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ORIGINAL ARTICLE

The amber theory of Lyme arthritis: initial description and clinical implications

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Abstract Lyme arthritis differs in many respects from other bacterial causes of arthritis. Based on an observation made for a patient with Lyme arthritis, we propose that the pathogenesis of joint swelling in Lyme arthritis is due to the introduction into the joint space of non-viable spirochetes or more likely spirochetal debris enmeshed in a host-derived fibrinous or collagenous matrix. This "amber" hypothesis can account for the clinical and laboratory features of Lyme arthritis and is amenable to experimental validation. Validation would directly impact the clinical management of patients with Lyme arthritis.

Keywords Arthritis · *Borrelia burgdorferi* · Lyme disease · Septic arthritis

Introduction

Arthritis is the most common extracutaneous manifestation in untreated Lyme disease patients in USA who present with skin infection due to *Borrelia burgdorferi* (i.e., erythema migrans) [1]. As with other causes of septic arthritis, Lyme arthritis is typically a mono- or oligo-articular arthritis of large joints [1]. However, many clinical features of Lyme arthritis are rather unique [1–3] and differ from those seen with pyogenic bacterial pathogens such as *Staphylococcus aureus* [4].

For example, Lyme arthritis has a delayed onset, occurring on average of about 6 months after onset of *B. burgdorferi* infection and presumably long after the joint was seeded by the spirochete through hematogenous dissemination (Table 1) [1]. Blood cultures are negative in patients with Lyme arthritis [5] and indeed are rarely positive in any Lyme disease patient in the absence of a concomitant erythema migrans skin lesion [5]. Erythema migrans has typically resolved long before patients develop joint swelling.

Furthermore, although synovial fluid samples from patients with Lyme arthritis often show evidence of *B. burgdorferi* DNA [6–12], both culture techniques [7, 9, 12–14] and limited data from other types of molecular analyses, such as testing for the presence of mRNA [7], indicate that viable spirochetes are nearly always absent from synovial fluid *before* antibiotic treatment is received. Consistent with the absence of viable spirochetes in synovial fluid, Lyme arthritis typically resolves spontaneously in days to months only to reappear again in the same or a different joint [1, 2, 15]. This process appears to be caused by the presence of viable *B. burgdorferi* cells in the joint but outside of the synovial fluid compartment, since such recurrences are effectively reduced in frequency by antibiotic therapy as demonstrated by multiple treatment trials [16], including one that was randomized, double-blind, and placebo-controlled [14].

In approximately 10 % of patients with Lyme arthritis, joint swelling will persist for months to years despite antibiotic treatment [1, 2, 8, 11, 15, 17–19]. Both the genetics of the host [15, 19] and the pathogen [8] appear to play a role in driving this outcome. PCR testing of synovial fluid will eventually show clearance of *B. burgdorferi* DNA in those cases in which it could be initially detected despite the

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Table 1 Features of Lyme arthritis

Occurs an average of 6 months after onset of cutaneous infection with *Borrelia burgdorferi* (i.e., erythema migrans) in untreated patients
Patients with Lyme arthritis are not spirochetemic at the time of onset of the joint swelling
DNA of *B. burgdorferi* (particularly plasmid DNA) can often be detected in synovial fluid of untreated patients with Lyme arthritis, but the synovial fluid is almost invariably culture negative. In addition, other molecular analyses suggest that viable spirochetes are absent from synovial fluid in untreated patients (e.g., absence of mRNA)
In most untreated patients, the arthritis will spontaneously resolve within days to months but typically will recur in the same or a different joint unless the patient is treated with antibiotic therapy
In genetically predisposed patients, particularly those infected with certain genetic subtypes of *B. burgdorferi*, Lyme arthritis of the knee may persist irrespective of antibiotic therapy, but may respond to NSAIDS, DMARDS, intra-articular steroids, or synovectomy. In these patients development of arthritis of other joints, however, is usually prevented by antibiotic therapy

persistence of synovitis [7, 17]. The synovitis generally improves with interventions directed against inflammation that are used to treat non-infectious forms of arthritis, such NSAIDs, DMARDs, and intra-articular steroids [17]. Synovectomy is sometimes performed if other measures fail to result in improvement. Ultimately, the synovitis will resolve spontaneously, but this may take several years. Although antibiotic therapy does not lead to resolution of the synovitis per se in such cases, development of Lyme arthritis of other joints is usually effectively prevented by antibiotic treatment [15, 17].

