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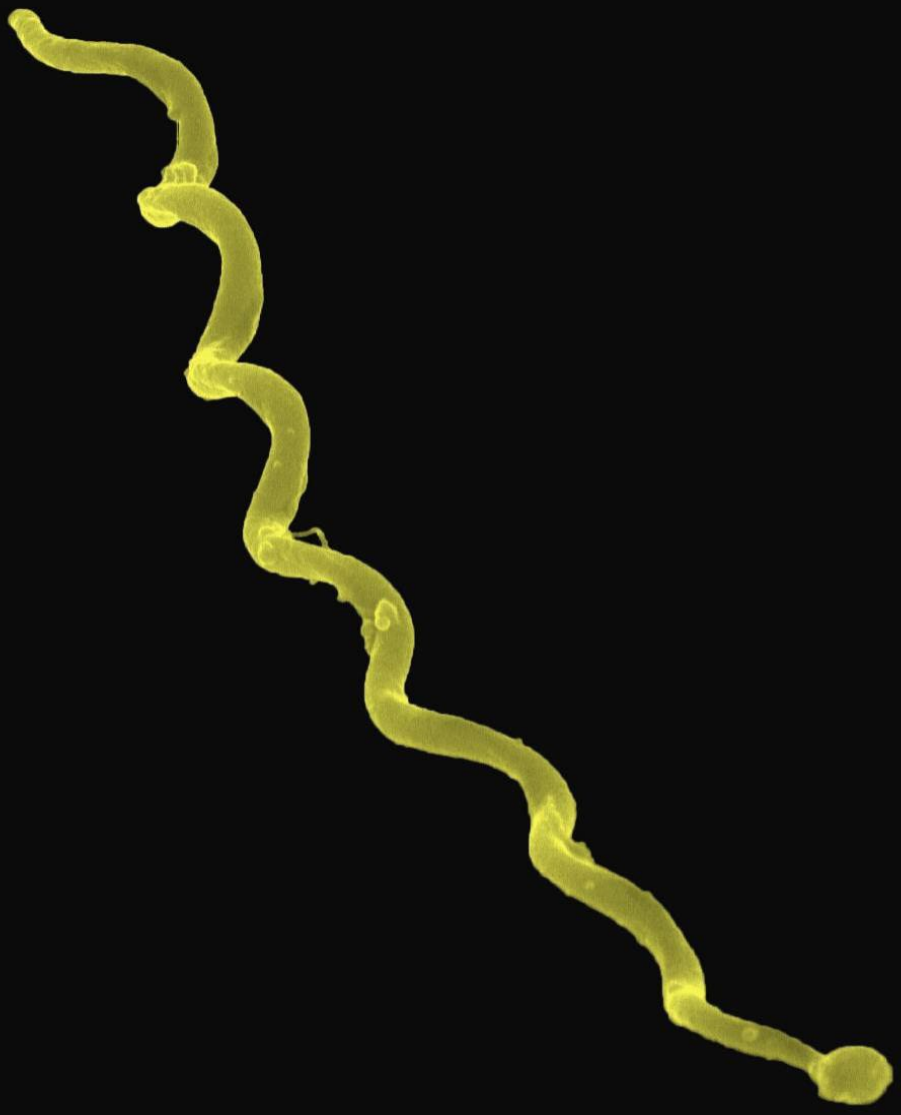

Current Efforts in Lyme Disease Research, 2023 Update

Original Report 2015

National Institute of Allergy and Infectious Diseases

Current Efforts in Lyme Disease Research

Table of Contents

Current Efforts in Lyme Disease Research

Introduction

Ticks can transmit a variety of disease-causing pathogens, including bacteria, viruses and parasites. Lyme disease, the most prevalent of the tick-transmitted infections in the United States, 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, and the pathogen that causes Lyme disease was identified in the early 1980s by scientists from NIAID and Stony Brook University. The bacterium, transmitted by a bite of an infected *Ixodes scapularis* tick (the blacklegged or deer tick), is now named *Borrelia burgdorferi* after Willy Burgdorfer, a NIAID scientist who co-discovered the pathogen with other scientists.

