

## SERONEGATIVE LYME DISEASE

Dissociation of Specific T- and B-Lymphocyte Responses to *Borrelia burgdorferi*

RAYMOND J. DATTWYLER, M.D., DAVID J. VOLKMAN, M.D., PH.D., BENJAMIN J. LUFT, M.D., JOHN J. HALPERIN, M.D., JOSEPHINE THOMAS, B.S., AND MARC G. GOLIGHTLY, PH.D.

**Abstract** The diagnosis of Lyme disease often depends on the measurement of serum antibodies to *Borrelia burgdorferi*, the spirochete that causes this disorder. Although prompt treatment with antibiotics may abrogate the antibody response to the infection, symptoms persist in some patients.

We studied 17 patients who had presented with acute Lyme disease and received prompt treatment with oral antibiotics, but in whom chronic Lyme disease subsequently developed. Although these patients had clinically active disease, none had diagnostic levels of antibodies to *B. burgdorferi* on either a standard enzyme-linked immunosorbent assay or immunofluorescence assay. On Western blot analysis, the level of immunoglobulin reactivity against *B. burgdorferi* in serum from these pa-

tients was no greater than that in serum from normal controls.

The patients had a vigorous T-cell proliferative response to whole *B. burgdorferi*, with a mean ( $\pm$ SEM) stimulation index of  $17.8 \pm 3.3$ , similar to that ( $15.8 \pm 3.2$ ) in 18 patients with chronic Lyme disease who had detectable antibodies. The T-cell response of both groups was greater than that of a control group of healthy subjects ( $3.1 \pm 0.5$ ;  $P < 0.001$ ).

We conclude that the presence of chronic Lyme disease cannot be excluded by the absence of antibodies against *B. burgdorferi* and that a specific T-cell blastogenic response to *B. burgdorferi* is evidence of infection in seronegative patients with clinical indications of chronic Lyme disease. (N Engl J Med 1988; 319:1441-6.)

THE best clinical marker of acute Lyme disease is a characteristic skin lesion, erythema chronicum migrans (ECM).<sup>1-4</sup> In many patients, initial infection progresses to chronic Lyme disease, a spectrum of clinical signs and symptoms characterized by persistent musculoskeletal, cardiac, and neurologic involvement.<sup>4-10</sup> Since *Borrelia burgdorferi* was discovered to be the etiologic agent of this infectious disease, the demonstration of specific antibodies to this spirochete has been considered to be the best indicator of exposure to the organism and has become a prerequisite for the diagnosis of chronic Lyme disease.<sup>11-15</sup> A vigorous humoral response against *B. burgdorferi* develops during the course of natural infection. In chronic Lyme disease, *B. burgdorferi* persists, inducing an ongoing inflammatory response.<sup>16,17</sup> The absence of a continuing humoral response in patients treated with antibiotics during the ECM phase of Lyme disease has been believed to indicate that the organism had been effectively eradicated.<sup>18-20</sup> However, many patients given oral antibiotics at the ECM stage have reported persistence of symptoms, including severe chronic fatigue, numbness of the extremities, memory loss, and joint pain.<sup>18-21</sup> In the absence of elevated levels of antibodies to *B. burgdorferi*, these symptoms have been attributed to a post-Lyme disease syndrome rather than considered as evidence of persistent infection and failure of the initial antibiotic regimen to treat the disease effectively.

In persons exposed to *B. burgdorferi*, an early, vigorous, and sustained specific T-lymphocyte response develops that often precedes a measurable antibody response.<sup>22,23</sup> In this study we used borrelia-specific

T-cell immune responses to document exposure to *B. burgdorferi* in patients with symptoms of chronic Lyme disease who lacked diagnostic levels of specific serum antibodies to the organism. It is noteworthy that all these patients had received oral antibiotics early in the course of infection. Thus, these findings exemplify an infectious disease in which removal of most of the microbial antigens at an early point in its course appears to abrogate the development of sustained humoral immunity despite evidence of persistent infection.

