

The Antibody Response in Lyme Disease

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We determined the antibody response against the *Ixodes dammini* spirochete in Lyme disease patients by indirect immunofluorescence and an enzyme-linked immunosorbent assay (ELISA). The specific IgM response became maximal three to six weeks after disease onset, and then declined, although titers sometimes remained elevated during later disease. Specific IgM levels correlated directly with total serum IgM. The specific IgG response, often delayed initially, was nearly always present during neuritis and arthritis, and frequently remained elevated after months of remission. Although results obtained by indirect immunofluorescence and the ELISA were similar, the ELISA was more sensitive and specific. Cross-reactive antibodies from patients with other spirochetal infections were blocked by absorption of sera with *Borrelia hermsii*, but titers of Lyme disease sera were also decreased. To further characterize the specificity of the humoral immune response against the *I. dammini* spirochete, ³⁵S-methionine-labeled spirochetal antigens were identified by immunoprecipitation with sera from Lyme arthritis patients. These polypeptides had molecular weights of 62, 60, 47, 37, 22, 18, and 15 kDa, and were not recognized by control sera. We conclude that the ELISA, without absorption, is the best method to assay the humoral immune response in Lyme disease, and we have identified methionine-containing spirochetal polypeptides that may be important in Lyme arthritis.

Although the *Ixodes dammini* spirochete, the causative agent of Lyme disease, has been isolated from skin at the site of erythema chronicum migrans (ECM), blood, and spinal fluid of patients, culture of the organism is a low-yield procedure [1,2]. Direct visualization of the spirochete in patient specimens has also been difficult [1]. Therefore, determination of antibody titers is currently the best laboratory method for diagnosis [1,3]. We report here the antibody response in Lyme disease determined by indirect immunofluorescence and a newly developed ELISA. In addition, we describe absorption studies of cross-reactive antibodies from patients with other spirochetal infections. Finally, to further characterize the immunospecificity of the antibody response in Lyme disease patients, we present data regarding the immunoprecipitation of *in vivo* ³⁵S-methionine-labeled *I. dammini* spirochetal antigens.

ANTIBODY RESPONSE BY IMMUNOFLUORESCENCE

Initially, antibody studies were done by indirect immunofluorescence alone. *I. dammini* spirochetes, strain G39/40 isolated from ticks in Connecticut, were fixed

to individual wells of microtiter slides by freezing at -70°C . Indirect immunofluorescence was performed by standard methods [1]. Each slide was overlaid with twofold dilutions of patient serum and subsequently with fluorescinated antihuman IgM or IgG. All slides were read blindly and titers were reported as reciprocals of dilution.

IgM and IgG antibody titers were done in 135 patients with Lyme disease and 80 control subjects (Fig. 1) [1]. The patients with Lyme disease included 80 people with ECM: 40 with ECM alone and 40 with ECM complicated by carditis and/or neuritis (30 patients) and often arthritis (25 patients). Twenty additional patients with neurologic or cardiac involvement and 35 with arthritis who did not have earlier samples taken were also studied. The control subjects included patients with active inflammatory arthritis, acute infectious mononucleosis, and healthy individuals from Connecticut and Georgia. Specific IgM titers in Lyme disease were highest during the third through the sixth week after onset of ECM. Titers gradually declined thereafter, but a significant number of patients still had titers >64 during neuritis, carditis, or arthritis. Specific IgG titers were lowest during ECM, increased during neuritis or carditis, and were highest months later when arthritis was present. Among the patients with neurologic, cardiac, or joint involvement, 94 percent had IgG titers >64 , and all 60 of the patients with arthritis had such titers. Of the 80 control subjects, only three with infectious mononucleosis had an IgM titer >64 and none had an IgG titer >64 .

ANTIBODY RESPONSE BY ELISA

To extend these findings, we developed an ELISA to measure specific IgM and IgG antibodies in Lyme disease [4]. *I. dammini* spirochetes, strain G39/40, were grown to late log phase in modified Kelly's medium [5], harvested by centrifugation, washed, and sonicated on ice. This lysate was used as the antigen for solid phase coating. The ELISA was performed by standard techniques [6]. Endpoint titers of

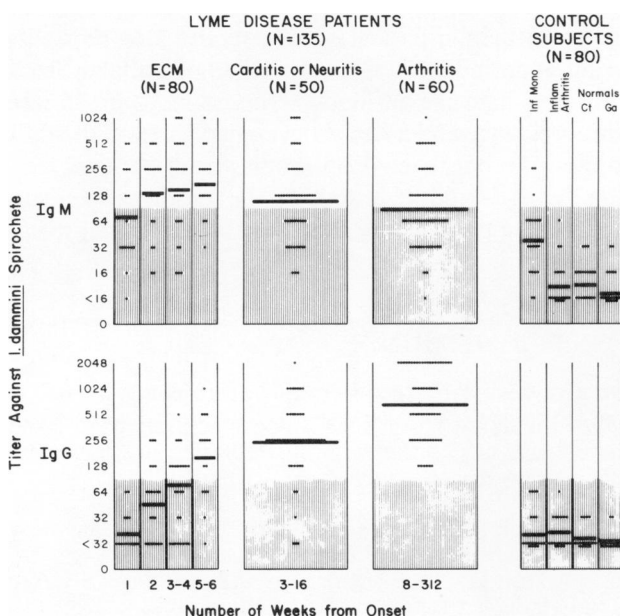


FIG. 1. Antibody titers against the *I. dammini* spirochete in serum samples from 135 patients with different clinical manifestations of Lyme disease, as compared with those found in 80 control subjects. The heavy bar shows the geometric mean titer for each group; the shaded areas indicate the range of titers generally observed in control subjects. Reprinted, by permission of the *New England Journal of Medicine* [1].

unknown sera were determined at optical density values that were two standard deviations above those of 17 normal control sera.

