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PROSPECTS FOR DEVELOPMENT OF AN EFFECTIVE LYME DISEASE VACCINE

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PROSPECTS FOR DEVELOPMENT OF AN EFFECTIVE LYME DISEASE VACCINE

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ABSTRACT

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Lyme disease is the most prevalent tick-borne disease in the world, and the annual number of cases continues to increase. By this measure, a vaccine to prevent the illness would be valuable. However, the first commercial attempt at a human Lyme disease vaccine failed to adequately protect people for an extended period of time, and also caused serious side effects in a small population of recipients. Though many different vaccine formulations have been proposed and evaluated since then, none have yielded results sufficient to warrant progression to clinical trials. This review provides historical perspective of early efforts and discusses current strategies that may ultimately prove more effective at providing vaccine-induced antibody-mediated protection.

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INTRODUCTION

Lyme disease is a tick-transmitted bacterial infection that annually affects thousands of people worldwide. The most common vectors are hard-bodied *Ixodes* spp. ticks including *I. scapularis* and *I. pacificus* in the US, and *I. ricinus* and *I. persulcatus* in Europe and Asia. The illness is caused primarily by infection with any of three genetically-distinct spirochetal bacteria within the *Borrelia burgdorferi* sensu lato complex. For example, Lyme disease cases in the US are most commonly caused by *Borrelia burgdorferi* sensu stricto (ss), while *B. afzelii* and *B. garinii* are responsible for most cases in Asian patients, and European patients can be infected with any of the three genospecies.

Despite several decades of effort to decrease prevalence, Lyme disease remains the most commonly reported vector-borne illness in the US. For example, there were 27,203 confirmed cases and 9,104 probable cases reported to the Centers for Disease Control (CDC) during 2013 (1), and the CDC believes the actual number of annual Lyme disease cases in the US may actually exceed 300,000 (2). In addition, Europe reports approximately 85,000 confirmed cases annually (3), and the actual number may also be several times greater. Therefore, current recommendations to quell Lyme disease; including avoiding areas of tick infestation, wearing protective clothing, utilizing insect repellants, performing tick checks after possible exposure, and landscape management, have had only limited effectiveness. This review highlights past efforts to develop and market a successful human Lyme disease vaccine and discusses recent findings that may

yield more effective strategies for providing antibody-mediated protection against the illness.

BACKGROUND

Historical Perspective

A Lyme disease-like illness was first reported in 1909 by the Swedish physician Arvid Afzelius, who described a European patient with a unique skin lesion termed erythema chronicum migrans (ECM) that developed after a tick bite (4). However, Lyme disease was not formally recognized as an illness until 1975, when Dr. Allen Steere reported the disease in a group of children who resided in Lyme, Connecticut (5). Subsequently, spirochetal bacteria that reacted strongly with immune sera from the afflicted children were recovered from *Ixodes* ticks (6, 7, 8), and shortly thereafter researchers recovered spirochetes from blood (9, 10), CSF (10) and skin biopsies (10) from additional patients who developed similar clinical abnormalities after a tick bite. Subsequent studies resulted in the classification of the spirochetes that caused the illness as *Borrelia burgdorferi* in honor of Dr. Willy Burgdorfer, who was primarily responsible for characterizing the original isolate (11).

Since that time, numerous genetically-distinct species within the genus *Borrelia* have been characterized and Lyme disease spirochetes are now collectively referred to as *Borrelia burgdorferi* sensu lato until they are formally speciated. The original type strain is classified as *Borrelia burgdorferi* ss, and this organism remains the primary cause of the illness in the US. However, Lyme disease cases caused by *B. bissettii* (12), *B.lonestarii* (13, 14), and *B. miyamotoi* (15, 16) have also been reported. In addition, Lyme disease cases in Europe and Asia may be caused by *B. burgdorferi* ss, but illnesses

from infection with *B. afzelii* (17) or *B. garinii* (18) are also common. Moreover, Europe and Asia harbor a far greater diversity of *Borrelia* spp., including at least 14 pathogenic genospecies recognized to date (19, 20), although human illness with most occurs relatively rarely. More significantly, the geographical range of *Borrelia* spp.-infected ticks continues to expand dramatically in both the US (21, 22) and overseas (20, 23, 24), and climate change is likely a significant factor (25).

