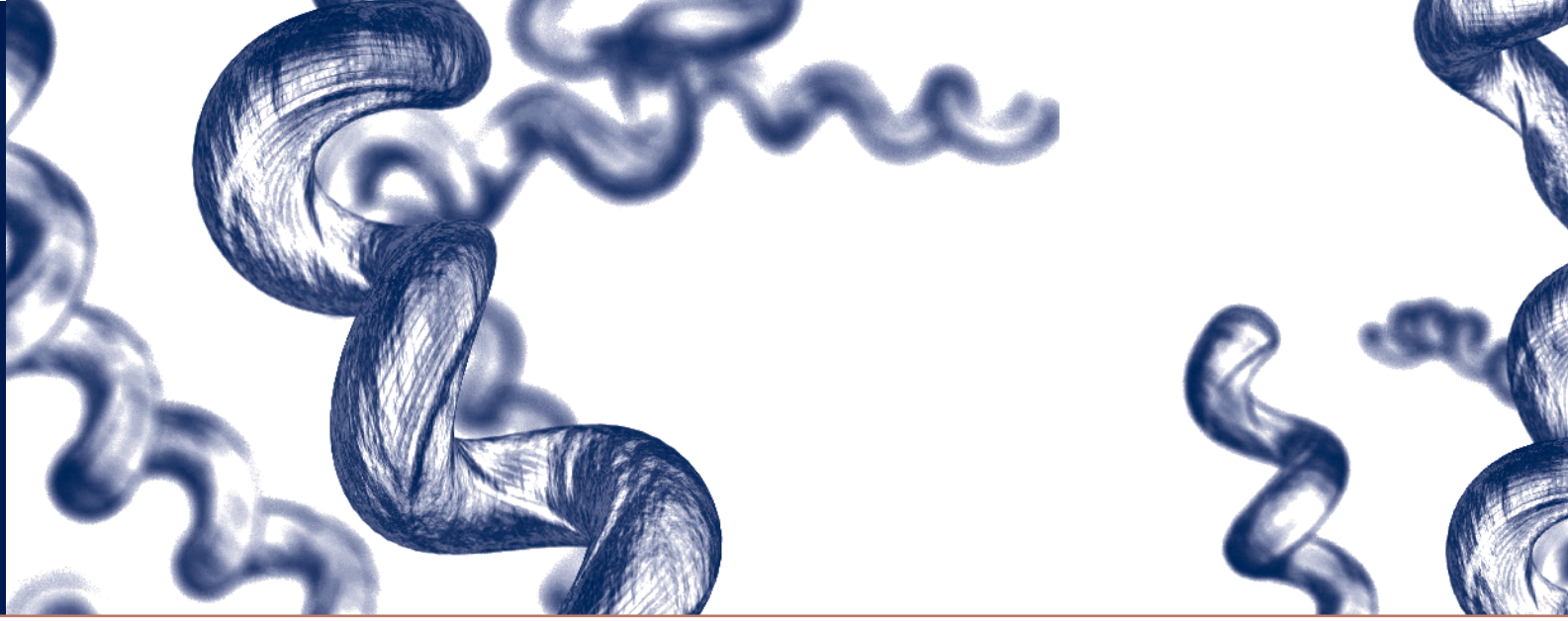

Global Lyme Alliance

# Does Chronic Inflammation cause PTLD?

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JANUARY 2021

LYME DISEASE IS AN INFECTIOUS DISEASE CAUSED BY BACTERIA CALLED *BORRELIA BURGDORFERI*. THESE BACTERIA INFECT VIA BITES OF THE BLACKLEGGED TICK, AND CAUSE A CONSTELLATION OF SYMPTOMS.

Early disease manifests as a localized skin rash at the bite site, which as disease progresses, expands into fever, headache, myalgia, and arthritis. The illness can be serious and debilitating, and in rare cases, fatal (Figure 1). When early symptoms such as skin rashes, chills, sweats, fatigue and headache prompt timely diagnosis and treatment, complete recovery is possible in many patients. This may prevent the arthritis, carditis, and neurological symptoms of later, disseminated disease, which is due to the migration of the bacteria from the initial skin site, through the blood circulation, to the nervous system, heart, and other organs (Figure 2).However, many Lyme patients do not receive proper diagnosis during early infection, due to the heterogeneity of symptoms and inaccuracy of the current blood test, which is the subject of a later white paper in this series. But even among those patients who are correctly diagnosed and treated according to current guidelines, there are some who continue to suffer symptoms like chronic pain, unremitting fatigue, and neurocognitive difficulties, for more than six months and sometimes years after treatment. Symptoms may vary between patients, and are often subjective in nature. Much of the mainstream medical community is only beginning to accept that persisting, long-term illness is a possible outcome of Lyme disease. What do we know about these patients, and why are they still sick?

Long-term, debilitating illness after treatment is known as post-treatment Lyme disease syndrome (PTLD), affecting an estimated 10-20% of antibiotic-treated Lyme patients[1]. It's suspected that patients who experience delays in diagnosis and treatment, and those with more severe early disease, are more likely to develop PTLD. With at least 329,000 people getting Lyme disease every year in the US[2,3], this is a sizeable population, and many can be sick for years. A Dutch study estimated an average 1.7 disability-adjusted years lost per patient, due to Lyme disease[4]. In the United States, the medical cost of treating Lyme disease was calculated at almost $3,000 per patient; this translates into more than $1 billion per year for all patients[5]. A recent study by GLA and Brown University used mathematical models to calculate that up to 2 million people could be living with PTLD by 2020[6] (Table 1). In such individuals, lost wages and productivity are inestimable, compounded by the derailment of education, careers, and family life.

It is not clearly understood whether PTLD is a result of continued replication of *B. burgdorferi*. Bacterial persistence in chronic conditions has been demonstrated in other human diseases, and untreated long-term syphilis, caused by another spirochetal bacterium, is also characterized by a constellation of progressive symptoms affecting multiple organ systems. However, the subject of persistent bacteria in Lyme disease is beyond the scope of this article and will be discussed in greater detail in another posting.

Alternatively, or perhaps in addition to bacterial persistence, an aberrant immune response resulting in chronic inflammation may be responsible for PTLD. Below, we consider the evidence that a dysfunctional immune response is a major cause of long-term symptoms in previously treated patients. This review will first summarize immunological events during acute, early Lyme disease, then explore recent findings of immune correlates of PTLD. A large body of research has used animal models, particularly experimentally-infected mice to study this question. However, for the sake of a concise discussion, here we focus on findings of human Lyme disease immunology. To understand immune functions during late disease, it's important to first review what happens during acute, early infection.

