

Review

Why is There Still no Human Vaccine Against Lyme Borreliosis?

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Lyme disease, transmitted by ticks, is a complex illness that can be difficult to diagnose but easy to treat in most early cases, yet difficult in its latest stage. Every year, infections with *Borrelia burgdorferi sensu lato* spirochetes cause thousands of new cases of illness around the world, including people with a normal immunological reaction. Prevention in the form of vaccines is difficult due to e.g. very high variability of *Borrelia* antigen proteins, which precludes the construction of an effective vaccine. After the withdrawal of the OspA vaccine (LYMERix) in the USA, despite promising results, no vaccine protecting humans against all pathogenic species from the *B. burgdorferi* s.l. group is available. Recent data indicate that an effective vaccine may require a combination of several antigens or multiple epitopes based on vector-borne proteins and several outer membrane proteins of *Borrelia*. With the discontinuance of Lyme vaccines, personal protective behavior and the avoidance of exposure in high-risk areas remain necessary resources of prevention.

Key words: *Borrelia burgdorferi sensu lato*, Lyme disease, adaptation strategies of spirochete, LYMERix vaccine, new approach to vaccine, prevention.

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Borreliosis, also known as Lyme disease or Lyme borreliosis (LB) is a tick-borne bacterial infection, in which the disease process extends to the skin and/or multiple internal organs and systems. Therefore infections in humans manifest different clinical symptoms including dermatological, neurological and rheumatological ones (STEERE 2001).

Etiological factors of this disease are some of the species belonging to the *Borrelia burgdorferi sensu lato* complex, transmitted by ticks, mainly of the *Ixodes* genus and it is the most commonly diagnosed disease transmitted by these arthropods. In the U.S. a pathogenic species *B. burgdorferi sensu stricto* is transmitted primarily by *I. scapularis*; in Europe the three most important pathogenic species, *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii* are transmitted by *I. ricinus* (WODECKA 2003; WODECKA *et al.* 2010). However, LB occurs worldwide, and differences in the frequency of occurrence of certain symptoms of the disease in the U.S. and Europe are attributed to differences in molecular structure of *Borrelia* (WEBER 2001). In North America this disease is manifested by symptoms that are different from

those seen in other parts of the world – there is a domination of multi-organ form affecting joints and CNS (Central Nervous System), occasionally with severe course (STANEK *et al.* 2011).

LB in its typical course manifests itself with skin lesions, so-called *erythema migrans*, often accompanied by nonspecific fever, myalgia, arthralgia and fatigue. Early diagnosed and treated borreliosis is curable by antibiotic therapy administered orally. However, patients whose treatment becomes complicated, especially when their nervous system is attacked, require several weeks of intravenous antibiotic treatment, which produces additional risks such as reaction to medication, treatment of coexisting opportunistic diseases, and complications associated with intravenous catheter (NIGROVIC & THOMPSON 2007). However, many LB patients and their primary care physicians remain unaware that they are infected, and patients may not respond to treatment (PLOTKIN 2011; STANEK *et al.* 2012; STEERE & LIVEY 2012). Importantly, severe consequences of LB can affect the skin, nervous system, joints and heart (STANEK *et al.* 2011, 2012) and successful antibiotic therapy

may not prevent reinfection (NADELMAN & WORMSER 2007). In addition, patients with the late-stage manifestation of arthritis may develop persistent joint inflammation that no longer reacts to antibiotics (STEERE & ANGELIS 2006). BOCKENSTEDT *et al.* (2012) using the mouse model of LB demonstrated that inflammatory *B. burgdorferi* components can persist near cartilaginous tissue after treatment for LB. The authors propose that these deposits could contribute to the development of antibiotic-refractory Lyme arthritis. EMBERS *et al.* (2012) infected *Rhesus* macaque with *B. burgdorferi* and administered aggressive antibiotic therapy 4-6 months later. Their results demonstrate that *B. burgdorferi* can survive antibiotic treatment and controlled post-dissemination in a primate host. Considering the difficulties and progression of LB in endemic areas, embarking on prophylactic programs would be the most effective way of intervention to prevent LB (BARRETT & PORTSMOUTH 2013).

