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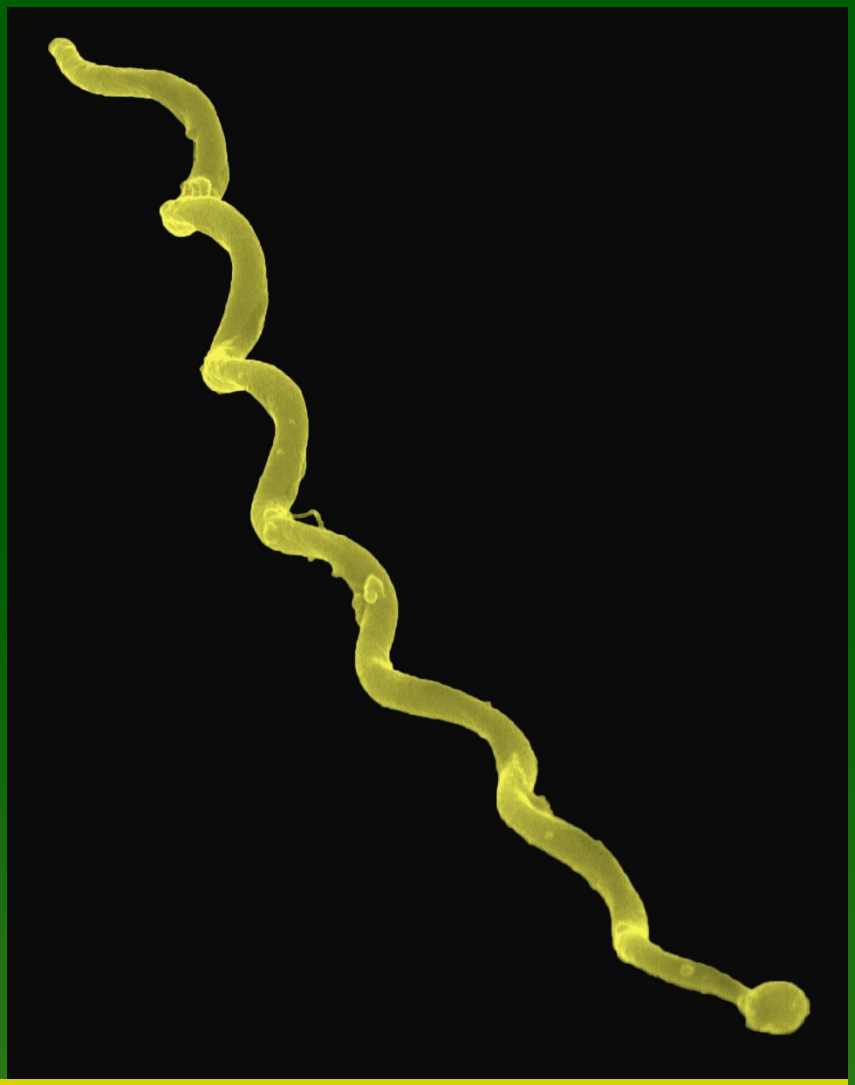

Current Efforts in Lyme Disease Research, 2017

National Institute of Allergy and Infectious Diseases

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Current Efforts in Lyme Disease Research

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Introduction

Ticks can transmit a variety of disease-causing pathogens, including those responsible for Rocky Mountain spotted fever, ehrlichiosis, anaplasmosis, babesiosis, tick-borne encephalitis, and Powassan disease. Lyme disease, the most prevalent of the tick-transmitted infections, is named after a town in Connecticut where a group of arthritis cases in children appeared in the early 1970s. By the mid-1970s, the symptoms of the disease were being described. The pathogen that causes Lyme disease was identified in 1981 by Willy Burgdorfer, Ph.D. and Alan Barbour, M.D., scientists at the Rocky Mountain Laboratories of the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), with their colleague Jorge Benach, Ph.D. at SUNY Stonybrook. The bacterium, transmitted by deer ticks, is now named *Borrelia burgdorferi* [1].

