food and Drug Administration; IL = interleukin; Osp = outer surface protein; PBS = phosphate buffered saline; PCR = polymerase chain reaction; Th = T helper cells.

Microbiology

B. burgdorferi sensu lato is a Gram-negative spirochete. At least three species are known to be human pathogens: *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii*. The taxonomy and molecular typing of *B. burgdorferi* have recently been reviewed in detail [11]. Whereas all three species occur in Europe, only *B. burgdorferi* sensu stricto occurs in the United States. It has been suggested that the different clinical manifestations were caused by distinct species of *B. burgdorferi* sensu lato. Based on DNA amplification, the late dermatologic manifestation acrodermatitis chronica atrophicans (ACA), which is observed in Europe but rarely, if ever, in the United States, was mostly but not exclusively associated with *B. afzelii* [12]. Neuroborreliosis is frequently but not exclusively caused by *B. garinii* [13], and all three species have been detected in synovial fluid samples from patients with Lyme arthritis [14].

The genome of *B. burgdorferi* sensu stricto (strain B31) has been sequenced. The *B. burgdorferi* genome contains 853 genes distributed on a linear chromosome of ~920,000 base pairs and at least 17 linear and circular plasmids with another ~530,000 base pairs [15]. *B. burgdorferi* does not contain the enzymes necessary for the production of lipopolysaccharide [15]. The *B. burgdorferi* genome instead contains ~130 genes coding for lipoproteins [15]. The lipid moiety is formed by the post-translational attachment of tripalmitoyl-*S*-glyceryl-cysteine. The outer surface proteins (Osps) are anchored to the spirochete's outer membrane via these lipid moieties. The Osps bind the Toll like receptor-2 and are potent activators of the innate immune system [16,17].

The Osps are differentially expressed in different hosts [18]. OspA and OspC are the most intensively studied *B. burgdorferi* Osps, and the change from OspA expression to OspC expression seems to be important for the migration of *B. burgdorferi* from the tick's midgut to the salivary gland and for the subsequent invasion of the mammalian host [19].

B. burgdorferi may persist in the host for many years and has been isolated from an ACA lesion more than 10 years after the initial symptoms [20]. *B. burgdorferi* can also reinfect the same host [21].

Clinical manifestations

The clinical manifestations of Lyme disease have been reviewed in a recent series of excellent reviews [21–23] and will be described here only briefly. The clinical manifestations of Lyme disease are frequently categorized as early localized disease (erythema migrans [EM]), followed days or weeks later by early disseminated disease (e.g. Bell's palsy, arthralgia/arthritis) and late disease (e.g. subtle encephalopathy, treatment-resistant Lyme arthritis).

Dermatological symptoms

EM is a slowly expanding erythematous papule or macule, often with central clearing, and is diagnostic for early Lyme disease. EM occurs within days or several weeks at the site of the tick bite and may be accompanied by flu-like symptoms. It is recognized in at least 80% of the patients with objective evidence of *B. burgdorferi* infection [21,22]. In Europe, ACA is a late dermatologic manifestation of Lyme disease.

Neurological symptoms

Approximately 10–15% of untreated patients with EM develop neurological symptoms of Lyme disease. Early neurological symptoms occur within weeks after the infection (early disseminated disease). The most common symptom is facial palsy, either unilateral or bilateral. Other early neurological symptoms include lymphocytic meningitis, mild encephalitis and mononeuritis multiplex. These symptoms typically resolve even in untreated patients [21,22].

Late or chronic neuroborreliosis occurs in approximately 5% of untreated patients. Typical manifestations include chronic axonal neuropathy and a subtle encephalopathy, which can occur after months or years of latent infection [21,22].

Cardiological symptoms

Less than 8% of untreated EM patients develop cardiological symptoms. The typical feature is a transient atrioventricular block of varying degrees [21,22]. In Europe, but not in the United States, *B. burgdorferi* has been isolated from endomyocardial biopsies from patients with dilatative cardiomyopathy [24].

