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# Review

# Cellular and molecular aspects of Lyme arthritis

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**Abstract.** Lyme disease is a multisystem illness initiated upon infection with the spirochete *Borrelia burgdorferi*. Whereas the majority of patients who develop Lyme arthritis may be successfully treated with antibiotic therapy, about 10% go on to develop arthritis which persists for months to years, despite antibiotic therapy. Development of what we have termed treatment-resistant Lyme arthritis has previously been associated with both the presence of particular major histocompatibility complex class II alleles and immunoreactivity to the spriochetal outer surface protein A (OspA). Recently, we showed that patients with treatment-resistant Lyme arthritis, but not patients with other forms of arthritis, generate synovial fluid T cell responses to an immunodominant epitope of OspA and a highly homologous region of the human-lymphocyte-function-associated antigen-1$\alpha_L$ chain. Identification of a bacterial antigen capable of propagating an autoimmune response against a self-antigen provides a model of molecular mimicry in the pathogenesis of treatment-resistant Lyme arthritis.

**Key words.** Lyme disease; arthritis; autoimmunity; molecular mimicry; OspA.

## Introduction

Lyme disease or Lyme borreliosis is a complex, multisystem infection caused by the tick-borne spirochete *Borrelia burgdorferi*. The infection is endemic in certain parts of North America, Europe and Asia. In the United States, where Lyme disease is the most common vector-borne disease, arthritis is a prominent late manifestation of the illness [1, 2]. About 10% of patients with Lyme arthritis develop what we have termed antibiotic treatment-resistant arthritis, which typically affects one or both knees for months to years despite treatment with multiple courses of antibiotic therapy [3–5]. In our experience, such patients have no detectable spirochetal DNA in synovial tissue or synovial fluid after antibiotic therapy, which suggests that the spirochete has been eliminated by this treatment [5–9].

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The majority of patients with treatment-resistant Lyme arthritis have alleles associated with rheumatoid arthritis, particularly HLA-DRB1*0401 or 0101 alleles [10]. Near the onset of prolonged periods of arthritis, such patients often produce high levels of immunoglobulin (IgG) antibody to outer surface protein A (OspA) of *B. burgdorferi* [6, 11]. Moreover, these patients often have OspA-specific T helper 1 (Th1) cells in their synovial fluid [12]. OspA peptide mapping studies in DRB1*0401 transgenic mice and in 0401-positive individuals identified a 12-amino-acid region of OspA ($OspA_{165-185}$) as the immunodominant epitope presented within the context of HLA-DRB1*0401. A gene bank search revealed one human protein, leukocyte function-associated antigen-1 (LFA-1), which had a sequence homologous with the dominant epitope of OspA and predicted binding by the 0401 molecule. It was then shown that synovial fluid T cells of patients with treat-

ment-resistant Lyme arthritis often responded to both spirochetal OspA and human LFA-1 [13]. Molecular mimicry, leading to autoimmunity in the joint, would explain the persistence of joint inflammation in Lyme arthritis after the apparent eradication of the spirochete from the joint with antibiotic therapy.

## Agent of Lyme disease

Three pathogenic species of *B. burgdorferi sensu lato* are known to cause human Lyme disease, *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii*. To date, all North American isolates belong to the species *B. burgdorferi sensu stricto*. All three species are found in Europe, though most isolates are *B. garinii* or *B. afzelii*. Only the latter two groups have been found in Asia [14, 15]. These differences presumably account for variations in the clinical picture of Lyme borreliosis seen throughout the world.

The complete genome of a prototypic *B. burgdorferi* strain (B31) was recently sequenced [16]. Interestingly, a large number of sequences account for predicted or known lipoproteins, including outer-surface proteins A through F. Several of these proteins are differentially expressed, and presumably help the spirochete to survive in markedly different arthropod and mammalian host environments [16]. The complexity of these expression patterns likely plays an important role in host immune reactivity.

