



COMMENTARY

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## Vaccination against Lyme disease: Are we ready for it?

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### ABSTRACT

Lyme disease is the most common tick-borne illness in the Northern hemisphere and is caused by spirochetes of the *Borrelia burgdorferi sensu lato* complex. A first sign of *Borrelia* infection is a circular skin rash, erythema migrans, but it can develop to more serious manifestations affecting skin, nervous system, joints, and/or heart. The marked increase in Lyme disease incidence over the past decades, the severity of the disease, and the associated high medical costs of, in particular, the persistent forms of Lyme disease requires adequate measures for control. Vaccination would be the most effective intervention for prevention, but at present no vaccine is available. In the 1990s, 2 vaccines against Lyme disease based on the OspA protein from the predominant *Borrelia* species of the US showed to be safe and effective in clinical phase III studies. However, failed public acceptance led to the demise of these monovalent OspA-based vaccines. Nowadays, public seem to be more aware of the serious health problems that Lyme disease can cause and seem more ready for the use of a broadly protective vaccine. This article discusses several aspects that should be considered to enable the development and implementation of a vaccine to prevent Lyme disease successfully.

### ARTICLE HISTORY

Received 7 August 2015  
Accepted 21 August 2015

### KEYWORDS

*Borrelia burgdorferi*; Ixodes ticks; Lyme disease; Lyme borreliosis; vaccine development; vaccination strategy

### Introduction

Ticks and Lyme disease have been around for thousands of years. In fact, the presence of bacteria causing Lyme disease, i.e. *Borrelia (B.) burgdorferi*, was demonstrated at an autopsy on a well-preserved 5,300-year-old mummy, also known as “Ötzi” or the “Iceman” found in 1991 in the ice of the Ötztal Alps on the Austrian-Italian border.<sup>1</sup> The disease was first described over 100 y ago in Europe by Buchwald and Afzelius.<sup>2</sup> The name “Lyme” disease came later and refers to a mysterious outbreak that occurred in the United States in the 1960-70s affecting children who developed skin rashes followed by arthritic condition; these children could recall being bitten by a tick in the region of Lyme, Connecticut. In 1982, the etiologic agent of Lyme disease was discovered by Willy Burgdorfer, who isolated spirochetes belonging to the genus *Borrelia* from the midguts of *Ixodes* ticks.<sup>3</sup>

Nowadays, Lyme disease or Lyme borreliosis is the most common tick-borne illness in the Northern hemisphere.<sup>4</sup> Erythema migrans is a frequent early manifestation of the disease that presents as a local skin lesion with reddish expanding borders and often a clearing center. Occasionally Lyme disease is accompanied by fatigue, fever, headache, arthralgia and/or myalgia. If left untreated, infected individuals can develop more serious manifestations affecting the skin, nervous system, joints, and/or the heart.<sup>4,5</sup> If provided timely, Lyme disease can be successfully treated with antibiotics. But, not all patients do

respond to antibiotic treatment and continue to have persisting symptoms.<sup>5,6</sup> In addition, people are often unaware of being bitten by a tick and ignore the first signs and symptoms that could help with early detection and treatment. Another problem is that Lyme disease is often difficult to diagnose. Serological tests determining the presence of specific antibodies against *B. burgdorferi* are generally used to support a clinical diagnosis, but are often negative in the initial 3 weeks after infection of the patient. On the other hand, false positivity of Lyme disease serologic tests may occur. Unfortunately, at present no licensed vaccine against Lyme borreliosis is available, and therefore appropriate clothing in tick-infested areas, using repellants and the early removal of attached ticks remain the most important preventative measures.