Why do healthy people suffer from Lyme disease?

The data indicate that every year *B. burgdorferi sensu lato* spirochetes cause thousands of new cases of infections in people worldwide. These are usually patients with a competent immune system, and despite an active immune response, spirochetes can maintain persistent infection in their bodies. Why then do healthy people succumb to borreliosis?

These bacteria adopt different strategies to survive in the body of an immunocompetent host from the time of infection until they eventually spread to various tissues (SINGH & GIRSCHICK 2004). The success of borreliae depends on the ability to colonize host tissues and the counterattack of defense mechanisms. The bacteria have a remarkable ability of avoiding the host immune response (SINGH & GIRSCHICK 2003, 2004). Changes in the synthesis of outer surface proteins (Osp) of borreliae are the first strategy to avoid destructive action of the host immune system (BROOKS *et al.* 2003). Osp genes encoding proteins of antigen character occur in a number of allelic forms within the *B. burgdorferi* s.l. species, which is undoubtedly related to the deception of the host immune system. Changes in surface antigens lead to the presentation of new antigens to the host immune system which thus far has not produced antibodies against them, and in turn, antibodies can no longer fulfill their role against primary antigens.

These extraordinary properties and the ability to infect different species probably arise from the specific structure of the genome of these bacteria. These features are thought to be associated with factors encoded in plasmids, which were found in large numbers in the genome of *Borrelia*

(GLÖCKNER *et al.* 2006), and which display high variability due to recombination with each other. Moreover, mutations are much more frequent in the plasmid DNA than in the chromosome which also greatly increases variability. The chromosome in the bacterium is a linear DNA molecule, described as the first of its kind in the world of bacteria. Its size is about 0.96 Mbp. Plasmids in *Borrelia*, 21 in total, are both circular (9) and linear (12) and are composed of approximately 613 000 bp. Linear plasmids were initially detected 20 years ago, first in yeast, then in bacteria including *Borrelia*. Such a large number of plasmids is not present in any other bacteria (FARLOW *et al.* 2002). Interestingly, there are unique plasmids in all three pathogenic species of the *Borrelia* genus containing less than 50% sequence similarity to any other plasmids. This phenomenon may be the reason behind the bacteria's ability to adapt to different environments, as well as their pathogenicity (GLÖCKNER *et al.* 2006).

Research has shown that pathogenic species of *B. burgdorferi* s.l. exist in three different developmental forms. These include the typical spherical and immobile cysts containing tightly coiled spirochetes, forms resistant to almost all antibiotics (ALBAN *et al.* 2000). Others are the L forms, i.e. spheroplasts devoid of a cell wall, insensitive to many antibiotics acting on the cell wall (MURSIC *et al.* 1996) used in borreliosis treatment, and finally the vesicular forms, so called bleb forms excreted outside the home cell and membrane gemmas (both forms contain DNA and surface antigens) (ALBAN *et al.* 2000). During infection, these three forms can turn into one another, which may result in ineffective treatment. There is also evidence that a spirochete can produce spore forms inside cells such as macrophages, lymphocytes and endothelial cells and in this way it can avoid the action of antibiotics and antibodies of the host. The high morphological variability of *B. burgdorferi* jointly with the variability of surface antigens not only enables spirochetes to avoid host defense mechanisms, but also aids the development of infection. However, the strategies of borreliae in this area are multifaceted, e.g. *erp* and *crasp* gene products inhibit the cytolytic activity of the host serum by binding the regulators of complement (KURTENBACH *et al.* 2002; SKOTARCZAK 2009). The aforementioned properties and the fact of avoidance of blood vessels during the life cycle of the vertebrate and the absence of specific symptoms of borreliosis make diagnosis as well as the design of vaccines very difficult.

Is a vaccine against borreliosis needed?