Typically, the first symptom of Lyme disease is a rash known as erythema migrans, which starts as a small red spot at the site of the tick bite and gets larger over a period of several days, usually forming a circular or oval-shaped rash. The rash can be all red, or it may have some clearing and look like a bull's eye. The rash can range in size and may be accompanied by other symptoms such as fever, headache, stiff neck, body aches, and fatigue. Although these symptoms may be similar to common viral infections, Lyme disease symptoms tend to last longer or may come and go over time. Untreated, the infection can spread, with additional rashes appearing at different sites on the body. The development of facial or Bell's palsy (weakness of the muscles on one or both sides of the face), meningitis, and/or heart involvement (light-headedness, fainting, shortness of breath, heart palpitations, or chest pain) may also occur. Additionally, some people who have Lyme disease may develop arthritis, usually in the large joints such as the knees [2, 3].

The diagnosis of Lyme disease is based on signs and symptoms of the infection, history of exposure to infected deer ticks, and laboratory tests. Serologic tests are most helpful for patients who have later manifestations of the disease, as it can take several weeks after infection for these tests to become positive. Patients with early Lyme disease should be treated without serologic testing [4].

In most individuals, Lyme disease is effectively treated with antibiotics. A small percentage of patients report a range of sometimes debilitating symptoms called Post-Treatment Lyme Disease Syndrome (PTLDS), which may continue years after treatment [5]. The reasons for these symptoms are unknown. Some evidence points to an autoimmune disease, perhaps triggered by the initial infection or by remnants of the original infection.

History of NIAID's Lyme Disease Research Program

Since Burgdorfer and Barbour's seminal discovery in 1981, NIAID has supported an extensive and diverse research portfolio that encompasses basic and clinical research studies on Lyme disease. These studies are conducted by extramural and intramural investigators, including intramural scientists at NIAID's Rocky Mountain Laboratories in Hamilton, Montana and at NIAID laboratories in Bethesda, Maryland.

The institute has remained at the forefront of Lyme disease research and continues to address fundamental questions essential for progress in the field. NIAID's many contributions include:

- Helping to sequence the entire genome of *B. burgdorferi* and improving overall molecular tools available for studying the organism and response to infection. Without these early tools, research on the basic biology and disease-causing properties of *B. burgdorferi* would have lagged far behind that of other bacteria.

- Sequencing the genome of the tick that transmits *B. burgdorferi, Ixodes scapularis* (https://www.ncbi.nlm.nih.gov/pubmed/26856261). This sequence is important for understanding the genetic basis of tick biology and tick/*Borrelia* interactions. Genomic data and bioinformatics tools for researchers are available in VectorBase (https://www.vectorbase.org/organisms/ixodes-scapularis).

- Funding and conducting basic research on disease transmission, including research on ticks and their role in the disease cycle, tick biology and behavior, and tick/pathogen and tick/host interactions.

- Funding and conducting fundamental studies on the human immune response to *B. burgdorferi,* identifying bacterial factors involved in that response, and studying disease pathogenesis. The identification of potential biomarkers of *B. burgdorferi* has informed current diagnostic tests and continues to be explored for development of improved tests.

- Addressing questions concerning the efficacy of long-term antibiotic therapy for the treatment of late or "chronic Lyme disease" through the support of three placebo-controlled clinical trials. The peer-reviewed, published results of these studies demonstrate that prolonged antibiotic therapy does not have a sustained benefit and that risks to the patient outweigh the benefits (https://www.niaid.nih.gov/diseases-conditions/chronic-lyme-disease).

- Investigating the control of Lyme disease and other tick-borne pathogens by better understanding vector interactions with the pathogen and vertebrate host. Tick control approaches may involve the use of repellents and treatment of wildlife and pets.