## Disease vector and its life cycle

The ticks of the *Ixodes ricinus* complex transmit the spirochete *B. burgdorferi* when they feed on various mammals. These ticks have larvae, nymph and adult stages. In the northeastern and midwestern United States, the vector tick is called *Ixodes scapularis* [17]. This tick preferentially feeds on the white-footed mouse *Peromyscus leucopus* during its larval and nymphal stages, and the deer *Odocoileus virginianus* during its adult stage. The life cycle of the spirochete depends on horizontal transmission. First, infected nymphs transmit the spirochete to mice in early summer. Then, in late summer, the infected mice transmit the spirochete to feeding larvae. These infected larvae eventually molt into infected nymphs that begin the cycle again the following year. The cycle may be interrupted when *I. scapularis* nymphs incidentally feed on other animal hosts, including humans. Transmission is greatest from May through July, during the peak period of questing of the nymphal ticks [18]. Although adult ticks are often infected with the spirochete, they transmit the infection less frequently.

In three endemic northeastern communities (Nantucket, Great Island and Martha's Vineyard), about 40% of the nymphal ticks are infected with *B. burgdorferi* [18]. Prior to feeding, the spirochetes are located primarily in the midgut of the tick [19]. Once the tick attaches to a host, the organisms undergo rapid growth, with a doubling time of nearly 4 h [19]. After $\sim$24–48 h of attachment, the spirochetes begin to migrate to the tick's salivary gland, from which they are injected into the mammalian host. Thus, the tick must remain attached for at least 24–48 h for transmission to occur. Ticks usually detach after about 96 h of feeding, and the remaining spirochetes once again reside solely in the tick midgut [19].

## Clinical picture of Lyme disease

Lyme arthritis was recognized as a separate entity in 1976 because of a geographic clustering of children in Lyme, Connecticut, who were thought to have juvenile rheumatoid arthritis [20]. It soon became apparent that Lyme disease was a multisystem illness that primarily affects the skin, joints, nervous system and heart [2, 21]. The illness usually begins in summer (stage 1) with a characteristic expanding skin lesion, erythema migrans, that occurs at the site of the tick bite. Within several days to weeks (stage 2), the spirochete may spread to many other sites, and after months to years (stage 3), sometimes following long periods of latent infection, it may cause persistent disease, most commonly affecting the joints or nervous system. Recommended antibiotic regimens for each stage are listed in table 1.

Months after the onset of illness, within the context of a strong cellular and humoral immune response to *B. burgdorferi*, about 60% of patients in the United States begin to experience intermittent attacks of joint swelling

Table 1. Current recommendations for antibiotic therapy in Lyme disease [60].

| | | |
|---|---|---|
| Stage 1: early, localized Lyme disease | | |
| Doxycycline (>8 years) | 100 mg po bid* | 2–3 weeks |
| Amoxicillin | (adult) 250–500 mg po qid | 2–3 weeks |
| | (children) 40 mg/kg/d divided dose (maximum 2 g/d) | 2–3 weeks |
| Erythromycin | (children) 30 mg/kg/d divided dose | 2-3 weeks |
| Stages 2 and 3: early disseminated and late Lyme disease | | |
| Doxycycline (>8 years) | 100 mg po bid | 4–8 weeks |
| Ceftriaxone | 2 g IV/IM qd | 4 weeks |
| | (children) 75–100 mg/kg/d IV/IM | |

and pain, primarily in large joints, especially the knee. However, both large and small joints may be affected, usually one or two joints at a time. Affected knees are commonly more swollen than painful. Attacks of arthritis generally last from a few weeks to months separated by periods of complete remission. Joint fluid white cell counts range from 500 to 100,000 cells/$mm^3$, most of which, in patients with high white cell counts, are polymorphonuclear leukocytes. Tests for rheumatoid factor and antinuclear antibodies are usually negative.