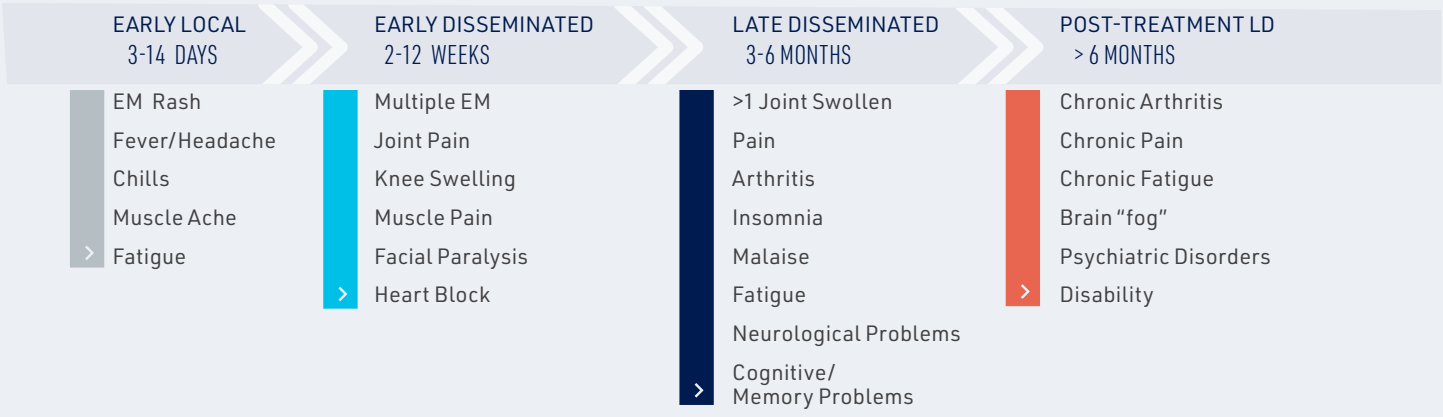



FIGURE 1. THE STAGES OF LYME DISEASE

Lyme disease early symptoms start at the bite site in most, but not all cases. An expanding rash known as *erythema migrans* (EM) may be accompanied by flu-like symptoms. If untreated, disease progresses through early and late disseminated stages, with new symptoms appearing. For those who are diagnosed and treated, 10-20% may continue to suffer debilitating symptoms, known as post-treatment Lyme disease.

# EARLY IMMUNE EVENTS IN B. BURGDORFERI INFECTION

During initial infection via tick bite, *B. burgdorferi* senses its new mammalian host. After leaving the tick vector, an arachnid, the bacteria must now survive changes in nutrients, oxygenation, pH, and temperature. The infecting bacteria also must survive the early host immune response. Early on, *B. burgdorferi* changes its gene expression, even before leaving the tick, to prime itself for survival and replication in the new environment. For example, it replaces outer surface protein A (OspA) on its outer membrane with OspC, which promotes early infection. The bacteria multiply in the skin, often causing *erythema migrans* (EM), a slowly expanding rash that sometimes forms a characteristic bull's eye lesion. Patients may also have atypical rashes not resembling a bull's eye lesion, they can have multiple EM lesions, or none at all. After a week or so, the bacteria spread via blood or lymph to other tissues, aided by bacterial flagella-powered motility, which is critical for disease progression[7]. In fact, genetically engineered non-motile *B. burgdorferi* are quickly contained by the immune response[8] and eliminated by phagocytic cells and other clearance mechanisms. Additional outer surface proteins, such as BBK32, aid directly in the spread of bacteria, since they interact with host proteins to promote adhesion and bacterial penetration into tissues[9]. Migration of *B. burgdorferi* from the skin to distant sites such as the joints, heart and central nervous system results in symptoms expanding into these tissues during the disseminated stages of the disease (Figure 2).

