Relationship between Immunity to *Borrelia burgdorferi* Outer-surface Protein A (OspA) and Lyme Arthritis

Allen C. Steere, Elise E. Drouin, and Lisa J. Glickstein

Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Antibiotic-refractory Lyme arthritis may result from *Borrelia burgdorferi*-induced autoimmunity in affected joints. Such patients usually have certain HLA-DRB1 molecules that bind an epitope of *B. burgdorferi* outer-surface protein A (OspA163–173), and cellular and humoral immune responses to OspA are greater in patients with antibiotic-refractory arthritis than in those with antibiotic-responsive arthritis. Recent work in a mouse model suggests that, during *B. burgdorferi* infection, OspA in genetically susceptible individuals stimulates a particularly strong Th1 response, which may be one of several factors that can help set the stage for a putative autoimmune response in affected joints. However, vaccination with OspA did not induce arthritis in this mouse model, and case and control comparisons in human vaccine trials did not show an increased frequency of arthritis among OspA-vaccinated individuals. Thus, a vaccine-induced immune response to OspA does not replicate the sequence of events needed in the natural infection to induce antibiotic-refractory Lyme arthritis.

*Borrelia burgdorferi*, the sole agent of Lyme disease in the United States, is particularly arthritogenic [1]. Months after early infection, if the infection is not recognized and treated with antibiotics, ~60% of patients experience intermittent or persistent joint swelling and pain, which is a late manifestation of the illness [2]. In Europe, where the infection is usually caused by *Borrelia afzelii* or *Borrelia garinii*, arthritis is a less common manifestation of the disease [1]. Lyme arthritis usually resolves after receipt of a 1- or 2-month course of oral doxycycline or a 1-month course of intravenous ceftriaxone therapy [3, 4]. However, in a small percentage of patients in the Northeastern United States, joint inflammation persists for >3 months after >8 weeks of oral antibiotics or >4 weeks of intravenous antibiotic therapy, or both; this condition is called antibiotic-refractory Lyme arthritis [5]. In such patients, polymerase chain reaction (PCR) results for *B. burgdorferi* DNA in synovial fluid, which are often positive prior to treatment, are usually negative at the conclusion of antibiotic therapy [5, 6], and the results have been uniformly negative in synovial tissue obtained months after antibiotic treatment [5, 7]. This suggests that synovitis in patients with antibiotic-refractory Lyme arthritis may persist after the near or total eradication of spirochetes from the joint with antibiotic therapy.

In 1990, it was reported that patients with chronic Lyme arthritis had increased frequencies of the HLA-DR4 and DR2 alleles [8]. Next, it was noted that patients with antibiotic-refractory arthritis were more likely to have cellular and humoral immune responses to *B. burgdorferi* outer-surface protein A (OspA) than were patients with antibiotic-responsive arthritis [9, 10], and the severity of joint swelling correlated directly with cellular and humoral immune responses to this spirochetal protein [11, 12]. In July 1998, during the same week that the results of the phase III trials of *B. burgdorferi* OspA vaccines were reported in the
ASSOCIATION OF ANTIBIOTIC-REFRACTORY LYME ARTHRITIS WITH HLA-DR MOLECULES THAT BIND B. BURGDORFERI OSPA165–173

In DRB1*0401-positive individuals, the 9 core amino acids of the immunodominant epitope of OspA are located in positions 165–173 of the protein [15]. To assess patients’ reactivity with this epitope, longer peptides containing amino acids from the peptide-flanking regions were used, because these amino acids influence both HLA-DR binding and T cell receptor recognition. However, for each study, the length of the OspA peptide was slightly different, which is the reason that the subscript numbers for this epitope vary.

Using molecular techniques, the frequencies of HLA-DRB1 alleles were determined in 121 patients with antibiotic-refractory or antibiotic-responsive Lyme arthritis, and in vitro binding of the OspA163–175 peptide to 14 recombinant DRB molecules was assessed [16]. In general, the DRB molecules that bound OspA163–175 (eg, DRB1*0401, 0101, 0404, and 0405 and DRB5*0101) were more common among patients with antibiotic-refractory arthritis, whereas those that did not bind it (eg, DRB1*0301, 1101 and 1104) were more frequent among patients with antibiotic-responsive arthritis (Figure 1). Altogether, 79% of the patients with antibiotic-refractory arthritis had at least one of the 7 known OspA peptide-binding DR molecules, compared with 46% of the patients with antibiotic-responsive arthritis (odds ratio [OR], 4.4; P < .001). Furthermore, the HLA-DR alleles associated with chronic Lyme arthritis in the previous study [8] were quite consistent with those associated with antibiotic-refractory arthritis in the current study [16].

