Protective Immunity and New Vaccines for Lyme Disease

Maria Gomes-Solecki,1 Paul M. Arnaboldi,2 P. Bryon Backenson,3 Jorge L. Benach,4 Christopher L. Cooper,5 Raymond J. Dattwyler,6 Maria Diuk-Wasser,7 Erol Fikrig,8 J. W. Hovius,9 Will Laegreid,10 Urban Lundberg,11 Richard T. Marconi,11 Adriana R. Marques,12 Philip Molloy,13 Sukanya Narasimhan,7 Utpal Pal,14 Joao H. F. Pedra,15 Stanley Plotkin,16 Daniel L. Rock,17 Patricia Rosa,18 Sam R. Telford III,19 Jean Tsao,20,21 X. Frank Yang,22 and Steven E. Schutzer23

1Department of Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee, USA; 2Department of Microbiology/Immunology, New York Medical College, New York, USA; 3New York State Department of Health, Albany, New York, USA; 4Department of Molecular Genetics and Microbiology, Stony Brook University, New York, USA; 5Molecular and Translational Sciences, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA; 6Department of Ecology, Evolution, and Environmental Biology, Columbia University, New York, USA; 7Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, USA; 8Department of Internal Medicine, Section of Infectious Diseases, Amsterdam Multidisciplinary Lyme Borreliosis Center, Amsterdam University Medical Centers, Academic Medical Center, The Netherlands; 9Department of Veterinary Sciences, Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, Wyoming, USA; 10Valneva Austria GmbH, Vienna, Austria; 11Department of Microbiology and Immunology, Virginia Commonwealth University Medical Center, Richmond, Virginia, USA; 12Lyme Disease Studies Unit, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA; 13Imugen, Norwood, Massachusetts, USA; 14Department of Veterinary Medicine, University of Maryland, College Park, Maryland, USA; 15Department of Microbiology and Immunology, University of Maryland School of Medicine, Maryland, USA; 16Department of Pediatrics, University of Pennsylvania, Philadelphia, Pennsylvania, USA; 17College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Illinois, USA; 18Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA; 19Department of Infectious Disease and Global Health, Tufts University, North Grafton, Massachusetts, USA; 20Fisheries and Wildlife and 21Large Animal Clinical Sciences, Michigan State University, East Lansing, Michigan, USA; 22Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana, USA; and 23Department of Medicine, Rutgers New Jersey Medical School, Newark, New Jersey, USA

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*; Borrelia; vaccines.
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 phylogenetic 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. *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 noncontagous 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

Acknowledgments. The authors thank Rebecca Leshan, PhD, and the Cold Spring Harbor Laboratory Banbury Center Staff for the support offered with organization and hosting of the meeting. The authors gratefully acknowledge that, throughout many years, research in this area was supported in part by the Intramural and Extramural Research Programs of the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Centers for Disease Control and Prevention, and the European Commission.

Disclaimer. The content of this publication does not necessarily reflect the views or policies of the New York State Department of Health or of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Army.

Financial support. The meeting referred to in the text was supported by a grant to the Cold Spring Harbor Laboratory Banbury Center from the Steven and Alexandra Cohen Foundation. Meeting sponsors had no participation in the content of the meeting, or in preparation of the manuscript.

