

ISOLATION OF *BORRELIA BURGDORFERI* FROM THE MYOCARDIUM OF A PATIENT WITH LONGSTANDING CARDIOMYOPATHY

GEROLD STANEK, M.D., JUTTA KLEIN, M.D.,
REGINALD BITTNER, M.D.,
AND DIETMAR GLOGAR, M.D.

LYME borreliosis (or Lyme disease) is a systemic disorder caused by the spirochete *Borrelia burgdorferi*, a newly discovered species of borrelia.^{1,2} The disease usually begins with a characteristic skin lesion; neurologic, cardiac, and joint involvement may develop weeks to months later. Some manifestations may last for years or even decades.³

Lyme borreliosis is transmitted by arthropods, especially by ticks of the genus *Ixodes* and by some tabanid species. The area in which it is endemic includes most of the United States,⁴ all of Europe,⁵ the European and Asian parts of Russia,⁶ China,⁷ and Japan.⁸ Cardiac abnormalities induced by Lyme borreliosis were reported even before the discovery of its etiologic agent.⁹

In this report we describe a patient who had had dilated cardiomyopathy for approximately four years. He had antibodies to *B. burgdorferi* on both enzyme-linked immunosorbent assay (ELISA) and immunoblotting. The high antibody concentration and the intense immunoblot reaction suggested a chronic form of Lyme borreliosis. Cardiomyopathy associated with *B. burgdorferi* infection was established by a positive culture of endomyocardial-biopsy tissue.

CASE REPORT

A 54-year-old man was admitted to our hospital with a diagnosis of dilated cardiomyopathy. He had been in excellent health until 1985. He had enjoyed hunting, hiking, and tennis. In the early spring of 1985 he had a severe influenza-like infection accompanied by a high temperature (up to 39°C), cough, generalized arthralgias, headache, and fatigue. There was no skin rash. The illness lasted about 14 days. The patient took co-trimoxazole (dosage unknown) for one week as prescribed by his general practitioner. Although the symptoms subsequently disappeared, an arrhythmia with supraventricular and ventricular beats was noted during routine checkups.

In April 1986 echocardiography revealed an enlarged left ventricle with overall hypokinesia and markedly reduced function. The valvular function was normal. Dilated cardiomyopathy was suspected. Radionuclide ventriculography disclosed a reduced ejection fraction of 39.7 percent in the left ventricle and 28 percent in the right ventricle. A 24-hour Holter electrocardiographic recording (ECG) documented a sinus rhythm with a supraventricular and complex ventricular arrhythmia of Lown Grade IVB, displaying frequent, multiform ventricular premature beats, with bigeminy, couplets, triplets, and nonsustained runs of up to six beats.

In July 1987 the patient reached a workload of 200 W during a standardized bicycle exercise test; the ECG showed a Lown Grade

From the Hygiene Institute (G.S.), the Third Department of Anatomy (R.B.), and the Department of Cardiology (J.K., D.G.), University of Vienna, Vienna, Austria. Address reprint requests to Dr. Stanek at the Hygiene Institute, University of Vienna, Kinderspitalgasse 15, A-1095 Vienna, Austria.

IVB ventricular arrhythmia. Cardiac catheterization confirmed the diagnosis of dilated cardiomyopathy. The left ventricle was highly dilated and hypokinetic. The left end-diastolic pressure was 22 mm Hg, and the pulmonary arterial pressures were elevated (systolic, 48 mm Hg; diastolic, 18 mm Hg; mean, 28 mm Hg). The cardiac index was 2.6 liters per minute per square meter. Coronary angiography showed only a small lesion in the right coronary artery, which was interpreted as a focal spasm induced by the catheter. Myocardial-perfusion imaging with thallium-201 and challenge with dipyridamole showed a diffuse, myopathic pattern without marked signs of redistribution.