General Morphology and Laboratory Growth of *Borrelia* spp.

Borrelia spp. spirochetes are helically-shaped microaerophilic organisms (26) with a cell wall composition similar to a typical gram-negative bacterium, but with a significantly lesser amount of lipopolysaccharide (LPS) that also lacks a lipid A moiety (27, 28). The organisms range from three to 20 μm in length, and each has 7 to 11 flagella in the periplasmic space that are anchored at each end in the protoplasm (29). Darkfield microscopy is most commonly used to visualize the organisms, since the spirochetes are not easily seen after gram staining because the unique cell wall does not readily incorporate the dyes.

Borrelia spp. are fastidious organisms that can be cultured in the laboratory by growth in BSK medium that is incubated at approximately 32ºC in the laboratory. However, slightly increased or decreased incubation temperatures (30, 31) or variations of essential ingredients such as bovine serum albumin (32) can have profound effects on the antigenicity of the cultured organisms.

Genetic Characteristics

The organisms typically harbor a small linear chromosome and up to 21 linear or circular plasmids (33) that contain genes coding for multiple proteins that are not essential under all growth conditions. The spirochetes also scavenge some essential nutrients from the surrounding host milieu (34), because they lack the capacity to synthesize individual amino acids. In addition, the linear plasmids are structurally unique since there are hairpin loops at each end that are composed of inverted repeats (35, 36). Additionally, the entire genome of *B. burgdorferi* ss strain B31 has been sequenced (34), which has fostered numerous investigations to characterize the functions and interactions of specific genes (33, 37-40).

Among the proteins synthesized from plasmids, are multiple lipidated outer surface proteins (Osp), designated OspA to OspF, which are expressed under varying environmental conditions. Although the specific function(s) for most remain unknown, researchers have shown that OspA is an adhesion protein that mediates attachment to epithelial cells within the tick midgut (38, 41, 42). In addition, OspC plays some undefined, but essential role in the ability of the spirochetes to establish infection in mammals (39, 43). One possibility is the protein also plays a role in adhesion (44). Other unique proteins include the basic membrane protein (Bmp) (45), which also plays an essential role in virulence (46), GMP synthase (47), telomere resolvase (48, 49), fibronectin binding protein (50), and decorin binding protein (Dbp), which fosters adherence to collagen-associated proteoglycan (38).

Regulated Expression of Osps

Borrelia ssp. increase or decrease the expression of some Osps in response to environmental stimuli (51). For example, OspA is the predominant outer surface protein expressed when the spirochetes reside in the midgut of unfed ticks (41, 52) where the spirochetes are typically bound to epithelial cells (53). However, the expression of OspA is decreased and the expression of OspC increases as the spirochetes acquire a bloodmeal (39, 54), because expression of OspC is necessary to release the spirochetes from the epithelial cells and assist with migration to the salivary glands (43, 55). The spirochetes then continue to express high levels of OspC as they enter the mammalian host and establish infection (56, 57). Interestingly, the duration and magnitude of OspC expression seems to vary widely among different mammalian hosts. For example, spirochetes that infect humans express high levels of OspC for an extended period of time, as evidenced by the high levels of anti-OspC antibodies that are a hallmark of early human Lyme disease (58, 59). In contrast, high levels of anti-OspC antibodies are only rarely detected after infection of mice (60) or canines (61), which suggests the increased expression of OspC is significantly more short-lived in these mammals.

