PERSPECTIVES

Lyme disease: the next decade

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Abstract: Although Lyme disease remains a controversial illness, recent events have created an unprecedented opportunity to make progress against this serious tick-borne infection. Evidence presented during the legally mandated review of the restrictive Lyme guidelines of the Infectious Diseases Society of America (IDSA) has confirmed the potential for persistent infection with the Lyme spirochete, *Borrelia burgdorferi*, as well as the complicating role of tick-borne coinfections such as *Babesia*, *Anaplasma*, *Ehrlichia*, and *Bartonella* species associated with failure of short-course antibiotic therapy. Furthermore, renewed interest in the role of cell wall-deficient (CWD) forms in chronic bacterial infection and progress in understanding the molecular mechanisms of biofilms has focused attention on these processes in chronic Lyme disease. Recognition of the importance of CWD forms and biofilms in persistent *B. burgdorferi* infection should stimulate pharmaceutical research into new antimicrobial agents that target these mechanisms of chronic infection with the Lyme spirochete. Concurrent clinical implementation of proteomic screening offers a chance to correct significant deficiencies in Lyme testing. Advances in these areas have the potential to revolutionize the diagnosis and treatment of Lyme disease in the coming decade.

Keywords: Lyme disease, *Borrelia burgdorferi*, L-forms, cysts, biofilms, proteomics

The gathering storm

Lyme disease is a controversial illness.[1,2] Over the past decade, two opposing camps have emerged in the controversy over this tick-borne illness. One camp is represented by the Infectious Diseases Society of America (IDSA), which maintains that Lyme disease is a rare illness localized to well-defined areas of the world.[3,4] According to IDSA, the disease is 'hard to catch and easy to cure' because the infection is rarely encountered, easily diagnosed in its early stage by means of accurate commercial laboratory tests, and effectively treated with a short course of antibiotics over 2 to 4 weeks. Chronic infection with the Lyme spirochete, *Borrelia burgdorferi*, is rare or nonexistent.[3,4]

The opposing camp is represented by the International Lyme and Associated Diseases Society (ILADS), which argues that Lyme disease is not rare and, because its spread is facilitated by rodents, deer, and birds, can be found in an unpredictable distribution around the world accompanied by other tick-borne coinfections that may complicate the clinical picture. According to ILADS, tickbites often go unnoticed and commercial laboratory testing for Lyme disease is inaccurate.[1,5] Consequently the disease is often not recognized and may persist in a large number of patients who are untreated or undertreated, requiring prolonged antibiotic therapy to eradicate persistent infection with the evasive Lyme spirochete.[1,5]

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The controversy over Lyme disease came to a head in November 2006 when IDSA released new guidelines severely limiting treatment options for patients with persistent Lyme symptoms.[3] The guidelines were so restrictive that the Attorney General of Connecticut initiated an unprecedented investigation into potential antitrust violations by IDSA, the dominant infectious disease society in the United States, in its formulation of the guidelines.[6–8] The investigation found significant conflicts of interest and suppression of data in the guidelines development process.[6,7] As a result, IDSA created a new scientific panel to review its Lyme guidelines in a process under the complete control of IDSA.[8,9] The review panel held a hearing in July 2009 that was broadcast live over the internet and featured more than 300 peer-reviewed articles and 1600 pages of analysis supporting the concept of persistent infection despite short-course antibiotic therapy of 2 to 4 weeks in patients with persistent Lyme disease symptoms.[8,9] Despite this extensive evidence, the IDSA review panel voted unanimously to uphold the flawed Lyme guidelines. This result was not surprising given that seven of the eight members of the review panel were members of IDSA, which selected the panel.[8,9]

Advances and contradictions

The unprecedented legal action against the IDSA Lyme guidelines reflected frustration over the widening gap between groundbreaking experimental evidence and entrenched clinical practices in Lyme disease.[8,9] The past decade witnessed significant advances in understanding the pathogenesis of *B. burgdorferi* infection.[10–16] The genome of *B. burgdorferi* was sequenced in its entirety, and the biologic and immunologic contribution of various genes was elucidated.[10–12] In particular, the mechanisms of "stealth pathology" utilized by the Lyme spirochete in evading the host immune response and establishing infection in diverse tissues was illuminated.[13–16] Animal models of Lyme disease in gerbils, hamsters, mice, dogs, monkeys, and horses provided evidence for persistent infection in various tissues following experimental transmission of *B. burgdorferi*.[17–30] In many of these models, infection persisted despite the equivalent of short-course antibiotic therapy.[19–27]