Lyme arthritis

Approximately 60% of untreated EM patients develop intermittent attacks of monoarticular or oligoarticular arthritis, primarily in large joints. Most patients with Lyme arthritis respond to antibiotic therapy; however, in ~10% of patients with Lyme arthritis, the inflammation persists despite antibiotic therapy [21,23]. The synovial lesion in treatment-resistant Lyme arthritis resembles that of other chronic arthritides such as rheumatoid arthritis, including the formation of germinal center like structures within the inflamed synovium [21]. The incidence of treatment-resistant Lyme arthritis is lower in children than in adults [25,26]. In Europe, both *B. burgdorferi* sensu stricto and *B. garinii* can cause treatment-resistant Lyme arthritis [27].

Patients who had been treated with steroids, either systemically or intra-articularly, before Lyme arthritis was diagnosed and the appropriate antibiotic treatment administered have an increased risk of developing treatment-resistant Lyme arthritis [26,28]. In addition, host factors may be crucial for the pathogenesis of treatment-

resistant Lyme arthritis. *B. burgdorferi* DNA can be amplified reliably from synovial fluid prior to antibiotic treatment [29]. In contrast, most patients with treatment-resistant Lyme arthritis yield consistently negative PCR results in synovial fluid after antibiotic treatment [29–31]. Whereas *B. burgdorferi* DNA can be amplified from synovial tissue in a minority of such patients [30], most patients yield negative results from both synovial fluid and synovial tissue [31]. It therefore seems possible that chronic synovitis is maintained in the absence of *B. burgdorferi*.

HLA-DR4 was found to be associated with a lack of response to antibiotic therapy [32,33]. Both the severity and the duration of Lyme arthritis are increased in patients with IgG antibodies against OspA [33,34]. *B. burgdorferi*-specific T cell lines from patients with treatment-responsive arthritis recognized OspA only infrequently. In contrast, T cell lines from patients with treatment-resistant arthritis preferentially recognized OspA [35], and these patients dominantly recognized certain OspA epitopes [36,37].

These findings are compatible with the idea that an HLA-DR4-restricted immune response against *B. burgdorferi* triggers an autoimmune synovitis, which persists even in the absence of *B. burgdorferi*. These pathophysiological considerations are discussed later.

Diagnosis

The Centers for Disease Control (CDC) have published a case definition for Lyme disease, which combines clinical and laboratory data and is used in the United States for public health surveillance [38]. Owing to the somewhat different clinical manifestations and the very different serologic response to *B. burgdorferi*, there are currently no generally accepted diagnostic criteria for Lyme disease in Europe.

The characteristic clinical findings, together with a history of tick exposure in areas where Lyme disease is endemic, supported by the detection of antibodies against *B. burgdorferi* by ELISA or western blot, are the most important elements for the diagnosis of Lyme disease [21–23,38].

Serology

Serological analyses for antibodies against *B. burgdorferi* are usually performed when Lyme disease is suspected. The recommended procedure is to first use an ELISA and then to use western blotting for the confirmation of positive ELISA results [21–23,38]. The development of an antibody response to *B. burgdorferi* infection in untreated patients is well described. IgM antibodies become detectable 3–4 weeks after the infection, peak after 6–8 weeks, and subsequently decline. IgG antibodies appear 6–8 weeks after the infection and remain detectable for many years [21]. It is important to note

that both IgG and IgM responses may persist for longer than 10 years even after successful antibiotic treatment [21].

The CDC issued recommendations for test performance and interpretation for the serologic diagnosis of Lyme disease [39]. The sensitivity, specificity and reproducibility of the currently available commercial tests are poor. Serological testing in general is therefore not clinically useful if the pretest probability of Lyme disease is less than 0.20 or greater than 0.80, and the American College of Physicians has issued recommendations for the cost-effective use of serological testing [40].

The situation in Europe is even more complicated because there all three species of *B. burgdorferi* sensu lato can cause Lyme disease. The serological response in European Lyme disease patients consequently differs from that in their American counterparts and there are currently no generally accepted criteria for the serological diagnosis of Lyme disease.

