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Immunologic Aspects of Lyme Borreliosis

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Immune responses to *Borrelia burgdorferi* infection are now well characterized. Following infection there is an early T cell response and a more slowly evolving B cell response. IgM antibodies appear first and are followed by IgG and IgA. Early antibodies are primarily against a 41-kilodalton flagellum-associated antigen; responses to other spirochetal antigens develop later. Serologic assays that use whole *B. burgdorferi* preparations are not always able to detect an early rise in antibodies above a background of cross-reactive antibodies present in most uninfected individuals. Moreover, some individuals with neurologic involvement who lack diagnostic levels of serum antibody to *B. burgdorferi* have high levels of the antibody in their cerebrospinal fluid. Specific T cell blastogenesis to *B. burgdorferi* can further document infection. Analysis of T cell subsets in Lyme arthritis demonstrates a marked decrease in the $CD4^+2H4^+$ subpopulation in the synovial fluid, although normal numbers of these cells are present in peripheral blood. Immunologic measurements are useful in evaluating and treating a wide array of patients who may be infected with *B. burgdorferi*.

Lyme borreliosis is a chronic infectious disease caused by the spirochete *Borrelia burgdorferi* [1–4]. As the experience with this illness grows, an increasing array of clinical manifestations are being associated with this spirochetal infection [4–9]. Early *B. burgdorferi* infection is frequently heralded by a characteristic skin lesion, erythema migrans (EM), and at times by a nonspecific flu-like illness [10–13]. Specific organ involvement becomes apparent as the disease progresses. Within 6–8 weeks after the onset of infection, up to 20% of patients develop acute neurologic or cardiac abnormalities [6, 14]. Later, as the disease evolves, a wide range of neurologic disorders (e.g., chronic meningitis, meningoradiculitis, encephalitis, and peripheral neuropathy) can develop [6, 8]. Moreover, if the disease is left untreated, approximately half of North American patients develop arthritis [15].

As in any chronic infectious disease, host responses are thought to play a major role in shaping the clinical expression of this illness. These responses include an early and vigorous T cell response to the presence of the Lyme spirochete and a more slowly evolving B cell response [16, 17]. Although spirochetes are difficult to detect, they have been demonstrated in tissues obtained from individuals with high levels of antiborrelial antibodies, a finding that indicates the presence of immunity alone does not guarantee eradication of this organism [18, 19]. Furthermore, persistent symptoms can occur even in patients treated with antibiotics [20, 21].

At present, because of the difficulty in isolating *B. burgdorferi* from the infected host, documentation of exposure to *B. burgdorferi* is dependent upon the demonstration of a specific immune response to this spirochete. However, early in the course of infection it is difficult to identify infected individuals with use of current serologic tests. In addition, the subsequent development of a mature humoral response can be aborted by early antimicrobial therapy [13, 17]. Cellular immune assays, which can often detect specific immunity within the first weeks of infection, are available in only a limited number of institutions.

Antibody Responses

Antibody responses to *B. burgdorferi* have been evaluated with use of immunofluorescent assays (IFAs), ELISAs, and immunoblots. As in other infectious diseases, it has been demonstrated that specific IgM responses to this microorganism appear first and that specific IgG develops later [13, 22, 23]. Specific IgM can usually be detected 3–4 weeks after the onset of infection; it peaks after 6–8 weeks and gradually declines thereafter. The IgM response at this stage of disease is directed primarily at the

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41-kDa flagellum-associated antigen. Occasionally, titers of IgM remain elevated throughout the course of the infection [23]. In part, this late IgM response may represent an expansion of the IgM response to newly expressed borrelial antigens. It has been demonstrated by Craft et al. [23] that specific IgM antibodies can develop to the 34-kDa OspB antigen later in the course of infection.

Specific IgG and IgA responses gradually increase during the second and third months of infection and, once established, may remain detectable for years. However, as in syphilis, prompt antimicrobial therapy aborts the development of a mature humoral response [13, 17]. Thus, patients treated before they develop a mature response often lack diagnostic levels of *Borrelia*-specific antibodies.

The failure of early antimicrobial therapy to completely eradicate the infection and the subsequent development of a chronic illness make this disease especially difficult to diagnose in patients who do not develop a mature antibody response. However, specific T cell and/or local humoral responses may be demonstrable in patients who lack diagnostic levels of circulating antibodies to *B. burgdorferi* [17, 24–26]. In contrast, individuals who have already developed a mature antiborrelia IgG response often remain seropositive after successful antibiotic therapy. In these patients a reduction in levels of specific antibody can be observed with time, but the absolute levels of antibody may remain above normal. Consequently, the presence or absence of circulating antibodies to *Borrelia* following antibiotic therapy is not a reliable indicator of cure.

