Persistent infection in chronic Lyme disease: does form matter?

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Abstract
Lyme disease remains a controversial illness. The controversy is based on a profound disagreement over the existence of persistent infection with the Lyme spirochete, *Borrelia burgdorferi*, and the ability of this persistent infection to cause chronic symptoms in patients who are untreated or undertreated for the initial spirochetal disease. In this article, we summarize evidence from animal models, human studies and in vitro experiments that support persistent spirochetal infection as the cause of chronic Lyme disease. Specifically, the role of cysts and biofilms in this process is outlined, and the need for better treatment options for patients with chronic Lyme disease is defined.

Keywords: Lyme disease, *Borrelia burgdorferi*, cysts, biofilms, animal models

Introduction
More than 35 years after its discovery, Lyme disease remains a controversial illness [1-4]. At the heart of this controversy lies a profound disagreement over the existence of persistent infection with the Lyme spirochete, *Borrelia burgdorferi*, and the ability of this persistent infection to cause chronic symptoms in patients who are untreated or undertreated for the initial spirochetal disease. Although the Infectious Diseases Society of America (IDSA) maintains that there is no “credible scientific evidence” for persistent infection with *B. burgdorferi* following 2-4 weeks of antibiotic therapy [3], a growing number of animal and human studies provide evidence for persistent infection as a cause of chronic symptoms in Lyme disease patients, thereby disputing the IDSA point of view [5] (Tables 1 and 2).

Review
A recently published monkey study by Embers et al., provides the best animal evidence for persistent infection as a model of chronic Lyme disease [6]. The study was conceived as an animal counterpart to the human trial by Klempner et al., that was published in 2001 [7], and the monkeys were treated with a regimen of intravenous ceftriaxone followed by oral doxycycline that was identical to the protocol used in the human trial. The results of this study showed that three-quarters of the monkeys failed treatment, and these animals had evidence of persistent infection in various tissues at necropsy using culture, immunofluorescence and polymerase chain reaction (PCR) techniques [6]. Equally important, the study showed that 25% of treated monkeys cleared their infection, thereby demonstrating antibiotic efficacy in some animals. This finding contradicts the negative treatment results reported by Klempner et al., that IDSA cites to support its belief that antibiotics are not effective in treating patients with persistent Lyme disease symptoms [8]. Although the monkey study was designed and conducted with the approval and funding of the National Institutes of Health, curiously it took a dozen years for the study to see the light of day while the IDSA continued its campaign to discredit the persistent infection theory of chronic Lyme disease [9].

In another recently published study, Bockenstedt et al., present a mouse model of *B. burgdorferi* infection that on the surface appears to contradict the monkey study [10]. Following infection, the mice were treated with subcutaneous ceftriaxone or doxycycline administered in drinking water. The authors arrive at the conclusion that non-infectious spirochetal “debris” gets deposited around the joints of these mice, and instead of being cleared by the reticuloendothelial system this “debris” is responsible for persistent inflammation in mouse tissues [10]. The “debris”, which contained both DNA and protein particles, could not be cultured, transmitted to other mice via ear transplants or to ticks that were allowed to feed on the mice (xenodiagnosis). This novel theory of non-infectious persistence of *B. burgdorferi* “debris” including the presence of DNA is highly speculative and contradicts previous experimental results. For example, Malawista et al., showed that *B. burgdorferi* DNA is rapidly cleared from culture-negative ear and bladder tissues of mice following prompt antibiotic treatment [11], and Lazarus et al., demonstrated that DNA from dead spirochetes is routinely cleared from mouse skin within several hours [12]. Furthermore the study methods of Bockenstedt et al., may have been insufficient to rule out persistent spirochetal forms of *B. burgdorferi*, since ear transplants are often negative following antibiotic treatment, and using an insufficient number of animals for xenodiagnosis may fail to demonstrate transmissible infection [13,14]. Of greater importance, there appear to be two alternative mechanisms of *B. burgdorferi* persistence that merit consideration in these mice.