Asymptomatic infection

The interpretation of serological data is further complicated by the fact that many inhabitants of areas endemic for Lyme disease are seropositive yet lack any history or symptoms of Lyme disease [21]. The frequency of asymptomatic infection may be considerable. In one Swedish study, more than one-half of the people who were seropositive by ELISA could not recall any symptoms suggestive of Lyme disease [41]. When the vaccine against Lyme disease was tested in a prospective clinical trial, 137 of the placebo recipients seroconverted on western blotting during the 2-year observation period. Twenty-eight of those recipients (20%) did not show any clinical symptoms of Lyme disease [42].

Culture, DNA detection

The culture of *B. burgdorferi* from patient specimens proves the diagnosis of Lyme disease but is difficult to perform and rarely attempted for diagnostic purposes [21]. PCR allows the reliable detection of *B. burgdorferi* DNA in synovial fluid [29] or synovial tissue [30] from patients with Lyme arthritis, and in the cerebrospinal fluid from patients with early central nervous system manifestations of Lyme disease [43]. However, there is considerable interlaboratory variation in the results and PCR detection of *B. burgdorferi* DNA is currently not considered a routine method [21,22].

Treatment

The Infectious Diseases Society of America has recently issued detailed treatment recommendations [44]. Briefly summarized, doxycycline (orally, 2×100 mg/day over 2–3 weeks) is the first choice in early infection (EM) if there are no contraindications [21,22,44]. For EM

patients, intravenous therapy offers no benefit over the recommended oral treatment with doxycycline [45].

Neurological symptoms, regardless of whether early (e.g. facial palsy) or late (e.g. encephalomyelitis), should usually be treated with ceftriaxone (2 g/day intravenously for 2–4 weeks). Acute neuroborreliosis usually resolves within several weeks following this treatment, whereas the resolution of chronic neuroborreliosis may take some months [21,22,44].

Cardiac disease (i.e. atrioventricular block) should be treated intravenously, similar to neuroborreliosis [21,22,44].

Lyme arthritis should be treated for 30–60 days with doxycycline (200 mg/day orally) or with ceftriaxone (2 g/day intravenously for 2–4 weeks) [21–23,44]. One study found the subsequent development of neuroborreliosis which required ceftriaxone treatment more frequently in those patients who had been treated with doxycycline than in those who had been treated with ceftriaxone [33]. If Lyme arthritis persists despite the appropriate antibiotic treatment and if PCR analysis of the patient's synovial fluid is negative, nonsteroidal anti-inflammatory drugs and possibly arthroscopic synovectomy can be beneficial [21,23].

Long-term outcome

A small percentage of patients who have had Lyme disease experience an array of chronic symptoms, including myalgia, arthralgia, fatigue, and cognitive impairment, that persist even after the appropriate antibiotic therapy of Lyme disease. Such symptoms may persist for many years, resulting in a severely diminished health-related quality of life for these patients [46,47]. This syndrome, which shares some features with chronic fatigue syndrome and fibromyalgia, has been termed 'chronic Lyme disease syndrome' or 'post Lyme disease'. The reasons for the persistence of these symptoms are currently unclear and could include persistent or slowly resolving infection, self-perpetuating chronic inflammation or autoimmunity after the spirochetes have been eradicated, and permanent tissue damage caused by the infection or the immune response against it. The frequency, diagnosis, pathophysiology, and appropriate treatment of 'post Lyme disease' have been a highly contentious issue for more than a decade. A number of clinical studies have recently tried to determine the long-term outcome of patients with Lyme disease. Overall, these studies indicate an excellent prognosis for people with Lyme disease [25,48–55]. Patients in whom antibiotic treatment for facial palsy had been delayed are more likely to develop chronic symptoms, indicating that early dissemination of the infection into the central nervous system might be a risk factor [49,53,55]. Children seem to be at lower risk than adults for 'post Lyme disease' [50,52]. Importantly, when persons with previous Lyme disease were compared with age-matched controls, pain and fatigue were reported at the same frequency in both groups [52–55].