The total number of patients who continue to have recurrent attacks of arthritis decreases by about 10–20% each year. However, attacks of knee swelling sometimes become longer during the second or third year of illness. It is usually during this period that approximately 10% of untreated patients develop chronic arthritis, defined as 1 year or more of continuous joint inflammation. The synovial histology in these patients, which shows synovial hypertrophy, vascular proliferation, fibrin deposition and a heavy infiltration of mononuclear cells, is typical of that found in all of the chronic inflammatory arthritides, including rheumatoid arthritis [22]. Furthermore, although *B. burgdorferi* DNA can usually be detected in joint fluid by polymerase chain reaction (PCR) prior to antibiotic treatment, in our experience, the PCR results are negative in synovium and synovial fluid after antibiotic treatment [5–9]. This suggests that Lyme arthritis may persist in certain individuals after the apparent eradication of the spirochete from the joint with antibiotic treatment. Thus, the question arises, Why does arthritis persist in these patients after the causative agent has been eradicated?

## HLA association with autoimmune diseases

Major histocompatibility complex (MHC) class II molecules play a critical role in the activation of the immune system. Polymorphisms within the genes encompassing the class II structure influence immune activation by at least two mechanisms. First, polymorphic amino acid residues on distinct class II proteins determine whether or not an individual peptide will bind and, therefore, be presented by a particular class II molecule displayed on an antigen-presenting cell (APC). Second, class II molecules regulate the developmental selection of T-cell-receptor (TCR) specificities in the thymus, thereby affecting the repertoire of T cells available to recognize foreign peptides in the context of MHC class II molecules. Given the fundamental role of MHC class II molecules in development of an immune response, it is not surprising that they have been implicated in numerous forms of immune dysfunction, most notably autoimmunity [23].

The first indication that treatment-resistant Lyme arthritis might be an autoimmune disease was a study of human lymphocyte antigen (HLA) alleles in patients with Lyme arthritis of brief, moderate or chronic duration [6]. An increased frequency of the HLA-DR4 specificity, determined by serologic typing methods, was found in patients with chronic disease [6]. In addition, HLA-DR4 specificity was associated with lack of response to antibiotic therapy [6]. More recently, using contemporary molecular techniques, the majority of patients with chronic, treatment-resistant Lyme arthritis were found to have alleles associated with rheumatoid arthritis, particularly HLA-DRB1*0401 or 0101 alleles [10]. The class II MHC alleles associated with rheumatoid arthritis have the same shared sequence in the third hypervariable region of the HLA-DRB1 chain that may be found in at least 15 different DRB1 alleles [23–25]. In rheumatoid arthritis, the cause of the disease and the critical peptides are not known. However, in Lyme arthritis, it is possible to ask, What antigens are these class II molecules presenting?

## Treatment-resistant Lyme arthritis and cellular and humoral immunity to OspA

Outer-surface proteins A and B (OspA and OspB), which are encoded by the same plasmid and share 56% sequence homology, are expressed primarily in the midgut of the tick [26]. As the tick feeds and migrates to the tick's salivary gland, OspA and OspB are downregulated and OspC is upregulated [26]. In cultured spirochetes, the expression of OspC can be induced simply by raising the incubation temperature from 32 to 37 °C [26]. However, if unfed ticks are incubated for up to 6 days at 37 °C, OspC induction does not occur [26]. Therefore, temperature alone does not result in modulation of Osp expression in vivo.

In people, a complex immune reaction develops in response to infection with *B. burgdorferi*, resulting in a gradual expansion of immunoreactivity to an increasing array of spirochetal proteins over a period of months to years [27]. In some patients with Lyme disease, an ephemeral antibody response may be found to OspA early in the infection, primarily of the IgM isotype. Over time, it has been shown that in approximately 70% of patients with Lyme arthritis, the final point of antibody response expansion is the development of IgG antibody to OspA and OspB of the spirochete [6, 11]. The onset of this response appears late in disease and typically occurs near the beginning of prolonged episodes of arthritis [6]. Furthermore, the risk for development of treatment-resistant Lyme arthritis has been shown to double when patients have HLA-DR4 specificity and generate an anti-OspA antibody response [11].