## METHODS

## Subjects

Patients were classified as either seropositive or seronegative on the basis of their serum antibody reactivity to *B. burgdorferi* in a standardized enzyme-linked immunosorbent assay (ELISA). Serum samples were considered positive if the concentrations of antibodies to *B. burgdorferi*, expressed as optical-density values, were more than 3 SD above the mean values for a panel of samples obtained from a group of healthy adults with no history of *B. burgdorferi* infection. Samples with optical-density values less than 3 SD above the mean were considered negative.

The present study was based on findings in 17 patients with signs, symptoms, and a history compatible with the diagnosis of chronic Lyme disease who had specific optical-density values in the negative range ( $< 3$  SD above the mean). All were from Suffolk County, New York, an area in which Lyme disease is highly endemic. Eighteen other patients with comparable signs, symptoms, and histories of chronic Lyme disease who had diagnostic levels of antibodies to *B. burgdorferi* on ELISA served as positive controls, and 17 healthy adults with no history of Lyme disease or any other rheumatic or immune disorders served as negative controls. The clinical manifestations of both the seronegative and seropositive patients are shown in Table 1. Of the 17 seronegative patients, 15 had well-documented histories of ECM, the best clinical marker of *B. burgdorferi* infection. In each of these patients an influenza-like illness had accompanied the ECM. The other two patients had a similar history of an influenza-like illness that occurred soon after a tick bite. All 17 patients had been treated with oral antibiotics for at least 10 days at the time of their initial illness. Twelve had received tetracycline (1 to 2 g per day), four penicillin (1 to 2 g per day), and one erythromycin (1 g per day).

At the time of their initial presentation at the Lyme Disease Clinic at our institution, all 17 seronegative patients had systemic

From the Department of Medicine (R.J.D., D.J.V., B.J.L., J.T.), the Department of Pathology (M.G.G.), and the Department of Neurology (J.J.H.), State University of New York at Stony Brook, School of Medicine, Stony Brook, N.Y. Address reprint requests to Dr. Dattwyler at the Division of Clinical Immunology, Health Sciences Center, SUNY at Stony Brook, Stony Brook, NY 11794-8161.

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symptoms, including fatigue, arthralgias, headaches, and cognitive dysfunction. Fever was not noted. The incidence of arthritis or a history of arthritis in this group (12 of 17 patients) was similar to that observed in the seropositive group (15 of 18 patients). In both the seronegative and the seropositive groups the arthritis was characterized by pain occurring when the affected joint was moved, periarticular soft-tissue swelling, synovial thickening, or small effusions. In all cases it was mild, oligoarticular, and nonerosive and its course contained remissions and relapses. The arthritis generally involved large joints, especially the knee. Nine of the seronegative patients had peripheral neuropathy. Four had entrapment neuropathies, documented by nerve-conduction studies, and seven had measurable abnormalities of peripheral nerves other than entrapment neuropathy, also documented by nerve-conduction studies. The neurophysiologic abnormalities of the peripheral nerves were indicative of an axonal neuropathic process. Demyelinating neuropathy was not observed. None of the patients had a history of recurrent infections. At the time of entrance into the study none were taking glucocorticosteroids or other immunosuppressive agents.

After the 17 seronegative patients received treatment with parenteral antibiotics (either penicillin [24 million units per day in divided doses for 10 days] or ceftriaxone [2 to 4 g per day for 14 days]), all had marked improvement. Objective evidence of active disease, including arthritis, resolved in all patients over a three-month period, with the exception of peripheral neuropathy, which resolved more slowly. None of the patients had a recurrent episode of arthritis or a symptomatic relapse during an eight-month follow-up period.