Lyme disease, and especially the possibility of chronic sequelae, continues to receive massive publicity, resulting in an irrationally exaggerated anxiety about the risk of chronic complications from Lyme disease [56]. Partly due to this anxiety there is an overdiagnosis of Lyme disease, and people with nonspecific symptoms such as myalgia, arthralgia, or fatigue are frequently misdiagnosed as having Lyme disease [56–59]. Even worse, these patients are frequently subjected to prolonged antibiotic treatment that can cause considerable and sometimes lethal side effects [60,61]. Two recent randomized, controlled clinical trials tested the efficacy of prolonged antibiotic treatment. One was performed in patients who were seropositive for antibodies against *B. burgdorferi*, and the other was performed in patients who were seronegative. Treatment consisted of either 2 g ceftriaxone intravenously daily for 30 days, followed by 200 mg doxycycline orally daily, or the matching intravenous and oral placebos. Ninety days of antibiotic treatment did not improve the symptoms more than 90 days of placebo [62]. Prolonged antibiotic treatment for suspected 'chronic Lyme disease syndrome' is therefore expensive, ineffective, burdened with side effects and should be avoided.

From infection to autoimmunity?
Lessons from Lyme disease

Abundant clinical, epidemiological, and experimental evidence suggest autoimmunity as a potential sequel of infection [63–65]. At the same time, one should not gloss over the paucity of evidence directly implicating infection as the cause of any of the important candidate autoimmune diseases. Are the late neurological manifestations of Lyme disease or the treatment-resistant form of Lyme arthritis autoimmune diseases triggered by the infection with *B. burgdorferi*? The possible immunopathology of the chronic neurological manifestations of Lyme disease has recently been reviewed [66]. In the present paper, we shall focus on the treatment-resistant form of Lyme arthritis. As already detailed (see 'Lyme arthritis'), clinical evidence suggests that the ongoing synovial inflammation in patients with treatment-resistant Lyme arthritis may be caused by factors other than persistent *B. burgdorferi* infection [29, 31–35]. Ideas about the path from infection to autoimmunity divide into two groups: antigen specific, and antigen nonspecific. For the first set of ideas, what is important is T cell epitopes. The hypothesis of molecular mimicry predicts that microbial peptide sequences occasionally mimic sequences in the host. Once T cells respond to the microbial peptide, the hitherto peaceful self-antigen is also recognized and disease ensues [64]. The antigen nonspecific set of ideas assumes inflammatory cytokines to be the major culprits. One example would be hypersensitivity that develops to traces of persistent antigen [63,65].

Molecular mimicry and the pathogenesis of treatment-resistant Lyme arthritis

Both HLA-DR4 and a T cell response against *B. burgdorferi* OspA are associated with the treatment-resistant form of Lyme arthritis [32,35]. Therefore, the HLA-DR4-restricted response to *B. burgdorferi* OspA has been suspected to trigger chronic synovitis via molecular mimicry. Compatible with this hypothesis, it was shown that T cells from the synovial fluid of patients with treatment-resistant Lyme arthritis recognized both an immunodominant OspA epitope and a highly homologous peptide derived from LFA-1 [67]. This is similar to the original report of cross-reactive T cells that recognize both a microbial peptide and a highly homologous self-peptide [68], which has since been confirmed by a number of investigators in different systems [69,70]. In several instances, autoimmunity was elicited by immunization of experimental animals with such cross-reactive peptides [71], albeit at much reduced incidence and severity or only at significantly higher antigen doses than the homologous self-antigen [64,65].