Lyme disease is caused by the spirochete bacteria *B. burgdorferi*, which are transmitted to humans by the bite of *Ixodes* ticks. The *B. burgdorferi sensu lato (B. burgdorferi s.l.)* group comprises all *Borrelia* species known to cause Lyme disease. The most common species in North America is *B. burgdorferi sensu stricto (B. burgdorferi s.s.)*.<sup>7</sup> Common *Borrelia* genospecies in Europe are *B. garinii*, *B. afzelii*, *B. burgdorferi s.s.*, *B. valaisiana* and *B. spielmanii*.<sup>7</sup> (Table 1). The three species, *B. burgdorferi s.s.*, *B. garinii*, and *B. afzelii*, are the most common pathogens causing Lyme disease and they are each associated with different clinical manifestations of chronic Lyme disease. *B. burgdorferi s.s.* seems to be the most arthritogenic; *B. garinii* is

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**Table 1.** Predominant *B. burgdorferi* s.l. spp., Ixodes tick spp and reservoir host animals in USA and Europe.

		<i>Borrelia</i> spp.	<i>Ixodes</i> spp tick	Reservoir host animal
USA <sup>7</sup>	northeast, north central western	<i>B. burgdorferi</i> s.s. <i>B. bissettii</i>	<i>I. scapularis</i> <i>I. pacificus</i>	white-footed mice; eastern chipmunks probably small mammals, such as rodents
Europe <sup>7</sup>		<i>B. garinii</i> , <i>B. afzelii</i> , <i>B. burgdorferi</i> s.s., <i>B. valaisiana</i> and <i>B. spielmanii</i>	<i>I. ricinus</i>	large variety of hosts including birds, rodents and other small-to-medium-sized mammals

considered to be the most neurotropic, whereas *B. afzelii* has been mostly associated with skin manifestations.<sup>5</sup> The high diversity of prevalent species within *B. burgdorferi* s.l. group in Europe leads to a wider variety of clinical manifestations in Europe compared to US. The number of *B. burgdorferi* infected ticks appears to be dependent on geographic location, may vary from one year to another and shows seasonal fluctuations; also the tick activity shows seasonal variation.<sup>5,8-10</sup> Furthermore, the infection rate of *B. burgdorferi* in ticks depends on density of all ticks and (*B. burgdorferi* positive) reservoir hosts, which in turn is dependent on the habitat and climate. Apart from insufficient preventative methods, threat of increased number of *Borrelia*-infected ticks as well as the expansion of geographic tick areas may further lead to an increased risk for Lyme borreliosis.<sup>8,9</sup>

As with other tick-borne diseases, the incidence of Lyme disease has a bimodal distribution with respect to age; rates seem highest among children 5-9 y of age and among adults 55-59 y of age.<sup>11</sup> Incidence estimates of Lyme borreliosis may strongly differ according to what disease characteristics are monitored. Thereby, uncertainties with respect to diagnoses play an important role. Underreporting and misclassification may be a result. Furthermore, very few countries have made Lyme borreliosis a mandatorily notifiable disease. The American surveillance system, based on notifications of observed cases, has been active since 1991. In Europe, most countries do not have national monitoring data available.<sup>12</sup> In the US, annually approximately 30,000 cases of Lyme disease are reported, but the actual number of cases could be far higher, since there are probably many unreported Lyme cases.<sup>13</sup> In 2013, the US Centers for Disease Control and Prevention (CDC) released a preliminary estimate of around 300,000 cases per year (i.e., 94 per 100,000 population) based on insurance claims, lab results and population surveys.<sup>13</sup> (Table 2). Geographically, the distribution of Lyme

cases in US is highly focal, with 10 states in the Northeast and upper Midwest accounting for 93% of all reported cases.<sup>11</sup> In Europe, approximately 85,000 cases of Lyme borreliosis are reported annually. However, this is probably also an underestimate as case reporting is highly inconsistent in Europe and many infections go undiagnosed.<sup>12</sup> Based on available data, the highest reported incidence of Lyme borreliosis in Europe is found in the central part, with an estimated incidence of 261 per 100,000 population in Germany,<sup>14</sup> 206 per 100,000 in Slovenia and 135 per 100,000 in Austria.<sup>15</sup> (Table 2). A marked increase in the incidence of Lyme disease has been reported over the past decades in Europe and US.<sup>12,15,16</sup> The increased incidence of Lyme borreliosis is alarming.