### ELISA for Antibodies to *B. burgdorferi*

The ELISA was performed as previously described,<sup>19</sup> with minor modifications. In brief, 96-well microtiter plates (Flow Laboratories, McLean, Va.) were coated with a sonicate of *B. burgdorferi* (5  $\mu$ g per milliliter) for 18 hours in 0.05 M sodium carbonate (pH 9.6) and then washed three times with phosphate-buffered saline (pH 7.2) containing 0.05 percent Tween 20 (PBS/Tween). Excess binding sites on the wells were blocked by postcoating the wells with 250  $\mu$ l of phosphate-buffered saline containing 1 percent bovine serum albumin. The plates were washed as described above. Serum from all three study groups was diluted to 1:500 in PBS/Tween. Aliquots of 0.2 ml were added to duplicate wells and incubated for one hour at 37°C. The plates were washed, and alkaline phosphatase-conjugated goat antihuman immunoglobulin specific for both light-chain and heavy-chain determinants was added to detect bound immunoglobulin of all isotypes. After incubation for one hour at 37°C, the wells were again washed three times in PBS/Tween. *p*-Nitrophenyl phosphate (Sigma, St. Louis) was added at a concentration of 0.66 mg per milliliter in 0.15 M sodium bicarbonate containing 1 mM magnesium chloride, to each well. Plates were incubated two hours at room temperature, and the reaction was stopped with 30  $\mu$ l of 3 N sodium hydroxide. The optical density of each well at 405 nm was measured on an ELISA reader (Bio-Tek, Winooski, Vt.).

### Immunofluorescence Assay

Indirect immunofluorescence assays for the detection of both IgM and IgG antibodies to *B. burgdorferi* were performed according to standard techniques. In brief, slides coated with *B. burgdorferi* (Diagnostic Technology, Hauppauge, N.Y.) were incubated with serum at serial dilutions beginning at 1:64. After 60 minutes at room temperature, the slides were washed three times in phosphate-buffered saline and then incubated for 30 minutes with fluorescein-labeled antihuman immunoglobulin, washed, and examined on a fluorescence microscope (Nikon-Photomat, Garden City, N.Y.).

### Electrophoresis and Immunoblotting

Qualitative antibody analyses were performed as previously described,<sup>24</sup> with slight modifications. In brief, *B. burgdorferi* organisms of the B31 strain were grown in BSK II media<sup>25</sup> and washed three times in phosphate-buffered saline. Spirochetes were resuspended in 2 ml of phosphate-buffered saline containing 10 mM ethylenedia-

minetetraacetic acid and 1 mM *p*-methylsulfonylfluoride and sonicated in an Ultra tip Labsonic System (Lab-Line Instruments, Melrose Park, Ill.) for five minutes. The protein concentration was measured according to the Bradford procedure.<sup>26</sup> The mixture was concentrated to 2.5 percent in sodium dodecyl sulfate and 2.5 percent in 2-mercaptoethanol, heated at 100°C for three minutes, and adjusted to a concentration of 0.4 to 0.6 mg of protein per milliliter. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed with a Mighty Small II apparatus (Hoeffer, San Francisco) at 60 V for two hours. Blots were left overnight at 4°C in TRIS buffer (50 mM TRIS-150 mM sodium chloride) containing 1 percent bovine serum albumin. They were then incubated with serum diluted with TRIS buffer for two hours at 37°C. Serum was assayed at dilutions of both 1:200 and 1:100. The blots were washed for 10 minutes with TRIS buffer containing 0.5 percent Tween-20 and then for 10 minutes in TRIS buffer alone. Bound immunoglobulin was detected with goat antihuman IgG, IgM, or IgA conjugated to alkaline phosphatase (Sigma). After a two-hour incubation at 37°C with a 1:5000 dilution of the conjugates, the blots were again washed in a two-step procedure and then incubated with the BICIP-Substrate System (5-bromo-4-chloro-3-indole phosphate-nitroblue tetrazolium; Kirkegaard and Perry Laboratories, Gaithersburg, Md.) for 20 minutes. The reaction was stopped by rinsing the blots in distilled water.