Lyme disease often begins with a circular or oval red rash called erythema migrans, which spreads from the bite site over several days. Other common symptoms include fever, headache, muscle and joint aches, and fatigue. Untreated, the infection can spread and lead to multiple rashes, facial or Bell's palsy (weakness of the muscles on one or both sides of the face), meningitis, and/or heart involvement. Later, individuals may develop Lyme arthritis, usually in the large joints such as the knees.

The diagnosis of Lyme disease is based on signs and symptoms of the infection, history of exposure to infected blacklegged ticks, and laboratory tests. Serologic tests, which detect an individual's antibody response to *Borrelia*, may take a few weeks after infection to become positive, highlighting the need for new diagnostic tests that can detect the earliest stages of infection.

In most individuals, Lyme disease is effectively treated with oral antibiotics. Even with treatment, approximately10-20 percent of patients report a range of continuing symptoms, collectively called Post-Treatment Lyme Disease Syndrome (PTLDS). The reasons for these symptoms are unknown, though causes such as autoimmune disease, persistent infection, coinfections, other illnesses, or chronic inflammatory processes triggered by non-living bacterial components have all been considered.

Even in the absence of known tick exposure or laboratory-confirmed *B. burgdorferi* infection, some individuals report extended, often debilitating symptoms attributed to Lyme disease. This "chronic Lyme disease" syndrome has been dismissed by some while defended by others. Regardless of the cause, the suffering is real, and additional research is necessary to elucidate the causes of these symptoms, including investigations of *B. burgdorferi* and any role the bacteria may play in long-term disability.

History of NIAID's Lyme Disease Research Program

Since the seminal discovery of *B. burgdorferi* 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

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 in addressing fundamental questions essential for progress in the field. NIAID's many contributions include:

- Helping to sequence the entire genome of *B. burgdorferi* and improving the molecular tools available for studying the organism and the host response to infection. Without these advances, 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,* in the northeastern, mid- Atlantic, and north-central United States: the blacklegged or deer tick, *Ixodes scapularis*. This updated and enhanced genome will enable a better understanding of the genetic basis of tick biology and tick/*Borrelia* interactions. Genomic data and bioinformatics tools for researchers are available in VectorBase.

- Supporting researchers to sequence the genome of the white-footed mouse, *Peromyscus leucopus*, the primary wild animal reservoir for *B. burgdorferi*.

- Supporting basic research on disease transmission, including research on ticks and their role in the disease cycle, tick biology and behavior, tick/pathogen and tick/host interactions, and the role of tick microbiome in pathogen development, as well as the effect of climate change on the distribution of ticks and the pathogens they transmit.

- Supporting fundamental research 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 PTLDS 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.

- Investigating the control of Lyme disease and other tick-borne pathogens by developing novel tick control approaches based on our understanding of tick interactions with the environment, the pathogens, and the vertebrate hosts. Tick control approaches may involve the use of repellents and the 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 hosts. Guided by the NIH Strategic Plan

for Tickborne Disease Research, this research is conducted by extramural and intramural investigators and spans basic science through human clinical research studies. Many projects are investigator-initiated efforts, but several are the result of targeted grant and small business initiatives. To encourage new grant applications that focus on advancing the priorities identified in the strategic plan, NIAID, along with other institutes, reissued a Notice of Special Interest (NOT-AI-23-013) Advancing Research for Tickborne Diseases. NIAID is committed to actively expanding the Lyme disease research portfolio to address high-priority areas such as persistence of infection after antibiotic treatment and the development of diagnostics for both early and late-stage disease.

NIAID uses all of these approaches to maintain a dynamic 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; tick biology and ecology; tick/pathogen and tick/vertebrate host interactions including the role of tick microbiome in pathogen development 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-stage Lyme disease.

Additional information on NIAID-funded Lyme disease research and tick-borne disease research is available on the NIAID Lyme disease 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. Project lists are available at the RePORT website 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 competitively awarded based on the NIH dual-level peer review process. The first level of peer review is conducted by a panel of scientific experts, and the second level of review by the NIAID Advisory Council before support is initiated. Research in the intramural program undergoes a rigorous, retrospective peer review process every four years by a Board of Scientific Counselors (BSC). The results of this review are used to determine future resource allocations. This process ensures that only highly meritorious science is supported.