All blood indexes, including the erythrocyte sedimentation rate, were normal. Tests for rheumatoid factor (the Rose-Waaler test and latex-agglutination test; Behring nephelometer analyzer), C-reactive protein (Behring nephelometer analyzer), circulating immune complexes (Behring nephelometer analyzer), and syphilis (Venereal Disease Research Laboratory test, Gamma Biologicals; and *Treponema pallidum* hemagglutination test, Fuji-rebio) were negative. An immunofluorescence assay revealed no antibodies against myocardial fibers. Frozen sections of rat myocardium were used as the substrate for the immunofluorescence assay. The patient's serum was first diluted 1:10, and then doubling dilutions were used. Goat antihuman IgG (the Fc region) conjugated with fluorescein isothiocyanate was used as the antibody (Atlantic Antibodies). Immunoglobulin levels, assessed with a Behring nephelometer analyzer, were within normal ranges.

The patient remained free of symptoms until June 1988, when he presented with increasing dyspnea and fatigue. The clinical examination revealed signs of moderate heart failure, with left ventricular enlargement as well as mild pulmonary congestion with basal rales. The liver was not palpable, and there was no peripheral edema. The chest film showed predominantly left-sided cardiac dilatation; the cardiothoracic ratio was 0.56. Treatment with diuretics and an angiotensin-converting-enzyme inhibitor was started. During a routine serologic screening in October 1988, an ELISA showed antibodies to *B. burgdorferi*. The ELISA was repeated and immunoblotting was performed; both were clearly positive.

An endomyocardial biopsy was performed, and the specimens were used for culture and histologic examination. Ceftriaxone (1 g per day) was administered intravenously for 14 days. The clinical findings remained unchanged during an observation period of six months.

RESULTS

Seroimmunologic Studies

The presence of serum antibodies to *B. burgdorferi* was determined by ELISA, with the supernatant of sonicated *B. burgdorferi* strain B31 used as the antigen. Peroxidase-conjugated goat antihuman IgG antibody (Nordic Immunological Laboratories) was used as the reactant. The results were analyzed with a 400 AT photometer (SLT Laboratory Instruments) at a wavelength of 492 nm. The results were considered positive if a serum sample in a dilution of 1:1000 exceeded an absorbance of 0.200. Blood samples were drawn from our patient on October 6, November 24, and December 12, 1988, and on January 19 and July 20, 1989. The absorbance values of the samples were 0.953, 0.985, 1.035, 0.995, and 0.841, respectively, indicating that the concentration of antibodies to *B. burgdorferi* was clearly elevated. The results obtained by ELISA were confirmed by immunoblotting. The absorbance values and the intense immunoblot reactions were comparable to those found in patients with culture-proven chronic manifestations of Lyme borreliosis,

such as chronic arthritis or acrodermatitis chronica atrophicans.

Culture

One endomyocardial-biopsy specimen (3 mm in diameter) was placed in 12 ml of BSK (Barbour-Stoermer-Kelly's II) medium¹⁰ immediately after removal and kept at 34°C for four weeks. Subcultures were made after the first day of incubation and every week thereafter. For the subcultures, 1 ml of the original medium was transferred under sterile conditions to 11 ml of new medium. Then, 1 ml of sterile medium was added to the original culture to adjust the volume. The growth of spirochetal organisms was assessed by dark-field microscopy ($\times 200$) every four days. Spirochetal organisms were observed in the original BSK medium after four weeks, and in the fifth subculture one week later. The organisms were harvested by ultracentrifugation and prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the proteins were separated according to their molecular weight with a Phast development unit (Pharmacia). The separated proteins of one gel were stained with Coomassie blue stain, and those of another were transferred to a nitrocellulose paper by diffusion blotting as described elsewhere.¹¹

The Coomassie blue stain revealed a strain of *B. burgdorferi* (Fig. 1) with the characteristic abundant major proteins but lacking a distinct outer-surface-membrane protein B of 34 kd and a protein C of 20 kd, which has been observed in some European isolates.¹² The myocardial isolate was recognized by the monoclonal antibodies H5332, H3TS, H9724, and H604 (kindly provided by Dr. Barbour, University of Texas, San Antonio).