Since spirochetes are part of the normal human flora, most individuals have antibodies to common spirochetal antigens [17, 27, 28]. In the course of human infection, the first antibody to *B. burgdorferi* is predominantly against a 41-kDa flagellum-associated antigen [28]. Epitopes on the antigen are shared by most spirochetes, and neither IgG nor IgM antibodies to this common spirochetal antigen are specific for *B. burgdorferi*. As the disease progresses, patients' antibody responses gradually evolve to encompass a broader range of antigens [17, 23]. However, *B. burgdorferi* shares a number of antigens with other spirochetes and several other bacteria [23, 28, 29]. The 60-kDa protein antigen of *B. burgdorferi* is a common bacterial antigen that is cross-reactive with a similar antigen found on many other bacteria, including gram-negative and gram-positive organisms, spirochetes, and archebacteria [29].

Currently available serologic assays that use whole *B. burgdorferi* preparations are not sufficiently sensitive to detect the early rise in antibodies above the high background of cross-reactive antibodies. Thus, serodiagnosis by either IFA or ELISA in the first few weeks of infection is not dependable, and there can be a high proportion of false-negative results [23]. Immunoblots can detect antibodies to *B. burgdorferi*, but specificity is a problem [28]. Using highly sensitive western blots, we found that >50% of a control population of individuals with no history of tick bite or EM and negative specific ELISA results had IgG antibody to the 41-kDa antigen. In addition, some of these individuals had antibodies to other spirochetal antigens, including the 25-, 31-, 34-, 60-, and/or 66-kDa peptides. This phenomenon has been noted by other researchers and likely contributes to the high background levels seen in IFA tests (often as high as 1:128 in normal control sera) [28].

Three separate groups of investigators have reported individuals who lacked diagnostic levels of specific antibody in their serum, yet had neurologic involvement and diagnostic levels of antibody in their CSF [24–26]. We have confirmed this finding in our laboratory. One possible explanation for this observation is that although commonly used oral antibiotics such as tetracycline or penicillin can effectively eradicate the bulk of *B. burgdorferi*, neither low-dose tetracycline nor low-dose penicillin enters the CNS in concentrations high enough to reach the reported MICs for the majority of *B. burgdorferi* strains [30–34]. Thus, spirochetes that reach this immunologically privileged site may remain viable and induce a local immune response. This explanation may not fully account for the observation of diagnostic levels of antibody in CSF but not in serum, since it is not clear that all these individuals received antimicrobial agents [26].

Cellular Immunity

A strong and specific T cell immune response to *B. burgdorferi* develops early in the course of infection, frequently preceding the development of a measurable antibody response [16, 17]. This cellular immune response, once developed, is sufficiently long-lasting that its measurement may be useful in the assessment of suspected Lyme borreliosis in patients in whom routine serologic tests are inconclusive. These studies can be carried out in most modern immunology laboratories. The proliferative response

to *B. burgdorferi* is assessed in a standard antigen assay. In our laboratory, infected individuals have average stimulation indexes of 17–20 [16, 17].

The inability of some investigators to demonstrate a vigorous cell-mediated immune response to *B. burgdorferi* can be explained by their failure to use the appropriate stimulating antigens, namely, whole *B. burgdorferi* or uncentrifuged lysates. These antigen preparations have T cell–precursor frequencies as low as 1:3,700, while supernates from centrifuged lysates have T cell–precursor frequencies ≥1:140,000 [17]. Two explanations have been offered for this difference. First, the T cell immunodominant antigens are membrane-associated antigens and are lost during centrifugation. Second, the particulate matter that separates out during centrifugation can be presented to T cells by phagocytic antigen-presenting cells better than the soluble antigens left in the supernates.

Neonatal Lyme Disease

In humans, *B. burgdorferi* is capable of infecting the fetus [35]. Sequelae (including abortion and fetal abnormalities) have been associated with infection [36, 37]. The time, incidence, and morbidity of in utero infection are not known. However, both humoral and cellular *B. burgdorferi*–specific responses can be detected in cord blood of previously infected neonates (authors' unpublished observations). In addition, *Borrelia*-specific antibodies have been found in the CSF of an infant with evidence of neonatal neurologic dysfunction whose mother had been infected in the second trimester. The mother, who was asymptomatic, had been treated with oral antibiotics and did not have diagnostic levels of antibodies to *B. burgdorferi* at the time of parturition (authors' unpublished observations). Effective therapy to eradicate borreliae on both the maternal and the fetal side of the placenta is essential, as persistent infection may be difficult to diagnose after the initial course of antibiotics.