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

Fundamental Studies of Pathogen Biology

NIAID supports multiple studies on the underlying biology of *B. burgdorferi*. This research helps scientists better understand how the pathogen infects the host, multiplies, survives, and ultimately causes human disease. An improved understanding of pathogen biology can lead to the identification of new targets for future diagnostics, therapeutics, and vaccines.

Examples of this research include:

- A comprehensive analysis of *B. burgdorferi* surface proteins and their cellular counterparts that could help scientists understand how the bacteria interact with human cells and tissues, which may lead to new targets for vaccines.

- Research on the proteins and cellular structures used by *B. burgdorferi* and related bacteria to travel through the body and affect multiple body sites.

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

- Investigations into how *B. burgdorferi* obtains critical nutrients within its human host, and on how critical survival genes are regulated within the bacteria.

- Research on the underlying genetics and proteins that enable the bacteria to adapt to the very different internal environment of ticks and humans, and to switch between them.

Studies on Persistence

NIAID also supports basic research projects to address key questions about why Lyme disease symptoms persist in some individuals, even after completion of treatment. In 2022, NIAID published a call for research to better understand persistent symptoms associated with Lyme disease (RFA-AI-22-046). Multiple awards under this opportunity were made in July 2023.

RFA-AI-22-046 is supporting research on the following topics:

- The bacterial genetic basis for persistence of *Borrelia burgdorferi.*
- Predictive markers for PTLDS.
- The role of the unique cell wall of *Borrelia burgdorferi* in PTLDS.
- The underlying mechanism of neurologic symptoms in PTLDS.
- Clinical, immunologic and metabolic factors that relate to symptom phenotypes in patients with PTLDS.

Other examples of research on persistence include:

- Investigations into how the bacteria respond to antibiotic stress.

- Research on how *B. burgdorferi* may trigger persistent symptoms in the absence of living bacteria, such as autoimmune or chronic inflammatory responses to bacterial components.

- Investigations of persistent infections using xenodiagnosis, a strategy that attaches live, disease-free, laboratory-bred ticks to people after they've completed antibiotic therapy, to see if *B. burgdorferi* can be detected in these ticks.

- Studies on the mechanisms behind Lyme arthritis, a late manifestation of untreated

Lyme disease.

Studies on Human Immunity to Infection

The immune system attacks different pathogens through various mechanisms, and pathogens in turn have evolved ways to partially evade those defenses. NIAID supports several research projects to better understand the interplay between the human immune system and *B. burgdorferi* as well as the mechanisms involved in pathogen evasion or control. These studies will provide insight into multiple areas of Lyme disease research including persistence of symptoms and potential new tools for combating or preventing disease. Some examples include:

- Research on bacterial factors that enable *B. burgdorferi* to evade multiple components of the immune system, such as antibody responses and critical elements of the early, innate immune response.
- Mechanisms of antigenic variation on the bacterial surface.
- Studies on how *B. burgdorferi* is cleared from the skin after infection.

Fundamental Studies of Tick Biology

NIAID supports basic research on *Ixodes scapularis* and other ticks that transmit the pathogens that cause Lyme disease and other infections. Ongoing efforts include:

- Research on tick feeding including identifying recent blood meals, which will shed light on the animal reservoirs responsible for maintaining *B. burgdorferi* in nature.
- Studies on the factors in tick saliva that affect transmission. These factors include proteins that block blood clotting and the pain response to a tick bite. This affects the skin immune response and may enhance the bacteria's chance of infecting humans.
- Studies on how ticks find their hosts, as well as studies characterizing *Borrelia* transmission and the possibility of interrupting the natural cycle of disease.
- Research on tick immunobiology and the role of the tick microbiome to better understand how *Ixodes* ticks recognize and respond to pathogens such as *Borrelia burgdorferi.*

Studies on Lyme Disease Diagnostic Testing

NIAID has long called attention to the need for improved Lyme disease diagnostics. Currently, no point-of-care diagnostic test is available that can detect early Lyme disease. Such a 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. NIAID efforts towards developing improved diagnostics include:

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

- Identifying and characterizing metabolic biomarkers and biosignatures for improved diagnostics. These studies may contribute to new methods for detecting Lyme disease, including diagnosis of early-stage disease, accurate staging of disease, or indications of successful treatment. For example, researchers are exploring ways to detect small-molecule metabolites in urine of early-stage Lyme disease patients, which would be very helpful in the clinic since urine is an easily obtainable sample.