Pathohistologic Studies

The biopsy specimens were fixed in 4 percent formalin and embedded in paraffin. Then, 8- μ m sections were stained with hematoxylin and eosin, according to the procedure of Steiner.¹³ The specimens were placed in silver nitrate solution for 12 hours.

The endocardium showed no signs of fibrosis or any other inflammatory response. The endomyxium was partially widened, and there was a slight proliferation of the interstitial connective tissue. There was subtle focal infiltration by lymphocytes and plasma cells. The walls of the small endomyxial vessels also showed thickening and infiltration by mononuclear cells. The diameters of the myocardial fibers showed unusual variation, ranging from atrophy to hypertrophy. Although the myonuclei were located centrally, as usual, most were extremely large and vesicular.

In sections stained with modified Steiner's silver stain, the myocardial cells appeared yellowish (Fig. 2). The cross-striation of the muscle fibers was well preserved, and lipofuscin granules were located in their usual perinuclear position. Connective-tissue cells stained less intensely than muscle fibers. The spirochetes were stained dark brown, almost black, and were seen mostly in the endomyxial space (Fig. 2A). One spirochete seemed to be attached to or even invading the surface of the myocardial cell (Fig. 2B). Some of the microorganisms had an irregular, stretched shape, due to the preparation process.¹⁴

DISCUSSION

Cardiac involvement in Lyme borreliosis was observed early in the study of the disease. Steere and colleagues described the stages of the systemic disease¹⁵ and reported on a series of patients, most of whom had conduction disorders of the heart.⁹ They also observed transient, mild left ventricular dysfunction in several patients and subclinical cardiomegaly in one patient.⁹ Marcus and colleagues observed spirochetal organisms in heart tissue from patients with myocarditis and pancarditis at autopsy.¹⁶ Our patient did not have the typical onset of Lyme borreliosis, with erythema migrans. The patient was frequently exposed to vectors of Lyme borreliosis because of his outdoor activities. The initial infection may be asymptomatic or nonspecific, with influenza-like symptoms; thus, erythema migrans is not a constant finding in Lyme borreliosis.

Our patient's major symptoms were high-grade

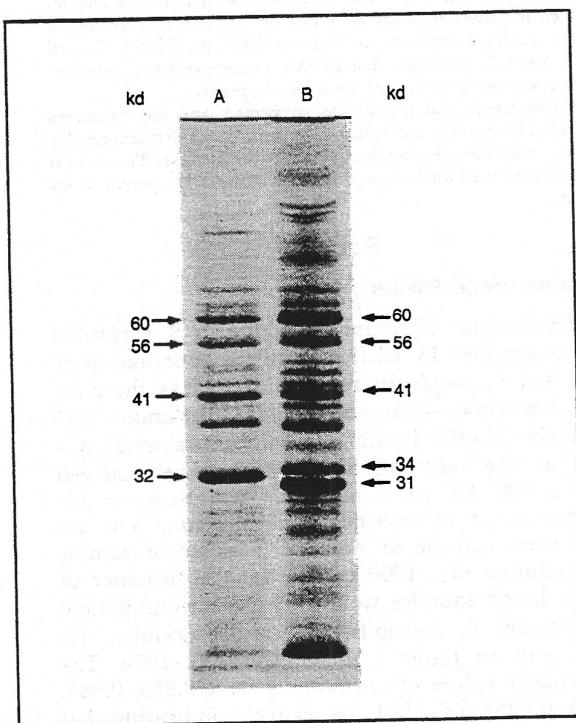


Figure 1. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of the *Borrelia* Strain Isolated from the Endomyocardial Biopsy Specimen (Lane A) and *B. burgdorferi* Strain B31 (Lane B).
The arrows indicate the abundant major proteins of both strains.