## Discussion

### Immunity to *Borrelia burgdorferi* s.l.

The innate immune response constitutes the primary line of defense against *B. burgdorferi* s.l. in which the complement system plays a crucial role. Unfortunately, many *Borrelia* strains can escape this by avoiding complement-mediated lysis via the *Borrelia* outer surface protein E (OspE).<sup>17</sup> The specific adaptive immune response against *B. burgdorferi* s.l. involves both humoral and cellular immune responses; both T-cell-dependent and T-cell independent immune responses lead to the production of *Borrelia*-specific antibodies that play an important role in the elimination of *B. burgdorferi*.<sup>18</sup> During acute infection, specific IgM antibodies directed against OspC and the flagellar protein (FlaB) have been detected.<sup>19,20</sup> In addition, other *Borrelia* proteins, including, the variable surface antigen (VlsE), basic membrane protein A (BmpA), decorin binding protein A and B (DbpA, DbpB), fibronectin-binding protein (BBK32), OspA, OspB and OspE are known

**Table 2.** Incidence of Lyme disease by region in USA and Europe

Location	Period	Lyme disease number (incidence) per year	Reference
USA	2013	Reported cases to CDC: 30,000 (9 per 100,000) Estimated cases by CDC: 300,000 (94 per 100,000)	13
Europe	2005	Reported cases: 85,000	12
Central Europe	2005	206 <sup>*</sup> per 100,000 (Slovenia)	15
Southern Europe	2005	135 <sup>**</sup> per 100,000 (Austria)	15
Western Europe	2007-8	261 per 100,000 (Germany)	14
	2005	<1 per 100,000 (Portugal and Italy)	15
	2010	132 <sup>***</sup> per 100,000 (The Netherlands)	16
	2005	16 per 100,000 (Belgium)	15
	2005	1 per 100,000 (England and Wales)	15

Methods used to acquire data differ per country, and are not always described.

\*estimate based on laboratory reports;

\*\*estimate based on physician survey;

\*\*\*defined as occurrence of erythema

to elicit antibody responses in natural infections.<sup>17,20-22</sup> Several of these antigens have proven to be useful in serologic tests for diagnosis of Lyme borreliosis. In a recent study, OspC IgM and VlsE IgG had the highest diagnostic value in patients with Lyme borreliosis.<sup>23</sup> Apart from antibodies against *Borrelia* proteins most patients with Lyme arthritis showed to have strong IgG reactivity against 2 glycolipids, MgalD (BbGL-II) and ACG (BbGL-I).<sup>24</sup> The specific antibody response that is responsible for protective immunity against natural infection, however, remains unknown. *Borrelia* can avoid attack by specific antibodies by the induction of resistance to the complement system, by changing the expression of the surface proteins or by antigenic variation.<sup>17,25-27</sup> On the other hand immunity, especially T cell immunity, against *Borrelia* may contribute to Lyme disease pathology, such as myocarditis and arthritis.<sup>28</sup>