Ongoing Research

The overarching goals of the NIAID Lyme disease research program are to develop better methods of diagnosing, treating, and preventing this disease in humans. To accomplish these objectives, the NIAID Lyme disease research portfolio includes a broad range of activities focusing on the pathogen, the vector, and the vertebrate host. These activities are conducted by extramural and intramural investigators and span basic science through human clinical research studies. Many of these projects are investigator-initiated efforts, but several are the result of targeted grant and small business initiatives that NIAID has used to actively grow the Lyme disease research portfolio to address high priority areas such as persistence of infection after antibiotic treatment and the development of both early- and late-stage diagnostics.

NIAID utilizes all of these approaches to ensure that it maintains a vibrant and multifaceted

Lyme disease research portfolio that addresses key basic, translational, and clinical research questions. The NIAID Lyme disease research portfolio includes systematic studies on microbial physiology; molecular, genetic, and cellular mechanisms of pathogenesis; mechanisms of protective immunity; vector biology and ecology; vector/pathogen and vector/vertebrate host interactions and influence on disease transmission; effect of co-infections with other tick-borne diseases; efficacy of different modes of antibiotic therapy; and development of more sensitive and reliable diagnostic tests for both early and late Lyme disease.

Additional information on NIAID-funded Lyme disease research and tick-borne disease research is available on the **NIAID website**. Detailed information about NIH funding for Lyme disease research is available on the NIH Categorical Spending page, accessible from the NIH Research Portfolio Online Reporting Tools (**RePORT**) website. Here, project lists are available that show funding amounts for each project and the NIH Institute or Center that has provided the support. These lists can be downloaded for further analysis. All funded extramural NIAID research projects are reviewed by a panel of scientific experts with a second level of review by the NIAID Advisory Council before support is initiated. This process ensures that only the highest quality science is supported.

The following summary highlights current major areas of research in NIAID's extramural and intramural Lyme disease research portfolio:

Studies on the Vector

NIAID supports basic research on *Ixodes scapularis*, the tick that transmits Lyme disease and other bacterial and arboviral infections. Ongoing efforts include research on tick feeding and factors in tick saliva that affect transmission. These factors include proteins that block blood clotting, the pain response to a tick bite, or other factors that may enhance the bacteria's chance of infecting humans. NIAID-supported researchers are also investigating the tick host-finding system and the development of ticks with reduced ability to transmit *Borrelia*, which could be used to interrupt the natural cycle of disease and its transmission to humans.

Studies on Pathogenesis

Studies on bacterial pathogenesis can help scientists understand how bacteria infect the host, multiply, and ultimately cause human disease. NIAID plays a key role in the support of basic pathogenesis studies to help understand how Lyme disease develops. Better understanding of disease pathogenesis can help to identify new targets for future diagnostics, therapeutics, and vaccines.

- A comprehensive analysis of *B. burgdorferi* surface proteins and their cellular counterparts could help scientists understand the unique dissemination characteristics and persistence of the bacterium.

- Structure/function studies of the Lyme bacterium flagellar motor may help scientists understand how *B. burgdorferi* and related bacteria travel through the body and affect multiple body sites.

- Studies aimed at understanding the surface composition of *B. burgdorferi* at the biochemical and molecular level may lead to novel ways of disrupting the interaction of the bacteria with the host or the generation of novel vaccine/diagnostic targets for future testing.

Studies on Persistence of Infection

NIAID also supports basic research projects to address key questions regarding persistence of infection in animal model systems (mice and non-human primates) and in human clinical studies.

- Studies of interactions between the tick and its vertebrate hosts, such as mice and deer, at different life cycle phases may reveal more about how *B. burgdorferi* evades the host immune system and provide clues on how to limit or prevent immune evasion.

- Analysis of an abundant bacterial surface protein (VlsE), known to vary its surface antigens, may help scientists understand how *B. burgdorferi* evades the host immune system.

- Real-time imaging analysis of *B. burgdorferi* infection in mice allows scientists to track the infection at the cellular level and to monitor treatment effectiveness.

- Xenodiagnosis is a strategy that uses the tick vector to detect the presence of *B. burgdorferi* in patients. A recent collaboration between NIAID scientists and Tufts Medical School showed the feasibility and safety of using laboratory-reared, disease-free larval ticks to search for persistence of *B. burgdorferi* infection after antibiotic therapy in Lyme patients. *B. burgdorferi* DNA was found in some ticks fed on patients, and a larger and more comprehensive study is underway to determine the significance of the finding.