Clinical Manifestations

Early after infection, *Borrelia* spp. localize in the skin (62), where most cause the formation of a lesion termed erythema migrans (EM) (63-65) that typically appears as an expanding red ring with central clearing (63, 66). Constitutional symptoms such as fever, fatigue, and arthralgia/myalgia (64, 65) are also common. If *B. burgdorferi* ss are not promptly eliminated by appropriate treatment with antibiotics, the organisms can then disseminate and cause secondary EM lesions (64, 65) or other clinical abnormalities that

suggest involvement of multiple organ systems including the heart (67) and facial nerves (68). Joint manifestations or more serious organ involvement can also occur in patients when the disseminated infections persist for months to years (69). In addition, a small number of long-term infections apparently trigger an autoimmune syndrome termed treatment-resistant Lyme arthritis (70-72) that continues to be problematic long after the spirochetes are eliminated by antibiotic treatment.

Other pathogenic species of *Borrelia* also cause characteristic EM lesions and constitutional abnormalities during the earliest stage of infection, but the manifestations vary widely after the spirochetes disseminate. For example, while *B. burgdorferi* ss most commonly colonize large joints (73, 74), *B. bissettii* is recovered almost exclusively from heart tissue (74, 75), *B. afzelii* most commonly infects the skin (73, 74), and *B. garinii* appears to have a propensity for nervous system tissue (73, 74). Therefore, a hallmark of *B. afzelii* infection is a unique skin lesion (73, 77) termed acrodermatitis chronica atrophicans (ACA) that manifests most often as chronic discoloration and thickening of skin tissue, and typical symptoms of infection with *B. garinii* are meningoradiculitis, lymphocytic meningitis, or cranial nerve palsy (73, 74, 78).

IMMUNE RESPONSES AFTER INFECTION

Cellular-Mediated Immunity

In addition to the innate immune defense mechanisms, infections with *Borrelia* spp. induce adaptive cellular immune responses typical of the responses induced by other bacterial infections. For example, spirochetal antigens presented by resident macrophages in turn induce secretion of cytokines that ultimately attract additional phagocytic cells that include more macrophages and natural killer cells (79, 80). Interestingly, the spirochetes are phagocytosed by a unique "coiling" mechanism, where the phagocytic cells undergo actin filament rearrangement to form pseudopods that wrap around and engulf the spirochetes (80, 81). In addition, the presentation of individual antigens via major histocompatibility complex (MHC) I or II activate Th-1 cytotoxic or Th-2 helper cells, respectively. The cytotoxic (Th-1) T-cells subsequently kill the spirochetes independent of phagocytosis, and the T helper (Th-2) cells induce proliferation of B-cells that ultimately produce antigen-specific antibodies (83-85)**.** Both the innate and adaptive cellular-mediated immune mechanisms are effective at eliminating the spirochetes, especially when they are targeting organisms in the bloodstream (86), but some apparently escape by sequestering in "immune-privileged" sites, such as skin or heart fibroblasts, joints, or CSF (87-89).

Antibody-Mediated Immunity

IgM antibodies are produced after the first few weeks of infection and antibodies specific for the flagellar protein, OspC, or a basic membrane protein (BmpA) predominate (90). After 3 to 4 weeks, the antibody response expands to include IgG antibodies against these and multiple other proteins. The IgG antibodies mainly function by one of two mechanisms. Most coat the surface of the spirochetes and "mark" the organisms for elimination by phagocytic cells (91, 92). However, a few, including antibodies specific for OspA (93, 94), OspB (93, 94), the 39-kD periplasmic protein, (95) or OspC (58), continue to function like IgM antibodies by inducing a complement cascade that results in formation of a membrane attack complex that lyses the spirochetes independent of phagocytosis (60, 96-98). This IgG antibody response, termed borreliacidal, has been the most effective at providing antibody-mediated immunity after vaccination (99, 100).

Interestingly, mechanisms that might prevent killing by the borreliacidal antibodies have been described**.** For example, some spirochetes have been shown to express complement regulator-acquiring surface proteins (CRASPs) (101-103) that prevent complement deposition on the spirochete surface by binding the complement cascade regulators H-factor or FHL-1/reconectin (104, 105). However, Lyme disease spirochetes that infect humans have not been shown to possess these types of mechanisms.