While progress was being made in research models of tick-borne disease, controversy raged over the clinical features of Lyme disease.[31–41] A growing number of studies highlighted persistent symptoms in patients following clinical infection with *B. burgdorferi*, but the pathologic mechanism of those symptoms remained murky.[31–36] The concepts of 'post-Lyme syndrome', 'post-treatment Lyme disease', and 'chronic Lyme disease' were hotly debated, and the issues of postinfectious autoimmunity versus persistent spirochetal infection remained unsettled despite numerous studies from Europe and the United States that documented failure of short-course antibiotic therapy and persistent *B. burgdorferi* infection in various tissues (Appendix 1).[33–36] The role of prolonged antibiotic therapy in patients with persistent Lyme symptoms was also debated based on conflicting study results involving a limited number of patients who had been symptomatic for long periods and had already failed similar treatment.[42–47] The generalizability of these studies to the majority of patients with persistent Lyme symptoms was also questioned.[47]

Evidence for chronic infection

The comprehensive review of the IDSA Lyme guidelines provided strong evidence for chronic spirochetal infection in patients with persistent Lyme symptoms (Appendix 1).[48–58] This evidence was supported by ongoing studies showing failure of 'standard' antibiotic therapy in mice infected with the Lyme spirochete.[20–24] Coupled with previous animal and human studies of persistent infection and antibiotic failure, this evidence underscores the importance of chronic infection in Lyme disease. It also raises many questions about the mechanism(s) and optimum therapy for persistent spirochetal illness.

Complementing the evidence in favor of chronic *B. burgdorferi* infection, clinical and experimental studies have shown that tick-borne coinfections may also have chronic phases.[59–67] In the past, reports of pathology due to *Babesia*, *Anaplasma*, *Ehrlichia*, and *Bartonella* species have focused on the fulminant acute forms of infection that are relatively easy to diagnose and often fatal in immunocompromised patients.[61,63,67] More recently, these organisms have been associated with chronic persistent infection in animal models and humans.[59–67] The presence of coinfecting organisms has been shown to enhance the symptoms and exacerbate the severity of Lyme disease.[68–73] Thus recognition of chronic coinfections supports the concept of unresolved illness due to persistent infection with the Lyme spirochete.

Renewed interest in cell wall deficient bacterial forms

Cell wall-deficient (CWD) bacterial forms were first described in 1935 by Klieneberger, who named them L-forms after the Lister Institute where she worked.[74] Subsequent research by Dienes showed that various bacteria could form CWD colonies and then revert back to bacillary morphology under appropriate conditions.[75] An extensive review by Domingue

and Woody highlighted the extent of CWD morphology in many bacterial strains and the potential role of these mutant bacteria to produce persistent infection and chronic diseases.[76] The confusing terminology used to describe CWD bacteria has hindered work in this field. While the term 'L-form' or 'spheroplast' describes CWD morphology in coccobacillary organisms, the term 'cyst' or 'round body' has been used to describe similar morphology in spirochetes.[76]

Margulis et al described CWD spirochetal forms in 1993.[77] Subsequently Preac-Mursic and colleagues demonstrated the formation and cultivation of *B. burgdorferi* 'spheroplast-L-form variants',[78] and Brorson et al showed that these forms, which he termed cysts or round bodies, could revert to viable spiral forms of the bacteria.[79,80] This observation has been confirmed by other investigators.[81–83] Although the pathogenicity of CWD borrelial forms has been questioned,[3] recent studies have suggested a link between CWD borrelia and neurodegenerative diseases,[84–86] and resistance of cystic forms to antibiotic therapy has been documented.[87–90] Recent advances in understanding molecular mechanisms of CWD bacterial formation has offered a glimpse at new treatment approaches to chronic Lyme disease.[90] Currently the only antibiotic that reliably targets the cyst form of *B. burgdorferi* is metronidazole or its derivatives,[87,88] while other agents have yielded negative or conflicting treatment results with cysts.[23,89,90] Given the potential importance of CWD forms in persistent *B. burgdorferi* infection, newer antibiotics aimed at this evasive mutant are desperately needed to eradicate chronic infection in Lyme disease.[85]