### Lymphocyte-Proliferation Assays

The lymphoproliferative response of peripheral-blood mononuclear cells to whole *B. burgdorferi* organisms was assessed during the initial evaluation for chronic Lyme disease. Proliferation assays were performed as previously described.<sup>22</sup> Mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) density-gradient centrifugation and washed three times. They were adjusted to a final concentration of  $1 \times 10^6$  cells per milliliter in RPMI-1640 supplemented with 10 percent human AB serum, 100 U of penicillin, and 100  $\mu$ g of streptomycin per milliliter, and 2 mM L-glutamine. A total of  $1 \times 10^5$  cells per well were pipetted into a 96-well microtiter plate (Costar, Cambridge, Mass.) and stimulated (in triplicate) with whole *B. burgdorferi* at a concentration of  $1 \times 10^6$  organisms per well. Control wells received medium only. Cell cultures were incubated in a humidified atmosphere of 5 percent carbon dioxide at 37°C. [<sup>3</sup>H]Thymidine (specific activity, 6.7 Ci per millimole [New England Nuclear, Boston]) was added to the wells (1  $\mu$ Ci per well) 18 hours before harvesting at five days. Cells were collected with a cell harvester (Skatron, Sterling, Va.) onto glass-fiber filters, and the radioactivity associated with the cells was counted in a liquid scintillation system (LS7500, Beckman Instruments, Fullerton, Calif.). Counts were expressed as disintegrations per minute (dpm). The triplicate values were averaged, and a stimulation index (SI) was calculated for each patient, according to the formula, SI = dpm (stimulated)/dpm (unstimulated).

### Statistical Analysis

The Student one-tailed t-test was used to compare the T-cell proliferative responses in the three groups tested.

## RESULTS

At presentation, 12 of the 17 seronegative patients had evidence of active arthritis on physical examination, and 9 had a peripheral neuropathy that was confirmed by nerve-conduction studies. All 17 reported that they had had severe chronic fatigue and recurrent headaches, 16 that they had had arthralgias, and 15 that they had had difficulty with short-term memory and cognition; all had objective evidence of active disease (Table 1). Eleven of the patients were evaluated with an extensive battery of neuropsychological tests,<sup>27</sup> including the California Verbal Learning Test (a test of memory) and the booklet version of the Category Test<sup>28</sup>; all 11 had evidence of intellectual

**Table 1. Characteristics of Patients Seropositive and Seronegative for Chronic Lyme Disease.**

	SEROPOSITIVE PATIENTS (N = 18)	SERONEGATIVE PATIENTS (N = 17)
Mean age (yr)	36.6 (16–72)	38.0 (10–65)
Sex (M/F)	9/9	11/6
History of ECM	14	15
Initial influenza-like illness	17	17
Initial treatment		
Tetracycline	1	12
Penicillin	1	4
Cephalexin	1	—
Erythromycin	—	1
Duration of illness (mo)*	8.4±2.5 (1–24)	17.6±5.4 (8–72)
Clinical manifestations		
Arthritis	15	12†
Peripheral neuropathy	7	9
Symptoms‡	18	17

\*Period (mean ±SD) from initial illness to diagnosis of chronic Lyme disease.

†Three other patients had a history of arthritis.

‡Includes headaches, fatigue, arthralgia, and cognitive dysfunction.

impairment. The results of serologic tests for antinuclear antibodies and rheumatoid factor, VDRL testing, and C1q binding assay, and serum protein electrophoresis patterns were all negative or normal.