Studies on Lyme Disease Diagnostic Testing

Currently, there is no point-of-care diagnostic test for Lyme disease that can substitute for laboratory-based testing. A point-of-care diagnostic would enable physicians to make more informed treatment decisions when a patient in a Lyme-endemic area presents with symptoms consistent with Lyme disease. In recent years, NIAID has published targeted research initiatives and utilized the Small Business Innovation Research funding mechanism to inform the research community of its interest in advancing research on new diagnostic tools for Lyme disease. For example, several projects supported under the request for applications, *Improved Diagnostic Capabilities for Select Biodefense and Emerging Pathogens* (RFA-AI-11-024), were transitioned to later-stage development in 2014 and are ongoing.

- The development and testing of a new cytokine-based immunoassay for Lyme diagnosis, if successful, could allow for earlier and more rapid diagnosis of Lyme disease.

- Metabolic biomarkers and biosignatures for improved diagnostics are being identified and characterized. These studies may contribute to new methods for detecting Lyme disease, including earlier-stage diagnosis, accurate staging of disease, or indications of successful treatment.

- Several investigators are working on the development of a new, rapid point-of-care Lyme diagnostic test using lateral flow technologies.

Studies on Lyme Vaccines

The first Lyme disease vaccine, LYMErix, was approved for use in humans in 1998 but was voluntarily withdrawn by the manufacturer in 2001 due to low public demand. NIAID was not directly involved in the design and implementation of the LYMErix vaccine trials. However, patents for cloning the genes used in making the vaccine, as well as knowledge of how certain antibodies contribute to protective immunity, were derived from basic research grants funded by NIAID. NIAID-supported efforts have continued to build upon these advancements and have helped to spur new vaccine approaches currently under investigation.

- Investigators are characterizing novel vaccine formulations and targets, including approaches that target proteins in tick saliva that are critical for the transmission of the Lyme bacteria to humans.
- Several research groups are investigating the potential of reservoir vaccines to reduce Lyme disease in humans. These oral "bait" vaccines are aimed at preventing mice from becoming infected, thereby interrupting the transmission cycle [6]. Some groups are field testing their products with support from the Centers for Disease Control and Prevention (CDC).
- Researchers have identified tick proteins that facilitate transmission of Lyme disease bacteria or that enhance survival of those bacteria in vertebrate hosts. Studies are ongoing to see if vaccines specifically targeting some of these proteins may be used as a strategy for an "anti-tick vaccine" to be used to prevent disease.

Autoimmunity and Lyme Disease

Understanding the mechanisms of inflammatory disorders that accompany PTLDS and/or antibiotic-refractory Lyme arthritis may inform the development of therapies to help those impacted by these symptoms.

- Auto-antigens, which are thought to initiate or perpetuate an autoimmune response, may be at least partially responsible for post-infectious Lyme disease symptoms. Investigators are screening multiple candidate auto-antigens, and one auto-antigen has been characterized.

- Scientists are characterizing the reactivity of nervous system antibodies, which seem to differ between those patients that develop PTLDS and those that do not. These studies

may lead to an understanding of why some patients continue to have ongoing symptoms and may open new avenues for treatment.

Clinical Studies

NIAID intramural researchers at the Bethesda, Maryland, campus run a research program that aims to advance scientific knowledge of Lyme disease and translate these advances into clinical practice. Patients with Lyme disease are studied at the NIH Clinical Center with the goals of ameliorating disease and improving prognosis, as well as increasing understanding of the laboratory diagnosis, clinical manifestations, and immunological responses associated with *B. burgdorferi* infection. More than 500 volunteers are currently enrolled in ongoing studies focused on:

- Evaluation, treatment, and follow-up of Lyme disease patients to assess clinical course and outcomes and define immune responses to infection.
- Identification of biomarkers of infection to aid development of new diagnostic tools.
- Investigation of potential causes of PTLDS.
- Exploration of potential persistence of infection after antibiotic therapy for Lyme disease.