- Utilizing new approaches to develop rapid point-of-care Lyme diagnostic tests, such as lateral flow technologies.

- Developing multiplex qPCR assays to simultaneously detect Lyme disease and coinfections such as babesiosis.

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 after reduced public demand. Since that time, the incidence and geographic distribution of Lyme disease has grown, and NIAID is currently supporting research to develop and evaluate new vaccine candidates for Lyme disease. In 2019, NIAID issued a Funding Opportunity Announcement focused on Tick-borne disease prevention (RFA-AI-19-037). Current research efforts, partially supported by RFA-AI-19-037, include:

- Identifying *B. burgdorferi* antigens that could be used in next-generation Lyme disease vaccines.

- Studying the potential of a "chimerotope" vaccine, modeled after a recently approved canine vaccine for use in the veterinary field.

- Pursuing novel vaccine formulations and targets, including proteins in tick saliva that are critical for the transmission of *B. burgdorferi* to humans. Such proteins may hold potential as antigens for "anti-tick" vaccines.

- Research on reservoir vaccines to reduce Lyme disease in humans. These oral "bait" vaccines are designed to prevent a known reservoir, deer mice, from becoming infected, thereby interrupting the transmission cycle. Some groups are field testing their products with support from the Centers for Disease Control and Prevention (CDC) as well.

Studies on Treatments for Lyme Disease

NIAID supports discovery and translational research efforts to identify and develop novel therapies that are specific to *B. burgdorferi*. While most cases of Lyme disease can be effectively treated with standard antibiotics, a more narrow-spectrum therapeutic agent could have the advantage of clearing the infection with less disruption of the patient's microbiome. NIAID also recognizes the importance of identifying appropriate therapeutic options for those patients who continue to experience symptoms after antibiotic treatment. Current research efforts include:

- Assessing activity of small-molecule inhibitors against cultured *B. burgdorferi* and characterizing the mechanism of action.

- Preclinical testing of a novel selective antibiotic in a mouse model of infection.
- Assessing the ability of existing, approved antibiotics to kill *B. burgdorferi.*
- Characterizing essential genes and/or molecular pathways in *B. burgdorferi* as new potential drug targets.
- Comparing the outcome of different treatment regimens for meningitis in the pediatric population.

Clinical Studies

NIAID intramural scientists on the Bethesda, Maryland campus conduct research 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 the understanding of the laboratory diagnosis, clinical manifestations, and human immune responses associated with *B. burgdorferi* infection.

More than 700 volunteers have been evaluated within 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 and development of new diagnostic tools.
- Investigation of potential causes of PTLDS.
- Exploration of potential persistence of infection after completion of antibiotic therapy for Lyme disease.
- Characterization of the immune response to tick bites in humans and the development of tick immunity.
- Information about these and other studies can be found on ClinicalTrials.gov, a registry and results database of publicly and privately supported clinical studies.

Recent NIAID Lyme Disease Scientific Advances

NIAID intramural scientists are involved in multiple basic and clinical research studies to advance our understanding of Lyme and other tick-borne diseases. NIAID continues to invest in research programs to increase the understanding of complex vector-host-pathogen interactions and their influence on Lyme disease, with the ultimate goal of identifying new targets for intervention.

Some recently published findings of NIAID intramural researchers are summarized below.

- **Can we better define post-treatment Lyme disease symptoms?**
  There is no standardized approach to define PTLDS. NIAID intramural researchers, in collaboration with extramural investigators, characterized patients with and without post-treatment Lyme disease symptoms and used statistical modeling to categorize patients and quantify the severity of their symptoms. While it still requires some optimization, this tool can be used to improve research and clinical care for patients with symptoms after treatment of Lyme disease.

- **What are common characteristics of Lyme neuroborreliosis patients?**
  In a retrospective observational study, NIAID intramural researchers described the features and outcomes of patients with early neurological Lyme disease presenting with facial palsy. The investigators showed that most patients presented in the summer months and had erythema migrans, which was frequently associated with systemic symptoms. These are important clues in the patient's history that help with early diagnosis. Most patients had an excellent recovery, and the recovery was similar for patients who received antibiotics alone or both antibiotics and corticosteroids.