Table 2 compares the results on ELISA in the seronegative and seropositive patients with Lyme disease. None of the 17 seronegative patients had levels of antibodies to *B. burgdorferi* that were more than 3 SD above the mean level in the negative controls. Immunofluorescence assays using whole *B. burgdorferi* organisms were also negative at serum dilutions above 1:64 (minimum positive titers in our laboratory,  $\geq 1:256$ ). Western blot analyses of solubilized whole *B. burgdorferi* antigens were performed to confirm the absence of specific anti-borrelia antibodies. Figure 1A shows IgG immunoblots for six seropositive patients. Their serum samples were assayed at a 1:200 dilution; all samples showed reactivity against distinct borrelia antigens. The samples differed in the number of antigens detected. The longer the Lyme disease was untreated in these seropositive patients, the greater the number of antigens against which their serum samples reacted (lanes d through f). Figure 1B shows representative IgG blots for five of the seronegative patients. Lane a represents serum from a seropositive patient that was run as a positive control. Lanes b through f represent samples from seronegative patients that were run at twice the concentration of the samples from the seropositive patients in order to detect low levels of borrelia-reactive antibodies. The most consistent indicator reactivity observed in the samples from the seronegative patients was a faint band corresponding to the 41-kd borrelia flagella antigen. Similar reactivity was observed in blots for 10 other seronegative patients that were run in separate assays (data not shown). This pattern of reactivity against spirochetal antigens, especially the 41-kd protein, was also observed in samples from the majority of controls (Fig.

1C). When the blots for the seronegative patients were stained for IgM or IgA antibodies, little specific reactivity was observed. A faint IgM band of reactivity against the protein associated with the 41-kd flagella was detectable in 2 of the 14 patients tested. Faint bands of IgA reactivity were also detected against the 41-kd and 66-kd antigens in all of five seronegative patients tested. This pattern of low-level reactivity of IgA and IgM antibodies was also observed in the normal controls and was readily distinguishable from the pattern observed in the seropositive patients, both in terms of the number of antigens recognized and the intensity of the bands. Thus, immunoblot analysis was consistent with the results obtained with the ELISA and the immunofluorescence assay, and demonstrated a profoundly blunted response to *B. burgdorferi* in the patients with clinically active disease.

T-cell immune responses specific for *B. burgdorferi* were assessed according to a standard proliferative assay using whole *B. burgdorferi* as the stimulatory antigen. These results are shown in Figure 2. Of note is the finding that the 17 seronegative patients with clinical Lyme disease had proliferative responses that were virtually identical to those of the seropositive patients. The stimulation index in the seronegative patients ranged from 2.7 to 58.0, with a mean ( $\pm$ SEM) of  $17.8 \pm 3.3$ . The average number of counts was 19,455 dpm. The group of 18 seropositive patients had similar blastogenic responses to *B. burgdorferi*, with a mean stimulation index of  $15.8 \pm 3.2$ . In contrast, the 17 healthy controls had stimulation indexes that ranged from 0.5 to 7.8, with a mean of  $3.1 \pm 0.5$ , and an average count of 2932 dpm. Statistical analysis did not demonstrate any significant difference between the seronegative and the seropositive patient groups. However, the proliferative responses in each patient group were significantly different from those in the normal control group ( $P < 0.001$ ).

## DISCUSSION

Infection with *B. burgdorferi*, the etiologic agent of Lyme disease, is associated with the development of a vigorous T-cell response to this organism.<sup>22,23</sup> In this study we have shown that a specific T-cell response can occur independently of a diagnostic humoral response to *B. burgdorferi*. Although our 17 chronically ill patients had clinical histories, signs, and symptoms

**Table 2. Reactivity for *B. burgdorferi* Antibodies on ELISA.\***

	NORMAL CONTROLS (N = 10)	SERONEGATIVE PATIENTS (N = 17)	SEROPOSITIVE PATIENTS (N = 18)
Mean serum antibody concentration	0.44	0.55	3.81
Standard deviation	0.19	0.24	0.62

\*Values are expressed as normalized values for optical density (donor value in subject  $\div$  [mean value among normal controls + 3 SD]).