Biofilms

Another mechanism of chronic infection involves the formation of biofilms.[92–98] These adherent polysaccharide-based matrices protect bacteria from the hostile host environment and facilitate persistent infection. Biofilms are responsible for a number of chronic infections, including periodontitis, chronic otitis media, endocarditis, gastrointestinal infection, and chronic lung infection.[92–98] Sapi and MacDonald raised the possibility of biofilm formation by *B. burgdorferi*, and subsequent work has demonstrated these spirochetal formations in culture and in the tick gut.[99,100] Combinations of borrelial cysts and putative biofilms have also been noted in patient skin biopsies using focus floating microscopy.[101] Biofilm formation is dependent on cyclic di-GMP expression,[102,103] and recent studies have shown that *B. burgdorferi* expresses this regulatory molecule.[104,105] Coordinated steps in the elaboration of biofilms have been demonstrated in other bacteria, and it remains to be seen whether similar molecular processes

occur in borrelial strains and whether these processes play a role in persistent infection.[106,107]

To date no antibiotic treatment exists for biofilm formation. However elucidation of the regulatory steps in the biofilm process should allow development of 'designer' antibiotics that interfere with this process.[106] It has recently been shown that mutations in genes that regulate biofilm development can interfere with the elaboration of new biofilms and also cause collapse of established biofilm colonies.[107] These findings indicate the potential effectiveness of newer antibiotics that target the biofilm regulatory process, suggesting a novel approach to treatment of Lyme disease and other chronic infections.[108,109]

Testing for Lyme disease

As we enter a new decade, clinical testing for Lyme disease remains abysmal.[110–115] The two-tier algorithm recommended by the Centers for Disease Control and Prevention utilizes a screening enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay followed by a confirmatory Western blot. Although this approach has a high test specificity, the sensitivity of the two-tier approach in Lyme disease patients tested at least 4 to 6 weeks after infection is only 44% to 56%, which is inadequate for a clinical diagnostic test and, by comparison, far below the 99.5% sensitivity of diagnostic HIV testing.[110,114,115] Furthermore, the misconception that two-tier testing is highly sensitive for Lyme disease patients with persistent arthritic or neurologic symptoms derives from a study that selected patients based on positive Lyme testing and then showed high levels of two-tier test positivity.[115] This circular reasoning is a systematic problem with the evaluation of Lyme testing.

There are a number of reasons for the inaccuracy of Lyme testing, including use of less antigenic laboratory spirochetal strains in the commercial test kits, elimination of important spirochetal target proteins from those kits, and lack of standardization of the commercial Lyme assays.[111–113] Gender bias may also be a factor: while chronic Lyme disease is reportedly more common in women, the two-tier test system yields positive results more often in men.[116] Although a newer ELISA targeting the conserved VlsE or C6 peptide of *B. burgdorferi* has been developed, this test system does not appear to be more sensitive than the two-tier approach.[117,118] While molecular testing has been useful for diagnostic confirmation and treatment monitoring in other illnesses, molecular testing for *B. burgdorferi* has been unreliable, and newer molecular techniques targeting tick-borne agents remain unproven and expensive.[119,120] Assays for more accessible

surrogate markers of Lyme disease have yet to be accepted by the general medical community.[121–125] Thus testing for Lyme disease remains problematic.

A newer approach to Lyme testing involves the use of proteomics.[126,127] Based on the known genetic make-up of the spirochete, numerous proteins can be generated in vitro and tested for antigenicity using Lyme patient sera. In this manner, novel target proteins can be identified, and conceivably new test systems based on these proteins can be developed without even knowing the function or location of the antigens within the spirochete.[126] Work on these proteomic-based test systems is already in progress, but extensive clinical validation will be required to bring those tests to market. Nevertheless the proteomic approach to Lyme testing holds great promise for more accurate serological diagnosis, and development of proteomic testing for tick-borne diseases provides a useful diagnostic model for other chronic and elusive infections. Beyond proteomics, novel test systems that exploit electromagnetic signals generated by bacterial DNA sequences may also prove to be effective in the diagnosis of chronic Lyme disease.[128,129]

Big pharma is watching

Until now, the pharmaceutical industry has steered clear of Lyme disease. There are a number of reasons for this avoidance, including the fear of entry into a controversial field and the perception that Lyme disease is easy to treat with short-course generic antibiotics. In simple terms, uncertainty about the disease and lack of profitable treatment options has limited pharmaceutical involvement in Lyme disease. This scenario is in stark contrast to the AIDS epidemic, where the prospect of billion-dollar antiviral sales propelled the pharmaceutical industry into a leading role in combating the pandemic.[130,131] In a more recent example, the development of effective (and lucrative) drug therapy for fibromyalgia has boosted the status of that previously maligned diagnostic entity and fostered unprecedented awareness of the condition in the medical community and among the lay public.[132] The lack of a similar dynamic in Lyme disease has been a significant roadblock to progress in treating the tick-borne illness.