Novel techniques such as combinatorial peptide libraries have more recently been used for the study of T cell cross-reactivity. Several different groups have demonstrated that T cell recognition of multiple different peptides occurs much more frequently than previously assumed and that sequence similarity is not a prerequisite for such cross-recognition [72–75]. One example is a human T cell clone that was raised against *B. burgdorferi* OspC and that recognized a large number of microbial and human peptides [74]. Another example showed HLA-DR4-restricted T cell hybridomas raised against OspA that recognized a large number of murine or human peptides, including some that are ubiquitously exposed to the immune system (e.g. a peptide derived from 4-1BB ligand) or known targets of autoimmune responses (e.g. peptides derived from Pro-Insulin or the SS-B antigen) [75]. Given this unexpected degree of T cell cross-reactivity, why does autoimmunity not occur much more frequently? Several regulatory mechanisms normally prevent cross-reactive T cells from causing injury [65]. First, both the microbial peptide and the self-peptide must be processed and presented by antigen presenting cells. Also, the self-antigen must be present at high enough concentrations and the T cells at high enough numbers. Third, the T cells must receive the appropriate co-stimulatory signals from antigen presenting cells to produce the proinflammatory cytokines required for tissue damage rather than protective cytokines. Fourth, the T cells must be capable of migrating to the tissue where the cross-reactive self-antigen is expressed and must escape immunoregulation [65]. Finally, some degree of autoreactivity seems to be necessary for the survival of naive T cells and possibly also for memory T cells [65].

The idea that cross-reactivity between one particular microbial peptide and one particular self-peptide is indica-tive of pathogenicity is thus probably too simple. Nevertheless, molecular mimicry remains one attractive hypothesis for the pathogenesis of autoimmune disease. For example, microbial peptides might help to maintain the pool of memory T cells specific for an autoantigen. Furthermore, persistent or recurrent infections could bring the number of autoreactive T cells over a critical threshold, thereby supporting the development of autoimmune disease.

Cytokines, innate immunity

Mononuclear cells or T cell clones derived from the synovial fluid of patients with Lyme arthritis produce a Th1 cytokine pattern [66,76,77], and one study reported a stronger Th1 dominance in patients with treatment-resistant arthritis than in patients with treatment-responsive arthritis [77]. In mice, *B. burgdorferi* can induce Th1 phenotype development in naive Th cells [78], and early data seemed to indicate an association of Th1 responses with susceptibility to arthritis in murine models (reviewed in [66]). More recently, however, it has become clear that there is no clear correlation between Th1 or Th2 responses and susceptibility to Lyme arthritis in murine models of the disease [79].

In addition, cytokines such as IL-1β, IL-6, IL-10, IL-11, or IL-12 that are produced by cells of the innate immune system have been implicated in the regulation of arthritis severity in patients or animal models (reviewed in [63,66]). *B. burgdorferi* lipoproteins such as OspA are mitogenic for B cells and potently activate cells of the innate immune system via their binding of Toll like receptor-2 [16,17]. *B. burgdorferi* thus induces, in host cells, the production of IL-1β, IL-6, IL-10, IL-12, and tumor necrosis factor-α (reviewed in [63,66]). *B. burgdorferi* OspA, via its activation of the innate immune system, also induces the differentiation of Th cells that co-express IL-17 and tumor necrosis factor-α both in mice and in humans [80]. IL-17 is a proinflammatory cytokine in candidate autoimmune diseases such as rheumatoid arthritis [81]. To date, microbial lipopeptides are the only currently known physiological stimulus for IL-17 production [80]. IL-17 in turn induces the production of other proinflammatory mediators such as IL-6 [81]. The reciprocal induction/enhancement of IL-17 and IL-6 could therefore contribute to infection-induced immunopathology of chronic inflammatory diseases such as treatment-resistant Lyme arthritis.

Altogether, it is likely that antigen-nonspecific mechanisms mediate, perhaps in synergy with antigen-specific mechanisms, the immunopathology that finally leads to treatment-resistant Lyme arthritis in susceptible patients.

Prevention
Exposure prophylaxis
Several more or less practicable prophylactic measures have been suggested to avoid tick bites, including the

complete avoidance of wooded areas, simple physical measures such as wearing long sleeves and long pants that are tucked into the socks, and drastic measures such as area application of insecticides [82]. Tick and insect repellents are frequently recommended. However, repellents that contain *N,N*-diethylmetatoluamide need to be applied every 1–2 hours to maintain effectiveness and their use has resulted in serious neurological complications, including encephalopathy, seizures, coma, and death in children [83,84]. Permethrin can be sprayed on clothing and kills ticks but should not be applied to skin due to its possible carcinogenicity [85]. In infested areas, daily careful screening for possible tick bites seems to be the most realistic and useful prophylactic measure. This is further supported by a number of experimental and observational data that indicate ticks need to be attached to the host for at least 24 to >50 hours for transmission of *B. burgdorferi* to occur [19,86–88]. Vigilance for tick bites is indicated not only to protect against *B. burgdorferi* infection, but also against other tick-borne infections [87].