Table 2. *B. burgdorferi* $OspA_{165–173}$ homology in LFA-1.

| *B. burgdorferi* $OspA_{165–173}$ | Y | V | L | E | G | T | L | T | A |
|---|---|---|---|---|---|---|---|---|---|
| *B. garinii* $OspA_{160–163}$ | F*/L | A/T | L | E | G | T | L | T | A |
| *B. afzellii* $OspA_{165–173}$ | F | T | L | E | G | K | V | A | N |
| $hLFA\text{-}1_{332–340}$ | Y | V | I | E | G | T | S | K | Q |

*Variation in the first two amino acids of this 9mer is seen among several isolates, consequently both are listed.

Thus, reactivity to OspA is a significant correlate of prolonged Lyme arthritis.

T cell lines from patients with treatment-resistant Lyme arthritis have previously been shown to preferentially recognize OspA, as compared with T cell lines from patients with treatment-responsive disease [28]. We and others have shown that Th1 cells dominate the localized immune response in the joints of patients with Lyme arthritis, as well as patients with RA or other chronic inflammatory arthritides [12, 29]. Interestingly, we were able to detect OspA-reactive Th1 cells exclusively in the synovial fluid of patients with treatment-resistant arthritis months to years after antibiotic treatment [12].

Demonstration of T cell involvement in the pathogenesis of severe destructive Lyme arthritis has been described in a hamster model of Lyme disease. Lymph node T cells from hamsters vaccinated with $10^8$ formalin-inactivated spirochetes in adjuvant are able to confer susceptibility to severe destructive arthritis when transferred into naive hamsters challenged with $10^6$ viable *B. burgdorferi* [30]. The same is not true when naive recipients are infused with either normal T cells and challenged with viable spirochetes, or when infused with primed T cells and challenged with dead *B. burgdorferi* [30]. These results imply that a gene product which is actively expressed by the spirochete when inside a mammalian host is involved in the T-cell-mediated propagation of destructive arthritis. Hence, T cells seem to be responsible for development of destructive arthritis as opposed to conferring protection from disease [31].

Attempts to map dominant T-helper-cell epitopes of OspA in humans with a variety of HLA.DR alleles [28], as well as in $H\text{-}2^k$ mice [32, 33], have been reported. Although several T cell epitopes of OspA have been identified [28], the number of patients tested to date is too small to draw conclusions regarding dominant epitopes for particular class II MHC alleles. In the $H\text{-}2^k$ mouse, a single, C-terminal peptide is able to prime the animal for a protective anti-OspA antibody response [32, 33].

Despite intensive investigations by many research groups, we still do not understand the cellular and molecular mechanisms which lead to antibiotic treatment-resistant Lyme arthritis in certain individuals. No murine model for treatment-resistant Lyme arthritis has been established to date. The observations that development of treatment-resistant Lyme arthritis is correlated with the presence of DR4 alleles and persistent OspA reactivity late in disease have led us to hypothesize that patients with treatment-resistant Lyme arthritis have progressed into an autoimmune state by developing a cross-reactive response between OspA and a self-antigen. In order to substantiate our hypothesis, we set out to identify an autoantigen involved in the development of this localized, chronic immune reaction.

## Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis

Using an algorithm designed by Hammer et al. [34] which predicts immunodominant epitopes able to be presented by DRB1*0401, we defined $OspA_{165–173}$ (see table 2 for peptide amino acid sequences) as the peptide most likely to be involved in eliciting an immune response [13]. These predictions were verified using murine class $II^{-/-}$ mice transgenic for a chimeric DRB1*0401 molecule capable of interacting with murine CD4 [13]. We then searched the Genetics Computer Group gene bank for human proteins containing homologous sequences. Only one peptide sequence of human origin was predicted to be presented by DRB1*0401, as defined by the class II binding algorithm, namely $LFA\text{-}1\alpha_{L332–340}$ [13]. This peptide is located extracellularly in the region of LFA-1$\alpha$L called the interactive or I domain. This region mediates binding interactions between LFA-1 and its ligand, intracellular adhesion molecule-1 (ICAM-1) [35–38].