Amber hypothesis

How might all of these observations be explained? Insights from a patient we encountered suggest a plausible explanation that has not been previously proposed. Synovial fluid obtained from the knee of this patient prior to antibiotic therapy was inoculated into Barbour–Stoenner–Kelly (BSK) medium. Spirochetes were seen on microscopic examination of the culture medium, which initially led us to believe that we had for the first time at our center successfully cultivated *B. burgdorferi* from synovial fluid. However, the visualized spirochetes were non-motile and appeared to be enmeshed in a matrix (chemically undefined); in addition, they could not be sub-cultured. Lack of success in subculture has also been noted previously in the only prior report of a "positive" synovial fluid culture in a US patient with Lyme arthritis [20]. It was concluded that the spirochetes observed in our patient were in fact dead but well preserved morphologically in a manner somewhat analogous to organisms in amber (in ordinary usage, the term "amber" refers to a translucent fossilized tree resin that may contain preserved insects, plants or animals).

This interpretation has led to what may be termed the "amber" hypothesis of Lyme arthritis, which can explain what has been observed clinically (Table 2). It is postulated that *B. burgdorferi* preferentially spreads through the blood stream to structures in close proximity to the joint space such as the synovial membrane, joint capsule, tendons and/or tendon sheaths, ligaments, cartilage or menisci, where some bacterial cells may become enmeshed in a host-derived fibrinous or collagenous matrix. *B. burgdorferi* is known to adhere to type 1 collagen, decorin and other components of the connective tissue extracellular matrix as reviewed elsewhere [3, 15, 21, 22]. If this enmeshed material eventually dislodges and enters the joint space, the spirochetal material may be released over time. Specific components of the spirochetal material, yet to be defined, might then stimulate an inflammatory response at least until the material is cleared or neutralized by the host, and possibly longer. This would be recognized clinically as Lyme arthritis. Entry into the joint space of encased spirochetes that appear to be morphologically intact is probably not essential in triggering an inflammatory reaction, particularly

Table 2 The amber hypothesis of Lyme arthritis

Posits that non-viable spirochetes or spirochetal debris become enmeshed in a chemically undefined matrix that dislodges into the joint space. This hypothesis can account for:

1. delayed onset relative to acquisition of infection
2. spontaneous recovery and recurrences without antibiotic therapy
3. absence of positive synovial fluid cultures
4. absence of evidence of viable spirochetes by other test methods
5. imbalance of plasmid DNA of *B. burgdorferi* compared with chromosomal DNA in synovial fluid
6. eventual resolution of an episode of synovitis after treatment with antibiotics but with the potential for development of recurrent synovitis in the same joint or in a different joint in a small minority of patients

in view of the apparent uniqueness of the observation made in our patient. Instead, introduction of enmeshed spirochetal debris is more likely the triggering factor. The process being proposed is analogous to what has been observed in certain experimental studies of animals [4, 23, 24].

In addition, since viable spirochetes cannot be found in synovial fluid based on the available evidence, it would appear that either non-viable spirochetes preferentially become enmeshed or during the enmeshing process, the spirochete becomes non-viable. Of relevance to this supposition, in other bacteria, there is evidence that not all binding to the extracellular matrix is mediated by molecules present on the surface of bacteria; instead, some molecules capable of binding might be released by the bacteria [25]. Therefore, it could be speculated that immunologically injured spirochetes might potentially elaborate or release such factors and thereby promote their encasement. The key presumption in terms of explaining certain of the clinical features of Lyme arthritis is that when encased, material of spirochetal origin (including nucleic acids) would be preserved longer than dead bacterial cells or debris not within such an encasement and thus account both for the observed delay in onset and the detection of borrelial DNA in synovial fluid. It is important to emphasize, however, that what is being suggested in the amber hypothesis is not biofilm production (the term "biofilm" may be defined as an aggregate of microorganisms adhering to a surface that is encased in an extracellular biopolymer). There is no convincing evidence at present that *B. burgdorferi* can elaborate biofilm, and furthermore, microorganisms remain viable in biofilms, which differs from what appears to be happening in Lyme arthritis.