Potential conflicts of interest. The following authors declare potential conflicts of interest: M. G.-S. (grants from the National Institutes of Health [NIH] and the Centers for Disease Control and Prevention; salaried president and CEO of Immuno Technologies, Inc; stockholder in US Biologic, Inc; patents 7605248, 7582304, and 20090297560A1; and consultant for Boehringer Ingelheim), P. B. B. (cooperative agreement), R. J. D. (salaried president and CEO of Biopeptides, Inc, and US patents), E. P. (former royalty from GlaxoSmithKline [GSK]); J. W. H. (grants from the European Commission, personal fees from Merck Sharp & Dohme, grants from Intravacc), R. T. M. (grants from Steven and Alexandra Cohen Foundation, NIH, and Zoetis; patents; royalties), P. M. (employment by Imugen), A. R. M. (consultant for Glaxo Loyale Alliance and American Lyme Disease Foundation), S. P. (consultant for Sanoﬁ, Merck, GSK, and Pfizer; unpaid consultant to Valneva), and S. R. T. (grants from the NIH; consultant for Fuller Laboratories, Meridian Biosciences, and Oxford Immunotec). The other authors report no potential conflicts. The authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Nelson CA, Saha S, Kugler KJ, et al. Incidence of clinician-diagnosed Lyme disease, United States, 2005-2010. Emerg Infec Dis 2015; 21:1625–31.
2. Benach JL, Bosler EM, Hanrahan JP, et al. Sirophetes isolated from the blood of two patients with Lyme disease. N Engl J Med 1983; 308:740–2.
3. Ackermann R, Kabatzi R, Boisten HP, et al. Ixodes ricinus spirochete and European erythema chronicum migrans disease. Yale J Biol Med 1984; 57:573–80.
4. Fikrig E, Barthold SW, Kantor FS, Flavell RA. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. Science 1990; 250:553–6.
5. Schäible UE, Wallach R, Kramer MD, et al. Immune sera to individual Borrelia burgdorferi isolates or recombinant OspA thereof protect SCID mice against infection with homologous strains but only partially or not at all against those of different OspA/OspB genotype. Vaccine 1993; 11:1049–54.
6. Steere AC, Sikand VK, Meurice F, et al. Lyme Disease Vaccine Study Group. Vaccination against Lyme disease with recombinant Borrelia burgdorferi outer-surface lipoprotein A with adjuvant. N Engl J Med 1998; 339:209–15.
7. Sigal LH, Zahradnik JM, Lavin P, et al. Recombinant Outer-Surface Protein A Lyme Disease Vaccine Study Consortium. A vaccine consisting of recombinant Borrelia burgdorferi outer-surface protein A to prevent Lyme disease. N Engl J Med 1998; 339:216–22.
8. Eschner AK, Mugnaini K. Immunization with a recombinant subunit OspA vaccine markedly impacts the rate of newly acquired Borrelia burgdorferi infections in client-owned dogs living in a coastal community in Maine, USA. Parasit Vectors 2015; 8:92.
9. Nigrovic LE, Thompson KM. The Lyme vaccine: a cautionary tale. Epidemiol Infect 2007; 135:1–8.
10. Comstedt P, Schuler W, Meineke A, Lundberg U. The novel Lyme borreliosis vaccine VLA15 shows broad protection against Borrelia species expressing six different OspA serotypes. PLoS One 2017; 12:e0184357.
11. Schwam TG, Pierson J, Golde WT, Dolan MC, Rosa PA. Induction of an outer surface protein on Borrelia burgdorferi during tick feeding. Proc Natl Acad Sci USA 1995; 92:2909–13.
12. de Silva AM, Telford SR III, Brunet LR, Barthold SW, Fikrig E. Borrelia burgdorferi OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. J Exp Med 1996; 183:271–7.
13. Bhatia R, Hillman C, Carraci V, Chefni BN, Tilly K, Rosa PA. Infection history of the blood-meal host dictates pathogenic potential of the Lyme disease spirochete within the feeding tick vector. PLoS Pathog 2018; 14:e1006959.
14. Grimm D, Eggers CH, Caimano MJ, et al. Experimental assessment of the roles of linear plasmin lip2p and lip2b-1 of Borrelia burgdorferi throughout the infectious cycle. Infect Immun 2004; 72:5938–46.
15. Pal U, Yang X, Chen M, et al. OspC facilitates Borrelia burgdorferi invasion of Ixodes scapularis salivary glands. J Clin Invest 2004; 113:220–30.
16. Bockenstedt LK, Hodzic E, Feng S, et al. Borrelia burgdorferi strain-specific OspC-mediated immunity in mice. Infect Immun 1997; 65:4661–7.
17. Earnhart CG, Marconi RT. An octavalent Lyme disease vaccine induces antibodies that recognize all incorporated OspC-type specific sequences. Hum Vaccin 2007; 3:281–9.
18. Brown EL, Kim JH, Reisenbichler ES, Hook M. Multicomponent Lyme vaccine: three is not a crowd. Vaccine 2005; 23:3687–96.
19. Garcia-Monco JC, Seidman R, Benach J. Experimental immunization with Borrelia burgdorferi induces development of antibodies to ganglossides. Infect Immun 1995; 63:4310–7.
20. Stöb S, Fingerle V, Wilcke B, et al. Acylated cholesteryl galactosides are specific antigens of borreliosis causing Lyme disease and frequently induce antibodies in late stages of disease. J Biol Chem 2009; 284:13326–34.
21. Crowley JT, Toledom AM, LaRocca TJ, Coleman JL, London E, Benach JL. Lipid exchange between Borrelia burgdorferi and host cells. PLoS Pathog 2013; 9:e1003109.
22. Hahn BL, Padmore LJ, Ristow LC, Curtis MW, Coburn J. Live attenuated borrelia burgdorferi targeted mutants in an infectious strain background protect mice from challenge infection. Clin Vaccine Immunol 2016; 23:725–31.
23. Duk-Wasser MA, Hoen AG, Csolo P, et al. Human risk of infection with Borrelia burgdorferi, the Lyme disease agent, in eastern United States. Am J Trop Med Hyg 2012; 86:320–7.
24. Arsnow I, Tsao JI, Hickling GJ. Nymphal Ixodes scapularis questing behavior explains geographic variation in Lyme borreliosis risk in the eastern United States. Ticks Tick Borne Dis 2019; 10:553–63.
25. Tsao JI, Wootton JT, Bunikis J, Luna MG, Fish D, Barbour AG. An ecological approach to preventing human infection: vaccinating wild mouse reservoirs inter-venes in the Lyme disease cycle. Proc Natl Acad Sci USA 2004; 101:18159–64.
26. Gomes-Solecki MJ, Brisson DR, Dattwyler RJ. Oral vaccine that breaks the transmission cycle of the Lyme disease spirochete can be delivered via bait. Vaccine 2006; 24:4440–9.
27. Scheckelhoff MR, Telford SR, Hu LT. Protective efficacy of an oral vaccine to reduce carriage of Borrelia burgdorferi (strain N40) in mouse and tick reservoirs. Vaccine 2006; 24:1949–57.
28. Meiries Rich L, Aroso M, Contente-Cuomo T, Ivanova L, Gomes-Solecki M. Reservoir targeted vaccine for Lyme borreliosis induces a yearlong, neutralizing antibody response to OspA in white-footed mice. Clin Vaccine Immunol 2011; 18:1809–16.
29. Richer LM, Brisson D, Melo R, Ostfeld RS, Zeidner N, Gomes-Solecki M. Reservoir targeted vaccine against Borrelia burgdorferi: a new strategy to prevent Lyme disease transmission. J Infect Dis 2014; 209:172–80.
30. Bensaci M, Bhattacharya D, Clark R, Hu LT. Oral vaccination with vaccinia virus expressing the tick antigen subolesin inhibits tick feeding and transmission of Borrelia burgdorferi. Vaccine 2012; 30:6040–6.