To test the hypothesis that $LFA\text{-}1\alpha_{L332–340}$ could act as a molecular mimic of $OspA_{165–173}$ and therefore behave as a candidate autoantigen specifically in patients with treatment-resistant Lyme arthritis, we tested patients for T cell reactivity to OspA, LFA-1 and the predicted immunodominant peptides. Th1 responses were restricted to patients with treatment-resistant Lyme arthritis. Importantly, patients with other forms of chronic arthritides who possessed DRB1*0401 alleles did not respond to these proteins, emphasizing the need for exposure to *B. burgdorferi* in order to trigger the response [13].

While the genetic predisposition for development of treatment-resistant Lyme arthritis has been correlated to DR4 and immunoreactivity to OspA, we cannot rule out other genetic, environmental and infectious factors which might play a role in the development of this prolonged form of arthritis. In our studies, we noted that some patients with treatment-resistant Lyme arthritis neither possess RA-associated alleles nor generate T cell responses to hLFA-1, possibly implicating additional autoantigens involved in disease development [13].

Interestingly, DR4-tg mice do not appear to be susceptible to development of treatment-resistant chronic Lyme arthritis [39, 40]. When we compared the sequence of human LFA-1$\alpha_{L332-340}$ to murine LFA-1$\alpha_{L332-340}$, subtle sequence differences were observed which might explain the inability of these mice to develop treatment-resistant disease. It is important to point out that *B. burgdorferi*-induced transient Lyme arthritis in rodents cannot be transferred with immune T cells in the absence of an infection. It is likely, therefore, that the mouse is unable to elicit an autoimmune reaction as a consequence of the inflammatory response to the spirochete. One possibility is that this species lacks the self-determinant which evokes the cross-reactive response. It may be this feature that renders the wild mouse a natural carrier of *B. burgdorferi*.

## Not all OspAs are alike: why treatment-resistant Lyme arthritis might only occur in patients infected with *B. burgdorferi sensu stricto*

In the United States all pathogenic isolates of *B. burgdorferi sensu lato* to date have been a single species, *B. burgdorferi sensu stricto* [41, 42]. A greater diversity of *B. burgdorferi sensu lato* species is found around the world: *B. garinii*, *B. afzelii*, and *B. burgdorferi sensu stricto* are found in Europe, and *B. japonica* is isolated to Asia [42–45]. This is thought to explain regional variations in the clinical manifestation of the disease. For instance, the neurologic deficit, meningoradiculitis, has been described in patients typically infected with *B. garinii*, whereas the skin manifestation of infection, acrodermatitis chronicum atrophicans, has been identified specifically in patients with *B. afzelii*. In contrast, Lyme arthritis has only been described in patients with *B. burgdorferi sensu stricto* infection. Furthermore, in the serologic analysis of patients infected with different species of *B. burgdorferi*, antibody reactivity to OspA and B was detected strictly in patients with *B. burgdorferi sensu stricto* induced Lyme arthritis [44]. Interestingly, *Borrelia* of different genotypes are not equally infectious. *B. garinii* and *B. afzelii* are able to infect nearly 100% of C3H mice challenged. This is in contrast to *B. japonica*, which averages an infection rate of only 20% [46].

We compared the $OspA_{165-173}$ sequences among the different species of *B. burgdorferi sensu lato*, hypothesizing that differences in this region might explain why infection with *B. burgdorferi sensu stricto* has been the only species described to elicit arthritic disease. Although both *B. garinii* and *B. afzelii* contain sequences homologous to *B. burgdorferi sensu stricto* $OspA_{165-173}$ (*B. garinii* $OspA_{160-168}$ and *B. afzellii* $OspA_{165\text{-}173}$), several residues are disparate, and the DR4-predicted binding scores are significantly lower than those of *B. burgdorferi* $OspA_{165-173}$ and hLFA-1a$_{L332-340}$. No homology was found within the OspA sequence of *B. japonica*. If cross-reactivity between $OspA_{165-173}$ and hLFA-1a$_{L332-340}$ is responsible for development of treatment-resistant Lyme arthritis, these species-specific sequence differences may explain why treatment-resistant Lyme arthritis has only been reported in response to infection with *B. burgdorferi sensu stricto*, and not with *B. garinii*, *afzelii or japonica*.