- **What is the long-term prognosis for pediatric Lyme disease patients?**
  Together with investigators from Children's National, NIAID intramural investigators characterized the long-term outcomes of pediatric Lyme disease patients in the Washington, DC area. This was a cross-sectional evaluation to assess parent- and patient-reported outcomes. Most participants reported full resolution of symptoms, with the majority recovering within 6 months after completing antibiotic treatment. At the time of study completion, 6% of children still experienced symptoms attributed to Lyme disease and 1% experienced symptoms significant enough to impair daily functioning. This study supports previous data that the overall prognosis for children to make a complete recovery from Lyme disease is excellent. For the small number of children who do not experience full recovery, more research is needed to better define the course and pathogenesis of their prolonged symptoms, as well as novel targeted therapies to relieve their suffering.

- **Can we improve the tools we use to detect the bacteria that cause Lyme disease?**
  Together with investigators at Columbia University, intramural investigators helped develop the TBD Capture Sequencing Assay (TBDCapSeq). The TBDCapSeq is a probe-based capture assay targeting the eleven most common tick-borne agents found in the US. For *B. burgdorferi* and *Babesia microti*, the sensitivity of TBDCapSeq was comparable and occasionally exceeded the performance of agent-specific quantitative PCR and resulted in 25 to > 10,000-fold increase in pathogen reads when compared to unbiased high-throughput sequencing. The TBDCapSeq could have a major impact in studies of tick-borne pathogens by improving detection and facilitating genomic research that was previously unachievable with standard sequencing approaches.

- **How can we measure and monitor responses to treatment?**
  Together with investigators at Tufts, NIAID intramural researchers showed that *B. burgdorferi* can accumulate intact phospholipids from its environment, and that antibody responses to host phospholipids occur in mice and patients with acute Lyme disease. Serial samples during treatment and recovery showed a marked decline in antibody titers, suggesting that these antibodies may be of value in monitoring the response to treatment.

- **Is xenodiagnosis a useful tool to detect whether *B. burgdorferi* can persist after treatment?**
  Whether *B. burgdorferi* can persist after antibiotic therapy is an area of ongoing debate. Studies in animals showed that *B. burgdorferi* DNA can be detected in tissues after antibiotic therapy and that xenodiagnostic ticks feeding can acquire the bacteria. To address this controversy, NIAID intramural scientists and researchers from Tufts and Yale University performed the first study in humans using xenodiagnosis with *I. scapularis* ticks to identify *B. burgdorferi*. The phase I study, published in 2014, showed the procedure was safe and well tolerated. A phase 2 xenodiagnosis study has been performed (see ClinicalTrials.gov identifier NCT02446626), in collaboration with Tufts University, New York Medical College, Mansfield Family Practice and Stony Brook University. Data from this study are currently being analyzed.

- **How can we accelerate fundamental research to enhance our understanding of *B. burgdorferi* biology?**
  Many components of the Lyme disease bacterium are unique and of unknown significance to pathogenesis. Targeted mutagenesis and complementation are important tools for studying genes of unknown function in the Lyme disease spirochete *B. burgdorferi.* Recently, a powerful new tool has been adapted to the study of spirochetes, allowing visualization of living cells. With this work, researchers have developed vital new tools for molecular genetic investigations in *B. burgdorferi*. This will enable the identification and characterization of essential genes and pathways in *B. burgdorferi*, which could become future targets for novel drugs and vaccines.

- **How can we prevent infection?**
  NIAID intramural researchers are also interested in the potential promise of an 'anti-tick' vaccine. Recruitment recently began for a clinical research protocol to study the human immune response to tick bites, with the ultimate goal of identifying potential vaccine targets (see ClinicalTrials.gov identifier NCT05036707). While still in the early preclinical research stage, this approach potentially offers a novel strategy to prevent Lyme disease and other tick-borne illnesses.

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 research is described here, highlighting the diversity of studies funded.