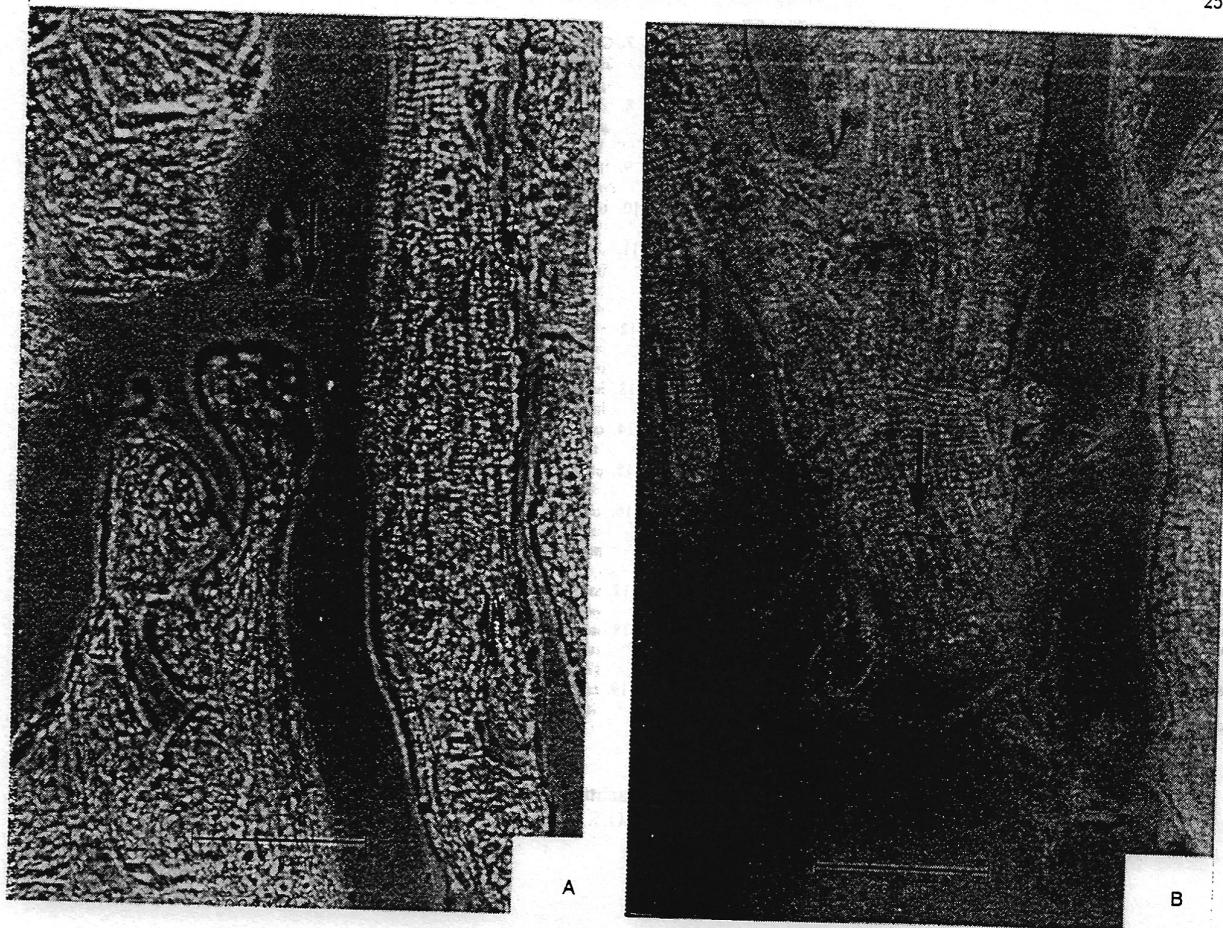


Figure 2. Human Myocardium Infected with *B. burgdorferi* (Modified Steiner's Silver Stain). A spirochetal organism is shown in the endomyocardial space (arrow, Panel A) and apparently within the myocardial cell (arrow, Panel B).

ventricular arrhythmias (Lown Grade IVB) followed by chronic heart failure, indicating dilated cardiomyopathy. Routine serologic screening for myocarditis revealed clearly elevated levels of antiborrelia antibodies. The high absorbance values found on ELISA and the results of immunoblotting point to a long-standing infection with borrelia.

