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Cultivation of *Borrelia burgdorferi* from Joint Fluid Three Months After Treatment of Facial Palsy Due to Lyme Borreliosis

COLLEAGUES—Lyme arthritis is a common manifestation of Lyme disease in the United States and in Europe [1, 2]. Thus far, *Borrelia burgdorferi* has been demonstrated in synovial biopsy specimens [3] and in joint fluid [4], but attempts to isolate and subculture the organism have failed. We report the case of a girl with Lyme disease, from whom we were able to isolate and propagate *B. burgdorferi* from joint fluid and to then perform serological tests on this isolate.

Case report. In October 1986, a 15.5-y-old girl was bitten by a tick in Austria. There was no local skin manifestation, such as erythema chronicum migrans, and she also never perceived any generalized symptoms such as malaise, fatigue, headache, musculoskeletal pain, or fever. Her history was free of any relevant disease.

At the end of January 1987, she was diagnosed with peripheral paralysis of the left facial nerve. Physical examination was otherwise normal, and she was afebrile; the patients' body weight was 58 kg. A complete blood count; her erythrocyte sedimentation rate; and radiographs of paranasal sinuses, mastoid processes, and petrous pyramids revealed normal findings. Repeated serological studies (table 1) confirmed the assumed *B. burgdorferi* etiology of facial palsy and excluded a viral etiology (i.e., herpes simplex virus, cytomegalovirus, Epstein-Barr virus, mumps virus, or coxsackievirus). Oral treatment with amoxicillin-clavulanate (625 mg, four times per day) was discontinued after 12 d because the patient developed a maculopapular rash. A two-week course of an oral corticosteroid (1 mg of betamethasone, two times per day) was prescribed. A lumbar puncture, performed because of only partial resolution of facial palsy, showed no pathological findings. Staining and culture attempts for *B. burgdorferi* in CSF were negative; the specific antibody titers are listed in table 1. During treatment with oral doxycycline (100 mg, two times per day) for two weeks, residual paralysis of the left facial nerve completely resolved, and the patient remained free of neurological symptoms.

Two months later, the patient developed isolated arthritis of the right knee without any preceding trauma. A laboratory examination showed the following: a peripheral leukocyte count of 7000 cells/mm³, with a normal differential; erythrocyte sedimentation rate, 27 mm/h; normal levels of creatinine and liver enzymes in serum; negative rheumatoid factors and antinuclear antibodies; and normal values for circulating immune complexes and complement analysis (CH50 and APH50). Using arthrocentesis, we collected 60 mL of cloudy joint fluid; microscopic analysis showed two to 11 granulocytes per visual field, a test for rheumatoid factor was negative, and a culture for *B. burgdorferi* was positive. The antibody titers against *B. burgdorferi* in serum and joint fluid are included in table 1. Ceftriaxone was administered intravenously in high doses (4 g per day), in the clinic, over a three-day period; thereafter, iv treatment was continued in normal doses (2 g per day), at the patient's home, for 11 d. Response to therapy was prompt; the patient's gonarthritis completely resolved by the end of the two-week course of ceftriaxone. The patient has remained healthy during the 11-mo follow-up period; her serum antibody responses to *B. burgdorferi* are given in table 1.

Serology (immunoperoxidase slide test). Binding of specific IgG and IgM antibodies to smears of *B. burgdorferi* (strain B31 or the patient's isolate [subculture number 2, B2]) on methanol-fixed slides was visualized by subsequent incubation with peroxidase-conjugated antibody to human IgG or IgM, followed by incubation with a chromogenic substrate. In this test the final titer is defined as the maximal $\log_2$ dilution of the sera in which *Borrelia* are distinctly demonstrable by light microscopy. As reported previously [5], this method gives identical results to the immunofluorescence test.

Culture. An aliquot of 0.5 mL of joint fluid from the affected knee was inoculated into a glass tube containing 5 mL of modified Kelly's medium (BSK II) [6]. The tube was closed tightly and incubated at 32 C. Every seven days, the culture was checked for growth of spirochetes by using dark-field microscopy. After four weeks, numerous motile spirochetes were detected and could successfully be subcultured by the technique mentioned above. The spirochetes reacted with monoclonal antibodies H 5332 (supposed to be specific for *B. burgdorferi* [7]) and H 9724 (specific for flagellae of *Borrelia* spp. [8]), as well as with all serum samples containing IgG antibodies against *B. burgdorferi* strain B31 that have thus far been tested in our laboratory (>20 samples).