31. Schuijt TJ, Hovius JW, van der Poll T, van Dam AP, Fikrig E. Lyme borreliosis vaccination: the facts, the challenge, the future. Trends Parasitol 2011; 27: 40–7.

32. Kung F, Kaur S, Smith AA, et al. A Borrelia burgdorferi surface-exposed transmembrane protein lacking detectable immune responses supports pathogen persistence and constitutes a vaccine target. J Infect Dis 2016; 213:1786–95.

33. Hofhuis A, Harms M, van den Wijngaard C, Sprong H, van Pelt W. Continuing increase of tick bites and Lyme disease between 1994 and 2009. Ticks Tick Borne Dis 2015; 6:69–74.

34. Merino O, Almazán C, Canales M, et al. Control of Rhipicephalus (Boophilus) microplus infestations by the combination of subolesin vaccination and tick autocidal control after subolesin gene knockdown in ticks fed on cattle. Vaccine 2011; 29:2248–54.

35. Coumou J, Wagemakers A, Narasimhan S, et al. The role of mannose binding lectin in the immune response against Borrelia burgdorferi sensu lato. Sci Rep 2019; 9:1431.

36. Aps LRMM, Piaptola MAF, Pereira SA, Castro JT, Santos FAO, Ferreira LCS. Adverse events of vaccines and the consequences of non-vaccination: a critical review. Rev Saude Publica 2018; 52:40.

37. Hviid A, Hansen JV, Frisch M, Melbye M. Measles, mumps, rubella vaccination and autism: a nationwide cohort study. Ann Intern Med 2019; 170:513–520. doi:10.7326/M18-2101.

38. Steere AC, Drouin EE, Glickstein LJ. Relationship between immunity to Borrelia burgdorferi outer-surface protein A (OspA) and Lyme arthritis. Clin Infect Dis 2011; 52(Suppl 3):s259–65.

39. Lathrop SL, Ball R, Haber P, et al. Adverse event reports following vaccination for Lyme disease: December 1998-July 2000. Vaccine 2002; 20:1603–8.

40. Wormser GP, Masters E, Nowakowski J, et al. Prospective clinical evaluation of patients from Missouri and New York with erythema migrans-like skin lesions. Clin Infect Dis 2005; 41:958–65.