In a search for other Borrelia epitopes with this HLA-DRB1 binding pattern, T cell epitopes were predicted in 17 known immunogenic proteins of B. burgdorferi using the T cell algorithm TEPITOPE, and the HLA-DR binding profiles of 15 candidate peptides were confirmed using in vitro binding assays [17]. In addition, the B. burgdorferi proteome was searched for proteins with sequence homology with OspA165–173. From this work, 1 B. burgdorferi peptide (BB0347392–404) was identified that had a strong refractory-arthritis–associated HLA-DR binding profile, and another peptide (GK297–306) shared sequence homology with OspA165–173. However, patients’ cells did not proliferate in response to either peptide, making it highly unlikely they were involved in refractory arthritis. Thus, recognition of OspA165–173 remains the only currently identified B. burgdorferi epitope associated with this disease course.

OSPA165–173-REACTIVE T CELLS AND OSPA ANTIBODY RESPONSES IN LYME ARTHRITIS

After 1998, a number of reports confirmed and extended observations about the association between OspA-reactive T cells and antibiotic-refractory Lyme arthritis. Using overlapping 20-mer peptides of the OspA protein, it was learned that patients with antibiotic-refractory arthritis often had T cells that proliferated and secreted interferon (IFN) \( \gamma \) in response to 4 or 5 OspA epitopes, including OspA163–175 [12]. Substitution analysis showed that valine (166) and threonine (172) were critical for immunogenicity of the OspA163–175 peptide [18, 19]. Consistent with this result, lymphocytes from 7 patients with antibiotic-refractory arthritis proliferated in response to B. burgdorferi OspA163–175 but not in response to the equivalent B. afzelii or B. garinii peptide [18], which has substitutions in positions 166 and 172, perhaps explaining in part why antibiotic-refractory arthritis has rarely been recognized in Europe.

Using newly developed tetramer technology, 50%–60% of DRB1*0401-positive patients with antibiotic-refractory or antibiotic-responsive arthritis had detectable OspA161–175-specific T cells in peripheral blood specimens; these cells were concentrated in synovial fluid, and the frequencies of the cells were greater in patients with antibiotic-refractory arthritis [20]. However, in 2 patients each with antibiotic-refractory or antibiotic-responsive arthritis, the frequencies of these T cells decreased to low or undetectable levels during or soon after antibiotic therapy, which was months before the resolution of synovitis in the patients with antibiotic-refractory arthritis (Figure 2). This suggested that persistent synovitis in these patients was not perpetuated directly by OspA161–175-specific T cells.
Similarly, in a recent study, ~70% of patients with either antibiotic-responsive or antibiotic-refractory arthritis had positive antibody responses to OspA, and the values were higher in those with antibiotic-refractory arthritis [21]. However, by the conclusion of antibiotic therapy, which was given for 1–2 months longer in the refractory group, antibody titers to OspA were more common among patients with antibiotic-refractory arthritis, whereas the other 7 DRB molecules, which showed negligible or no binding of the peptide, were more common among patients with antibiotic-responsive arthritis. Reproduced with permission from Steere et al [16].

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Figure 1. Correlation of the relative binding affinity of the Borrelia burgdorferi outer-surface protein A (OspA)163–175 peptide for 14 HLA-DR molecules and percentage allele frequencies of these HLA-DR molecules in patients with antibiotic-refractory Lyme arthritis (antibiotic-refractory patients) or antibiotic-responsive Lyme arthritis (antibiotic-responsive patients). Seven of the 14 DRB molecules, which showed strong-to-weak binding of OspA163–175, were more common among patients with antibiotic-refractory arthritis, whereas the other 7 DRB molecules, which showed negligible or no binding of the peptide, were more common among patients with antibiotic-responsive arthritis. Reproduced with permission from Steere et al [16].

Figure 2. Correlation of the frequency of Borrelia burgdorferi outer-surface protein A (OspA)161–175–specific T cells in peripheral blood in serial samples from 4 patients with antibiotic-responsive Lyme arthritis (antibiotic-responsive patients) or antibiotic-refractory Lyme arthritis (antibiotic-refractory patients). The 2 patients with antibiotic-responsive arthritis were first evaluated prior to antibiotic therapy, and both responded to a 1-month course of oral doxycycline. The 2 patients with antibiotic-refractory arthritis were first seen 1 and 4 months after the start of antibiotic therapy. Patient 3 (Pt#3) was treated with a 2-month course of oral doxycycline and a 1-month course of intravenous ceftriaxone. Patient 4 (Pt#4) received a 1-month course of doxycycline, followed 3 months later by a 1-month course of intravenous ceftriaxone. The cross-hatched areas show the amount of joint swelling, and the lines with closed circles show the frequency of OspA161–175–specific T cells. In both patients with antibiotic-responsive arthritis and those with antibiotic-refractory arthritis, the frequencies of OspA161–175–specific T cells decreased during the period of antibiotic therapy, whereas joint inflammation persisted for months longer in patients with antibiotic-refractory arthritis than in patients with antibiotic-responsive arthritis. Reproduced with permission from Kannian et al [20].
decreased to similar levels in both the patients with antibiotic-responsive arthritis and those with antibiotic-refractory arthritis (Figure 3). Thus, consistent with the negative PCR results for B. burgdorferi DNA in joint fluid after antibiotic treatment [5, 7], the decrease in cellular and humoral responses to OspA after the conclusion of antibiotic therapy in both the antibiotic-refractory and antibiotic-responsive groups suggests that synovitis persisted after the period of infection. Moreover, neither OspA-reactive T cells nor OspA antibody would appear to be directly involved in post-infectious synovial inflammation.