compatible with the diagnosis of active Lyme disease, their levels of antibodies to *B. burgdorferi* as measured by ELISA or by immunofluorescence assay were indistinguishable from those of normal controls. This lack of reactivity was confirmed by Western blot analysis. Serum from these 17 patients primarily exhibited only weak reactivity to the 41-kd flagella antigen, an antigen sharing epitopes with many other spirochetes,

including both other borrelia species<sup>20</sup> and treponema species. In contrast, 14 of the 17 patients had a marked T-cell response to this organism. Three patients had low T-cell responses (stimulation indexes of 2.7, 4.1, and 4.9) that overlapped with the range of the normal controls. Each of these three patients, however, had a history of ECM that had been observed by a physician, early antibiotic therapy, a clinical course compatible with Lyme disease, and improvement after administration of parenteral antimicrobial agents known to be effective against *B. burgdorferi*. A similar range of T-cell reactivity was also observed in the seropositive patients. Once established, this T-cell reactivity in both seronegative and seropositive individuals was reproducible and persistent. None of the 17 patients had a history suggestive of a primary immune defect, although the possibility of a specific abnormality in the ability to respond to discrete spirochetal antigens or an immunoglobulin-subclass deficiency was not ruled out.

The disorder in these seronegative patients reflected a dissociation between T-cell and B-cell immune responses, in which the cell-mediated arm of the immune response was intact yet the humoral portion of the response to *B. burgdorferi* appeared to be blunted. This diminished antibody response is in contrast to the T-cell anergy commonly observed in several chronic infections (e.g., infection with *Mycobacterium leprae* or *M. marinum*, filariasis, and some chronic fungal infections<sup>29-33</sup>). It has previously been found that some patients who received antibiotics for ECM had low or undetectable levels of anti-borrelia antibodies.<sup>15,20</sup> This condition may be analogous to syphilis, in which early antimicrobial therapy can abort the development of a measurable humoral response. Since help from T cells is usually required to produce a vigorous antibody response to protein antigens and to augment the response to most carbohydrate antigens,<sup>34</sup> the development of a measurable T-cell response probably occurs before an antibody response becomes fully manifest. In previous studies, we showed that patients with ECM can mount specific T-cell responses to *B. burgdorferi* before antibodies to this organism become detectable by routine ELISA.<sup>22,23</sup>