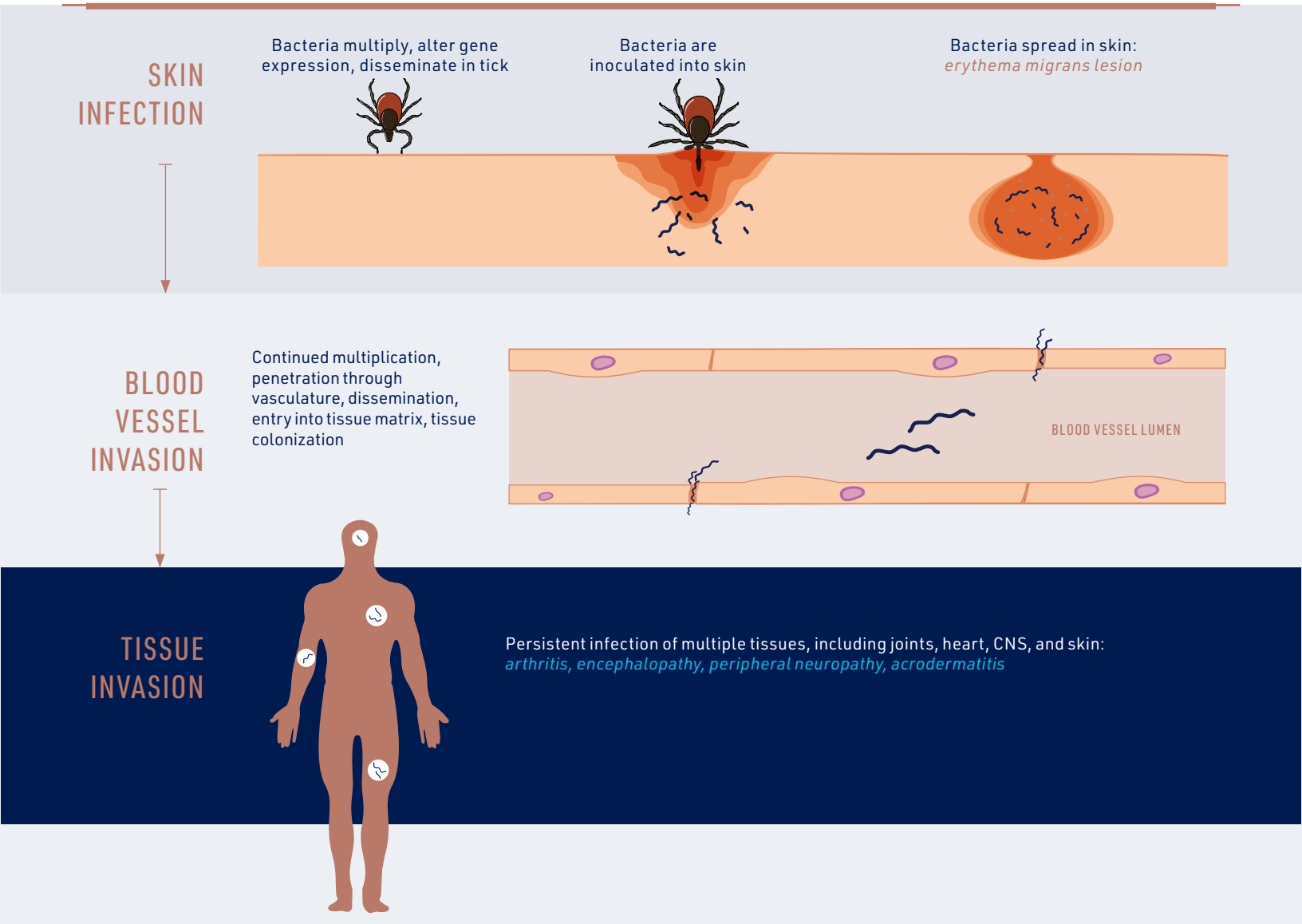



FIGURE 2

Infectious events during early and disseminated Lyme disease. Spirochetes are inoculated in the skin through the bite of an infected hard-shelled *Ixodes* tick. CNS, entral nervous system. PNS, peripheral nervous system. Modified from reference 34. Coburn J. et al., 2013, Trends in Microbiology 21 (8): 372-379. With permission from Elsevier.

# ACUTE IMMUNE RESPONSE

During early infection, the host immune response counters the invasion with both innate and acquired immunity. Biopsies of EM rashes often show accumulated neutrophils, dendritic cells, monocytes, macrophages, and T cells, all evidence of the recruitment of immune cells to the site of infection[10]. Antigen-presenting cells (APCs) like dendritic cells and monocytes sense the presence of *B. burgdorferi*. This can be, though is not limited to, recognition of *B. burgdorferi* lipoproteins by receptors on the surface of APCs, which promotes cytokine secretion into the infected tissues, blood, and lymph[11]. Initially, these are mostly pro-inflammatory, such as tumor necrosis factor (TNF), interleukin 1 (IL-1), IL-2, IL-6, and type 1 interferon (IFN). Chemokines, which signal and recruit immune cells, such as CXCL1, CXCL9, CCL3 and CCL4 are also elevated. IL-23, which promotes a Th17 response against extracellular pathogens, is increased during acute infection and is higher in patients who have more symptoms alongside an EM rash compared to those with EM rash alone[12].

| INCIDENCE SCENARIO | FAILURE RATE | 2016 | | 2020 | |
|---|---|---|---|---|---|
| | | DETERMINISTIC | SIMULATION | DETERMINISTIC | SIMULATION |
| A | | | | | |
| | 10% | 68, 603 | 69,011 (51,796 – 89,312) | 81,713 | 81,509 (61,141 – 105,591) |
| | 20% | 137, 207 | 138, 540 (114,456 – 164,408) | 163,426 | 163,705 (135,095 – 193,979) |
| B | | | | | |
| | 10% | 668,303 | 671,876 (511,989 – 866,523) | 792,572 | 790,411 (601,992 –1,017,496) |
| | 20% | 1,336,607 | 1,351,180 (1,126,160 – 1,608,309) | 1,585,145 | 1,590,259 (1,323,334 – 1,893,234) |
| C | | | | | |
| | 10% | 754,468 | 758,776 (575,431 – 980,601) | 969,020 | 967,822 (732,074 – 1,249,030) |
| | 20% | 1,508,937 | 1,523,869 (1,268,634 – 1,809,416) | 1,938,041w | 1,944,189 (1,619,988 – 2,304,147) |

TABLE 1

Estimated numbers of people in the US with post-treatment Lyme disease (PTLD) in 2016 and 2020, based on treatment failure rates of 10% or 20%. Three incidence rate scenarios were modeled. The deterministic figure is an approximation based on inputs as described in the original article. The simulation figure derived from the mathematical model shows medians and coverage intervals. Modified from DeLong A. et al., 2019, BMC Public Health, 19:352-359. Used under terms of the Creative Commons Attribution 4.0 International License, http://creativecommons.org/licenses/by/4.0/

An informative study led by Dr. Mark Soloski at Johns Hopkins University focused solely on patients during acute infection[13]. They found that patients' inflammatory biomarker profiles fell into two categories, which the researchers termed "mediator-high" and "mediator-low". In the former group, T cell cytokines like CXCL9, CXCL10, and CCL19 were increased in sera from patients one month after diagnosis of an EM rash compared to healthy controls. Inflammatory markers like C-reactive protein (CRP) and serum amyloid A were also increased. In the mediator-low Lyme patients, these cytokines and inflammatory proteins were closer to normal controls. What was novel about this study was that when patients were sorted according to their immune mediator type, the mediator-high group was more likely to be positive for anti-*Borrelia* antibodies than mediator-low (78% vs. 40%) individuals. In addition, the high mediator group had more symptoms pre-treatment than the low mediator group (mean of 8.5 vs. 4.2). Together with high CXCL9, CXCL10, and CCL19, the mediator-high group also had elevated liver enzymes, suggestive of disease occurring in this organ as well. These findings, obtained with funding support from GLA's predecessor organization, Lyme Research Alliance, attest to the complexity of the acute response to *Borrelia* infection. But beyond this, they also demonstrate divergent mechanistic pathways that determine disease course and symptom severity. And when bacterial populations are subject to the selective pressure of the host immune system, only those with the greatest fitness survive and continue to spread and replicate. In a mouse model of infection, it was found that the major challenges faced by the bacteria at the site of infection had a profound effect on the surviving population of

bacteria[14], which would go on to spread to distal tissues and organs. These studies highlighted the importance of the early immune response as a barrier to systemic bacterial colonization. But it also suggested that identifying the genetic bacterial variants that are eliminated, as well as those which survive the initial infection, may be a fruitful path to investigate how bacteria establish a foothold in their new host, and continue to replicate.