IgM and IgG antibody titers were determined in 12 patients with severe Lyme disease in whom serial sera were available from a prolonged period: generally during early disease (ECM), during neuritis and/or arthritis, and after remission. Forty-one serial specimens from these 12 patients were tested. Controls included 40 patients with rheumatoid arthritis, systemic lupus, acute infectious mononucleosis, and normal subjects. None of the controls, including ten patients with infectious mononucleosis, had IgM or IgG titers ≥ 200 ; titers > 200 were therefore considered elevated. In patients with Lyme disease, specific IgM titers were highest during ECM, and 11 of the 12 patients had titers > 200 . Titers declined thereafter, but eight patients had elevated titers during neuritis (4 to 16 weeks after onset of ECM) and four still had elevated titers during arthritis (4 to 29 months after onset of ECM). Specific IgG levels were lowest during ECM, increased during neuritis, were highest months later when arthritis was present, and remained high after 2 to 16 months of remission. Nine of the patients had IgG titers > 200 during neuritis, and all had such titers during arthritis.

Most recently, we also examined the antibody response by ELISA in 32 patients seen early after onset of ECM. All were treated with antibiotics and did not develop neurologic, cardiac, or joint disease. Ten patients had mild illness, usually ECM alone, and twenty-two had more severe illness — multiple skin lesions, musculoskeletal symptoms, and/or abnormal liver function tests. Three patients with mild disease (ECM alone) had an elevated IgM response during acute disease or convalescence, and only one had an elevated IgG response. In contrast, patients with more severe disease were more likely to have positive IgM responses, although twelve patients still had titers ≤ 200 during acute disease or convalescence. IgG responses were often low during acute disease, but seven patients had a positive response during convalescence.

We conclude from these data that the IgM response, although often absent in early localized Lyme disease, is generally present in patients with ECM who are more ill. The maximal response occurs after three to six weeks, and then declines. However, it may persist in later disease. Although the mechanism of the continued elevation of specific IgM is unclear, its persistence implies ongoing antigenic stimulation of the immune system, perhaps by an intact spirochete. The IgG response, often absent in early disease, is nearly always present during neuritis, carditis, or arthritis, and may remain elevated after months of remission. Either antibody response can be aborted by early antibiotic therapy.

COMPARISON OF ELISA AND IMMUNOFLUORESCENCE

To compare our ELISA and immunofluorescence tests, we determined IgM and IgG titers by both methods on 41 samples from Lyme disease patients. Although there was a significant correlation of titers between the two methods, the ELISA was more sensitive and specific than immunofluorescence. In particular, the false-positive IgM responses seen in certain infectious mononucleosis samples by immunofluorescence were not found in the same samples when tested by ELISA.

CORRELATION OF TOTAL AND SPECIFIC IgM

We also investigated the correlation between total and specific IgM levels in Lyme disease patients. In 41 serum samples from various stages of Lyme disease, specific

IgM, determined by ELISA, correlated directly with total IgM ($r = 0.62$, $p < 0.001$, slope = 101). Since total serum IgM reflects disease activity [7], this finding implies that the persistence of specific IgM antibodies may also be associated with more severe disease. In contrast, there was no correlation between total and specific IgG.

CROSS-REACTIVE ANTIBODIES

Next, to look for cross-reactive antibodies in other spirochetal infections, we determined IgG titers by ELISA in sera from patients with louse-borne relapsing fever, syphilis, or leptospirosis, and compared them to titers in Lyme disease patients. Using the *I. dammini* spirochetal lysate as the solid-phase antigen, serologic cross-reactivity against the *I. dammini* spirochete was found in these spirochetal infections, particularly relapsing fever and syphilis. After adsorption with a sonicate of *Borrelia hermsii*, similar to the FTA-adsorbed test for syphilis [8], sera from patients with relapsing fever, syphilis, and leptospirosis generally had a fourfold or greater reduction in titer, compared to unabsorbed sera. Titers of Lyme disease sera were also lowered by absorption, although the decrease was usually twofold. Thus it currently seems impractical to use this adsorption step as a refinement of our original ELISA procedure. Lyme disease can usually be distinguished clinically from relapsing fever, syphilis, and leptospirosis. If confusion exists in separating Lyme disease from syphilis, VDRLs are positive only in syphilis [9].

POLYPEPTIDES OF THE LYME SPIROCHETE

Preliminary attempts to characterize the immunospecificity of the IgG antibodies in Lyme patients were also made. *I. dammini* spirochetes (strain G39/40) in 7.5 ml of modified Kelly's medium were pulse-labeled for 12 hours by the addition of 1 mCi of ^{35}S -methionine at late log phase growth. After collection by centrifugation and after extensive washing in cold 0.01 M PBS/5mM magnesium chloride, the organisms were sonicated on ice. Intact organisms and large cellular debris were then removed by centrifugation at 10,000 g for 30 minutes at 4°C. The ^{35}S -methionine-labeled crude supernatant fraction, after immunoprecipitation with patient sera and staphylococcal protein A [10], was then subjected to polyacrylamide gel electrophoresis [11], followed by 2,5-diphenyloxazole fluorography and autoradiography. By this method, spirochetal polypeptides with monomeric molecular weights of 62, 60, 47, 37, 22, 18, and 15 kDa were identified with sera from Lyme arthritis patients. Control sera from normal patients and patients with active inflammatory arthritis did not precipitate these antigens. These preliminary experiments demonstrate the spirochetal antigens responsible for the IgG response in chronic Lyme disease and may provide a basis for the delineation of the most important antigens of Lyme arthritis.

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