Information about these and other studies can be found on ClinicalTrials.gov, a registry and results database of publicly and privately supported clinical studies of human participants. For information on NIAID-conducted studies, refer to the study title or ClinicalTrials.gov identifier provided in the table below:

Study Title	ClinicalTrials.gov Identifier
Xenodiagnosis After Antibiotic Treatment for Lyme Disease	NCT02446626
Evaluation, Treatment, and Follow-up of Patients with Lyme Disease	NCT00028080
Evaluation of Lyme Disease: Clinical, Microbiological, and Immunological Characteristics	NCT00001539
Analysis of Lyme Disease Lesions	NCT00132327

Recent NIAID Lyme Disease Scientific Advances

NIAID intramural scientists at the Rocky Mountain Laboratories utilize molecular approaches in conjunction with experimental models to analyze *B. burgdorferi*. Of particular interest is how *B. burgdorferi* adapts to the distinct environments it encounters in the tick vector and mammalian

host. These basic research studies provide knowledge about key components of the Lyme disease bacterium that are important for tick-borne transmission and infection of mammals, including humans. Some recently published findings of NIAID intramural researchers are summarized below.

- **What kind of quality of life can Lyme disease patients expect long term?**
  Long-term follow up of Lyme patients enrolled in an NIH natural history study showed that health scores increased to be greater than or equal to the national average by end of follow-up, regardless of Lyme disease stage and severity at diagnosis [7].

- **How does *B. burgdorferi* ensure its surface allows for success in diverse environments*?***
  B. burgdorferi produces specific membrane proteins to effectively infect and persist in diverse organisms. Researchers found *B. burgdorferi* has a specific protease, called FtsH, which ensures that membrane proteins are properly formed and active when needed and removed if improperly folded or dysfunctional. Cells depleted of FtsH die in laboratory conditions and cannot colonize mice or ticks, revealing an absolute requirement for this protease to ensure the bacteria's survival. This work provides insight into *B. burgdorferi* viability and could lead to the identification of novel therapeutic targets [8].

- **Why do Lyme disease bacteria change what they look like at different stages of the infectious cycle?**
  B. burgdorferi cycles between mammalian and tick environments that vary over time. *B. burgdorferi* survival in these diverse conditions requires adaptation, a hallmark of which is a change in the composition of its outer surface. Researchers assessed whether the predominant surface proteins made by *B. burgdorferi* at different points of the infectious cycle were interchangeable and found that while some could substitute for each other, others could not. Moreover, their data confirm that the surface protein OspA both protects spirochetes within ticks from mammalian antibody and serves an additional role during tick colonization [9].

- **How does *B. burgdorferi* change as it moves between mammal and tick?**
  Transmission of *B. burgdorferi* between the tick vector and a mammalian host requires the bacterium to sense and adapt to these distinct environments. Researchers investigated how and where BBD18, a regulatory protein they had previously identified, contributed to this adaptive process. They found that this key regulator played a critical role during bacterial acquisition by a feeding tick by shutting off the program needed by the Lyme disease bacteria in the mammal and switching on a different program needed to colonize the tick vector. These studies provide insight into how *B. burgdorferi* regulates its adaptive response during transition from mammalian host to tick vector [10, 11].

- **Can we facilitate laboratory investigations of *B. burgdorferi?***

Many components of the Lyme disease bacterium are unique and of unknown significance to pathogenesis. While molecular genetics provides a powerful means of investigating novel and potentially important elements of bacterial pathogens, the tools currently available for such studies in *B. burgdorferi* are somewhat limited. Targeted mutagenesis and complementation are important tools for studying genes of unknown function in the Lyme disease spirochete *Borrelia burgdorferi.* Researchers have developed a convenient and widely applicable tool for molecular genetic investigations in *B. burgdorferi* [12].

NIAID also supports extramural scientists who are investigating multiple aspects of Lyme disease. This includes basic, translational, and clinical studies. Some of the most recent extramurally-supported research is described here, highlighting the diversity of studies funded.