So, it's obvious that the early immune response is complex and has multiple actors. Akin to a military force, there are specialized cells and proteins that function in different ways and at different times. They may act to directly kill bacteria or recruit other cells to do the job. Inflammatory cytokines signal the defensive state and ramp up cellular and humoral responses. As infection progresses, the complement system and other proteins that promote bacterial lysis, or destruction, are activated. With the right signals, immune cells are induced to differentiate, proliferate, and perform their antimicrobial defensive functions. Ultimately, their goal is to contain, kill, and clear the infecting pathogen. But what controls these forces? Regulation is by different signaling pathways, which are the product of activation or repression of specific genetic networks.

# GENETIC CONTROL OF THE ACUTE IMMUNE RESPONSE

A thorough understanding of biological phenomena would include knowing the genetic controls that explain the observations. Cells carry out their activities because genes encoded in their DNA instruct them to perform their functions, and this is true for all cells, not only those of the immune system. DNA instructions are then transcribed into RNA, which serves as the template for specific protein synthesis. It is these proteins which carry out highly specialized functions. These can be chemical messengers (e.g. cytokines, chemokines), intermediaries between cells (e.g. antibodies), or as structural elements (e.g. cell membranes or receptors on immune cells). So, it's important to learn how genetics are involved in the Lyme disease response.

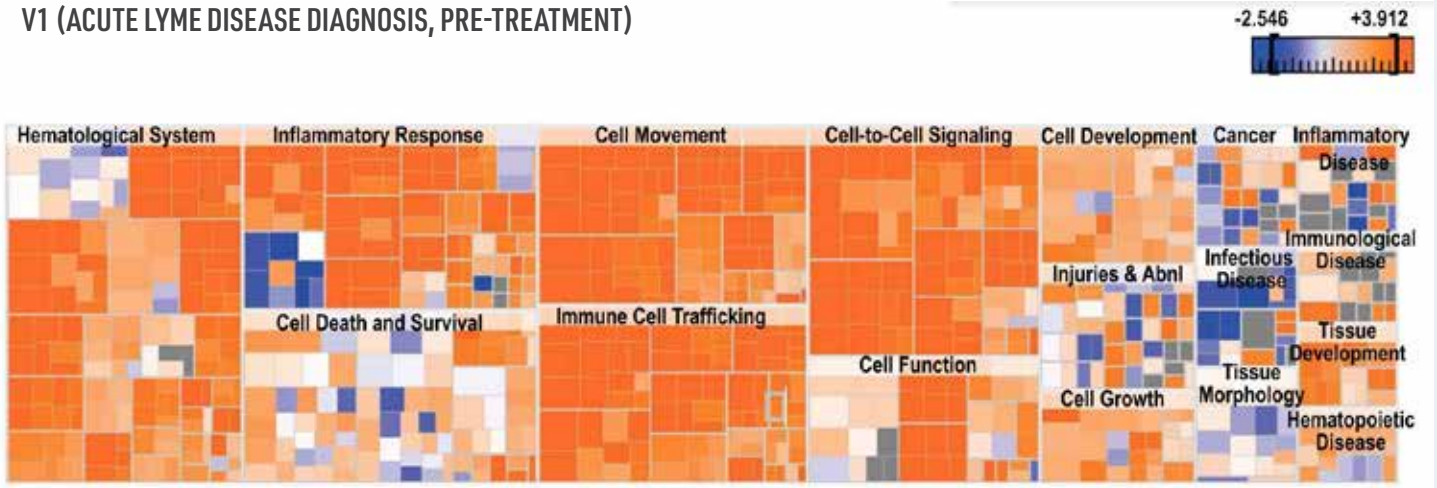
A key study examining genetic controls in acute Lyme disease was published by Dr. Charles Chiu of University of California, San Francisco[15]. His study, funded by GLA, compared gene transcription in the peripheral blood mononuclear cells (PBMCs) of Lyme patients with that of healthy controls. These cells provide a snapshot of immune cells circulating in the blood, and when drawn from patients at different time points during the progression of Lyme disease, show the evolution of the immune response. Importantly, Lyme patients' PBMCs were compared with those of healthy people, as well as with patients suffering from other diseases. Genetic pathways involved with activation of inflammatory responses and immune cell movement were upregulated in acute Lyme infection relative to healthy controls, and these remained activated even after antibiotic treatment. In total, 847 genes were upregulated, while 388 were downregulated in patients with EM rashes. Among these differentially expressed genes were those of proinflammatory cytokines and biomarkers of inflammation, as well as some anti-inflammatory cytokines like IL-6 and IL-10. The complete collection of expressed genes is known as the transcriptome (Figure 3).

Six months after treatment, the Lyme disease transcriptome in PBMCs did not completely return to baseline compared to controls, with 686 total differentially regulated genes. Some of these differential gene expression patterns were common to other chronic immune-mediated illnesses. Systemic lupus erythematosus (SLE), chronic fatigue syndrome, and rheumatoid arthritis shared between 9-18% of differentially-expressed genes with PTLD, suggesting some common features between the four conditions. Another interesting finding was the identification of four genes that were differently expressed in PTLD patients compared to Lyme patients who returned to health. These genetic analyses showed a specific immune response during acute *B. burgdorferi* infection that persisted even after antibiotic treatment and beyond, giving clues to why some patients continued to be ill. In addition, these results suggest biomarkers that may be identified, which will improve early diagnosis, before antibodies are made.