Every year the number of diagnoses of borreliosis increases throughout the world. Most cases are

observed in the northeastern states of the U.S., in Central and Eastern Europe and in western and eastern Asia (mainly northern Japan) (HASHIMOTO *et al.* 2007; CHINMOY & SCHWARTZ 2011). These are regions of endemic occurrence of the disease associated with tick inhabited areas. In 2009 the U.S. Center for Disease Control and Prevention (CDC) reported about 30 thousand cases confirmed as Lyme disease and more than 8.5 thousand cases suspected of suffering from the disease, while for example in 2000, only 17 730 cases were reported in the U.S. In 2008, 85 500 patients, including 65 500 in Europe, 16 500 in North America, 3 500 in Asia and 10 000 in North Africa (HUBÁLEK 2009) were estimated to have suffered every year from the world's most common disease transmitted by ticks. HENRY *et al.* (2011) and SCOTT *et al.* (2012) state that the number of reported cases across Europe and North America is probably significantly underestimated.

In Europe, the largest number of Lyme disease cases (between several hundred to several thousand) have been reported in Germany, Austria, Slovenia, Switzerland, Sweden and Western Russia (WEBER 2001; RAUTER & HARTUNG 2005). In Poland, according to the National Institute of Hygiene, in 2009 there were 10 332 confirmed and suspected cases of Lyme disease (morbidity 27.08), in 2010-2011 (morbidity 23.62), to 12 779 in 2013 (morbidity 33.2). In the first half of this year there were more than 5.3 thousand cases of Lyme disease compared to approx. 4.2 thousand cases in the same period of the year 2013. However, it is estimated that the number of infections with spirochetes in this country may be even tenfold higher than the official statistics show since only hospitalized cases are recorded.

To address the question raised above, not only the statistical data of the incidence or number of diagnosed cases should be considered, but also the diagnostic difficulties of this disease and, above all, quality of life of people suffering from Lyme disease. BARRETT and PORTSMOUTH (2013) in their latest findings conclude that vaccination would be the most effective intervention to prevent LB not only because LB incidence is on the rise, but also because its geographical distribution is spreading, and is predicted to continue to do so, concurrently with an increasing overlap between humans, ticks and their reservoir hosts. Furthermore, changes in climate are factors influencing the increase of the prevalence of Lyme borreliosis. NARDELLI *et al.* (2009) point out that the rise of borreliosis to the rank of an endemic disease, as well as difficulties in diagnosis and its continuing - despite prevention - increase in incidence, make the search for an effective vaccine against Lyme disease worth any effort (NARDELLI *et al.* 2009), and not doing so is

a serious blunder and negligence of public health (PLOTKIN 2011). However as PLOTKIN (2011) notes, the construction of a vaccine is possible provided that infectious disease specialists and epidemiologists will demand it.

Why was the LYMERix vaccine withdrawn?

Intensive development of a vaccine against borreliosis took place in the U.S. during the '90s of the 20th century (NARDELLI *et al.* 2009). The vaccines were tested using whole cells of bacteria, live attenuated vaccines from flagellar mutants, recombinant OspA-based vaccine, subunit vaccine containing OspC protein, or DNA vaccine based on the ospA gene. Other potential candidates for a vaccine were a decorin-binding protein, P35 protein with a mass of 35 kDa and P66 protein (WANG *et al.* 1999). The first vaccine (recombinant Lyme vaccine, LYMERix™) was registered in the U.S. in 1998, however, after less than 5 years it was withdrawn from the market because of a lack of interest (PLOTKIN 2011; POLAND 2011). The lack of interest of this vaccine was due to i.a. serious concerns about side effects that FDA revealed (SMITH *et al.* 2002) and a class action lawsuit (STRICKER & JOHNSON 2014) as well. Thus, concerns about its safety might have contributed to its withdrawal. Another reason might have been insufficient recommendation from the CDC and inadequate training of doctors in proper usage (NIGROVIC & THOMPSON 2007; PLOTKIN 2011). However, despite the fact that it has been more than 10 years since that time, and more than 300 thousand people have developed Lyme disease in the U.S. every year (the latest CDC statistics), a human borreliosis vaccine is still unavailable.