A consistent observation has been that by the time patients develop Lyme arthritis, they are already strongly seropositive [18]. This could simply be due to the fact that Lyme arthritis occurs late in the course of infection. Alternatively, a robust immunologic response may be an important contributing factor in the pathogenesis of Lyme arthritis, since by the time arthritis has developed, the humoral immunologic response is considerably stronger and broader than at earlier time points [26, 27]. Perhaps, the most straight forward role of a vigorous immunologic response would be to substantively enhance the ensuing inflammatory process (see Experimental support: amber hypothesis subsection below) [3, 15, 18]. It is of interest, however, to speculate about less intuitively obvious pathogenetic mechanisms. For instance, with certain other bacteria, it has been suggested that some antibodies to the microorganism might paradoxically strengthen the binding of bacterial cells to components of the extracellular matrix [25]. If this were found for *B. burgdorferi*, an argument could be advanced that a strong immunologic response would serve to promote the encasement of spirochetal material. In either circumstance, if a strong immunologic response was demonstrated to play an essential role in the pathogenesis of Lyme arthritis, the duration of time required for it to develop could be a contributing factor in explaining why synovitis occurs as a late clinical manifestation. Of relevance to the apparent uniqueness of arthritis relative to the other clinical manifestations of Lyme disease, it also seems likely that either the spirochetal molecules that are stimulating the inflammatory process and/or the host responses in the joint space are different from those associated with inflammation at other sites in *B. burgdorferi* infection, such as skin or subarachnoid space, since granulocytes rather than lymphocytes or plasma cells are the predominant cell type in synovial fluid [1, 3, 15].

In the amber hypothesis, it is further postulated that enmeshed spirochetal material would preferentially form in untreated patients rather than as a result of spirochetal killing due to antibiotic therapy. Although a precise explanation for why this should occur is not clear, injury from antibiotics is likely to be very different from injury due to the host's immune response, such that it can be hypothesized that the likelihood of future enmeshed spirochetal material might be considerably less from antibiotic treatment. The observed high degree of efficacy of antibiotic therapy in preventing subsequent episodes of synovitis in patients with Lyme arthritis is strong evidence against a significant role for antibiotic injury in promoting further spirochetal encasement [14, 16, 28].

It is also proposed that without the spontaneous formation and dislodgement into the joint space of encased spirochetal material, there would be no joint swelling despite the presence of viable *B. burgdorferi* cells in nearby anatomic compartments of the joint, possibly even as close as in the synovium. Exactly why viable *B. burgdorferi* cells might not enter and persist in the joint space and thereby cause joint inflammation is not entirely clear, but the evidence to date has consistently failed to demonstrate the presence of viable organisms in this location in patients [7, 9, 12–14], which served as the impetus for postulating the amber hypothesis. Thus, the likely role of antibiotic therapy in patients with Lyme arthritis is to eradicate the spirochete from the joint sites that were originally seeded and thereby prevent further development of matrix-enmeshed spirochetal material. If the amber hypothesis is correct, it would be predicted that some patients would develop recurrent joint swelling after the completion of antibiotic therapy. This would logically follow because antibiotic therapy would not be expected to have any effect on matrix-enmeshed spirochetal material that had already formed. Consistent with this hypothesis, subsequent episodes of joint swelling have in fact been observed in a minority of patients who have been treated with antibiotics [14, 28].

In the presence of predisposing genetic factors of the host and/or the spirochete, this "sterile" inflammation, once initiated,

might persist on a more chronic basis [1, 2, 15, 17]. When prolonged joint inflammation develops, it could occur simply because of persistence of inflammatory spirochetal molecules or alternatively because of immunologic dysregulation [3, 15], or both.

Experimental support: amber hypothesis

Evidence has existed for a long time that non-intact spirochetal material containing nucleic acids is likely to be present in the joint space of patients with Lyme arthritis. In attempting to explain why chromosomal DNA of *B. burgdorferi* could be detected in synovial fluid much less frequently than plasmid DNA, investigators had previously hypothesized that membranous vesicles or blebs from the spirochete containing only plasmid DNA were shed into the joint space [12].

It is also well established experimentally for other microorganisms that neither viable nor even intact bacterial cells are required for the development of acute and chronic joint inflammation in animal systems [4, 29–31]. In particular, it has been recognized that introduction of certain cationic molecules into a joint may cause arthritis, but the nature of the microbial inflammatory material can be quite varied. Bacterial DNA itself may be inflammatory when introduced into a joint because of the presence of unmethylated CpG moieties; in bacterial DNA, unlike in mammalian DNA, the C residue in the CpG dinucleotide is typically unmethylated [32].

Using an experimental rat model, it has previously been shown that the introduction of certain outer surface lipoproteins of *B. burgdorferi* directly into a joint will cause severe arthritis and that the inflammatory potential of such material can be enhanced in animals previously immunized with the same material [23, 24]. According to the investigators, the latter findings may have been due to an antigen-induced allergic arthritis. The *B. burgdorferi* genome contains >150 genes encoding putative lipoproteins making this class of proteins of considerable interest in the pathogenesis of joint inflammation in Lyme arthritis [23, 24]. In addition, it should be kept in mind that not all spirochetal lipoproteins are surface exposed, suggesting that intact spirochetes would not be essential in causing this inflammatory reaction.