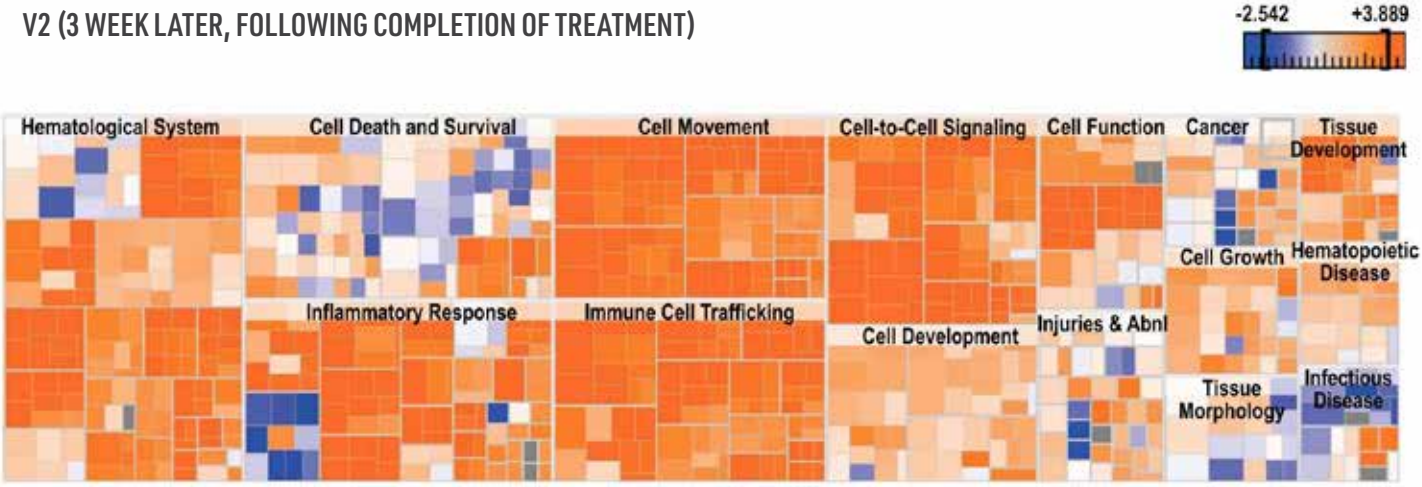
But there also is individual patient variation at the genetic level that may determine the course of Lyme disease. The innate immune system is responsible for rapid, preprogrammed defensive responses to invasive pathogens. Innate responses typically occur before the mobilization of acquired immune responses, which require more time for adaptation to the specific pathogen. The toll-like receptors (TLRs) are proteins on the surface of immune cells, that when bound by bacterial proteins, elicit swift inflammatory responses. A manifestation of advanced, disseminated Lyme disease in some patients is arthritis that is unresponsive to antibiotics, known as antibiotic-refractory Lyme arthritis. These patients, when infected with a specific strain of *B. burgdorferi*, had a higher frequency of 1805GG genetic variants of the TLR1 gene[16]. In contrast, patients with EM rashes or antibiotic-responsive arthritis, both of which are earlier and less severe stages of disease, tended to have 1805TG or 1805TT versions of TLR1. The patients with 1805GG had higher blood levels of IFNγ, CXCL9, and CXCL10, indicative of a stronger inflammatory response than in patients with 1805TG or 1805TT polymorphisms. Even when blood cells were isolated from the 1805GG group, their cells responded in vitro to *B. burgdorferi* with higher levels of cytokines than the 1805TG or 1805TT patients. For other TLR genes, such as TLR2 and TLR5, there was no association between gene polymorphisms and disease severity or outcome. This analysis showed that individual genetic variation in the early immune response is an important determinant of severity of symptoms and, ultimately, disease outcome.

These and other studies give clues regarding what genetic pathways to investigate in individuals who transition from acute infection to disseminated disease to persistent illness. However, further studies to identify more genetic controls in the response to Lyme

## V1 (ACUTE LYME DISEASE DIAGNOSIS, PRE-TREATMENT)




## V2 (3 WEEK LATER, FOLLOWING COMPLETION OF TREATMENT)

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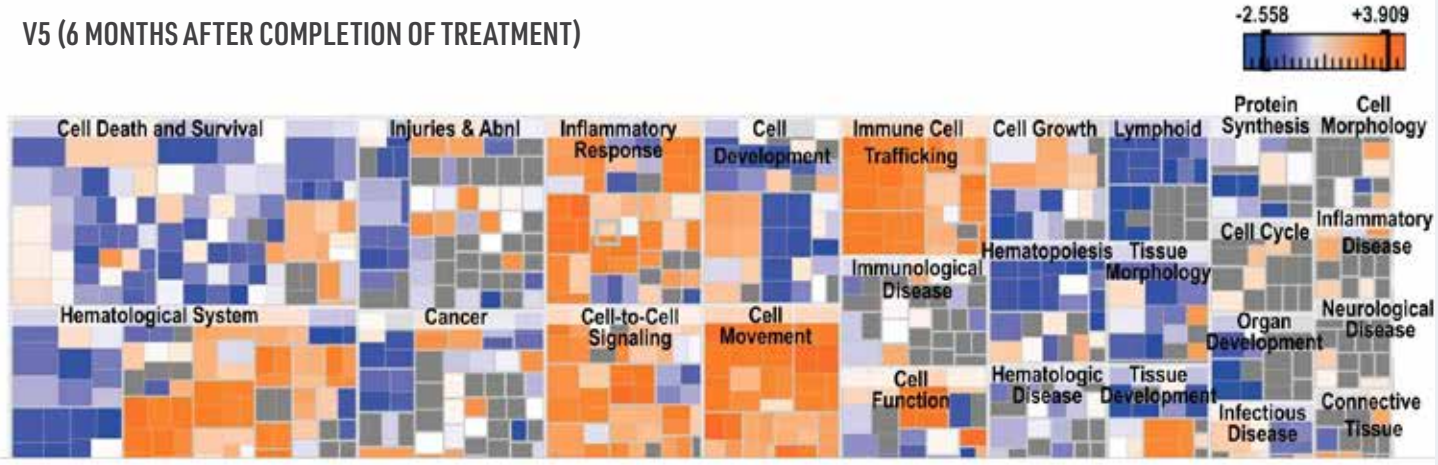


FIGURE 3

Differential gene expression in Lyme disease patients at different time points in their disease. Genetic pathways are either activated (orange) or inhibited (blue). Modified from Bouquet J. et al., 2016, mBio 7:e00100-00116. Used under terms of Creative Commons CC BY license.

disease, as well as during normal symptom resolution after treatment, will clarify why a subset of patients continue to suffer long-term illness. It may also pave the way for a precision medicine approach to treating Lyme disease, where one day it may be possible to identify patients who may be at higher risk for more severe illness. These individuals may require different or more aggressive first-line therapy during acute infection, than those who are predicted to return to health.

# PROGRESSION TO PTLD

## DEFINING PTLD

Antibiotics used to treat Lyme disease are typically bacteriostatic, which means they prevent bacterial replication, but do not directly kill them the way bactericidal antibiotics would. With bacteriostatic treatments like doxycycline, a healthy immune system is presumed to contain and kill the bacteria. However, if the acute response does not contain the infection, bacteria continue to replicate, and inflammation continues. Lyme patients who go on to develop PTLD have more symptoms during early infection, and are symptomatic for longer duration during acute illness, than those who return to health. Delays in treatment are also associated with PTLD.