The LYMERix vaccine was based on recombinant surface protein A (OspA) of *B. burgdorferi* s.s. ZS7 strain and was developed by a European company, GlaxoSmithKline, like the second vaccine ImmuLyme (PasteurMerieux-Connaught). Preclinical studies in mice demonstrated that OspA protein induced a long-term protective response (FIKRIG *et al.* 1992). The resulting antibodies were sufficient to bind and neutralize living *Borrelia* spirochetes within the tick gut with antibodies against OspA while it was sucking mouse blood, thus effectively preventing skin infections (DE SILVA *et al.* 1996).

The LYMERix vaccine was for individuals aged 15-70, while ImmuLyme for individuals aged 18-92. The only contraindications in use of the vaccine were pregnancy or inappropriate age. The vaccine was especially recommended for people living or working in wooded areas or grasslands, where they could be infected with *B. burgdorferi* by ticks. Although its main target group consisted

of forest and farm workers, the vaccine was recommended for anyone hiking, climbing, hunting or fishing in risk areas (HUGHES & GUBLER 1999). Both vaccines had been tested in clinical trials on a sample of >10 000 people, yet only LYMERix was introduced to the public. The LYMERix vaccine was registered for use in 3 doses and was effective for about 80% of vaccines after administration of the third dose with aluminum hydroxide as adjuvant (STEERE & KRAUSE 1998). The drawback of this vaccine was that the protective immunization correlated only with a high titer of antibodies against OspA after immunization, and that approximately 5% of vaccines developed an insufficient response in the form of antibodies against the OspA protein. This was associated with decreased expression of TLR cell surface receptor (ALEXOPOULOU *et al.* 2002). Thus, high levels of antibodies did not persist long after vaccination and additional vaccination was necessary to maintain the protective titer (STEERE & KRAUSE 1998).

As the vaccine was effective for 80% of vaccines, the remaining 20% could still develop Lyme disease even after the third dose of vaccine (STEERE & KRAUSE 1998). Moreover, only three doses of vaccine provided full protection, so the time from the first dose (the second after a month) to the third after 12 months did not protect against infection. An additional drawback was the lack of testing in young children, which is a population at high risk for borreliosis (GROSECLOSE *et al.* 2004). And finally, another flaw was that the efficacy of this vaccine was only for the *Borrelia* species dominant in North America.

After approval of the vaccine by the U.S. Food and Drug Administration (FDA) on 21st December 1998, its entry into clinical practice was slow, for various reasons, including costs, which were often not refunded by insurance companies. Nevertheless, for example, in the period from December 1998 to July 2000 more than 1.4 million doses were distributed (SHEN *et al.* 2011). However, hundreds of vaccinated people soon began to report musculoskeletal and neurological side effects associated with the vaccine and a class action lawsuit against GlaxoSmithKline was filed (STRICKER & JOHNSON (2014). It was established that one of the OspA protein domains (OspA165-173 epitope) showed great similarity to a human protein fragment present in lymphocytes [(hLFA)-1 α L326-345], and in consequence antibodies which form after vaccination recognize both OspA proteins and human proteins.

It should be noted that the vaccine was withdrawn before Phase IV marketing reports were filed, and these reports probably would have revealed more difficulties with the vaccine. Nevertheless the U.S. FDA and CDC Agency have not

found any link between vaccines and patient complaints. Despite the lack of evidence that the complaints were caused by the vaccine, sales dropped and LYMERix was withdrawn by GlaxoSmithKline from the U.S. market in February 2002 (NIGROVIC & THOMPSON 2007).

The fate of LYMERix vaccines has been described in the medical literature (in Nature) as “a cautionary tale” and the withdrawal of the LYMERix as a case in which “pressure of unjustified public fears on vaccine developers went beyond reasonable safety considerations”.

Many people think that the negative reception of the vaccine against Lyme disease will suppress any future efforts in its development. Significantly, many of the flaws of the vaccine were known and predictable even before its acceptance and introduction. These threats must be considered before construction of a new, effective and safe vaccine against human Lyme borreliosis (STRICKER & JOHNSON 2014). However, it must be noted that this vaccine had been tested and available only in the U.S. where only one pathogenic species, *B. burgdorferi sensu stricto* has been recorded. Therefore, vaccines against *B. burgdorferi*, produced in the U.S. are ineffective in Europe. Significant serological differences have been found between strains of bacteria occurring in different geographical areas because surface proteins are encoded by rapidly evolving plasmids, as discussed above. A vaccine designed for application in Europe should include a mixture of proteins of *B. burgdorferi* having antigenic properties obtained on the basis of genetic material isolated from strains of bacteria found in Europe.

