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VIEWPOINTS

Protective Immunity and New Vaccines for Lyme Disease

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Lyme disease, caused by some *Borrelia burgdorferi sensu lato*, is the most common tick-borne illness in the Northern Hemisphere and the number of cases, and geographic spread, continue to grow. Previously identified *B. burgdorferi* proteins, lipid immunogens, and live mutants lead the design of canonical vaccines aimed at disrupting infection in the host. Discovery of the mechanism of action of the first vaccine catalyzed the development of new strategies to control Lyme disease that bypassed direct vaccination of the human host. Thus, novel prevention concepts center on proteins produced by *B. burgdorferi* during tick transit and on tick proteins that mediate feeding and pathogen transmission. A burgeoning area of research is tick immunity as it can unlock mechanistic pathways that could be targeted for disruption. Studies that shed light on the mammalian immune pathways engaged during tick-transmitted *B. burgdorferi* infection would further development of vaccination strategies against Lyme disease.

Keywords. Lyme disease; *Borrelia burgdorferi*; *Borreliella*; vaccines.

Lyme disease, also known as Lyme borreliosis, continues to grow both in terms of incidence and geographic spread. Like a number of other bacterial diseases, subsequent reinfections can occur due to lack of lasting immunity. Thus, individuals can get Lyme disease more than once if bitten by an infected tick. This compels the development of novel, broadly effective vaccines to control this vector-borne illness. Epidemiologic studies by the Centers for Disease Control and Prevention estimated that approximately 300 000 human cases of Lyme disease occur yearly in the United States [1]. In Europe, it is estimated that there are at least 100 000 annual cases. With the establishment of *Borrelia burgdorferi* as the etiological agent of Lyme disease in the United States [2, 3] it became evident that an array of clinical syndromes described in Europe were manifestations of the same disease due to infection with bacteria belonging to the *B. burgdorferi sensu lato* species complex.

This article developed following a series of meetings at the Cold Spring Harbor Laboratory Banbury Center to assess diagnostics (18–21 September 2016) as well as immunity and vaccine development to prevent Lyme disease (29 October–1 November 2017). The participants were from industry, academia, and government, with extensive experience in clinical and public health aspects, eco-epidemiological determinants of Lyme and other diseases, as well as development of vaccines (domestic animal, reservoir- and vector-targeted, and human). There was no intent to take a vote, or consensus, during the meeting; rather, there was discussion of research findings that support the best pathways forward. What emerged was a recognition among all participants that an effective vaccine is an important individual and public health tool to use in the United States and Europe.

Discussion of new vaccine candidates and strategies was centered around host immunity and the triad comprising the bacteria, the tick, and vertebrate reservoirs: how the bacteria can be targeted by additional vaccine candidates for direct application to humans and animals, how to disrupt transmission within the

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agents that maintain the enzootic cycle of *B. burgdorferi* (the tick and the reservoir), and how these indirect strategies would impact incidence of *B. burgdorferi* infection in accidental hosts (humans and domestic animals). The focus of discussion was on methods and approaches that can have practical use. Distinctions were made between vaccines that are achievable in the near future and those that are in preliminary developmental stages.

TARGETING THE SPIROCHETE IN THE VECTOR: OUTER SURFACE PROTEIN A

Two vaccines based on the outer surface protein A (OspA) of *B. burgdorferi* were developed in the 1990s [4, 5]. Fairly similar adjuvanted compositions were tested in clinical trials in humans [6, 7] and dogs [8]; vaccination reduced the risk of Lyme disease, thus demonstrating that immunization is a powerful intervention tool. Although effective, use of this vaccine in the general population was low and it was eventually discontinued by the manufacturer in 2002 [9]. Nevertheless, a second-generation OspA vaccine containing 6 different serotypes [10] entered a phase 2 clinical trial recently.

The discovery of the mechanism of action of OspA demonstrated that a vaccine administered to a mammalian host (eg, mouse) could effectively remove pathogenic bacteria from the tick vector [11, 12]. Further, the human clinical trials proved, for the first time in the history of bacterial vector-borne diseases, that a vaccine designed to eradicate a pathogen within the vector could prevent disease in humans. As such, it was the concept that catalyzed the development of new strategies to control Lyme disease that could bypass direct vaccination of the human host.