Immunization
A number of *B. burgdorferi* proteins including OspA [89,90], OspB [91], OspC [92], P35 and P37 [93], and decorin binding protein A [94] can induce protective antibody responses in experimental animals. Among these proteins, only recombinant lipidated OspA is currently licensed by the Food and Drug Administration (FDA) as a human vaccine. The OspA vaccine has a unique mode of action. When a tick feeds on an OspA-immunized host, the serum antibodies against OspA kill the spirochetes in the tick's midgut [95]. These antibodies also block the migration of *B. burgdorferi* from the tick's midgut to the salivary glands at titers that are considerably lower than those needed to kill the spirochetes. The OspA vaccine is thus an arthropod-specific transmission-blocking vaccine [96].

Two vaccines consisting of recombinant lipidated OspA have been studied in large clinical trials [42,97], each involving more than 10,000 participants. Both studies had very similar designs and outcomes. A total of 10,936 subjects aged 15–70 years who lived in 10 different states of the United States where Lyme disease is endemic participated in the study that led to the approval of LYMErix™ by the FDA [42]. Of these subjects, 5469 received the vaccine consisting of recombinant lipidated OspA adsorbed to aluminum hydroxide in PBS and 5467 received placebo (i.e. alum in PBS without OspA). Injections were given at entry, 1 month later and 12 months after the first injection. Within the first year of the study, 22 of the vaccine recipients and 43 of the placebo recipients developed definite Lyme disease (i.e. clinical symptoms indicative of Lyme disease [usually EM] plus seroconversion). Within the second year, 16 vaccine recipients and 66 placebo recipients had definite Lyme disease [42]. The vaccine efficacy was therefore 49% (95% confidence

interval, 15–69%) in the first year. In the second year, after the booster injection, the vaccine efficacy increased to 76% (95% confidence interval, 58–86%). Within the vaccine group, two subjects had asymptomatic infection (seroconversion without clinical symptoms) in the first year of the study and none in the second year. In contrast, 13 of the placebo recipients had seroconversion in the first year and 15 in the second year of the study. The vaccine's efficacy was correlated with the antibody responses to the protective epitope of OspA, which is recognized by a monoclonal antibody called LA-2 [42]. The vaccine recipients reported significantly more soreness, redness, or swelling at the injection site (~25%) than the placebo recipients (~8%). Within the first 30 days after receiving the injections, there were also more systemic side effects such as arthralgia, headache, myalgia, fatigue, or fever in the vaccine group (19.4%) than in the placebo group (15.1%). In contrast, there was no difference between the two groups in the frequency of late systemic side effects such as arthralgia [42].

The other clinical trial, which tested a slightly different OspA preparation and involved a total of 10,305 subjects, had very similar results [97]. Both vaccines were thus found to be safe and effective in the prevention of Lyme disease, and one vaccine (LYMErix™) was approved in 1998 by the FDA for use in persons older than 16 years. The current immunization schedule consists of three injections of recombinant lipidated OspA at baseline, and 1 and 12 months later. The CDC published recommendations for the use of the OspA vaccine [98].

The duration of protection is currently unknown and additional booster injections may be necessary. The incidence of Lyme disease is higher in children than in adults [7–9], and tetracyclins are contraindicated in small children. The OspA vaccine is immunogenic in children [99].

Previous studies both in patients with Lyme arthritis and in experimental animal models raised the possibility that an immune response against OspA might be detrimental and could perhaps trigger autoimmune synovitis [34,35,67, 100,101]. In the two aforementioned clinical trials, however, there were no significant increases in the frequency of arthritis in vaccinees [42,97]. Very rare side effects of any vaccine would not be detected even in careful clinical studies, as recently illustrated by experience with a rotavirus vaccine [102]. The fact that ~1.5 million doses of the OspA vaccine have been administered and there was no post-licensure discovery of a serious adverse effect should assuage fears that the vaccine might provoke arthritis.