According to the American team (SHEN *et al.* 2011) consisting of representatives of the CDC and the National Vaccination Institute from Washington, an effective vaccine adequately tested in a large population from the increased risk group will be very beneficial and useful in the prevention of Lyme disease. But such a vaccine must not only demonstrate a high standard of safety and efficacy, but also low cost and public acceptance.

New strategies in the construction of a vaccine

The failure of LYMERix inspired investigations into alternative strategies for LB vaccine development (BARRETT & PORTSMOUTH 2013). Work has started on a second-generation, multivalent OspA LB vaccine, designed to provide protection against almost all *B. burgdorferi* s.l. strains associated with human disease worldwide. Because in Europe and Asia LB is caused by a number of *Borrelia* species which encode antigenically divergent OspA proteins (in the USA, LB is caused only by *B. burgdorferi* OspA-1) a global vaccine requires

the inclusion of several antigenic variants of OspA (LIVEY *et al.* 2011). The vaccine contains protective epitopes from OspA serotypes 1-6, in which the hypothetical risk of T-cell cross-reactivity has been eliminated by replacing the putative cross-reactive OspA-1 epitope with the corresponding OspA-2 sequence. In preclinical studies, a single recombinant OspA containing protective components from two different OspA serotypes (1 and 2) induced antibody responses that protected mice against infection with either *B. burgdorferi* (OspA-1) or *Borrelia afzelii* (OspA-2) (LIVEY *et al.* 2011). Studies with this multivalent recombinant OspA vaccine demonstrated protection of immunized mice against infection with *B. burgdorferi*, *B. afzelii*, *B. bavariensis* and *B. garinii* (BARRETT & PORTSMOUTH 2013). Additionally, efficient antibodies were stimulated not only against the six OspA types targeted by the vaccine but also against other species of *Borrelia*, including *B. spielmani*, *B. valaisiana*, *B. lusitaniae* and *B. japonica*, indicating that the vaccine has the potential to prevent LB worldwide. Phase I/II dose-finding studies have been initiated to examine the safety and immunogenicity of the vaccine in an adult population (BARRETT & PORTSMOUTH 2013). Similarly, COMSTEDT *et al.* (2014) present an approach which allows the generation of a hexavalent OspA-based vaccine to potentially protect against a wide range of globally distributed *Borrelia* species causing LB. The experiments of WRESSNIGG *et al.* (2014) are also promising, they examine the safety and immunogenicity of a multivalent OspA vaccine in seronegative healthy adults or seropositive for previous *B. burgdorferi sensu lato* infection. The data show that using multivalent OspA vaccine is well tolerated and immunogenic in individuals previously infected with *B. burgdorferi sensu lato*.

Furthermore, there are currently discussions concerning the construction of an effective vaccine. New strategies in designing a vaccine against Lyme disease are based, among others, on the fact that *B. burgdorferi* spirochetes are transmitted by ticks, and that these spirochetes utilize a tick protein to stabilize the infection (SCHUIJT *et al.* 2011a). These include the immunization of a mixture of different *Borrelia* surface proteins (SCHWAN & PIESMAN 2000), immunization with tick proteins inducing the immune response at the site of a tick bite and/or inside the tick while sucking blood resulting in the interruption of feeding and detachment of the tick (SCHUIJT *et al.* 2011b). Another strategy is immunization with tick proteins that interfere with the host defense response, for example with complement fixation or directly affecting the borreliae. Another consideration is immunization with ticks' salivary proteins leading to the modulation of local host response, such as

the induction of Th-1 cells (ZEIDNER *et al.* 1996; POLJAK *et al.* 2012) or a combination of tick salivary proteins and antigens of *Borrelia* (e.g., Salp15 protein) (NARASIMHAN *et al.* 2007; HOVIUS *et al.* 2008; DAI *et al.* 2009).