We conclude from the history of this patient that he had become infected about five years earlier. The influenza-like infection occurred in the early spring and was treated with an antibiotic that is known to be ineffective against Lyme borreliosis. This period, however, does not necessarily represent the initial manifestation of Lyme borreliosis.

After the endomyocardial biopsy, the patient was treated with ceftriaxone (1 g per day intravenously). There was no improvement but also no further clinical deterioration. We did not determine the in vitro susceptibility of the myocardial isolate against ceftriaxone, but it is well documented in the literature and our own results (unpublished data) show that all isolates of *B. burgdorferi* tested thus far are highly sensitive to ceftriaxone. Therefore, it seems very un-

likely that this borrelia isolate was resistant. The uncertain effect of antibiotic treatment of cardiac involvement in Lyme borreliosis has also been observed by Olson and colleagues.¹⁷ It is well known, however, that the chronic arthritis, chronic encephalomyelitis, and acrodermatitis chronica atrophicans that occur in Lyme borreliosis may not respond well or immediately to antibiotics.³ The slight reduction of the absorbance values of the serum specimen analyzed by ELISA six months after treatment may represent a therapeutic effect.

Our patient was assumed to have cardiomyopathy associated with *B. burgdorferi* infection on the basis of ELISA and immunoblotting results. Although these results strongly suggest Lyme borreliosis, they cannot prove it. Several studies have demonstrated that antibodies to *B. burgdorferi* are found frequently in persons who are constantly exposed to tick bites (e.g., forestry workers) and have no clinical signs of Lyme borreliosis.¹⁸ Spirochetal organisms have not been isolated from such persons. Thus, the isolation of a spirochetal organism from an affected organ seems to prove its etiologic role. Nevertheless, one may also consider

the possibility, remote though it may be, that the cardiomyopathy and the *B. burgdorferi* infection were coincidental. To our knowledge *B. burgdorferi* has not previously been isolated directly from the myocardium of a patient. Furthermore, the organisms were visualized by a modified silver stain,¹³ and histologic staining revealed a subtle but clear lymphocyte infiltration as well as perivascular infiltrates, as described by others.¹⁹

We are indebted to Ms. Cornelia Köchl and Ms. Gabriele Schaden for technical assistance and to Dr. Christoph Holzinger for performing the endomyocardial biopsies.