### Vaccines

At present, there is no licensed vaccine available for Lyme borreliosis. Vaccine companies GlaxoSmithKline (GSK) and Pasteur Merieux Connaught had both developed vaccine against Lyme disease, resp. LYMERix and ImuLyme. Both vaccines consisted of the recombinant OspA protein and successfully entered phase III clinical trials starting in 1994-1995.<sup>29,30</sup> In these studies, LYMERix and ImuLyme were given to respectively 10,936 subjects (15-70 years) and 10,305 subjects (18-92 years); 2 injections were administered 1 month apart and a booster dose was given at 12 months. In the first year, after 2 vaccine doses, efficacy with respect to prevention against Lyme disease was estimated to be 49% for LYMERix and 68% for ImuLyme. In the second year, after 3 doses, the vaccine efficacy was estimated to be 76% for LYMERix and 92% for ImuLyme. Both vaccines had acceptable rates of local or systemic side effects.<sup>29,30</sup> Immediately after the publication of the clinical data, a report was published showing that Lyme arthritis was associated with immune reactivity toward the OspA protein.<sup>31</sup> During long-term infection, it was shown that *Borrelia* was able to re-express OspA and OspB in the joints leading to OspA/B-specific antibody responses that correlated with severe and prolonged Lyme arthritis.<sup>20</sup> A cross-reactive response between OspA/B and the LFA-1 self-antigen was suggested to be responsible for the observed Lyme-associated arthritis. Although it was hypothesized that an OspA-based vaccine might have similar effects, data from the clinical study with LYMERix showed no evidence for this. In 1998, LYMERix was licensed by the US Food and Drug Administration (FDA) under the condition to perform post-marketing surveillance to identify rare adverse events, such as polyarthritis.<sup>32</sup> ImuLyme, for unpublicized reasons, was not applied for licensure. Short after the licensure of LYMERix, the Advisory Committee on Immunization Practices (ACIP), which develop recommendations on how to use vaccines in the US, came out with a very cautious recommendation for the use of LYMERix, which is rather exceptional for a licensed vaccine. Even for the highest risk groups, defined in terms of individual exposure to tick-infested habitats, the ACIP advised to "consider" vaccination instead to issue a clear recommendation for using the vaccine.<sup>33,34</sup> The media's focus on the presumed arthritis risks of

the vaccine undermining the benefits resulted in a negative public's perception of the vaccine, which resulted in a reduced uptake of the vaccine. The FDA's evaluation of the post-marketing safety data, obtained from more than 1.4 million vaccine doses showed, however, no scientific evidence that the vaccine caused arthritis or any other harm. Nevertheless, these findings supporting vaccine safety did not lead to a revival of the vaccine's demand.<sup>32</sup> As a consequence, in 2002 LYMERix was voluntarily pulled off the market by GSK due to poor sales.<sup>33</sup> The withdrawal followed various lawsuits that claimed the vaccine could cause arthritis.<sup>33</sup> Since then, no other company has offered up an alternative.

### Vaccine development

OspA based vaccines, such as LYMERix and ImuLyme, works in a different way as traditional vaccines; it kills *B. burgdorferi* in ticks, not in humans.<sup>33,35,36</sup> OspA is expressed by spirochetes inside the tick, but after the uptake of a blood meal by the tick, OspA protein is downregulated.<sup>37</sup> Therefore, spirochetes entering the hosts' skin are in an OspA negative state, although in a prolonged state of infection OspA can be re-expressed.<sup>20,37</sup> Vaccine-induced antibodies kill the spirochetes within the tick's midgut.<sup>36,38</sup> Although, there is also evidence suggesting that OspA antibody binding to the surface of spirochetes blocks transmission to the host by a mechanism that does not require bacterial killing.<sup>39</sup> Disadvantage of this action mechanism of the vaccine is that a secondary antibody response and clonal expansion of the OspA-specific B cells will not occur upon invasion of the bacteria, because of the OspA negative status of the bacteria upon entry in the host. One might wonder whether the waning vaccine-induced OspA antibody response, without the boosting effect of natural infection, is still sufficient to kill the bacteria inside the tick long time after vaccination. Probably, additional vaccine booster doses would be necessary to maintain protective antibody titers. Both LYMERix and ImuLyme contained a single OspA protein derived from one species of *B. Burgdorferi s.l.*, i.e. *B. Burgdorferi s.s.*, the predominant species of the US. However, OspA is antigenically heterogeneous and OspA protective immunity is largely type-specific; a candidate vaccine designed to confer broad protection against Lyme disease globally must therefore contain several antigenic variants of OspA.<sup>4,40</sup> Recently, a double-blind, randomized, dose-escalation phase I/II study in adults was performed to investigate the safety and immunogenicity of a novel multivalent OspA vaccine that can potentially be an effective intervention for prevention of Lyme borreliosis in Europe and the USA, and possibly worldwide.<sup>4</sup> The vaccine appeared also to be well-tolerated and immunogenic in individuals previously infected with *B. burgdorferi s.l.*<sup>40</sup>