## Molecular mimicry and Lyme arthritis: the model

Based on our results defining the DRB1*0401-restricted OspA and hLFA-1$\alpha_L$ immunodominant epitopes, as well as prior work describing production of Th1 cytokines in patients with treatment-resistant Lyme arthritis, we propose a model describing how an autoimmune to response to LFA-1 my result after infection with *B. burgdorferi*. As mentioned earlier, LFA-1 is an adhesion molecule found on lymphocytes, with highest expression on activated T cells. Its primary function is as a mediator of cell-cell interactions during an inflammatory response [36, 47, 48]. It is most abundant on T and B cells, with highest expression on activated T lymphoblasts [36, 47–49]. LFA-1 is also present on macrophages after stimulation with lipopolysaccharide or the Th1 cytokine, interferon (IFN)-$\gamma$ [36, 47, 48]. The ligand for LFA-1, ICAM-1, is upregulated on fibroblasts, endothelial cells and synoviocytes when activated with interleukin (IL)-1, IFN-$\gamma$, tumor necrosis factor $\alpha$ (TNF-$\alpha$) and of particular importance, *B. burgdorferi* [35, 36, 48–53]. Hence, the modulation of ICAM-1 expression via cytokine production in an inflammatory response to *B. burgdorferi* in particular would not only facilitate margination and extravasation of T cells to the site of inflammation. It would also promote adherence of the localized T cells to connective tissue cells and thereby increase efficiency of T cell functions [36, 47, 48]. How this could ensue into a 'vicious cycle' of inflammatory reaction, recruitment and propagation of a local autoinflammatory response via cross-reaction of OspA reactive T cells with LFA-1 is described below.

First, *B. burgdorferi* enters the host and disseminates to multiple tissues by mechanisms under current investigation. Months later, a highly inflammatory immune response develops in the joint which, as we and others have shown, is dominated by Th1 IFN-$\gamma$-producing cells reactive against OspA [12, 29]. We propose that the high local concentration of IFN-$\gamma$ results in upregulation of LFA-1 and ICAM-1 [47, 53] on synoviocytes and synovial fibroblasts, as well as increased expression of MHC class II molecules on the local professional and nonprofessional APCs [54]. Others have shown that enhanced antigen presentation, including class II presentation of endogenous self-peptides, can occur during a prolonged immune response [55, 56]. In our model we would speculate that the combination of elevated LFA-1 expression on T cells and macrophages plus MHC class II upregulation on APCs results in increased LFA-1 peptide presentation by macrophages and synoviocytes which have processed either endogenous and/or phagocytosed LFA-1 [55, 56]. Hence, propagation of arthritis is initiated, such that even after elimination of the spirochetes by antibiotic therapy, the OspA-primed T cells remain activated by stimulation with LFA-1. The continued release of pro-inflammatory cytokines by these activated T cells and macrophages would then result in tissue damage and joint destruction [57].

Our model of molecular mimicry between OspA and LFA-1 provides one explanation of how chronic inflammation might persist in the absence of spirochetes and why antibiotic therapy is an ineffective treatment for such patients. This is the first model of an arthritic autoimmune disease where the initiating bacterial antigen and the persistent autoantigen are known.

## The search for additional autoantigens

Recently, two important breakthroughs in the search for peptide autoantigens have been described. First, the original DRB1*0401 algorithm has recently been expanded to encompass all of the DR class II family. Using DNA microarrays, Stuniolo et al. have been able to develop virtual matrices capable of predicting peptide-binding specificities for essentially all promiscuous DR ligands [58]. This technology has the potential to describe promiscuous candidate T cell epitopes involved in various autoimmune diseases where the initiating antigen is not known. A second method described by Hemmer et al. utilizes T cell clones isolated from a patient with chronic neuroborreliosis in conjunction with positional scanning peptide combinatorial libraries and biometric data analysis to identify candidate ligands. This method also demonstrates the promiscuity of T cell epitopes while at the same time maintaining a preference for peptides derived from the primary infecting organism [59]. These technological advances will greatly aid in the development of targeted immunotherapies for treatment of autoimmune diseases.


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