To meaningfully identify immunological differences between Lyme disease patients according to disease stage, careful documentation of clinical presentation and symptoms, along with meticulous collection of patient samples at different time points is needed. Then, patient samples can be analyzed and patterns of immune response can be observed. A few research groups, some of whom are funded by GLA or collaborate with GLA awardees, have undertaken this painstaking work. One such project is the landmark SLICE study, or Study of Lyme Immunological and Clinical Events, directed by Drs. John Aucott and Mark Soloski at Johns Hopkins University. Typically, skin biopsies of EM rashes are taken at the first visit, before the initiation of antibiotic treatment, along with blood samples and detailed clinical history. All patients are then treated with antibiotics, and follow-up clinic visits and blood draws are scheduled one month, two months, six months, one year, and two years following treatment. These patient samples and data form the basis of research that aims to dissect the differences between Lyme patients who recover and those who continue to be sick. A key element of their work has been defining PTLD[17], which is complicated by the subjective nature of some symptoms. No broadly accepted single case definition exists, but it is recognized by many evidence-based experts to be a multi-organ syndrome[18] that affects a significant portion of patients treated for Lyme disease[1,19]. The challenge in characterizing PTLD is the lack of an objective, quantifiable biomarker that differentiates these patients from other stages of Lyme disease. However, this may change soon, given promising advances in studying the immune state of PTLD patients.

# ANTIBODIES AND PTLD

Antibodies are proteins that have multiple functions during immune responses. Because they specifically recognize target antigens, they take time to be elicited and are one outcome of the adaptive or acquired immune response. One example of an antibody's function is to act as an opsonin, which after binding directly to a pathogen, promotes its recognition and digestion by phagocytes. On virus-infected cells, the binding of an antibody signals cytotoxic lymphocytes to kill cells, thus limiting the spread of progeny virus.

B cells produce antibodies, and specialized activated B cells known as plasmablasts have recently been identified as important in determining whether patients treated with doxycycline either return to health, or continue to have persistent symptoms[20]. Even before receiving treatment, more EM patients who initially had a higher percentage of plasmablasts as a total of all B cells ended up recovering after treatment, compared to those with lower numbers of plasmablasts. In addition, the plasmablasts of those who returned to health had, during early infection, antibodies that targeted more *Borrelia* proteins than those who eventually had persistent symptoms. Moreover, when the *Borrelia*-specific antibodies were tested in vitro against *Borrelia* grown in culture, they inhibited the growth of bacteria, indicating they are functional. Collectively, these results suggest that an initial, strong plasmablast response, with more antibodies targeting the pathogen, was associated with resolution of symptoms after treatment.

A study funded by GLA and led by Dr. Armin Alaedini at Columbia University documented elevated anti-neural antibodies

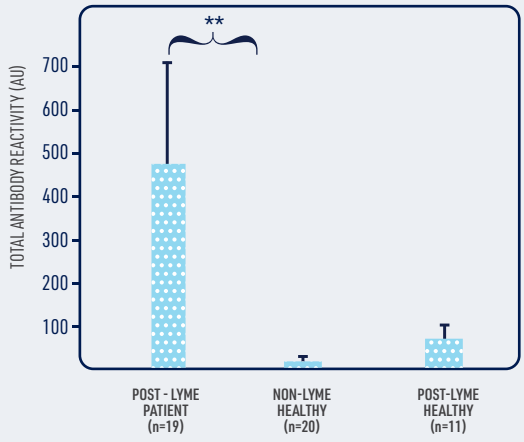


FIGURE 4.

Anti-neural antibodies in Lyme patients vs. healthy controls. ** indicates $p<0.01$, a statistically significant difference. Modified from Jacek et al., 2013, J Neuroimmunol 255:85-91. With permission from Elsevier.

in late Lyme patients (Figure 4). In almost half of patients with objectively scored memory loss, these were elevated, compared to 18.5% of Lyme-recovered and 15% of healthy controls[21]. Anti-neural antibodies were present even in patients with physician-diagnosed history of Lyme disease, who lacked antibodies against *B. burgdorferi*. This suggests that seroconversion due to actively replicating bacteria was not directly implicated in the presence of anti-neural antibodies. For patients reporting pain and cognitive dysfunction, anti-neural antibodies were elevated and similar to levels found in patients suffering from SLE, a multi-system autoimmune disorder[22]. How these anti-neural antibodies are elicited isn't understood. It's possible that *B. burgdorferi* infection may act as a polyclonal autoreactive B cell activator, turning on antibody production in a nonspecific way[23]. Alternatively, neural injury caused by the infection and invasion of the brain by spirochete bacteria may itself trigger antibody production. Whether anti-neural antibodies have a direct role in the disease process or are an outcome is also not understood and should be pursued as a research topic, given the high rate of neurological symptoms in PTLD patients.

# EVOLUTION OF ANTIBODIES AGAINST VlsE

During the progression of Lyme disease, *B. burgdorferi* uses a complex genetic recombination strategy to mutate VlsE[24], one of its outer surface proteins. This results in a constant race to make antibodies that recognize a changing target antigen and is one way that the bacteria can evade the host response. GLA-sponsored studies, again by Dr. Armin Alaedini's group, analyzed the antibody response to VlsE in Lyme patients during different stages of the disease. He found that anti-VlsE antibodies, like their target, evolved with progressive illness[25]. In earlier stages of the infection, the portions of VlsE that anchor the protein to the bacterial surface are buried, and thus are inaccessible to the immune system. This part of VlsE, known as the membrane-proximal (MP) domain, becomes more exposed as disease progresses, which indicates that the bacteria change with time. Antibodies recognizing the MP domain are more likely to be elicited in patients with late stage disease than earlier. This suggests that patients with more advanced disease continue to react to a changing pathogen and identifies MP domain-specific VlsE antibodies as a potential indicator of late-stage disease. Expanding these studies to see whether these antibodies diminish with a return to health would be interesting, because it would suggest that the ongoing immune response fades.

# INFLAMMATION & PTLD

Inflammation is a normal response to infection and tissue damage, and is typically characterized by recruitment of immune cells, followed by multiple activities that contain and kill the pathogen. Once the danger has passed, inflammation subsides. However, if inflammation continues, tissue damage may result and perpetuate inflammatory signaling, even after pathogens have been eliminated. Chronic inflammatory diseases are exemplified by autoimmune disorders, and some parallels with persistent symptoms in Lyme patients have been observed.