SEARCHING FOR CROSS-REACTIVE T AND B CELL RESPONSES TO OSPA

After the original report of potential cross-reactivity between the OspA and hLFA-1 epitopes [15], OspA163–175-specific T cells were cloned using tetramer reagents [22], and the clones were tested for cross-reactivity with the hLFA-1 peptide [23]. When stimulated with the OspA peptide, nearly all of the clones proliferated and secreted IFN-γ and interleukin13. In contrast, LFA-1αL326–345 stimulated only ~10% of these clones to proliferate, and they only secreted interleukin13. Thus, the LFA-1 peptide behaved as a partial agonist for only a small percentage of the OspA163–175-reactive clones, and these clones secreted only an anti-inflammatory cytokine. When the binding of these peptides was assessed in vitro using recombinant HLA-DR molecules, the HLA-DRB1*0401 molecule bound both the OspA and LFA-1 peptides, but the DRB1*0101 molecule, which is also associated with antibiotic-refractory arthritis, bound only the OspA peptide and not the LFA-1 peptide [24]. Taken together, these findings cast doubt as to whether the hLFA epitope could serve as a meaningful autoantigen in antibiotic-refractory Lyme arthritis.

In recent years, better characterization of the human proteome has permitted a more thorough search for human proteins with sequence homology with OspA163–175. In a recent search of the published human genome, the 2 peptides with the greatest sequence homology with OspA165–173 were from a protein called MAP kinase activator with WD-repeats (MAWD-BP280–288), which had identity with 8 of the 9 core amino acid residues, and from a protein called tetraspanin7 (T-span760–68), which had identity with 6 residues [25]. In comparison, LFA-1αL332–340 and 12 other human proteins had epitopes with 5 identical residues to the OspA epitope. MAWD-BP was shown to be expressed in synoviocytes derived from a patient with rheumatoid arthritis, and LFA-1 would presumably be expressed on T cells in joints, but T-span7 was not expressed by either synoviocytes or lymphocytes [25]. Although a minority of patients had T cell–proliferative responses to 1 or more of these 3 self peptides, reactivity was always less than that with the spirochetal peptide (Figure 4). Moreover, in a search for linked T and B cell responses to these putative autoantigens, antibody...
responses to MAWD-BP [25] or hLFA-1 (unpublished data) were not identified.

In an attempt to recapitulate relevant antigen-binding specificities in synovial lesions, Ghosh et al [26] generated recombinant antibody probes derived from B cells in patients’ lesions. Using a panel of intra-articular probes, as well as antibody fragments derived from patient peripheral blood samples, cytokeratin 10, which is cross-reactive with OspA, was identified as a target ligand. However, only 3 (20%) of 15 patients with antibiotic-refractory Lyme arthritis had slight antibody responses to this protein.

In summary, several human proteins have been identified that contain cross-reactive T or B cell epitopes with B. burgdorferi OspA. However, cross-reactive immune responses to a given human protein could only be demonstrated in a minority of patients with antibiotic-refractory arthritis, and even in these patients, the cross-reactive responses were usually weak. More importantly, 30%–40% of patients with antibiotic-refractory arthritis lacked OspA immune responses, and even in patients who had such responses, the frequency of Ospa165–173 reactive T cells decreased soon after antibiotic treatment. Although it is not possible to look for a structural homologue of the OspA epitope, these findings suggest that molecular mimicry does not explain the association between OspA immunity and antibiotic-refractory Lyme arthritis.

LESSONS FROM A MOUSE MODEL OF ANTIBIOTIC-REFRACTORY LYME ARTHRITIS

Iliopoulou et al [27] recently developed a mouse model of human B. burgdorferi–induced, antibiotic-refractory arthritis. In this model, both the human HLA-DR4 transgene, which is associated with antibiotic-refractory arthritis, and lack of the CD28 co-receptor, which leads to dramatically reduced numbers of T regulatory cells (Treg) [28], were necessary for persistent synovitis after antibiotic treatment of the infection. In contrast, when the DR4-Tg was replaced with the DR11-Tg, which is associated with antibiotic-responsive arthritis, the mice did not develop post-treatment synovitis [29]. Neither the DR4-Tg nor the absence of the CD28 co-receptor, by itself, led to persistent arthritis after treatment [30, 31]. These outcomes in mice are reminiscent of the findings in human patients with antibiotic-refractory Lyme arthritis [16, 32] and are consistent with the infection-induced autoimmunity hypothesis of this illness.