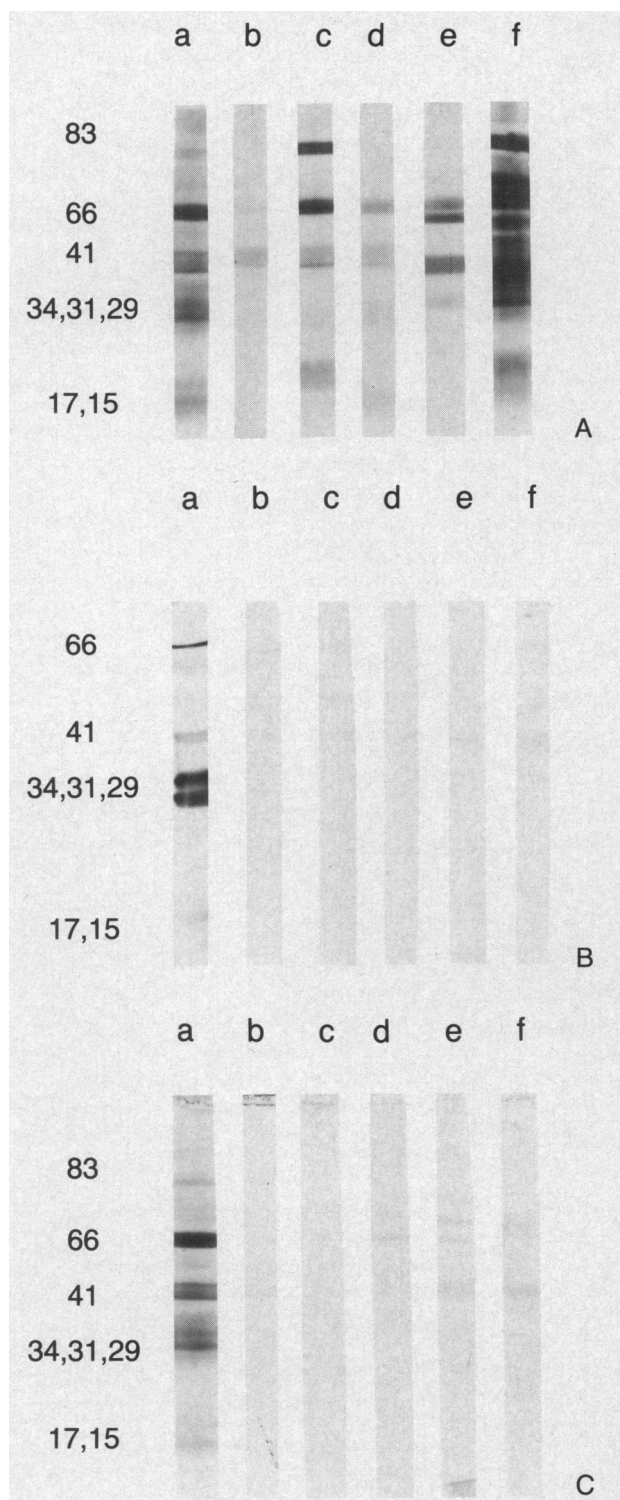


Figure 1. Immunoblots of IgG Antibodies to *B. burgdorferi* Antigens.

*B. burgdorferi* proteins were transferred to nitrocellulose and probed with diluted serum (either 1:200 for ELISA-positive samples or 1:100 for negative samples). Bound IgG was detected by goat antihuman gamma-chain antibody conjugated to alkaline phosphatase. Blots were developed with the BICIP-substrate system.

Panel A shows blots for six patients with chronic Lyme disease who were seropositive on ELISA. Panel B shows representative blots for five of the seronegative patients (lanes b through f), and Panel C immunoblots of serum from five of the normal controls (lanes b through f). Lane a in both Panel B and Panel C represents serum from a seropositive patient, shown for comparison.

Molecular-weight markers are shown to the left.

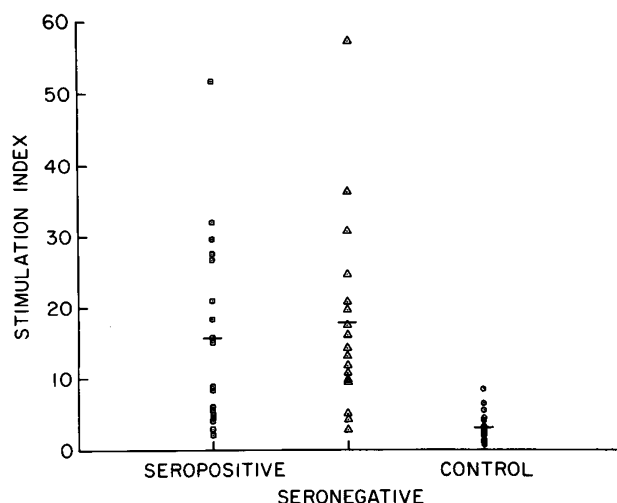


Figure 2. Lymphoproliferative Responses to *B. burgdorferi*.

Isolated peripheral-blood mononuclear cells were stimulated with whole *B. burgdorferi* organisms as outlined in the Methods. The cells were cultured in flat-bottomed microtiter wells, at a concentration of  $10^5$  cells per well, pulsed with  $1 \mu\text{Ci}$  of [ $^3\text{H}$ ]thymidine for 18 hours, and harvested on day 5. Values for the stimulation index are shown on the abscissa. The groups studied were patients with positive values on ELISA for *B. burgdorferi* ( $n = 18$ ), patients without serologic evidence of Lyme disease ( $n = 17$ ), and normal controls ( $n = 17$ ). Bars represent the arithmetic mean response in each group.

