COMMENTARY

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The Clinical Relevance of Studies on *Borrelia burgdorferi* Persisters

In North America, Lyme disease is principally caused by *Borrelia burgdorferi* sensu stricto, hereafter referred to as "*B. burgdorferi*." It is acquired by the bite of an infected Ixodes tick. The most common clinical manifestation is a skin lesion, referred to as "erythema migrans," which is due to cutaneous infection with *B. burgdorferi*. Other objective manifestations may involve the nervous system, heart, or joints. Treatment with antibiotics typically resolves the objective clinical manifestation. Accompanying subjective symptoms, such as fatigue and joint or muscle pain, often persist for many weeks. Patients with such subjective symptoms lasting 6 months or more are often referred to as having "post-treatment Lyme disease symptoms." Such prolonged symptoms occur in approximately 10% of US patients treated for erythema migrans.

One theory advanced to explain the long-term persistence of symptoms is failure of the initial course of antibiotic therapy to eradicate fully *B. burgdorferi* cells. Why or how residual bacterial cells might result in persistence of nonspecific symptoms, in the absence of a localized inflammatory lesion at the site of the residual infection, is not known. However, the possibility that post-treatment symptoms are due to persistent *B. burgdorferi* infection has been explored in several placebo-controlled, antibiotic retreatment studies. The results of 5 such clinical trials failed to provide evidence of convincing clinical benefit or that the risk/benefit ratio favored this therapeutic approach.[1,2] Because considerable improvement (up to 38%) was observed among placebo controls, this suggests that persistent symptoms are often reversible.[1] Some of these studies also attempted to establish evidence of persistence of *B. burgdorferi* by culture or molecular methods.[1] None were successful.

Despite the lack of evidence of persistent infection and the absence of a discrete inflammatory focus of infection expected for infections caused by *B. burgdorferi*, other indirect approaches have been examined to validate the assumption of persistent infection in patients with post-treatment symptoms. One is based on in vitro studies demonstrating persistence of viable *B. burgdorferi* in cultures treated with antibiotics.[3,4] This form of persistence has been seen with many other species of bacteria.[5] Such "persisters," after isolation and recultivation in vitro, however, are no more resistant to the killing effects of the antibiotic studied than they originally were; thus, they are neither antibiotic resistant mutants nor appear to be biologically altered in any other way from the original bacterial strain.[5] Various mechanisms have been proposed to account for this form of persistence[6,7]; however, none have been confirmed experimentally.

Furthermore, this form of persistence in vitro has not been observed consistently with *B. burgdorferi* and appears to be highly dependent on the particular laboratory conditions used. One condition is the requirement for a large inoculum of bacterial cells, numbers that may be less relevant—if relevant at all—to what occurs in vivo.[8] For example, in patients with meningitis due to Lyme disease, so few spirochetes are present in the subarachnoid space that both culture and polymerase chain reaction are negative in the majority of cases, before any treatment with antibiotics. In addition, and of greater importance, is that the in vitro conditions required to demonstrate the presence of "persisters" fail to account for the role of the humoral and cellular effects of the host's immune system. Because the protective effects of the host's immune system play a decisive role in curing or limiting infections in vivo, it is impossible to evaluate the clinical significance of "persisters" observed in in vitro experiments. Moreover, the in vitro phenomenon of "persisters" as described earlier is pertinent only to the cidal effects of antibiotics. Except for certain infections, for example, infective endocarditis, inhibitory effects of antibiotics are sufficient to cure bacterial infections. Many currently used antibiotics exhibit only bacteriostatic effects when used in vitro and in vivo, yet are highly effective clinically.

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Multiple studies have investigated whether *B. burgdorferi* might persist in infected animals after antibiotic therapy.[9-12] Several approaches have been used to assess persistence. They include culture; the polymerase chain reaction to detect DNA and RNA; quantitative polymerase chain reaction to determine if the number of borrelial cells is changing over time; whether ticks that fed on treated animals become infected (xenodiagnosis) and, if they do, whether they are capable of transmitting infection to uninfected animals; whether tissue samples obtained from antibiotic-treated infected animals cause infection after transfer to uninfected animals; whether antibody levels to *B. burgdorferi* change over time after antibiotic treatment; and various methods to visualize spirochetes in the tissues of antibiotic-treated animals. A major limitation of most of these studies is the failure to treat animals with doses of antibiotics that approximate the antibiotic exposure that would be expected in humans receiving standard treatment regimens.[9] In addition, most of these studies failed to measure the antibiotic blood levels achieved in infected animals at even a single time point.[9] The results of these studies have been highly variable. Some have claimed that viable cells were found,[9,11] whereas others have found evidence only of bacterial cellular debris.[10] Most of the studies that have claimed to demonstrate viability did not base this assessment on the ability to grow *B. burgdorferi* in culture. In one study, in which infected mice were treated with only 5 days of an antibiotic, culture of the entire mouse failed to reveal the presence of viable *B. burgdorferi*.[12] In addition, none of these studies have demonstrated that what was assumed to be a persistent infection was associated with tissue inflammation in the originally infected animals or that tick or tissue transfer of putative residual borrelia to uninfected animals induced inflammation.[9] One finding that emerged provided additional support for the hypothesis that the pathogenesis of Lyme arthritis might be related at least in part to *B. burgdorferi* cellular debris in or near joint spaces.[10,13]

Whether *B. burgdorferi* persists in some antibiotic-treated patients in the United States with clinically resolved Lyme disease has not been established or completely excluded. We do know that patients with recurrences of erythema migrans skin lesions are typically newly infected with a different strain of *B. burgdorferi* that was acquired from another tick bite.[14] As noted earlier, there is no evidence to date to indicate that "persisters" are present in patients with post-treatment Lyme disease symptoms.[1] If "persisters" were present in patients with persistent symptoms, what would be the mechanism responsible for causing symptoms in the absence of residual inflammation, because *B. burgdorferi* is not known to produce exotoxins.[15]

It cannot be overemphasized that the complete elimination of infection is seldom used as the benchmark for success in the treatment of other infectious diseases. Resolution of the objective manifestations of the infection and lack of relapse, rather than the complete elimination of viable bacteria, are of primary concern. Experience with latent tuberculosis has been highly instructive in providing evidence that persistence per se causes no symptoms, and if latent disease becomes active it is associated with a site of inflammation.

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