FIGLEROWICZ *et al.* (2013) searched for a component for an animal vaccine against *Borrelia* and used TROSPA protein from *I. ricinus*, the predominant vector of *B. burgdorferi* s. l. in Europe, and OspA from three bacterial species also typical for Europe: *B. garinii*, *B. afzelii* and *B. burgdorferi* s.s. TROSPA from *I. scapularis* was confirmed to be crucial for the colonization of the tick gut by these bacteria and interacts with the *Borrelia* outer surface protein A (OspA) (PAL *et al.* 2004). Because bacterial outer surface proteins differ depending on geographical location, FIGLEROWICZ *et al.* (2013) cloned the TROSPA gene from *I. ricinus* and three OspA genes from three *Borrelia* species and observed that the recombinant TROSPA formed complexes with three OspA proteins and that these proteins from different bacterial species had various abilities to bind TROSPA. Additionally, *I. ricinus* recombinant TROSPA showed immunogenic properties to induce an immune response in rats, thus, it seems to be a good candidate component for an animal vaccine against *Borrelia* in Europe as well as in other part of the world.

Thus, in the future, the construction of an effective vaccine against *Borrelia* should be based on a combination of vaccinogenic factors consisting of multiple *Borrelia* antigens, antigens of ticks, or a combination of both of them, causing a synergistic immune response against *Borrelia* and against ticks. Such an approach may not only be applicable to the prevention of transmission of *B. burgdorferi* from the tick to the host but can also be applied in the prevention of transmission of any pathogens transmitted by arthropods (SCHUIJT *et al.* 2011a).

How to protect yourself while waiting for a vaccine

Preventive measures are primarily aimed at reducing the number of new cases and the number of patients with late-stage Lyme disease (CHINMOY & SCHWARTZ 2011). The public should learn to be observant when entering areas of high frequency of ticks, or even to avoid such areas (SCHUTZER *et al.* 1998), because they are habitats of both ticks and their hosts. Maintaining personal safety involves wearing protective clothing like long sleeves and long trousers, application of repellents, and inspecting the body for ticks after exposure to these arachnids in order to remove them (HAYES & PIESMAN 2003). In addition, prevention should be based on an environmental assessment, i.e. knowl-

edge of the prevalence of ticks and their infection extensification, an assessment of flora and climatic conditions; however the effect of this action will be proportional to the awareness of the population in this area (HAYES & PIESMAN 2003).

Studies conducted in Connecticut in the U.S. in 2008 showed that the use of protective clothing and repellents significantly reduced the cases of acute borreliosis (VAZQUEZ *et al.* 2008). However, the same studies did not show such a relation in the case of a routine search of the body following exposure to ticks in endemic areas. Research has shown that nymphs remain anchored longer in the skin of children than adults (FALCO *et al.* 1996; CDC 2010). This may result from disproportions in the number of bitten children and adults, because children are indisputably a higher risk group. Despite the controversial effectiveness of body search for ticks, BHATE & SCHWARTZ (2011) recommend this procedure as well as the removal of ticks, especially in endemic areas. BHATE & SCHWARTZ (2011), in contrast to the WHO and CDC, recommend a quick pull of the tick from the skin without rotating it, without chemical adjuvants and without cryotherapy. After removal, the primary place of clinging should be disinfected with hot water and soap. Chemoprophylaxis in patients who know that they have been bitten by a tick, even in areas designated as endemic for Lyme disease, is still debated.

Conclusions

The negative reception of the first vaccine against Lyme disease has undermined efforts for further vaccine development. Nevertheless, several approaches leading to an effective vaccine are being discussed at present. According to an American team consisting of representatives of the CDC and the National Vaccine Institute, an effective vaccine properly tested and evaluated in a large population at increased risk will be very beneficial and useful in the prevention of Lyme disease. However, it must be characterized by a high standard of safety, efficacy, low cost and public acceptance. In anticipation of the vaccine, personal protection limiting exposure to ticks is recommended.

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