The precise mechanism of the observed humoral anergy has not yet been delineated. However, each of the 17 seronegative patients was given antibiotics early in the course of infection. Although the standard antibiotic therapy for Lyme disease appears to eradicate the organism in most instances,<sup>18</sup> we have found that in some patients the response to treatment is incomplete and late sequelae develop.<sup>21</sup> In the seronegative patients whom we describe, it may well be that antibiotic therapy resulted in the elimination of most spirochetes at a critical early stage of the immune response, before a sustained B-cell response had developed. A precedent for the abrogation of a mature antibody response by early treatment exists not only in *Treponema pallidum* infection<sup>35</sup> but also in Rh incompatibility, in which the infusion of antibody to the Rh antigen clears the antigen and selectively inhibits the maternal antibody response.<sup>36</sup> Current concepts of immune responsiveness hold that T cells initially recognize foreign antigen presented by cells expressing Class II major histocompatibility antigens, such as macrophages and dendritic cells.<sup>37</sup> After recognition, antigen-specific T cells proliferate and mature to become effector cells. These effector T cells can then interact with other cells in the immune system that express the same HLA-D-region gene products. In antigen-specific systems, an additional requirement for cell-cell interactions is the presence of antigen to provide a close physical linkage between the T effector cell and the other cells. Antigen-specific B cells, having both dense Ia and specific antigen receptors on

their surface, are exquisitely suited to present antigen to T cells.<sup>38</sup> In the absence of antigen, cognate recognition is not established and T cells and B cells do not interact effectively. Thus, according to this model of immune interaction, after an initial proliferative response by B cells that is independent of the response by T cells, the continued presence of the antigen is required for the development of a mature humoral response.

There is an apparent inconsistency in this model of seronegative Lyme disease and the generally held concept of the pathogenesis of chronic Lyme disease. If the cognate recognition of B cells by T cells fails to occur because the spirochetes have been removed, how can continued disease activity be explained? Although all the patients described were given antibiotics that could effectively eradicate the bulk of *B. burgdorferi* organisms from most sites of infection, the central nervous system and perhaps other privileged sites may not have received adequate treatment. Currently recommended regimens of oral tetracycline and penicillin fail to produce drug concentrations in the central nervous system that are high enough to reach the mean inhibitory concentrations for the majority of strains of *B. burgdorferi*.<sup>21,39,40</sup> Thus, spirochetes reaching this immunologically privileged site may remain viable despite standard therapy. A similar phenomenon has been observed in patients in whom therapy believed to be curative for *T. pallidum* infection was administered yet in whom active neurosyphilis later developed.<sup>41-43</sup> Consistent with chronic central nervous system infection is our recent observation that some patients with chronic Lyme disease have appreciable abnormalities of the white matter on magnetic resonance imaging.<sup>27</sup> In addition, three separate groups of investigators have reported local production of anti-borrelia antibody in the central nervous system in patients with neurologic symptoms of Lyme disease in the absence of diagnostic levels of serum antibodies.<sup>44-46</sup>

In summary, the diagnosis of chronic *B. burgdorferi* infection should be considered in any patient with a history of ECM and signs and symptoms compatible with chronic Lyme disease. Serologic assays remain the best tests for screening for exposure to *B. burgdorferi*. Although the true incidence of seronegative disease is unknown, it is not common. Seronegative patients represent less than 5 percent of the more than 200 patients with Lyme disease who are referred to our group each year. Assessment of T-cell blastogenesis should be limited to symptomatic patients who have been exposed to ticks, who have objective evidence of active disease, who have received antibiotics early in the course of their infection, and who have nondiagnostic levels of antibody systemically and locally. Although chronic fatigue is a common finding in chronic Lyme disease, we do not believe that chronic fatigue as an isolated symptom should be considered to be indicative of *B. burgdorferi* infection. It is important to diagnose chronic

Lyme disease with its accompanying neurologic and rheumatologic sequelae correctly, since this systemic spirochetosis can be effectively treated with appropriate intravenous antibiotics.<sup>41,47-49</sup> However, it is also important to remember that immunologic tests, whether they measure antibodies or cellular immune responses, are indicators of exposure and do not by themselves prove that the patient has an active infectious process.

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