The heterogeneity of the *Borrelia* species causing Lyme borreliosis makes the search for conserved antigens providing broad protection challenging. Especially, when considering that *Borrelia* has a very complex biology and alters expression of outer surface proteins according to temperature, pH, and other environmental stimuli. Apart from OspA, alternative *Borrelia* outer membrane proteins have been evaluated as vaccine antigens in various studies. In contrast to the OspA/B proteins, which are downregulated by the spirochetes when transmitted

to the host, other outer membrane proteins are upregulated within the host, including OspC, BBK32, RevA/B, DbpA/B or Erp proteins.<sup>26</sup> These proteins may serve as alternative candidates for protein-based vaccines. Several studies in mice have described the protective capacity of immunization with one of these proteins, but so far none of these potential vaccine candidates have entered phase II/III trials. An OspC vaccine had entered clinical phase I/II studies, but showed to develop erythema and swelling at the injection site in approximately half of the individuals.<sup>26</sup> Apart from *Borrelia* proteins, glycolipids, such as acylated cholesteryl galactosides (ACGal), have also been suggested as potential vaccine candidates.<sup>41</sup>

The saliva of ticks contains a mixture of pharmacologically active compounds that modulate the hosts' defense responses to the benefit of the tick feeding process. These saliva compounds are differentially produced during tick feeding and comprise inhibitors of blood coagulation, vasodilators and immunomodulatory substances as well as compounds preventing itching and pain.<sup>42</sup>

Salp15, is such a salivary protein, that seems to have various functions including inhibition of CD4+ T cell activation and binding to the OspC protein, which seems to be a strategy of the spirochete to escape from the hosts' innate and adaptive immune response against OspC. Another identified molecule found in the saliva of *I. scapularis* with complement inhibitory activity is Isac. Immunization with adenoviral-vectored Salp15 and Isac proteins showed a 60% reduction of *B. burgdorferi* spirochetes in the heart of mice after infected tick challenge.<sup>43</sup> This suggests that these tick salivary proteins are also potential vaccine candidates whether or not in combination with *Borrelia*-derived proteins and/or glycolipids.<sup>43,44</sup>

### Other developments

Ticks seem to prefer some individuals to others as hosts. Knowledge about the mechanism behind this difference is currently lacking, but could be useful in the development of preventive measures against tick bites. Tick resistance is another observed phenomenon, which seems not unusual in different animal species. It has been described that mice can be made tick resistant by multiple tick bites occurring over time; prior infestation with pathogen-free ticks induced a host response which provided protection against infection caused by ticks positive for *B. burgdorferi*.<sup>45</sup> This implies that the induction of tick resistance in humans (even in the absence of specific immunity to spirochetes) may be an alternative manner to prevent transmission of *B. burgdorferi* or other tick-transmissible pathogens. At present, the mechanism responsible for the induction of tick resistance is unknown and further research is needed to be able to use this as possible intervention strategy.

Vaccination of wildlife reservoirs is another approach to reduce human Lyme disease risk. Anti-tick vaccines targeting other tick species already exist in the veterinary field and have successfully been used to reduce tick-fever (babesiosis) in cattle. Whether anti-tick vaccines can also be used to (locally) eradicate *Ixodes* ticks, and prevent human tick-borne diseases remains to be established.<sup>44</sup> Recently, the efficacy of an oral bait vaccine based on the OspA protein was assessed in reservoir host animal, white-footed mice. Data from this laboratory

study suggests that oral immunization of wildlife reservoirs of *B. burgdorferi* with an OspA-based vaccine might be feasible and may ultimately lead to a risk reduction of human Lyme disease.<sup>46</sup> Field studies to test the efficacy in a wildlife setting will be a challenging next step. A disadvantage of such an approach is that there may be different preferred host species for different local tick strains. In Europe, *B. burgdorferi s.l.* is transmitted by *I. ricinus* ticks that are carried by a large variety of hosts including birds and small-to-medium sized mammals.<sup>7</sup>