An example is increased interferon alpha (IFNα) activity in the sera of patients with a history of Lyme disease and objective memory impairment. Blood sera taken from such patients induced higher levels of IFNα-responsive genes, IFIT1 and IFI44 RNA after incubation with reporter cells, compared to sera from Lyme recovered patients[21]. This suggests that IFNα activity is higher in memory-impaired PTLD patients compared with Lyme-recovered. IFNα is a cytokine that is released by various immune cells, such as macrophages, T cells, microglia, astrocytes, and neurons. Before the advent of direct-acting antiviral drugs, IFNα was historically used as a treatment for hepatitis C virus, and was associated with side effects of fatigue, muscle pain, and cognitive effects, all of which are symptoms of PTLD. How elevated IFNα may cause neurological symptoms or possible neurotoxicity needs further study. However, these findings suggest at least that elevated IFNα levels may be an important biomarker that contributes to the PTLD profile.

A study of European patients with neurological Lyme disease compared chemokine levels in sera and cerebrospinal fluid (CSF) before and after antibiotic treatment. Such a study would give insights about immune cell recruitment, because chemokines are signaling molecules actively involved in bringing immune effector cells to the sites of infection. Identifying chemokine profiles during acute infection and whether they change after therapy reveals immune processes during recovery or lack thereof. Pre-treatment, Lyme patient sera had higher CXCL8, CXCL11, CXCL12 and CXCL13 than in control subjects. The researchers also documented higher CXCL8, CXCL11, CXCL12, and CXCL13 in the CSF of neuroborreliosis patients compared to healthy controls, suggesting increased inflammatory processes in the central nervous system[26]. After treatment with ceftriaxone, CSF cytokine levels subsided, which may indicate potentially useful treatment markers. However, they remained above those of healthy controls. These findings have been corroborated by other studies that implicated some of these chemokines as potentially involved in the pathogenesis of neurological Lyme[27]. Mechanistic experiments, perhaps using animal models, may help to clarify the role of chemokines in neurological disease.

A specialized immune response that controls infections by pathogens like bacteria or fungi is the Th17 response. This centers around a subset of T cells known as type 17 helper cells. These cells are activated during infection, and if they continue to drive inflammation after the infection is cleared, a pathologic inflammatory disorder may occur. Th17 helper T cells are thought to be important in the development of several inflammatory diseases, such as rheumatoid arthritis, psoriasis, and inflammatory bowel disorder. Investi-

gation of their possible involvement in persistent Lyme disease symptoms was described in a study of Slovenian patients with EM rashes, who were monitored for a year after treatment[12]. IL-23 is a cytokine that promotes the expansion of Th17 helper T cell populations. After testing patient sera, a subset of those with high IL-23 at study entry were retrospectively identified as having reported more symptoms during acute infection, as well as at six and 12 months after treatment compared to those with low IL-23. High IL-23 responders also were more likely to have positive *Borrelia* bacterial cultures at study entry, suggesting a more robust early infection and less effective bacterial killing. This in turn, would potentially drive IL-23 production higher, which ultimately was associated with more severe symptoms both early and persisting long-term. This study also identified antibody responses against endothelial cell growth factor (ECGF), as a correlate with high IL-23 and persisting symptoms. The production of an antibody against a "self" protein might be further evidence of autoimmunity; whether it is causative of symptoms or a result of infection is not known. And, one caveat of this study was that patients were likely infected with *B. afzelii* or *B. garinii*, European species of *Borrelia*, which are known to elicit more varied symptoms and immune responses than the predominant North American species, *B. burgdorferi*.

The IL-23 findings raised the intriguing possibility that its elevation and continued production might be predictive of an impending transition to PTLD. The SLICE study monitored cytokine profiles of 76 patients with EM rashes, and compared them with 26 healthy control patients. Researchers identified high CCL19 levels at one month following treatment as a biomarker associated with the transition to PTLD. In fact, they calculated that CCL19 levels greater than 111.67 pg/ml in the blood at one month after treatment as 82% sensitive and 83% specific for the later development of PTLD[28] (Figure 5). Such individuals were more than 12 times more likely to develop PTLD than those below the cutoff. This association was statistically significant, even after controlling for age, sex, number of EM lesions and anti-*Borrelia* antibodies. CCL19 is a chemokine, produced mostly in secondary lymphoid tissues, and it recruits immune cells such as T cells, B cells, and dendritic cells. It promotes the development of immune microenvironments and enhances cellular activities geared toward killing and clearing pathogens, so it was not surprising to see elevations during acute infection. IL-23 and CCL19 have both been implicated in Th17 cells in a mouse model of encephalomyelitis[29], so this may be one possible pathway to investigate further. In the mouse model, CCL19 mRNA, which is translated into CCL19 protein, is elevated during early *Borrelia* infection[30].

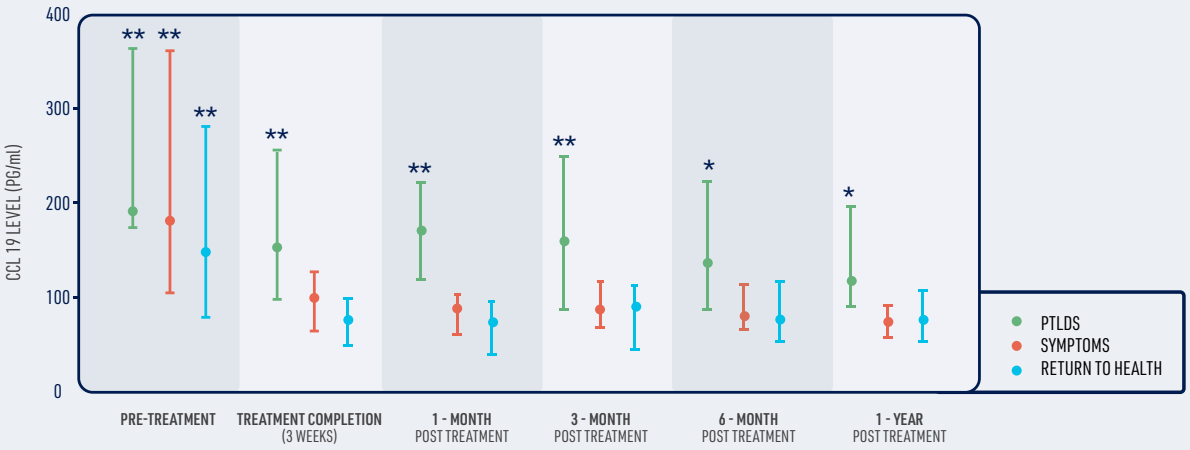


FIGURE 5.