Discussion. Optimal antimicrobial therapy for Lyme borreliosis has not yet been defined. In vitro susceptibility tests have shown that ceftriaxone and erythromycin are more active against *B. burgdorferi* than are tetracyclines or penicillins [9]. Oral therapy for erythema chronicum migrans, the early manifestation of Lyme borreliosis, is usually successful with either penicillin, tetracycline, or erythromycin, although isolated instances of failure to prevent further disease manifestation have been reported [1, 10]. Parenteral high-dose treatment has been recommended for neurological and rheumatologic complications of Lyme borreliosis [1, 11].

This report gives further evidence that oral two-week courses with amoxicillin-clavulanate or doxycycline, administered in doses recommended for treatment of primary Lyme borreliosis [1], may be inadequate for facial palsy due to Lyme disease. Despite clinical resolution of paralysis, subsequent arthritic complication occurred. To our knowledge, this is the first report of the successful isolation of *B. burgdorferi* from synovial fluid and the subsequent propagation through serial passage. This positive culture strongly suggests that the spirochetes were not eradicated by the initial antimicrobial regimens. Lack of meningeal inflammation could have prevented adequate drug penetration to the site of infection. The potential effect of the concomitant corticosteroid therapy on the inflammatory process remains speculative. Other possible explanations of treatment failure, such as insufficient patient compliance or reinfection by *B. burgdorferi*, were excluded by close medical and parental supervision. The observed apparent cure of borrelia arthritis by using iv ceftriaxone therapy confirms earlier observations [11]. Moreover, the present report indicates that pathogenesis of Lyme arthritis includes not only immunologic reactions but also direct synovial invasion by *B. burgdorferi*, as previously demonstrated by using the silver-impregnation method in synovial biopsy specimens [3].

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Please address requests for reprints to Dr. Urs B. Schaad, Division of Pediatric Infectious Diseases, Department of Pediatrics, University of Berne, Inselspital, CH-3010 Berne, Switzerland.

Table 1. Symptomatology, treatment, and diagnostic findings of the *B. burgdorferi* infection.

Date	Symptoms	Treatment (no. of days, route)*	Specimen	IgG antibody titers†		Culture‡
				B31	B2	
1/29/87	Facial palsy	Amoxicillin-clavulanate (12, po)	Serum	1:64	1:128	ND
2/18/87	Facial palsy	Betamethasone (14, po)	Serum	1:256	1:128	ND
2/25/87	Facial palsy	Doxycycline (14, po)	CSF	<1:2	<1:2	Negative
5/5/87	Gonarthritis	Ceftriaxone (14, iv)	Serum	1:64	1:64	ND
			Joint fluid	1:64	1:64	Positive
6/11/87	None	None	Serum	1:64	1:256	ND
8/11/87	None	None	Serum	1:128	1:128	ND

* Po = perorally.

† IgM antibodies were never detected in significant concentration. *B. burgdorferi* B31 = routine laboratory strain, B2 = patient's isolate.

‡ ND = not done.

Comparison of the patient's serum IgG antibody titers against *B. burgdorferi* B31, the routinely used laboratory strain in Europe, and *B. burgdorferi* B2, the isolated pathogen, revealed that the antibody response to B2 occurred earlier in the course of infection and, therefore, might allow prompter serological diagnosis. It is likely that the serologically relevant epitopes of B2 and B31 are not identical. Further experiments concerning analysis of the antigenic components in different strains of *Borrelia* isolated from patients or ticks from the same geographic region, as well as antigen selection for optimal serological diagnosis of Lyme borreliosis, are needed [7, 8, 12].

Juerg Schmidli, Thomas Hunziker,
Pierre Moesli, Urs B. Schaad
Departments of Dermatology and of Pediatrics,
University of Berne, Berne, Switzerland

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