Lyme Disease and Specific T Cell Populations

Fluorescent-activated cell sorter (FACS) studies with use of monoclonal antibodies to specific cell-surface markers have revealed no change in the absolute number of T cells (CD3), inducer cells (CD4), or suppressor cells (CD8) in peripheral blood taken from patients during any stage of Lyme disease [16]. Similarly, there is no change in the CD4/CD8 ratio; as is true for normal subjects, about half of the CD4 population in infected individuals are mature helpers (previously designated inducers of help [$CD4^+4B4^+$]) and half are less mature (previously designated inducers of suppression [$CD4^+2H4^+$]) [38]. Thus, FACS analysis of peripheral blood T cells does not appear to be helpful in the assessment of patients with *B. burgdorferi* infection.

Although Lyme disease is not associated with alterations in the number or ratios of T cells in the peripheral blood, it is associated with specific alterations in the response to T cell mitogens such as concanavalin A (Con A) and phytohemagglutinin (PHA) [16, 17]. Con A and PHA are known to stimulate different T cell populations; Con A preferentially stimulates $CD4^+2H4^+$ cells [39].

In early Lyme disease there is an increased immunologic responsiveness to both Con A and PHA [16]. However, in later stages there is a marked diminution of response to Con A, although the response to PHA remains similar to that seen in the early stages of the illness [16, 17]. In late Lyme disease there is also a reduction in natural killer cell activity [16]. Whether these alterations in immune response play a role in the various clinical manifestations of this illness remains to be determined. Although these changes are consistent, they are not specific for Lyme disease and are not usually included in routine clinical tests.

Synovial Fluid Studies

Examination of the synovial fluid of patients with active Lyme arthritis, unlike examination of the blood, does reveal some striking differences in both absolute numbers and percentages of certain T cells. There is a marked decrease in the number of $CD4^+2H4^+$ cells [38]. These observations parallel almost exactly the findings in synovial fluid of patients with rheumatoid arthritis [40], namely, markedly reduced numbers of the $CD4^+2H4^+$ subpopulation in the presence of a higher inducer/suppressor ratio.

The finding of a marked decrease in the $CD4^+2H4^+$ subpopulation in synovial fluid in both Lyme arthritis and rheumatoid arthritis suggests that the two diseases may share one or more basic pathophysiologic mechanisms, possibly the preferential migration of mature antigen helper ($CD4^+4B4^+$) cells into an area of active immune response. Selective migration could produce an imbalance in the normal im-

munoregulatory process. Abnormalities in normal immunoregulatory mechanisms have long been thought to play a role in rheumatoid arthritis, and the recent recognition of the decrease in inducer-of-suppression cells offers an explanation for many of the observed immunologic derangements. A decrease in this cell population would be expected to provide the imbalance that could permit helper forces to predominate.

Synovial biopsies have also indicated marked similarities between the two diseases [18]. For example, patients with chronic Lyme borreliosis have synovial cell hyperplasia and lymphocytic and plasmacytic infiltration similar to those observed in patients with classic rheumatoid arthritis. In addition, studies of the mononuclear cells from synovial fluid of patients with rheumatoid arthritis have demonstrated a reduced response to Con A and depression of suppressor activity.

Summary

The diagnosis of Lyme borreliosis in the absence of EM is dependent on the demonstration of a specific immune response to *B. burgdorferi* in an appropriate clinical setting. Unfortunately, *B. burgdorferi* shares many antigens with other spirochetes, and the currently available serologic assays cannot detect the early rise in antibodies against the background of cross-reactive antibodies. Attempts to increase the sensitivity of these assays have not been universally successful [41]. The enrichment of antigen mixtures to increase the amount of the 41-kDa antigen and the use of that antigen preparation in an ELISA result in enhanced sensitivity, especially for serum IgG to the 41-kDa antigen [26]. However, the ability to diagnose early CNS infection is not significantly improved by this method [26].

As the disease progresses, the ability to accurately diagnose exposure to *B. burgdorferi* by ELISA improves markedly. In untreated individuals the diagnosis of late Lyme borreliosis cannot be made without the demonstration of antibody to *B. burgdorferi* in either blood or CSF. T cell assays can demonstrate a specific immune response both earlier than assays for antibody and in those individuals whose humoral responses have been blunted by early antibiotic therapy. However, T cell assays are not widely available.

New approaches must be undertaken to further improve our ability to diagnose *B. burgdorferi* infection. Unlike the diagnosis of most bacterial infections, which is based on isolation of the organism, that of *B. burgdorferi* infection is dependent on the demonstration of a specific immune response to the organism. Methods to detect the presence of *B. burgdorferi* itself must be developed. However, the demonstration of an immune response to *B. burgdorferi* does not by itself prove a cause-and-effect relation between a patient's symptoms and an active infection. In addition, a more complete understanding of the immune response to this spirochete is needed to define the role of the immune response in the pathophysiology of the systemic infectious disease.

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