- **Why are different species of *Borrelia* associated with different symptoms?**
  Different species of *Borrelia* colonize different tissues and cause distinct manifestations of Lyme disease. For example, *B. burgdorferi* is associated with arthritis, while late-stage infections with *B. afzelii* and *B. garinii* commonly involve dermatologic and neurologic symptoms, respectively. There are several proteins on the surface of *Borrelia* that allow them to stick to host cells and tissues, and it is thought that variation in these outer surface proteins may account for the differences seen between species. One protein that varies significantly between different species is OspC. NIAID-supported researchers found that

different OspC proteins bind different components of the extracellular matrix, which is a network of molecules that line the tissues of the body. They found that different OspC variants promote the infection of distinct tissues in a mouse model of Lyme disease. This work highlights the importance of the OspC protein in determining which specific symptoms are associated with infection by different *Borrelia* species.

- **Can biochemical differences be detected in patients with early and post-treatment Lyme disease symptoms?**
  Molecular-level changes that occur during infection can be detected in patient blood. To better understand these changes, NIAID-supported investigators completed a comprehensive analysis to identify biochemical differences in the blood of patients with early localized Lyme disease and early disseminated Lyme disease, compared to healthy individuals. Their findings provided insights into specific molecular pathways that are affected by *B. burgdorferi* infection. Work from the same group used a similar methodology to identify metabolites that can distinguish patients with post-treatment Lyme disease symptoms from clinically cured patients over time. Studies like these provide important insights into the pathogenesis of Lyme disease. Further, the identification of biomarkers unique to different stages of disease could provide the basis for diagnostics that can inform patient treatment.

- **How do *Borrelia* and other spirochetes achieve their unique 'corkscrew' swimming movements?**
  Many bacteria move using external flagella, thread-like structures that move to propel the bacterium forward. *Borrelia* bacteria are classified as spirochetes, which have a distinctive spiral shape. Interestingly, while spirochetes have flagella, they are located internally beneath the outer cell membrane, and anchored on either end of the bacterium. Molecular motors coordinate the rotation of these internal flagella, allowing the spirochetes to move forward in a corkscrew-like fashion. Many research efforts have attempted to characterize the molecular mechanisms underlying this unique motility. For example, a recent NIAID-supported study used a combination of genetics and powerful imaging techniques to characterize some of the components of the motor, shedding light on the assembly and function of this unique molecular structure.

- **What can we learn about Lyme disease transmission from studying *I. scapularis* immune pathways?**
  If we can understand how a tick's immune system works, we may be able to harness it against *Borrelia burgdorferi* and control bacterial transmission to the vertebrate hosts. In this publication, the authors established that a mammalian immune molecule called interferon gamma, when ingested by ticks in the bloodmeal, not only triggers the tick immune response but also is crucial for tick metamorphosis and organ development. This interaction is controlled by a tick receptor called Dome1. By understanding how Dome1 regulates tick immunity and developmental processes, it could be targeted to inhibit or block these essential tick pathways.

- **How do molecular components of the tick gut impact tick biology and *B. burgdorferi* transmission?**
  After being taken up by a tick during a blood meal, *B. burgdorferi* spends most of its time

in the tick gut. The bacterium has evolved to survive in this environment, including interacting with the thick, mucus-like layer called the peritrophic membrane (PM), which helps protect the cells lining the gut. NIAID-supported investigators identified a protein, PM_CBP, which is essential to the integrity of this barrier. They showed that immunizing ticks with antibodies against PM_CBP could substantially reduce both acquisition of *B. burgdorferi* by the tick, and transmission of the bacteria from ticks to mice. The data suggested that the anti-PM_CBP antibodies disrupt the peritrophic membrane, suggesting that this may expose the bacteria to a more hostile environment within the tick gut. A second NIAID-supported study from the same research group showed that antibodies against another tick gut protein reduced *B. burgdorferi* survival in the tick. Such studies help further our fundamental understanding of tick biology and tick-pathogen interactions. They could also ultimately inform novel strategies, such as anti-tick vaccines, to prevent the spread of Lyme disease and other tick-borne illnesses.

- **Can we identify an antibiotic that selectively kills *Borrelia*?**
  While standard antibiotics can effectively treat most Lyme disease cases, they also disrupt the microbiome, which is important to maintaining human health and immunity. A drug that specifically kills Lyme-causing bacteria without affecting the microbiome would be ideal. In work that was partially supported by NIAID, researchers screened soil-dwelling bacteria that produce compounds with antibacterial properties. They were able to identify and purify a compound that was potently active against *Borrelia burgdorferi* and related bacterial species, but not against most other bacteria including those commonly found in the gut microbiome. This work and other NIAID-supported efforts have the potential to improve the available treatment options for patients suffering from Lyme disease.