Serum CCL19 levels in post-treatment Lyme disease patients, showing median and interquartile range. * indicates $p<0.05$, with ** indicating $p<0.01$. Modified from Aucott et al., 2016, Clin Vacc Immunol 23:757-766. With permission from the American Society for Microbiology.

Applying our knowledge of inflammatory processes in other disease scenarios is potentially useful to studying PTLD. C-reactive protein (CRP) is a protein produced by the liver, and elevated in patient blood during infection, inflammation, and tissue damage. With respect to its diagnostic potential, CRP is a biomarker of pathologic processes, and may help promote wound healing and antibacterial activity by phagocytes. Serum amyloid A (SAA) is another acute-phase protein, that when elevated, is implicated in some chronic immune diseases such as rheumatoid arthritis. In a study funded by GLA, CRP and SAA were both found to be elevated in patients with EM rashes during early Lyme disease, compared to healthy controls[31] (Figure 6). In patients with early and late neurological symptoms who underwent ultimately successful antibiotic treatment, CRP and SAA levels subsided to near-healthy levels. However, in patients with still more advanced disease -- antibiotic-refractory arthritis and PTLD -- they observed elevated CRP but not SAA. This suggests a resurgence of CRP with continued disseminated illness, but not SAA. An important result was when PTLD patients were compared to Lyme patients who had returned to health. CRP remained significantly higher in the PTLD patients than in Lyme patients who had recovered. Thus, CRP levels were high in early infection, diminished during the first stages of dissemination

of disease, then resurged in antibiotic-refractory Lyme arthritis and PTLD patients.

A strength of the CRP study was that the researchers also examined anti-*Borrelia* antibody responses in the same patient samples. During early infection and with EM rashes, antibody levels were low, and CRP was high. Antibody levels increased in patients with progressively disseminating disease and the advent of neurological symptoms. The authors proposed that very early in infection, high CRP correlated with high levels of bacteria in the skin and blood. However, with dissemination of the bacteria and clearance from blood, and a developing antibody response, CRP levels receded. Only in much later disease, such as antibiotic-refractory Lyme arthritis and PTLD, a different and not entirely understood inflammatory mechanism caused CRP to again increase, despite the absence of bacteria in the skin or blood, and irrespective of high antibody.

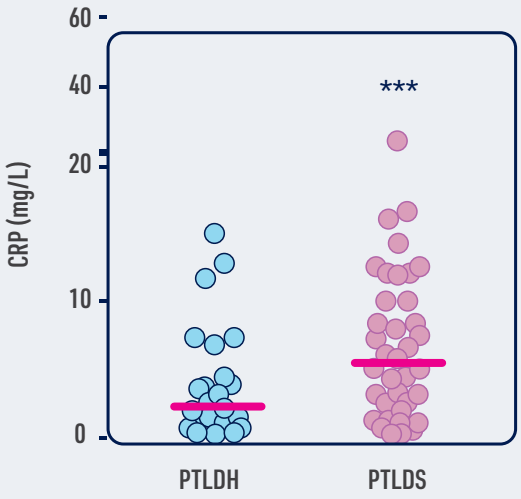


FIGURE 6.

Serum C-reactive protein in patients with post-treatment Lyme disease who returned to health (PTLDH) and those who remained sick (PTLDS). Modified from Uhde et al., 2016, Clin Infect Dis 63:1399-1404. With permission from Oxford University Press.

The authors were cautious in interpreting their data and pointed out that it isn't known if CRP plays a direct role in causing the symptoms or pathology of PTLD. Further studies will show whether CRP can be used as a reliable treatment endpoint or to help define disease stage. However, the findings were important in showing that careful, clinically-defined characterization of patients reveals potentially quantifiable biomarkers that may be useful in understanding immunopathology at different stages of Lyme disease.

Neurological symptoms are frequently associated with the early disseminated and later phases of Lyme disease. Chronic pain, cognitive deficit, "brain fog", and sleep disruption have all been reported as lasting more than six months after acute infection in a subset of patients, and these can be profoundly debilitating persistent symptoms. In vitro experiments with brain microglial cells have found that incubation with spirochetes or their debris causes these cells to produce inflammatory cytokines and chemokines. However, demonstrating immune activation in the brains of PTLD patients is more difficult and costly. Recent work reported positron emission tomography (PET) scans on 12 Lyme patients and 19 healthy controls to address this question[32]. Study subjects were injected with a radiolabeled tracer molecule that measures levels of translocator protein (TSPO), a molecule that is produced by activated microglia and astrocytes. Eight regions of the brain were scanned and the TSPO levels were calculated. In all brain regions studied, the Lyme patients had higher volumes of TSPO than the healthy controls. Each patient had fatigue and at least one cognitive symptom, such as difficulty finding words. However, the healthy controls were historical, and cognitive data was lacking in this group. This prevented full comparison of controls with Lyme patients. Overall, these findings are supportive of a hypothesized role of neuroinflammation in PTLD, but analysis of more patients and comparison with recovered Lyme patients is needed to further understand the processes involved in long term symptoms. In addition, correlating cognitive changes with brain imaging in Lyme vs. healthy controls in real time would be informative.

# CONCLUSIONS

An estimated **2 million individuals** are living with PTLD in 2020, making it a significant public health concern[6]. The cost of care for PTLD is likely to be profound, due to the chronicity of symptoms, their severity, and the large number of affected individuals. Due to the lack of a case definition or objective quantifiable biomarkers, public and private insurance does not recognize PTLD or bear the costs of this treatment, which are paid by patients themselves. Better diagnostic tests to identify both acute Lyme disease as well as the transition to PTLD are urgently needed. Additionally, we need better definition of biomarkers that could be used as measureable treatment endpoints in clinical trials, which would lead to improved treatment. Since immune dysfunctions accompany long-term symptoms, more understanding of immune responses in both people who have recovered, as well as those who continue to be ill would help clarify the process of disease. Hopefully, with improved definition of persistent symptoms[33] and more effective treatment, few, if any, will progress to PTLD.

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