TARGETING THE SPIROCHETE IN THE HOST

Many strains of *B. burgdorferi* are maintained in the same local populations of infected mice and ticks, and host responses to 1 strain do not prevent infection with a different strain. It was recently found that the blood from a seropositive host profoundly attenuates the infectivity of homologous bacteria within the tick vector without killing them, thus preventing superinfection by homologous bacteria while facilitating transmission of heterologous *B. burgdorferi* strains [13]. In this section, we discuss how lipid immunogens, outer surface proteins, and live-mutant vaccines have been investigated for their potential to induce protective immune responses to *B. burgdorferi* infection and how any new Lyme disease host-targeted vaccines need to account for species and strain variability. One understudied area that would further the development of new vaccine candidates against Lyme disease is the understanding of the mammalian immune pathways engaged during tick-transmitted *B. burgdorferi* infection.

Outer Surface Protein C and Other *B. burgdorferi* Proteins

Outer surface protein C (OspC) of *B. burgdorferi* has been long considered as a vaccine candidate against Lyme disease. Synthesis of OspC is induced during the blood meal while spirochetes reside in the tick midgut, and it is required by *B. burgdorferi* for host colonization [11, 14, 15]. Antibody-mediated immunity to OspC can prevent dissemination of homologous *B. burgdorferi* to the host [13, 16] during early infection. However, due to OspC diversity, such protection is strain specific. Over 30 distinct OspC phyletic types have been identified worldwide. Vaccine candidates based on OspC have evolved from inclusion of a single OspC variant to laboratory-designed proteins composed of isolated linear epitopes from multiple OspC types [17]. A dual vaccine antigen composed of OspC (epitope chimeric protein—chimeritope) and OspA has been approved by the US Department of Agriculture to prevent clinical manifestations associated with infection by *B. burgdorferi* in canines. The efficacy of OspC chimeritope vaccines (without OspA) has not yet been assessed in humans or mice. Other *B. burgdorferi* proteins such as decorin-binding protein A (DbpA) and fibronectin-binding lipoprotein (BBK32) have been tested in multiplexed combinations containing 1 OspC variant; a cocktail composed of these 3 proteins proved to be partially protective against needle-inoculated *B. burgdorferi* in mice [18].

Lipid Immunogens

The lipid rafts of the outer membrane of *B. burgdorferi* are mostly associated with lipoproteins that assist this organism in its adaptation to different hosts. The role of cholesteryl glycolipids of *B. burgdorferi* has been studied in mice, as well as in humans, and they were shown to be immune-reactive. However, antibodies to *B. burgdorferi* glycolipids reacted with gangliosides endogenous to mammalian human and murine cells, and the reverse was also shown to be true [19, 20]. This bidirectional cross-reactivity could complicate the development of *B. burgdorferi* glycolipids as immunogens. Further, it is unknown if antibodies to the *B. burgdorferi* glycolipids are protective. These are essential questions that need to be answered as there are advantages to exploring this system for vaccine development. Cholesteryl glycolipids are components of the vesicles that are shed by *B. burgdorferi* in culture, and these vesicles can be harvested and examined for their protein cargo [21]. Vaccines from attenuated *B. burgdorferi* could be developed using extracellular vesicles in their native form, or synthesized in the laboratory with modified *B. burgdorferi* glycolipids and a set of specific immunogenic polypeptides in the proper orientation for antigen recognition. This approach could exploit the properties of the glycolipids as adjuvants and the polypeptides as the main immunogens. This is a novel area that can be explored further for its basic biology ramifications and potential application to human vaccines.

Live Mutant Vaccines

Live-attenuated mutant vaccines have been proven to be effective for immunization against several contagious infectious

diseases. In terms of *B. burgdorferi* infection, live-attenuated flagella-less and *p66* mutants of *B. burgdorferi* can elicit partial or fully protective immunity in mice [22]; these mutants are also more effective than killed bacteria. Although such live mutants are incapable of establishing infection in mammalian hosts, this approach is unlikely to be used for human applications. Nevertheless, it could lead to identification of some individual targets with protective efficacy to develop new recombinant vaccine candidates; further, these mutants could be used to develop additional reservoir-targeted or other animal vaccines.

TRANSMISSION-BLOCKING VACCINES

Ecological approaches to reduce tick density, to decrease *B. burgdorferi* burden in ticks, and to eliminate transmission dynamics have been explored. Transmission-blocking vaccines, composed of reservoir-targeted and anti-tick vaccines, are promising tools to reduce Lyme disease. Deployment of effective transmission-blocking strategies as public health tools to control the incidence of human disease hinges on our understanding of the eco-epidemiologic determinants that inform potential Lyme disease risk or exposure, and on development of proper delivery vehicles for the vaccine.