- **Could a vaccine provide resistance to tick bites?**
  Some animals that are repeatedly exposed to tick bites develop 'resistance' in the form of an inflammatory immune response at the site of the tick bite that impacts tick feeding and attachment. Based on this observation, NIAID-supported researchers developed an mRNA-based vaccine designed to deliver a cocktail of 19 antigens that are found in tick saliva. When uninfected ticks were allowed to feed on immunized guinea pigs, they exhibited severely impaired feeding, and began to detach after 48 hours. In a second experiment using *B. burgdorferi* infected ticks, the researchers found that the vaccine also protected the animals from *B. burgdorferi* transmission.

- **Could persistent pieces of the bacteria be the cause of PTLDS in some people?**
  Lyme disease is generally responsive to antibiotic therapy. However, a subset of patients reports a wide range of symptoms that continue for months to years after treatment. Using a mouse model of Lyme disease, NIAID-supported researchers found that infectious bacteria are rapidly eliminated after antibiotic treatment, but that inflammatory, immunogenic antigens can persist near cartilage after treatment and might contribute to persistent symptoms.

- **Could PTLDS in some people be caused by an autoimmune or inflammatory response?**
  While Lyme arthritis can develop in individuals that did not receive antibiotic treatment for their infections, it can also develop in individuals that were treated with antibiotics. One

hypothesis is that post-treatment Lyme disease symptoms are caused by autoimmune and/or inflammatory responses that continue even after the infection is cleared. NIAID-funded researchers characterized patients with post-infectious Lyme arthritis who had various systemic autoimmune joint diseases that developed after antibiotic treatment. They reported that patient symptoms improved upon treatment with anti-inflammatory therapies. The same research group also performed RNA sequencing of joint tissue in post-infectious Lyme arthritis patients and found that they had increased markers of inflammation, and decreased expression of genes involved in tissue repair. This result suggested that the immune response in these patients may prevent appropriate tissue repair and maintenance long after completion of antibiotic treatment and clearance of active infection.

- **How does coinfection with other tick-borne infections impact pathogenesis?**
  Babesia microti is a parasite transmitted by the same tick species that transmits *Borrelia burgdorferi*, and people can become infected with the two pathogens at the same time. Recently, researchers used a mouse model of the two infections to investigate how each pathogen influences the other. They showed that infection with the *B. microti* parasite enhanced the severity of Lyme disease-like symptoms in the mice, while *B. burgdorferi* appeared to limit the growth and effects of *B. microti*.

- **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. NIAID-supported investigators have recently isolated and purified the biochemical machinery involved in RNA transcription. This led to the development of a new research tool that will facilitate studies of *B. burgdorferi* gene expression to better understand this important process. Certain proteins in the bacterium act as 'dimmer switches,' controlling the extent to which different genes are 'turned on' (i.e., transcribed and made into protein) in response to various environmental cues. Researchers are working to identify these regulatory proteins and characterize their functions. For example, multiple recent studies have explored the role of the regulatory protein DksA in enabling *Borrelia* to adapt to the mammalian host. One group characterized which genes were up- or down-regulated by DksA. A second study more specifically defined the interactions between DkSa and other proteins using a biochemical system. The research groups characterized environmental stressors, including acidity and high-salt content, that DksA responds to. Both studies also demonstrated that DksA is essential to the ability of *B. burgdorferi* to infect mice.

- **Can *Borrelia* colonize the central nervous system?**
  Lyme disease can lead to various neurologic symptoms, including central nervous system manifestations. Using a mouse model of infection, NIAID-supported researchers recently demonstrated that several *Borrelia* species can colonize the dura mater—the thick outer membrane that covers the brain and spinal cord. Colonization of the dura mater was associated with a local inflammatory immune response. Interestingly, an inflammatory response was also observed inside the brain, even though the bacteria themselves were not able to infiltrate beyond the dura mater. These responses may contribute to the various pathologies observed in Lyme disease patients.