PREVIOUS EFFORTS AT A HUMAN LYME DISEASE VACCINE

Soon after *B. burgdorferi ss* was identified as the causative agent of Lyme disease, researchers confirmed that passive immunization of serum from challenged animals (106, 107) or active immunization with killed *B. burgdorferi* ss (108) provided the recipient animals with antibody-mediated protection against a subsequent challenge with the organisms. Additional studies confirmed the protection was due primarily to OspA-specific IgG antibodies (7, 109, 110) that provided protection by borreliacidal activity (60, 96, 97, 111, 112). In response, the commercial companies Glaxo Smith Kline and Pasteur Merieux Connaught focused their efforts on developing an OspA-based vaccine for humans.

The initial products were a combination of recombinant (r) OspA derived from *B. burgdorferi* ss strain ZS7 and aluminum hydroxide adjuvant (LYMErix, SmithKline Beecham, Philadelphia, PA) (113, 114) or a non-adjuvanted rOspA derived from *B. burgdorferi* ss strain B31 (ImuLyme, Pasteur Merieux Connaught, North York, Ontario, Canada) which both produced a protective borreliacidal antibody response (114**,** 115). However, Lovrich et al. (116) showed that the immunity provided by the anti-OspA borreliacidal antibodies was limited to only *Borrelia burgdorferi* ss because of significant heterogeneity among the OspA from other *Borrelia* genospecies (116). Therefore, the products were not expected to provide comprehensive protection, especially in Europe or

Asia where Lyme disease caused by infection with *B. garinii* or *B. afzelii* also predominates.

More significantly, Schwan et al. (39) showed that OspA was expressed only by the spirochetes that were attached to endothelial cells in the tick midgut, and the protein was downregulated to an undetectable level almost immediately after the infected ticks began acquiring a human blood meal (112, 114, 117). Therefore, the effectiveness of an OspA-based vaccine was dependent on a sustained level of borreliacidal antibodies that would enter the tick coincident with acquiring a blood meal and kill the spirochetes in the midgut before OspA expression was decreased (118, 119). In addition, the lack of OspA expression in the vaccine-recipients eliminated the possibility for enhanced protection via an immunologic memory response. As further complication, the anti-OspA antibody response also triggered an "autoimmune phenomenon" in a small number of vaccine-recipients (72, 120, 121, 122) with histocompatibility leukocyte antigen (HLA)-DR4 or HLA–DR2 haplotypes, apparently because the anti-OspA antibodies also bound human lymphocyte function associated antigen 1 (hLFA 1).

Despite these concerns, the products were evaluated in large, randomized, double-blind, placebo-controlled studies, and the initial findings in the majority of recipients were encouraging. For example, LYMErix prevented Lyme disease-associated symptoms in 76% of recipients (114) and ImmuLyme provided effective protection in 68% and 92% of recipients after 2 or 3 dosages, respectively (115). However, the recipients were evaluated for only a few months after administering the vaccine and Parenti et al. (123) subsequently showed that the anti-OspA borreliacidal antibody response remained detectable after vaccination for only a few months. Therefore, long-

term protection became dependent on regular boosters. Despite the significant findings, the Food and Drug Administration approved the introduction of LYMErix to the marketplace in 1998, but poor sales, weak endorsement from the CDC, the difficult vaccination schedule, safety concerns, and even a class action lawsuit contributed to severely limit demand, and the product was quickly removed from the marketplace. In addition, the failed commercialization of LYMErix contributed to halt additional development of ImuLyme.

OTHER STRATEGIES FOR INDUCING ANTIBODY-MEDIATED IMMUNITY BY VACCINATION

Elimination/Prevention of Infection in Reservoir Hosts

The demise of LYMErix and ImuLyme caused a redoubling of efforts to prevent human Lyme disease by vaccination. One intriguing possibility that continues to generate interest is vaccinating reservoir hosts, most notably the white-footed mouse (*Peromyscus leucopus)*, to prevent the feeding immature *I. scapularis* ticks from becoming infected with Lyme disease spirochetes while they obtain a bloodmeal. In support, researchers showed that vaccinating *P. leucopus* with rOspA by needle (124) or administering the vaccine formulation via gavage (125) significantly reduced the rate of spirochetemia in recipient mice after intradermal needle-challenge with *B. burgdorferi* ss. In addition, vaccinating laboratory mice by introducing rOspA via food pellets also significantly decreased spirochetemia after challenged with infected *I. scapularis* ticks (126). Despite these promising results, however, the daunting task of administering an OspA-based vaccine to large populations of wild mice has greatly dampened enthusiasm and subsequent large-scale studies have not been forthcoming.