Eco-epidemiological Determinants

Lyme disease risk is geographically clustered and is determined by the complex interaction among the environmental hazard represented by the density of infected host-seeking *Ixodes scapularis* nymphal ticks, people's behavior influencing exposure to the hazard, and people's ability to intervene to reduce disease risk or severity. A near-nationwide map (except for California) of this hazard in the United States was drawn from data collected in 2004–2007 based on large-scale field collections of host-seeking *B. burgdorferi*–infected *I. scapularis* nymphs [23]. This map shows regions of high hazard in the Northeast and Upper Midwest, with lower hazard in the South, generally corresponding to observed geographical patterns of Lyme disease incidence in humans. Regional differences in incidence are further explained by distinct wildlife host communities and differences in nymphal *I. scapularis* host-seeking behavior in the North versus the South [24]. Updated, accurate maps of the expanding environmental hazard, as well as further research on the determinants of Lyme disease risk at multiple spatial and temporal scales, are required to best optimize, target, implement, and evaluate the efficacy of a vaccine. Furthermore, accurate maps of possible Lyme disease spread will help clinicians in border regions stay vigilant and are critical for clinical decision making.

Reservoir and Vector-targeted Vaccines

Vaccines aimed at animal reservoirs affect the natural enzootic cycle and reduce hazard by decreasing the number of infected vectors. This hypothesis was first tested in the United States by subcutaneous vaccination of wild white-footed mice (*Peromyscus leucopus*) with purified recombinant OspA and subsequent determination of reductions in nymphal infection prevalence the year after treatment [25]. Deployment of reservoir-targeted vaccines as part of integrated pest-management interventions is strictly dependent on the development of oral vehicles for delivery of the immunogen [26–28]. OspA-based vaccines are effective against most species and strains of *B. burgdorferi* (ie, heterologous challenge). A 5-year field trial of an orally delivered *P. leucopus*–targeted transmission-blocking vaccine showed that OspA-specific seropositivity in resident *P. leucopus* mice led to reductions in infection prevalence of the nymphal ticks collected in those field sites (23–76%) in a cumulative time-dependent manner [29]. Other outer surface proteins of *B. burgdorferi* (BB0405, BBA52, BBI39) and tick antigens (subolesin, salivary proteins, tick salivary lectin pathway inhibitor, tick histamine release factor) have been evaluated as potential transmission-blocking vaccine candidates [30–32]. Some of these candidates are fully protective against homologous challenge, some are partially protective against heterologous challenge, but unlike OspA, none of the new candidates are fully protective against heterologous challenge with tick-transmitted *B. burgdorferi*. The European Commission funded a consortium, designated Anti-tick Vaccines to Prevent Tick-borne Diseases in Europe, of 7 institutes to identify and characterize tick proteins involved in feeding and pathogen transmission. A subset of the transmission-blocking and anti-tick vaccine candidates that were identified are currently undergoing efficacy studies in experimental animal models of tick-borne Lyme disease [33].

Tick Immunity

A compelling argument for the role of tick immunity was the demonstration that targeting the tick antigen subolesin could be used for the control of *Rhipicephalus* (*Boophilus*) *microplus* tick infestations in cattle [34]. Laboratory models of nonreservoir hosts such as guinea pigs and rabbits develop a strong immune response to tick salivary proteins and reject ticks upon repeated tick infestations, a phenomenon coined as "tick immunity." Anecdotal and epidemiological evidence suggests that humans who are frequently exposed to tick bites might also develop resistance to ticks. *Mus musculus*, a laboratory model of the natural reservoir host, does not develop resistance to *I. scapularis* upon repeated tick infestations. Studies to address this dichotomy in host–vector interactions suggest that the salivary transcriptome and proteome are different in mouse- and guinea pig–fed *I. scapularis,* and that these differences might guide distinct host immune responses. Further, several genes are similarly expressed by *I. scapularis* when feeding on diverse hosts and likely represent the core set of functions critical for feeding [35]. These findings reveal a new insight into vector–host

interactions and provide a new model to better understand tick functional genomics. Perhaps it is the core proteome that needs to be deciphered to determine whether these proteins might be targeted with a vaccine to disrupt tick feeding and consequently thwart the transmission of *B. burgdorferi* and other pathogens.

RATIONAL DESIGN APPROACHES FOR VACCINE DEVELOPMENT

Modern vaccine approaches are evolving with rapid-on-demand flexibility and are based on rational design. Of note is the generation of novel adjuvant molecules with potent immunostimulatory properties resulting in an increase in the US Food and Drug Administration–approved adjuvant-containing vaccines (eg, Cervarix and Shingrix, GlaxoSmithKline). Moreover, the application of contemporary molecular and genetic approaches has guided the coherent design of protein- and peptide-based antigens to target immunodominant epitopes, retain cross-reactivity properties to pathogen families, or remove potential self-reactivity. Finally, the advancement in nucleic acid–based vaccines (eg, DNA and RNA) with improved delivery and immunogenicity provides a platform for the delivery of rationally designed antigens. Together, such considerations for future development of vaccines against Lyme disease could be applied to newly defined antigens, or to previously defined immunogens that failed to provide sufficient efficacy or safety profiles.