REFERENCES

- Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease: a tick-borne spirochosis? *Science* 1982; 216:1317-9.
- Steere AC, Grodzicki RL, Kornblatt AN, et al. The spirochetal etiology of Lyme disease. *N Engl J Med* 1983; 308:733-40.
- Steere AC. Lyme disease. *N Engl J Med* 1989; 321:586-96.
- Ciesielski CA, Markowitz LE, Horsley R, Hightower AW, Russell H, Broome CV. The geographic distribution of Lyme disease in the United States. *Ann N Y Acad Sci* 1988; 539:283-8.
- Stanek G, Pletschette M, Flamm H, et al. European Lyme borreliosis. *Ann N Y Acad Sci* 1988; 539:274-82.
- Dekonenko EP, Steere AC, Berardi VP, Kravchuk LN. Lyme borreliosis in the Soviet Union: a cooperative US-USSR report. *J Infect Dis* 1988; 158:748-53.
- Ai CX, Wen YX, Zhang YG, et al. Clinical manifestations and epidemiological characteristics of Lyme disease in Hailin county, Heilongjiang Province, China. *Ann N Y Acad Sci* 1988; 539:302-13.
- Kawabata M, Baba S, Iguchi K, Yamaguti N, Russel H. Lyme disease in Japan and its possible incriminated tick vector, *Ixodes persulcatus*. *J Infect Dis* 1987; 156:854.
- Steere AC, Batsford WP, Weinberg M, et al. Lyme carditis: cardiac abnormalities of Lyme disease. *Ann Intern Med* 1980; 93:8-16.
- Barbour AG. Isolation and cultivation of Lyme disease spirochetes. *Yale J Biol Med* 1984; 57:521-5.
- Stanek G, Jurkowitsch B, Köchl C, Burger I, Khanakha G. Reactivity of European and American isolates of *Borrelia burgdorferi* with different monoclonal antibodies by means of a microimmunoblot technique. *Zentralbl Bakteriol Mikrobiol Hyg* (in press).
- Wilske B, Preac-Mursic V, Schierz G, Busch KV. Immunological and immunological analysis of European *Borrelia burgdorferi* strains. *Zentralbl Bakteriol Hyg* [A] 1986; 263:92-102.
- Steiner G. Modified silver stain of microorganisms in tissues. *Am J Clin Pathol* 1950; 20:489-90.
- Macdonald AB. Human fetal borreliosis, toxemia of pregnancy and fetal death. *Zentralbl Bakteriol Hyg* [A] 1986; 263:189-200.
- Steere AC, Bartenhagen NH, Craft JE, et al. Clinical manifestations of Lyme disease. *Zentralbl Bakteriol Hyg* [A] 1986; 263:201-5.
- Marcus LC, Steere AC, Duray PH, Anderson AE, Mahoney EB. Fatal pancarditis in a patient with coexistent Lyme disease and babesiosis: demonstration of spirochetes in the myocardium. *Ann Intern Med* 1985; 103:374-6.
- Olson LJ, Okafor EC, Clements IP. Cardiac involvement in Lyme disease: manifestations and management. *Mayo Clin Proc* 1986; 61:745-9.
- Münchhoff P, Wilske B, Preac-Mursic V, Schierz G. Antibodies against *Borrelia burgdorferi* in Bavarian forest workers. *Zentralbl Bakteriol Hyg* [A] 1987; 263:412-9.
- Reznick JW, Braunstein DB, Walsh RL, et al. Lyme carditis, electrophysiologic and histopathologic study. *Am J Med* 1986; 81:923-7.

©Copyright, 1990, by the Massachusetts Medical Society
Printed in the U.S.A.

THE NEW ENGLAND JOURNAL OF MEDICINE is published weekly in the English language by the Massachusetts Medical Society (Waltham, MA, USA). Material printed in the *Journal* is covered by copyright. No part of this reprint may be reproduced or transmitted in any form without written permission. All rights reserved. Direct permission requests to the Permissions Department at the USA subscription office. Editorial office: 10 Shattuck Street, Boston, MA 02115, USA. **SUBSCRIPTIONS:** Subscription offices: 1440 Main Street, Waltham, MA 02154-1649, USA; P.O. Box 1123, Commerce Court Postal Station, Toronto, Ontario M5L 1K1, Canada; and Saxon Way, Melbourn, Royston, Herts SG8 6NJ, UK. Subscription prices: USA: \$79 per year (interns, residents \$47 per year; students \$41 per year). Outside USA: Canadian dollars drawn on a Canadian bank: C\$122 per year (interns, residents C\$88 per year; students C\$73 per year). Pounds sterling drawn on a UK bank: £75 per year (interns, residents, and students £51 per year). US dollar payments accepted at current rate of exchange pro rata to the pound. Payments and correspondence for all subscriptions delivered to Japan should be sent directly to Nankodo Co., Ltd., 42-6, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan. Rates are subject to change without notice. Sample copies available on request.

SG8 6NJ, UK. Subscription prices: USA: \$79 per year (interns, residents \$47 per year; students \$41 per year). Outside USA: Canadian dollars drawn on a Canadian bank: C\$122 per year (interns, residents C\$88 per year; students C\$73 per year). Pounds sterling drawn on a UK bank: £75 per year (interns, residents, and students £51 per year). US dollar payments accepted at current rate of exchange pro rata to the pound. Payments and correspondence for all subscriptions delivered to Japan should be sent directly to Nankodo Co., Ltd., 42-6, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan. Rates are subject to change without notice. Sample copies available on request.