Another possible strategy to prevent the development of Lyme disease is antibiotic prophylaxis following a tick bite. The effect of a single-dose doxycycline prophylaxis following a tick bite has been investigated.<sup>47</sup> However, the 6-week follow-up period in this clinical study was too short to assess the preventative effect on the development of late Lyme disease. For prophylactic antibiotic treatment, the risks of adverse events as well as the problem of antibiotic resistance (of other bacteria) need to be considered, and should not be given undue weight.

### Vaccination against Lyme disease: Are we ready for it?

If left untreated, symptoms of Lyme disease can last for many years leading to serious and chronic health problems.<sup>4,5</sup> Apart from untreated patients, approximately 10% to 20% of patients that have been treated with the recommended course of antibiotics may continue to have persisting symptoms for years.<sup>5,6</sup> In some cases, symptoms may be severe, chronic and adversely affect health-related function. For this reason, Lyme disease should not be considered a minor disease. Additionally, persistent form of Lyme disease is associated with high medical costs. In a recent study, it was estimated that the total medical costs attributable to Lyme disease in the US was estimated to range between \$712 million – \$1.3 billion each year.<sup>48</sup> The marked increase in Lyme disease incidence over the past decades in Europe and US requires measures to get it under control. Lyme disease prevention has focused traditionally on reducing human exposure to the bites of infected ticks by avoidance, followed by prompt tick removal and/or landscape management.<sup>11</sup> Although these methods are generally safe and inexpensive, their effectiveness remains uncertain, and rates of compliance are generally poor.<sup>11</sup> Vaccination would be the most effective intervention for prevention of Lyme borreliosis, but at present no vaccine is available for human use. Implementation of vaccination against Lyme disease is most relevant for groups with high exposure risk. Therefore, efficacy in this group should be considered first.

Failed public acceptance led to the demise of the monovalent OspA-based vaccines. The question that remains is whether the public is nowadays willing to accept a vaccine against Lyme disease. It seems that the public is presently more concerned about Lyme disease than when the first Lyme vaccine was marketed. The major reason for the public's reluctance to the use of the first vaccine was probably caused by the media's focus on the alleged risks of the vaccine, which undermined the benefits of the vaccine. The negative Lyme vaccine sentiment, including the lawsuits that were filed because of the alleged arthritis side effects of the vaccine, seemed to have hampered the development of other candidate vaccines up to now. The fact that the vaccine was not intended for use in children has also been

suggested to have played a role in the low uptake of the vaccine.<sup>35</sup> Perhaps, parents will be more inclined to vaccinate themselves and their children, if there is a pediatric vaccine against Lyme disease available. Furthermore, the persistence of the vaccine-induced immunity was unknown. Consequently, it was unclear whether and how many repeated booster doses were needed during life. This may also have had impact in the resistance to get vaccinated.<sup>35</sup>

Nevertheless, it is important that the public will be made aware of the serious health problems that Lyme disease can cause. In addition, it will be important to inform the public accurately about what can be expected from the vaccine in terms of effectiveness, long-term protection and side effects. National health organizations and infectious diseases experts could play an important role in that, providing clear and concise information and unequivocal recommendations for the use of the vaccine. Hopefully, vaccine developers and/or producers have sufficient confidence that a (broadly protective) Lyme vaccine can be successfully marketed. Perhaps the chance of success is greater if the vaccine is first released on the European market; the incidence rates as well as the fear of getting Lyme disease is high in various European countries. In addition, the public's resistance to a Lyme vaccine is expected to be lower in Europe than in US, there seems to be in advance no negative attitude against Lyme vaccination in Europe. Summarizing, at present we seem more ready than ever for the use of a broadly protective vaccine against Lyme disease.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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