PUBLIC EDUCATION, PUBLIC HEALTH PERSPECTIVES OF VACCINATION, AND VACCINE TRIALS

The development of new vaccines provides a great opportunity to educate colleagues and the public about advantages and hurdles of their application. In particular, how is good efficacy defined, what is cost-effective, what are the expected side effects, how is their causal significance assessed, and what are the acceptable risk–benefit ratios? Vaccines have been used successfully for hundreds of years against contagious diseases such as influenza, measles, smallpox, and pneumococcus. When the pathogen is highly contagious, vaccines are most effective when a large population is vaccinated, creating herd immunity, and leading to the protection of the individual and of the community. A small but vocal part of the public has had concerns that vaccines can cause severe adverse effects and have opposed mandatory use [36]. An instructive example is the public association of autism following vaccination with the mumps, measles, and rubella vaccine, which resulted from the publication and widespread lay-press commentary of a now retracted peer-reviewed paper that has been scientifically disproved for over 20 years [37], without improvement in adverse public perception. The OspA vaccine is another example that illustrates how public concern arose from a hypothesis that was disproved over a decade ago by the Lyme disease scientific community. In the Lyme disease case, several studies showed no difference in adverse effects between vaccinated individuals and placebo controls [38], thus corroborating previous published findings [39]. These studies may have encouraged the development of alternative formulations of the OspA vaccine currently undergoing clinical trials [10]. Furthermore, research into barriers of Lyme disease vaccine acceptability could be helpful in maximizing the potential for such a vaccine, if and when another comes to market.

As new vaccine efficacy trials begin for Lyme disease, it is important to recognize factors that may lead to false conclusions of vaccine failure. These include rashes that are very similar in appearance to erythema migrans produced by the bite of the lone star tick that causes a disease of unknown etiology, the southern tick–associated rash illness [40]. Furthermore, atypical erythema migrans rashes may be mimicked by a spider bite or drug eruption. Other factors to consider are infections than can cause cross-reactivity in certain Lyme disease serologic tests (eg, syphilis, rheumatoid arthritis, severe periodontitis, and relapsing fever caused by *Borrelia miyamotoi*). Non–*B. burgdorferi* infections that cause seroconversion would by themselves be a false indicator of vaccine failure.

Last, it should be highlighted that Lyme disease is a noncontagious vector-borne infection; consequently, the disease may develop if an infected vector feeds on a host. A vaccine directed against the causative agent *B. burgdorferi*, or against the tick vector that transmits this bacteria, will only protect the vaccinated person; thus, in this case, herd immunity does not apply toward protection of the community. In contrast to the public health goal of protecting the population against a highly contagious disease, which is often mandated by government officials in most Western countries, Lyme disease vaccination is an individual's personal choice, advisable to people at risk. A decision to be vaccinated should be based on scientific evidence such as risk of exposure in areas where the infected vector is present and the disease is endemic. The concept of personal immunization against a noncontagious disease versus widespread vaccination to prevent the spread of a contagious infection should be part of public education.

CONCLUSIONS

The rise and spread of Lyme disease, strain-specific immunity, and the fact that individuals can get Lyme disease more than once when bitten by an infected tick, compels and complicates the development of novel effective vaccines to control this vector-borne illness. These countermeasures include decreasing the number of infected ticks in the environment and protection of the individual. Vaccines are proven preventive measures against contagious and noncontagious infectious agents; the first-generation vaccine significantly reduced Lyme disease risk in vaccinated humans and continues to do so in companion canines. One of the most important observations

early on during Lyme vaccine development was that vaccination of the host produced an antibody response that effectively reduced infection in the vector upon tick feeding. This paradigm fostered the development of new approaches for control of Lyme disease and other vector-borne infectious agents focused on upstream blockage of the pathogen, before it can reach the host. It is plausible to envision the development of multiantigenic hybrid vaccines targeted both to the offending microbe(s) and to the vector carrier. We are now positioned at a crossroad where advanced technologies allow for the application of new genetic strategies for immunization, possible identification of new immunogens, and repurpose of proven vaccine candidates not only for humans but also for domestic animals and environmental reservoirs.

Notes

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