- **Why do different *Borrelia* strains behave differently?**
  The Lyme disease bacteria spread to targeted tissues within both tick and mammalian hosts, but strain-specific differences in their distribution within the host and ability to cause disease have been observed. Researchers identified molecules on the surface of the Lyme bacteria that are involved in attaching to different tissue structures within mammalian and tick hosts. They discovered that variations in those proteins can help direct where the bacteria go following infection. These studies may help explain different, strain-specific clinical manifestations of Lyme disease [13]. NIAID-funded research continues to explore the spirochetal factors that allow for the persistence, maintenance, and dissemination of the pathogen in the tick vector [14]. Researchers have also been investigating the role of proteins, such as P66 and its integrin-binding function, in the transmigration and dissemination of *B. burgdorferi* [15].

- **How prevalent is *Borrelia miyamotoi*?**
  Borrelia miyamotoi, a bacterium that causes relapsing fever, is transmitted by the same ticks as Lyme disease and occurs in all Lyme-endemic areas of the United States. Investigators conducted epidemiologic studies in the Northeastern United States and found that *B. miyamotoi* infection may be common in southern New England and that some *B. miyamotoi*-infected individuals falsely test positive for Lyme disease [16].

- **Can biomarkers lead to a better diagnostic test for early Lyme disease?**
  Lyme disease can be difficult to diagnose early in infection because current laboratory tests look for antibodies that take time to rise to detectable levels. In response to a specific NIAID solicitation for better early-stage Lyme diagnostics, researchers looked for biomarkers, which are non-antibody molecules that arise in response to infection, to see if a subset could be identified that were specific for Lyme disease. The first publication from that study reports a group of biomarkers that can differentiate between Lyme and a group of other diseases with high sensitivity, and that could accurately indicate Lyme disease in many blood samples that had tested negative through the currently accepted two-stage antibody test [17]. In addition, NIAID-funded researchers are developing a microfluidics-based point-of-care diagnostic for Lyme disease that shows promise for early-stage

diagnosis [18].

- **Which *B. burgdorferi* genes are switched on, and when?**
  Understanding genetic control of the Lyme bacteria life cycle is important for developing new diagnostic, treatment, and prevention measures. Scientists recently conducted a comprehensive study to determine which *B. burgdorferi* proteins are produced in tick nymphs and larvae as well as in bacteria that had been adapted to mammalian hosts. The results showed clear differences in which *B. burgdorferi* genes are switched on in response to different developmental stages and environmental clues, providing the first detailed molecular blueprint of the carefully regulated Lyme bacteria life cycle. These studies provide the framework for further research on the genes critical for *B. burgdorferi* survival and pathogenesis [19].

- **Why do symptoms persist in some people after antibiotic treatment?**
  Lyme disease is generally responsive to antibiotic therapy. In some patients, however, musculoskeletal symptoms may continue for months after treatment. Although ongoing infection is considered an unlikely explanation for persistent symptoms or disease, it cannot be definitively excluded. This is because *B. burgdorferi* is difficult to detect by culture, except in early infection when the tell-tale erythema migrans rash is present. NIAID-supported researchers used a mouse model to suggest that inflammatory, immunogenic antigens, but not infectious bacteria, can persist near cartilage after treatment and might contribute to persistent symptoms [20].

  In collaboration with NIAID intramural scientists, researchers have discovered that elevated C-Reactive protein responses are associated with antibiotic-refractory Lyme arthritis and PTLDS, indicating that inflammatory mechanisms may be different from those in active infection [21].

  Researchers are also continuing to search for persistence of infection after antibiotic therapy for Lyme disease. NIAID scientists, in collaboration with researchers from Tufts University and New York Medical College, are investigating xenodiagnosis using live, disease-free, laboratory-bred ticks to see if Lyme disease bacteria can be detected in people after completing antibiotic therapy. Researchers are also studying whether persistence is more common in people who continue to experience symptoms, such as fatigue and joint pain. An initial study showed that xenodiagnosis was safe and well-tolerated. The results of the study were published in 2014. A phase 2 xenodiagnosis study is now open (see ClinicalTrials.gov, identifier NCT02446626) [22].