These investigators also evaluated the effects of OspA vaccination alone in this model. Without B. burgdorferi infection of joints, the mice did not develop arthritis [31]. However, T cells from draining lymph nodes from OspA-vaccinated DR4 transgenic mice (DR4-Tg) had significantly greater production of IFN-γ than DR11-Tg mice (Figure 5A). Conversely, DR11-Tg mice had higher antibody titers to OspA than did DR4-Tg mice (Figure 5B). There is a strong correlation between Lyme arthritis severity and IFN-γ in mouse models [33–36], and patients with antibiotic-refractory Lyme arthritis have significantly higher levels of IFN-γ in joint fluid than do patients with antibiotic-responsive arthritis [37].

Although T cell reactivity with OspA epitopes was not examined in mice, this experience provides a possible explanation for the association between Ospa165–173 and antibiotic-refractory Lyme arthritis. According to this hypothesis, reactivity with the Ospa165–173 peptide, the immunodominant epitope
EXPERIENCE WITH VACCINATION WITH OSPA AND ARTHRITIS

Consistent with the lack of joint inflammation from OspA vaccination in the murine model [31], observations in human subjects also suggested that OspA vaccination did not cause antibiotic-refractory Lyme arthritis. During the SmithKline Beecham (now GlaxoSmithKline) phase III Lyme disease trial, which had >10,936 participants, several subjects developed inflammatory arthritis, but these individuals were found in both the vaccine and placebo groups, suggesting that their arthritis was not due to OspA vaccination [13]. In a pediatric safety and immunogenicity study, 1 child had transient ankle swelling after dose 2, which resolved and did not recur after dose 3 [39]. After licensing of the OspA vaccine in December 1998, 2 patients were reported to have transient oligoarticular arthritis after vaccination [40], but this experience seemed to be similar to that noted in the vaccine trials. On the basis of post-marketing surveillance data from the Centers for Disease Control and Prevention and the US Food and Drug Administration Vaccine Adverse Reporting System, the frequency of reports of inflammatory arthritis or rheumatoid arthritis after vaccination was thought to be below the expected level of these diseases in an unvaccinated population [41]. Furthermore, an analysis of HLA-DR profiles and OspA immunity in a subgroup of these patients was not suggestive of a vaccine-induced process [42].

Because OspA vaccination followed soon after by B. burgdorferi infection led to destructive arthritis in a hamster model [43], it was also suggested that vaccination with OspA might be associated with more-severe arthritis if a person subsequently acquired B. burgdorferi infection. In the GlaxoSmithKline trial, 3 of 10,936 participants had Lyme arthritis after vaccination, but they were probably infected prior to vaccination [13]. Similarly, in the Pasteur Meurieux Connaught trial, 3 of 10,305 participants had Lyme arthritis during the follow-up period [14]. Apparently, arthritis in all of these patients responded to antibiotic therapy. In a study of 30 patients with previous Lyme disease, those with a previous history of Lyme arthritis did not have a recurrence of arthritis after vaccination [44]. Although the numbers were small, OspA vaccination in human subjects did not appear to increase the risk or severity of Lyme arthritis if a person subsequently acquired B. burgdorferi infection.

In conclusion, factors known to be associated with the development of antibiotic-refractory Lyme arthritis include more frequent infection of joints with certain inflammatory strains of B. burgdorferi [45], HLA-DR alleles that bind an epitope of OspA (OspA165–173) [16], high joint fluid levels of inflammatory cytokines and chemokines [37], and low numbers of Treg in joint fluid [32]. In addition, we hypothesize that T cell reactivity with OspA165–173 is one of several factors that may lead to a vigorous Th1 inflammatory response in infected joints, which may help to set the stage for a putative autoimmune response. This, rather than molecular mimicry, may be the reason for the association between OspA immunity and antibiotic-refractory arthritis.

Despite this association, it is clear that a vaccine-induced immune response to OspA does not replicate the sequence of events needed in B. burgdorferi–infected joints to induce antibiotic-refractory Lyme arthritis. It remains possible that a vigorous inflammatory response to OspA vaccination may uncover latent autoimmunity in genetically susceptible individuals, as might happen with other types of vaccination or with certain infections. However, if this happened with OspA vaccination, it must have been a rare event that was below the level detectable by case and control comparisons in phase III vaccine trials or in studies of patients who received the vaccine commercially.

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