Further study of the amber hypothesis

The "amber" hypothesis is critically dependent on the accuracy of the data that demonstrates the absence of replicating cells of *B. burgdorferi* in the joint space. It should be emphasized that although this has been a consistent finding by different investigators and by different assay methods, the evidence is still limited [7, 9, 12–14]. Conceivably, inoculation of larger volumes of synovial fluid could lead to more positive cultures, as has been the experience with cultures for *B. burgdorferi* from blood [33].

Among the numerous animal models of Lyme arthritis, the mouse model has been the most extensively studied [34]. However, this model does not faithfully reproduce what has been observed clinically and has not provided evidence that would explain the episodic and delayed onset of arthritis seen in humans. Instead, the animal model that seems to most closely resemble the condition in humans is tick-transmitted *B. burgdorferi* infection of specific pathogen-free beagle dogs. Similar to humans, in this model, untreated dogs develop episodic and self-limited bouts of synovitis with fibrinosuppurative inflammation following an incubation period of 2–5 months [35–37]. And as in humans, antibiotic therapy is highly effective in preventing further episodes of joint inflammation, even if the dogs are immunosuppressed by corticosteroids [36]. In the dog studies, it was also noted that *B. burgdorferi* could be isolated from joint capsules of both lame and clinically normal dogs, suggesting that although viable spirochetes are indeed present in the joint area, their presence alone was not sufficient to cause joint inflammation [35]. Furthermore, in dogs with synovitis, the affected joint capsule was edematous and neutrophils were found both below the synoviocyte layer and between synoviocytes [37]. In future studies using the dog model, it would be of particular interest to determine systematically the frequency of positive cultures for *B. burgdorferi* from joint fluid per se.

It may be of importance in the design of future experimental studies intended to determine which spirochetal components can elicit an inflammatory response when directly introduced into joints, to bear in mind that *B. burgdorferi* cells grown in in vitro cultures are phenotypically different from those growing in vivo. Therefore, it would be of particular interest to study *B. burgdorferi* cellular material derived from in vivo grown spirochetes. Also, in these experiments, it may be of particular relevance to investigate animals which have mounted a robust immune response from longstanding infection. If joint inflammation is elicited, the next step should be a systematic search to identify which specific spirochetal components are responsible for this reaction.

Why is the amber hypothesis potentially relevant to the care of patients?

Patients with Lyme arthritis are treated with a 4-week course of oral antibiotics, which is longer than what is customarily prescribed for most of the other clinical manifestations of Lyme disease [16]. Furthermore, if the arthritis is not completely resolved at the end of treatment, then such patients are retreated with either another 4-week course of oral antibiotics or with a 2- to 4-week course of parenteral antibiotic therapy [16]. Persistent arthritis at 4 weeks, however, has been observed in at least 42 % of treated patients [14, 28]. Thus, a considerable proportion of patients with Lyme arthritis are regularly exposed to at least 8 weeks of antibiotic therapy that

may place them at increased risk for adverse events. If the amber hypothesis is correct, these patients are unlikely to benefit from retreatment with antibiotics and would be more appropriately treated with anti-inflammatory medications, such as intra-articular injections of corticosteroids. The preferred treatment strategy for such patients should be determined based on a randomized clinical treatment trial.

Conclusions

Available evidence suggests that Lyme arthritis is not caused by active infection within the joint space per se, in contrast to the experience with most other causes of bacterial arthritis. In what may be termed the "amber hypothesis" of Lyme arthritis, we propose that through hematogenous dissemination, *B. burgdorferi* infects anatomic structures in close proximity to the joint space such as, for example, the joint capsule. Over time, non-viable spirochetes or more likely spirochetal debris becomes enmeshed in a host-derived fibrinous or collagenous matrix. This material may then enter the joint space where eventually spirochetal material is released that causes joint inflammation, analogous to what has been observed in experimental animal systems. A pre-existing robust immunologic response is also likely to be important in pathogenesis. The well-established efficacy of antibiotic therapy is probably due to the eradication of viable spirochetes from the sites in the joint that were originally seeded and thereby prevent or substantively reduce future entry of non-viable but inflammatory spirochetal material into the joint space. If the amber hypothesis is correct, many patients with Lyme arthritis are being overtreated with antibiotics.

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Competing interests None

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