Prevention of Tick-Feeding

Another promising possibility is to develop a vaccine that stimulates antibodies that in turn prevent ticks from feeding, especially since the strategy would also provide protection against other tick-transmitted human pathogens such as *Anaplasma* spp., *Ehrlichia* spp., or *Babesia microti* (127). The current leading candidates are formulations

that induce antibodies specific for subolesin, a protein that is conserved among many tick species (128, 129), including *I. scapularis*, that helps "anchor" the tick to the mammalian host (128, 130-132). In support, De la Fuente et al. (129) showed that vaccinating laboratory mice with a vaccine that induced anti-subolesin antibodies significantly reduced transmission of *Anaplasma phagocytophilum* from feeding *I. scapularis* ticks. Similarly, Bensaci et al. (127) demonstrated that vaccinating laboratory mice with a Vaccinia virus that expressed recombinant subolesin antigens also induced an antibody response that prevented significant numbers of questing ticks from feeding**.** Based on these early successes, the approach has generated enthusiasm for more comprehensive feasibility studies, and several are ongoing.

Antibody-Mediated Immunity by Vaccination with Other Proteins

Researchers have also pursued vaccination with other *Borrelia* spp. proteins, including several that are expressed in the mammalian host, since they would then also conceivably induce an effective immunologic memory response. For example, vaccination with decorin binding protein (Dbp) A induces antibodies that are effective against a subsequent needle-challenge with Lyme disease spirochetes (133, 134). Similarly, vaccination with the fibronectin binding protein BBK32 (135-137) or the porin protein P66 (138, 139) have induced antibody responses sufficient to protected laboratory mice from subsequent needle- or tick-challenges**.** An important caveat, however, is that comprehensive protection by vaccination with each of these proteins has been confounded by significant inter- and/or intra-species heterogeneity, and the result has been that more comprehensive studies that include evaluating the ability of vaccination

with the antigens to stimulate effective antibody memory responses have not been forthcoming.

Induction of Antibody-Mediated Immunity by Vaccination with Multiple Proteins

Because of the extreme heterogeneity among individual *Borrelia* spp. proteins, more recent studies have focused primarily on evaluating protection after vaccination with multi-antigen vaccines (140). For example, Hanson et al. (141) showed that vaccinating mice with a combination of OspA and DbpA provided antibody-mediated protection that was effective against a needle challenge with a 100-fold higher concentration of *B. burgdorferi* than was achieved with either protein alone. Similarly, Brown et al. (142) showed that vaccination with a combination of DbpA, BBK32, and OspC antigens induced antibodies that protected laboratory mice (86-94%) from needle-challenge with multiple *Borrelia* genospecies. Despite these promising results, however, additional studies have not been forthcoming. One possibility may be ongoing concerns of the longevity of antibody-mediated protection without an effective immune memory response. In addition, demonstration that the expression of surface proteins by the spirochetes in laboratory mouse models differs significantly from the proteins expressed during human infection (30, 31, 32, 58-60) may also be confounding additional study.

FOCUS ON OSPC

Suitability for Antibody-Mediated Immunity

Several observations suggest that OspC may be the most effective vaccinogen. Most notably, high levels of OspC are expressed in the tick as the spirochetes migrate to the salivary glands (43, 55), and also during the early stages of mammalian infection (56, 57, 143, 144). In fact, Lyme disease spirochetes that have lost the ability to express the protein are non-pathogenic to humans (56, 57). Therefore, borreliacidal antibodies specific for OspC could be expected to provide protection in the tick and the human host. Moreover, there are multiple epitopes within OspC that induce borreliacidal antibodies (58, 60, 145, 146).