In his Commentary on the mouse study, Alan Barbour proposes the alternative hypothesis that cell-wall deficient L-forms, or cysts, may be responsible for *B. burgdorferi* persistence in these animals [15]. He notes that these cystic structures,
which Bockenstedt et al., observed in their infected animals (Figure 2 in their study [10]), have been described as a persister mechanism employed by many bacteria, including *B. burgdorferi* [16-24]. The study authors claim that these are not true cysts because they form too fast, appearing in minutes rather than hours or days. However, Brorson and Brorson have demonstrated that cysts of *B. burgdorferi* may develop in minutes under appropriate culture conditions [25]. Thus the observation of Bockenstedt et al., supports *B. burgdorferi* cyst formation in their mouse model, and this cyst formation appears to be a better explanation for spirochetal persistence compared to the "debris" that the authors postulate.

As noted above, the methods employed by Bockenstedt et al., may not have been sufficient to exclude persistent spirochetal forms in their animals. Persistent viable organisms may have been hidden in biofilms, the adherent polysaccharide-based matrices that protect bacteria against the host immune system and antibiotic therapy [1]. Biofilms of *B. burgdorferi* have been demonstrated *in vitro* by Sapi et al. [26]. These biofilms may take the form of "debris" on intravital microscopy, and they may contain organisms that are non-cultivable but still viable and prone to reactivation [26,27]. Biofilms of *B. burgdorferi* would also be consistent with the "amber hypothesis" proposed as a mechanism of persistent Lyme disease symptoms [28]. Persistor spirochetes in biofilms could explain the experimental results of Bockenstedt et al., and would offer a more plausible explanation than the "debris" hypothesis for the reasons outlined above.

**Conclusions**

Like most aspects of Lyme disease, the role of cysts and biofilms in persistent *B. burgdorferi* infection has been controversial [1,27,29]. However, the study by Bockenstedt et al., and the open-minded commentary by Barbour may open up new vistas on this fascinating aspect of bacterial persistence. Whether chronic Lyme disease arises from persisting spirochetal forms hidden in biofilms (as suggested by the monkey study of Embers et al., and the experimental work of Sapi et al.) or from cell wall-deficient cysts (L-forms) of *B. burgdorferi* (as suggested by the mouse study observations of Bockenstedt et al., and the interpretation of Barbour), persisting forms of bacteria require treatment. To date the treatment options for these bacterial persisters are extremely limited, but their recognition dictates a more aggressive approach to eradication of Lyme disease using combination antibiotic therapy modelled on treatment regimens for tuberculosis and HIV disease [2]. The fact that *B. burgdorferi* shares resistance genes with pathogenic mycobacteria supports the need for

<table>
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<th>Study/Year/ Reference</th>
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<td>Monkeys</td>
<td>PCR</td>
<td>3 months</td>
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</tr>
<tr>
<td>Embers et al., 2012 [58]</td>
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<td>Histology, PCR</td>
<td>1-4 years†</td>
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</tbody>
</table>

*PCR, polymerase chain reaction; LN, lymph node.
**Time from initial infection to final positive testing point.
†Detectable *B. burgdorferi* following antibiotic treatment.
this therapeutic approach [30,31]. It remains to be seen which form of *B. burgdorferi* is the true culprit in chronic Lyme disease and which form of treatment is most efficacious in clearing both forms of bacteria from patients [32-38].

### List of abbreviations

- PCR: Polymerase chain reaction
- LN: Lymph node
- Bx: Biopsy
- CSF: Cerebrospinal fluid
- IDSA: Infectious Diseases Society of America
- ILADS: International Lyme and Associated Diseases Society

### Competing interests

Raphael B. Stricker serves without compensation on the medical advisory panel of QMedRx Inc. He has no financial ties to the company. Lorraine Johnson has no conflicts to declare.

### Authors’ contributions

Raphael B. Stricker and Lorraine Johnson meet criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE). Both authors made substantial contributions to the conception and design of the article and were involved in the analysis and interpretation of data. Both authors were involved in all stages of manuscript development and have approved the final version.

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### References


74. Hunfeld KP, Ruzic-Sablic E, Norris DE, Klaiczy P and Strle F: In vitro susceptibility testing of Borrelia burgdorferi sensu lato isolates cultured from patients with erythema migrans before and after antimicrobial


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