Progress in understanding the various aspects of Lyme disease outlined above should encourage the pharmaceutical industry to assume a more active role in the Lyme arena. The evidence for chronic infection with the Lyme spirochete and coinfecting organisms supports a greater need for antibiotic therapy in this disease beyond the 2 to 4 weeks specified in the discredited IDSA guidelines.[133,134] The need for more

effective treatment of this chronic infection in turn supports the use of more complex (and lucrative) antibiotic regimens in Lyme disease. In a similar vein, targeting CWD forms of *B. burgdorferi* and biofilm formation offers the prospect of new antibiotic approaches to the disease, with an exciting opportunity for innovative therapeutics and increased profits. Development of antibiotic agents that target spirochetal CWD forms and biofilms may also provide valuable insight into the treatment of other chronic infections. The development of more reliable testing for Lyme disease based on proteomics will help to define the population in need of these innovative therapies. More reliable standardized testing will also assure reimbursement for newer Lyme therapies from third party payors.

Conclusions

In summary, extensive evidence now shows that persistent symptoms of Lyme disease are due to chronic infection with the Lyme spirochete in conjunction with other tick-borne coinfections. The mechanisms of chronic infection appear to involve CWD forms of the spirochete and biofilm formation, and these infectious processes are attractive targets for future drug development. Institution of more reliable Lyme testing based on proteomics should dispel uncertainty over the presence of the disease and facilitate targeting of patients who require treatment. The opportunity for the pharmaceutical industry to develop new drugs targeting novel infectious processes in a well-defined patient population will lead to broader recognition and more effective treatment of Lyme disease over the next decade.

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Appendix

Appendix 1 Evidence for persistent infection following treatment of Lyme disease[a]

Study/reference	Study origin	Persistence of *B. burgdorferi* shown by	Sample source
Weber et al[1]	Europe	Histology	Brain, liver (autopsy)[b]
Schmidli et al[2]	Europe	Culture	Synovial fluid
Cimmino et al[3]	Europe	Histology	Spleen
Preac-Mursic et al[4]	Europe	Culture	Skin Bx, CSF
Pfister et al[5]	Europe	Culture	CSF
Strle et al[6]	Europe	Culture	Skin Bx
Preac-Mursic et al[7]	Europe	Culture	Iris Bx
Haupl et al[8]	Europe	Culture	Ligament Bx
Strle et al[9]	Europe	Culture	Skin Bx
Preac-Mursic et al[10]	Europe	Culture	Skin Bx, CSF
Oksi et al[11]	Europe	Culture	CSF
		PCR	Brain Bx
		PCR	Brain (autopsy)
Priem et al[12]	Europe	PCR	Synovial Bx/fluid
Oksi et al[13]	Europe	Culture, PCR	Blood
Breier et al[14]	Europe	Culture	Skin Bx
Hunfeld et al[15]	Europe	Culture	Skin Bx
Hudson et al[16]	Australia	Culture, PCR	Skin Bx
Steere et al[17]	USA	Histology	Synovial Bx
Kirsch et al[18]	USA	Histology	LN (autopsy)
Liegner et al[19]	USA	Histology	Skin Bx
		PCR	Blood
Battafarano et al[20]	USA	Histology, PCR	Synovial Bx/fluid
Chancellor et al[21]	USA	Histology	Bladder Bx
Nocton et al[22]	USA	PCR	Synovial fluid
Shadick et al[23]	USA	Histology	Brain (autopsy)
Masters[24]	USA	Culture	Blood
Lawrence et al[25]	USA	PCR	CSF
Bayer et al[26]	USA	PCR	Urine
Nocton et al[27]	USA	PCR	CSF

Notes: [a]All patients had received a minimum of 2 to 4 weeks of antibiotic therapy; [b]Mother treated with antibiotics during pregnancy; newborn died.
Abbreviations: PCR, polymerase chain reaction; Bx, biopsy; CSF, cerebrospinal fluid; LN, lymph node.

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