Because of these properties, investigators confirmed the ability of vaccination with OspC to protect laboratory mice 100% from infection with Lyme disease when challenged with spirochete-infected nymphs (147). However, the protection was limited to challenge with only the *Borrelia* strain that the OspC was derived from (109, 148, 149). In addition, subsequent studies (145, 150-152) confirmed considerable amino acid variability in the OspC protein, even among *B. burgdorferi* isolates from the same geographic region (153), was responsible for the limited protection. Therefore, the extreme heterogeneity of OspC cast considerable doubt on the widespread utility of an OspC-based product.

Strategies to Overcome the Heterogeneity of OspC

Despite the general lack of enthusiasm, however, some researchers continued to investigate the feasibility of strategies to overcome the heterogeneity of OspC. One approach was to incorporate multiple protective epitopes from within OspC proteins from multiple *Borrelia* genospecies into a single vaccine, and then evaluate the ability of such a vaccine to provide comprehensive protection. In support, Earnhardt and Marconi (154) formulated a chimeric protein comprised of peptides that corresponded to 8 linear epitopes within multiple OspC proteins and showed that the immune serum from mice vaccinated with the chimera produced complement-activating antibodies that bound multiple *Borrelia* spp..

Another option was to identify a single epitope that is conserved among multiple pathogenic *Borrelia* spp. In support, researchers (60, 155, 156) identified the epitope recognized most reliably by human antibodies, which was a section located within the last 7 amino acids nearest the C-terminus of OspC (AESPKKP), and showed the antibodies formed against the epitope were borreliacidal (59, 60). More significantly, by analyzing the existing *ospC* sequences in the BLAST database, the investigators confirmed the region was absolutely conserved among the pathogenic *Borrelia* spp. (60, 155, 157). Moreover, the epitope is reliably expressed on the surface of OspC-expressing spirochetes (155).

Significant Issues Impeding Further Evaluation of OspC

Despite the aforementioned possible solutions for overcoming the heterogeneity of OspC, however, further evaluations have not been forthcoming because of two significant issues. Most notably, *Borrelia* spp. express large amounts of OspA and only small amounts of OspC while growing in traditional laboratory BSK medium (158). Therefore, in vitro studies to evaluate the ability of anti-OspC antibodies to bind intact spirochetes are difficult to perform. Moreover, while OspC expression can be increased during laboratory culture by manipulating the incubation temperature (30, 31) or pH of the growth medium (31), the increased expression of OspA apparently still hinders the ability of OspC antibodies to bind (159). In addition, in contrast to human infection (39, 41, 54), *Borrelia* spp. express little or no OspC while infecting laboratory mice (60), so in vivo protection studies using a mouse animal model are also difficult to interpret**.** One possibility to overcome this shortcoming is to utilize rhesus macaques, since they have been shown to mount a robust immune response to many of the same epitopes as humans, including OspC (160-162), but factors such as exorbitant costs and logistical issues have apparently prevented their use.

SUMMARY

Lyme disease is the most prevalent vector-borne disease in the United States, and the annual number of worldwide cases continues to increase (19-21). A vaccine that provides reliable, long-term protection would therefore be extremely valuable. Several strategies for inducing effective antibody-mediated immunity by vaccination, including developing products that induce antibodies in the reservoir host to kill the spirochetes or stimulate the production of antibodies in human recipients that prevent ticks from feeding, are being pursued. However, the difficulty of effectively vaccinating large numbers of reservoir hosts and the rigor of evaluating a strategy to prevent tickbites continues to significantly hinder progress. Alternatively, researching have pursued the more traditional approach of developing a vaccine that induces a human antibody response that provides long-term, comprehensive protection against Lyme disease spirochetes. However, this task also continues to be hampered by several confounding factors that include the extreme intra- and interspecies heterogeneity, an inability to culture spriochetes in the laboratory that express surface proteins at similar levels as occurs during infection in the human host, and the lack of an inexpensive laboratory animal model where the antigenicity in vivo more closely mimics the antigenicity of the spirochetes during human infection.

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