A Role for T Cells in the Pathogenesis of Treatment-Resistant Lyme Arthritis

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LYME BORRELIOSIS: NATURAL HISTORY AND PATHOLOGY

Lyme borreliosis is a chronic multisystem disease caused by the tick-borne spirochetes *Borrelia burgdorferi sensu stricto, B. garinii,* and *B. afzelii* (1–3). Months after the disease's onset, about 60% of untreated North American patients develop arthritis. The synovial lesion in patients with chronic Lyme arthritis is similar to that found in other forms of chronic inflammatory arthritis, including rheumatoid arthritis. There is a lymphoplasmacellular infiltrate, intense HLA-DR and -DQ expression throughout, and the majority of T cells are CD4 positive (4). While most patients with Lyme arthritis are cured with antibiotic therapy, about 10% have persistent arthritis for months or even several years after antibiotic treatment (2,5). Patients may thus be divided into two groups: those with treatment-responsive arthritis, defined as arthritis resolving after the appropriate antibiotic treatment, and those with treatment-resistant arthritis, which persists after treatment (5).

TREATMENT-RESISTANT LYME ARTHRITIS: PERSISTING INFECTION OR IMMUNOPATHOLOGY?

Two hypotheses, which are not mutually exclusive, can explain treatment-resistant Lyme arthritis: persistent infection and infection-induced immunopathology.

Arguments in favor of persistent infection include:

- The antibody-response against *B. burgdorferi,* including IgM-production, expands for months or even years after the infection (6,7).
- Local production of antibodies in the cerebrospinal fluid in the absence of a detectable antibody response in the serum (8) indicates that *B. burgdorferi* may persist at "privileged sites".
- Late manifestations of Lyme disease can be cured with antibiotic treatment (2).
- Disease-severity has been linked to spirochete burdens in murine models of Lyme arthritis (9).
- *B. burgdorferi* has been detected occasionally in the synovium or ligaments of patients with Lyme arthritis (4,10).

In addition, the host response to *B. burgdorferi* is likely to play a role in the pathogenesis of Lyme arthritis. While Borrelia DNA can be amplified reliably from pretreatment samples of synovial fluid (SF), most patients with treatment-resistant arthritis yield consistently negative polymerase chain reaction (PCR) test results in synovial fluid after antibiotic treatment (11,12). The ongoing synovial inflammation in patients with treatment-resistant arthritis may thus be caused by factors other than persistent *B. burgdorferi* infection.

In mice, susceptibility to *B. burgdorferi*-induced arthritis has been mapped to the major histocompatibility (MHC) locus (13,14). In patients with Lyme arthritis, presence of the HLA-DR4 specificity was reported to be associated with a lack of response to antibiotic therapy (15). Later, both HLA-DR4 specificity and the level of IgG reactivity against a particular *B. burgdorferi* antigen, the outer surface protein A (OspA), were found to be risk factors for treatment-resistant Lyme arthritis (5).

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THE CELLULAR IMMUNE RESPONSE TO *B. BURGDORFERI*

B. burgdorferi does not induce a completely protective immune response; reinfection is possible, and Lyme arthritis coexists with specific and strong humoral and cellular immune responses to *B. burgdorferi*. While the serological response has been extensively studied, comparatively little is known about the T cell response (for a review see Ref. 7). If T cells have a role in the pathogenesis of treatment-resistant Lyme arthritis, two competing hypotheses need to be considered. First, patients with treatment-resistant arthritis might lack the particular T cells that recognize and eradicate *B. burgdorferi* (in other words, they have what is referred to as a "hole" in the T cell repertoire). In such patients, *B. burgdorferi* may persist, maintaining chronic synovial inflammation. Although this possibility cannot formally be excluded, data from murine models make it unlikely (16,17). Alternatively, the T cells of patients with treatment-resistant Lyme arthritis might maintain chronic inflammation. This hypothesis leads to the testable prediction that the T helper cell response to *B. burgdorferi* in patients with treatment-responsive Lyme arthritis differs from that in patients with treatment-resistant arthritis. We examined T cell lines (TCLs) derived from the synovial fluid (SF) or peripheral blood (PB) of patients with treatment-responsive or treatment-resistant arthritis and found that OspB was recognized at high frequency by all TCLs. Other proteins were recognized by TCLs at lower frequency. In contrast, OspA was the antigen most frequently recognized by TCLs from patients with treatment-resistant arthritis, and the antigen least frequently recognized by TCLs from patients with treatment-responsive arthritis (18). Furthermore, we found that 31 TCL from three different patients with treatment-resistant Lyme arthritis dominantly recognized an OspA peptide epitope (T. Kamradt et al., manuscript in preparation). These findings support the hypothesis that the T cell response to OspA is involved in the pathogenesis of chronic synovial inflammation.

Further evidence of immunopathology in Lyme arthritis comes from an animal model: hamsters vaccinated with formalin-inactivated *B. burgdorferi* and later challenged with *B. burgdorferi* developed T cell-mediated severe, destructive arthritis under certain experimental conditions (19).