- **How important is antigenic variation in Lyme disease?**
  Recent studies on a *B. burgdorferi* surface protein, VlsE, highlight its importance in maintaining the Lyme bacteria life cycle in nature. These studies also describe the significant role played by antigenic variation, or changes in VlsE, in maintaining infections in mammalian hosts [23].

- **Does high cholesterol place you at increased risk of Lyme disease?**
  Because Lyme bacteria utilize cholesterol from their host to help construct their own membranes, researchers looked at whether individuals with lipid disorders such as high cholesterol might be at increased risk of Lyme disease. Using two different mouse models, the investigators determined that genetic conditions leading to elevated serum cholesterol may be a risk factor for increased severity of disease. Dietary cholesterol did not appear to lead to the increased risk, and because high cholesterol can stem from multiple genetic factors, additional work will be needed to identify the mechanism behind the results [24].

- **How do the Lyme bacteria cause arthritis?**
  A research group studying the factors leading to and sustaining Lyme arthritis identified a genetic locus in mice linked to type I interferon that is associated with *B. burgdorferi*-induced arthritis [25]. The same group also recently began characterizing the role of microRNAs, which are small molecules that help to regulate which genes are switched on and off, in regulating Lyme arthritis [26]. Other important research in this area is focused on the various systemic autoimmune joint diseases that may follow Lyme disease to determine the best course of treatment for these patients [27].

NIH Collaborations with Other Federal Agencies and External Organizations

NIH participates in the Department of Health and Human Services (HHS) Lyme and Other Tick-Borne Diseases Working Group along with representatives from the Office of the Secretary of HHS, CDC, and the U.S. Food and Drug Administration (FDA) to facilitate coordination and planning among participating agencies. The Working Group convenes twice a year to review the state of the science in Lyme disease research and has conducted four public webinars over the past few years to brief the scientific and patient communities on topics of interest, including the state of diagnostics, the persistence of infection in animal model systems, emerging tick-borne diseases, and vaccine research and development efforts.

NIH is partnering with the CDC, FDA, university researchers, and industry to develop improved diagnostics using the CDC Lyme disease serum repository for new test validation. The repository contains serum from Lyme disease patients and other disease etiologies that can be used as positive and negative controls. NIH provided support for the development of the repository, which is being used as a reference standard for the evaluation and calibration of new and existing assays [28].

Funding Opportunities and Research Resources

NIAID maintains a funding opportunities website to inform the research community about current grant opportunities and contract solicitations: https://www.niaid.nih.gov/grants-contracts/opportunities. In addition, NIAID has a comprehensive set of product development services and research tools and technologies to facilitate development and evaluation of

vaccines, diagnostics, and therapeutics: http://www.niaid.nih.gov/labsandresources/resources/dmid/Pages/default.aspx. These services make critical data, expertise, standardized research materials, and state-of-the-art technologies available to eligible investigators worldwide at no charge. The purpose of these resources is not to assist researchers in developing a particular product from start to finish, but rather to lower the financial risk to product developers by providing limited, but critical, information to fill specific gaps in the product development pipeline. Currently, NIAID is utilizing its preclinical resources to develop an improved methodology for culturing *B. burgdorferi*, which grows slowly and is very difficult to culture, particularly from patient samples. By developing better ways to grow the bacteria, NIAID hopes to enable faster and more efficient laboratory research on the pathogen.

Future Plans/Directions

NIAID's Lyme disease research portfolio will continue to encompass basic, translational, and clinical research studies, as well as studies on the tick vector and tick/pathogen interactions. Specifically, efforts will continue to be centered on the study of basic biology and pathogenesis of *B. burgdorferi*, which will help gain insight into Lyme disease. Research areas will include persistence of infection after antibiotic treatment using a variety of animal models, clinical studies, and improvement of Lyme disease diagnostics.

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