In times of economic pressure on health care providers, it is prudent to ask whether the Lyme vaccine is cost-effective. In the study that led to the FDA approval of the OspA

vaccine, 166 doses had to be administered to prevent one infection with *B. burgdorferi* (seroconversion with or without clinical symptoms of Lyme disease). The clinical trials were performed in areas of the United States in which Lyme disease is endemic [42,97]. In areas in which the incidence of Lyme disease is lower, more doses of the vaccine would have to be administered to prevent one infection. At an estimated cost to the pharmacist of $61.25 per dose [103], the study data translate into more than $10,000 per prevented infection. Treatment of patients who present with early symptoms of Lyme disease with doxycycline orally is cheap and effective. On the contrary, vaccination will also prevent late manifestations that are more difficult to treat and may occur if early manifestations such as EM are not recognized. Furthermore, the cost to prevent one infection could be much lower than the calculated $10,000 if the protection afforded by the vaccine lasted for more than 2 years, which is possible. Finally, the potentially immense costs of overdiagnosis and overtreatment for suspected Lyme disease could be prevented by vaccination. Thus, it is currently difficult to determine whether the Lyme vaccine is cost-effective. In an attempt to calculate the cost-effectiveness of vaccinating against Lyme disease, a group of authors from the CDC concluded that vaccination was cost-effective for people whose annual risk to contract Lyme disease exceeds 1% [104].

The OspA vaccine is not available in Europe. Lyme disease in the United States is exclusively caused by *B. burgdorferi* sensu stricto, whereas in Europe *B. burgdorferi* sensu stricto, *B. garinii,* and *B. afzelii* all cause Lyme disease. There is considerable OspA sequence heterogeneity among European isolates [105], thus preventing the effectiveness of an OspA vaccine that is based on one single sequence from *B. burgdorferi* sensu stricto [106]. A polyvalent vaccine, composed of several different OspC, is currently undergoing clinical trials in Europe.

Secondary prevention?
If a person who is not immunized against Lyme diseases recognizes a tick bite, should they receive antibiotic treatment? Even in areas where Lyme disease is endemic, such as Connecticut, the risk of developing Lyme disease following recognized tick bites is only 1–3% [103,107, 108]. Based on this low risk and on the possible side effects of treatment with doxycycline, the Infectious Diseases Society of America has issued a recommendation that tick-bitten persons should not routinely receive conventional antibiotic treatment [44]. A recent study demonstrated that a single dose of 200 mg doxycycline orally was 87% effective in preventing Lyme disease if administered within 72 hours after the tick bite [103]. Only 3% of the placebo recipients in this study, which was performed in Westchester County, New York, where the incidence of Lyme disease is arguably the highest in the world, developed Lyme arthritis. Thus, 40 doses of doxycycline had to

be administered to prevent one case of Lyme disease. Again, this number would be considerably higher in areas with a lower incidence of Lyme disease. In addition, clinical experience shows that most cases of Lyme disease result from unrecognized tick bites. This would further decrease the effect of prophylactic doxycycline administration on the incidence of Lyme disease.

Summary and outlook
The development of an effective vaccine against Lyme disease and the improved understanding of the long-term outcome of Lyme disease can be considered the most important advances in the field over the past several years. The sequencing of the *B. burgdorferi* genome has opened the door for an increased understanding of the biology of *B. burgdorferi* and its rapid adaptation to different hosts and environments.

Treatment for patients with acute manifestations of Lyme disease is well established and effective. Understanding the pathophysiology of chronic neuroborreliosis and treatment-resistant Lyme arthritis are both major challenges and a prerequisite for the eventual development of effective treatments for these conditions. While there is little doubt that infection-induced immunopathology has an important role in treatment-resistant Lyme arthritis, the jury is still out on the exact pathways that lead from infection to chronic inflammation and possibly autoimmunity. As in many other areas of immunology, a period of intense research on antigen-specific T cells seems to be supplemented now by a view on the innate immune system's role in mediating both protection and immunopathology.

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