POSSIBLE MECHANISMS OF T CELL-MEDIATED PATHOLOGY

How could a *B. burgdorferi*-triggered T cell response induce or maintain chronic synovitis? One might speculate that *B. burgdorferi* contains a superantigen, activating potentially autoreactive T cells bearing particular T cell receptor Vβ-elements (20,21). As yet, there is no evidence for a *B. burgdorferi* superantigen.

Another possible mechanism is the "bystander-activation" of potentially autoreactive T cells in the joint. *B. burgdorferi* is a strong inducer of the proinflammatory cytokines IL-1 (22) and TNF-α (23). Inflammatory agents, such as infection (24,25), bacterial toxins (20), or IL-1 (26), can interfere with T cell tolerance. According to this model the acute synovial inflammation caused by infection with *B. burgdorferi* activates self-reactive T cells within the synovium, where they overcome self-tolerance.

In the light of our findings (18), the most tempting hypothesis would be the existence of an arthritogenic epitope on OspA. Crossreactivity of the OspA epitope with a self epitope would then lead to autoreactivity via molecular mimicry (27). A recent search of the Gen-Bank, EMBL, and Swissprot databases did not reveal significant sequence homologies between OspA and any human proteins. Obviously, those databases include only a minority of possible self-antigens. Furthermore, a recent study has shown that simple sequence alignment may not identify molecular mimicry (28). T cell clones from multiple sclerosis patients, specific for an immunodominant epitope of myelin basic protein (MBP), were used to identify the structural requirements for MHC binding and TCR recognition of the peptide; databases were then searched for potentially cross-reactive peptides. Of the seven bacterial and viral peptides which activated the MBP-specific T cell clones, only one was identified by sequence alignment. This study suggests that molecular mimicry could be identified in Lyme arthritis by using OspA-specific T cell clones from patients with treatment-resistant Lyme arthritis to determine the structural requirements for the binding of an immunodominant OspA epitope to HLA and for the recognition of the peptide-HLA complex by the TCR.

If molecular mimicry between OspA and a self-epitope exists, one would have to ask why the patients' T cells are not tolerant of the cross-reactive arthritogenic epitope on OspA. As described above, unlike OspA, OspB is frequently

recognized by TCLs from patients with either form of Lyme arthritis (18). We therefore hypothesize that a mechanism of "epitope spreading", in which the T cell progressively recognizes more epitopes on a protein antigen during an immune response (29), occurs in some patients. The scenario suggests that OspB is an immunodominant antigen for patients with Lyme borreliosis. In most patients, the T cell response to OspB remains focused on OspB epitopes that are not highly homologous to OspA. In a minority of patients, however, chronic antigenic stimulation results in the spreading of T cell recognition from OspB epitopes that are not homologous to OspA to OspB epitopes which are similar to OspA epitopes. Indeed, two T cell clones which recognize a peptide epitope contained on both OspA and OspB have been identified (30). Continued antigenic stimulation by *B. burgdorferi* then leads to intramolecular epitope spreading, eventually reaching the putative arthritogenic OspA epitope. This hypothesis makes the prediction, currently being tested, that TCLs from patients with treatment-resistant Lyme arthritis recognize a broad variety of OspB epitopes. In contrast, TCLs from patients with treatment-responsive Lyme arthritis would recognize OspB epitopes that share little homology with OspA.

OTHER MECHANISMS FOR IMMUNOPATHOLOGY

All of this could reasonably be regarded as prolegomena to any future hunt for the infectious trigger of rheumatoid arthritis. However, even if, as we hypothesize, T cell recognition of an arthritogenic epitope on OspA plays a role in the pathogenesis of treatment-resistant Lyme arthritis, other factors must be involved (18). Cytokine production is an obvious possibility. Upon stimulation with *B. burgdorferi*, T cell production of interferon-γ occurs in arthritis-susceptible mice, while IL-4 is produced in arthritis-resistant mice (31). Clearly, T cell cytokines are not the only compounds involved; the balance of IL-β and IL-1β receptor antagonist is disturbed in the synovial fluid of patients with treatment-resistant Lyme arthritis but not in patients with treatment responsive Lyme arthritis (32). Similar findings have been made in other chronic inflammatory diseases, such as rheumatoid arthritis (33). Furthermore, *B. burgdorferi* is mitogenic for B cells (reviewed in Ref. 7) and might be costimulatory for incompletely activated T cells (34).

DEVELOPMENT OF A VACCINE AGAINST LYME DISEASE

Transfer of immune serum, mAbs against OspA, and vaccination of mice with recombinant *B. burgdorferi* antigens protect rodents from experimental infection with homologous strains of *B. burgdorferi* (reviewed in Ref. 7). Clinical trials using recombinant OspA as a vaccine against Lyme disease are currently under way and the first reports were optimistic (35). However, both the vaccine-induced T cell-mediated immunopathology in hamsters (19) and our own data on T cell recognition of OspA (18) raise the spectre of immunopathology. Since Lyme borreliosis is cured with antibiotic therapy in most patients, the benefits and potential risks of immunizing people with OspA should be weighed carefully, pending a better understanding of the protective and disease-enhancing aspects of the immune response.

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