- **Could studying non-immune biomarkers be the key to developing a reliable**

diagnostic for early Lyme disease?
Currently available diagnostics focus on detecting immune system response to *Borrelia* species, but a group of NIAID-funded scientists recently tried a different approach. These scientists were able to identify 45 biomarkers the distinguished individuals with early Lyme disease from endemic and nonendemic controls. The 45 biomarkers are all linked to metabolic processes that are known to be altered during Lyme disease. In the future, the group will evaluate whether the identified biomarkers can distinguish between patients with Lyme disease and those with diseases that have similar clinical presentations to Lyme disease.

- **Could biomarkers for Lyme disease provide the basis for a rapid point-of-care test?**
Rapid, sensitive diagnostics for Lyme disease are needed. Recent work by both NIAID intramural investigators and extramurally-supported researchers aims to develop a point-of-care test. The researchers first screened a set of known Lyme disease biomarkers for their ability to indicate infection. They then tested for the top three biomarkers in samples from people with early Lyme disease, from healthy individuals from areas where Lyme disease is endemic, and from people with Lyme arthritis. These data were compared to results obtained using the standard two-step testing procedure. Overall, the new set of biomarkers was more sensitive than standard Lyme disease tests. While the assay will require more refinement and testing, these promising results open the possibility of developing a point-of-care test.

- **Are monoclonal antibodies an effective prevention strategy for Lyme disease?**
Monoclonal antibodies are a promising preventative strategy for many infectious diseases. However, one challenge for their use as a prophylaxis against Lyme disease is that the antibodies would need to remain in circulation at high enough levels to provide protection for the entire tick season (a 6- to 8-month window). The *Borrelia* outer surface protein A (OspA) antigen is known to induce an effective immune response against Lyme disease. Along with the Department of Defense Tick-Borne Disease Research Program, NIAID provided support for a study which demonstrated that antibodies modified for increased stability had a longer half-life in non-human primates, and that administering the monoclonal antibody effectively protected the primates from infection. Based on this preclinical work, researchers conducted a Phase 1 clinical trial to assess the safety and blood levels of the modified antibody, sponsored by MassBiologics of UMass Medical School. Data from this study are currently being analyzed.

NIH Collaborations with Other Federal Agencies and External Organizations

NIH participates in the Department of Health and Human Services (HHS) Tick Borne Disease Partners along with representatives from the Office of the Secretary of HHS, CDC, and the Food and Drug Administration (FDA) to facilitate coordination and planning among participating agencies. The group convenes twice a year to review the state of the science in Lyme disease research and has conducted 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 ticks and tick-borne pathogens, as well as vaccine research and development efforts.

NIAID actively participated in the HHS Tick Borne Diseases Working Group, which was established by Congress in 2016 as part of the 21st Century Cures Act to help ensure interagency coordination. The final report to Congress was submitted in February 2023. NIAID is also currently collaborating with the CDC and other HHS agencies in the development of the National Vector-borne Disease Strategy as mandated in the Kay Hagan Tick Act. In addition, the Institute is a collaborator in the HHS LymeX Innovation Accelerator.

NIH is partnering with the CDC, FDA, university researchers, and industry to develop improved diagnostics using the CDC Lyme disease serum repository for new and existing assay 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.

Funding Opportunities and Research Resources

NIAID maintains several websites to inform the research community about current grant opportunities and contract solicitations. In 2022, NIAID's Division of Microbiology and Infectious Diseases published RFA-AI-22-046, 'Understanding Persistent Signs and Symptoms Attributed to Post-Treatment Lyme Disease (RO1 Clinical Trial Not Allowed).' Applications received in response to the RFA will be reviewed in March 2023, with selected applications to be funded later in fiscal year 2023.

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. These resources for researchers 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 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. NIAID's Strategic Plan for Tickborne Disease Research will guide the focus of ongoing and future research efforts. Priority areas will include fundamental research on the biology and pathogenesis of *B. burgdorferi* and its interactions with the tick vector, as well as translational research efforts aimed at advancing better detection and diagnostics, developing novel vaccines and other prevention strategies, and improving treatment for Lyme disease. Research on treatments for Lyme disease will incorporate efforts to assess new therapeutic strategies, and NIAID will also continue to focus on the development of tools and resources for researchers to accelerate research efforts.