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First Isolation of *Borrelia burgdorferi* from an Iris Biopsy

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The persistence of *Borrelia burgdorferi* in six patients is described. *Borrelia burgdorferi* has been cultivated from iris biopsy, skin biopsy, and cerebrospinal fluid also after antibiotic therapy for Lyme borreliosis. *Lyme Serology:* IgG antibodies to *B. burgdorferi* were positive, IgM negative in four patients; in two patients both IgM and IgG were negative. Antibiotic therapy may abrogate the antibody response to the infection as shown by our results. Patients may have subclinical or clinical disease without diagnostic antibody titers. Persistence of *B. burgdorferi* cannot be excluded when the serum is negative for antibodies against it.

Key Words: Lyme borreliosis—*Erythema migrans*—Antibiotic therapy

Lyme borreliosis, the most widespread disease transmitted by ticks, and caused by the spirochetal organism *Borrelia burgdorferi* (1) is characterized by various clinical stages, including dermatologic, neurologic, cardiac, rheumatologic, and ocular manifestations (2). Overlapping symptomatology of these stages is possible. Disease usually begins with the characteristic localized skin lesion *Erythema migrans* at the site of the tick bite, whereas the later phases—weeks, months to years after the primary infection—is marked by a disseminated infection.

With respect to ocular manifestations, conjunctivitis, keratitis, iritis, uveitis, vitritis, endophthalmitis, ischemic optic neuropathy, optic neuritis, oculomotor palsy, and retinal vasculitis have been reported (3–10). The diagnosis in all reported cases was made by clinical signs and serological tests for antibody to *B. burgdorferi*. This case report presents a patient in whom *B. burgdorferi* was first isolated from an iris biopsy. Additionally we report about the isolation of *B. burgdorferi* after corticosteroid and antibiotic therapy in patients with latent disseminated Lyme disease and interesting ophthalmological findings.

PATIENTS AND METHODS

Patients

See Table 1.

Serological Tests

Antibodies to *B. burgdorferi* in blood and cerebrospinal fluid (CSF) were determined by indirect immunofluorescence test (IFT) as described previously (11). To avoid unspecific false positive reactions, the test samples were absorbed with *Treponema phagedenis*. Antibody titers ⩾1:64 were regarded as significantly elevated, titers of 1:32 as borderline. Intrathecal production of antibodies against *B. burgdorferi* was assessed by comparing the CSF/serum ratio of enzyme-linked immunosorbent assay (ELISA) IgG values (units per milliliter) with the CSF/serum ratio of total IgG (CSF/serum index). A CSF/serum index of <2 was considered normal and >2 was considered elevated.

Bacteriological Examination

The iris biopsy and the samples of CSF and skin biopsy were examined for *B. burgdorferi* by darkfield microscopy and by culture in MKP medium as previously described (12,13). The cultures were incubated at 33°C for ⩾5 weeks and examined weekly by darkfield microscopy and subcultures.

Isolates were identified with monoclonal antibodies L321F11 and L221F8 by Western Blot (14). The susceptibility of the strains to different antibiotics were tested by MIC (minimal inhibitory concentration) using in vitro test in tube (13). Tests for monoculture of the isolates were done on solid media PMR agar (15).

Other Laboratory Examinations

The CSF was examined for white blood cells and total protein. Isoelectric focusing was used to determine oligoclonal IgG bands in the serum and CSF. Concentration of albumin and IgG in serum and CSF were determined by kinetic nephelometry. Serological examinations for syphilis and rheumatoid factor were performed.

RESULTS

The clinical and microbiological data are presented in Table 1.

TABLE 1. *Clinical and microbiological findings*

Patient no/age	Disease		Treatment	Antibodies to *B. burgdorferi*				Isolation of *B. burgdorferi* from
				Serum		CSF		
	Systemic	Ocular		IgM	IgG	IgM	IgG	
1/24	None	Panuveitis-Iridocyclitis	Corticosteroid Doxycycline	NE	593 U*	ND		Iris biopsy
2/55	*E. migrans*	Iritis-uveitis	Doxycycline Corticosteroid Ceftriaxone	NE	NE	ND		Skin biopsy
3/17	None	None	Cefotaxime	NE	1:64°	NE		CSF
4/60	None	Painful eyes	Ceftriaxone	NE	1:64°	NE		CSF
5/62	Arthralgias lymphadenopathy	Conjunctivitis	Penicillin	NE	NE	NE		CSF
6/25	Radicular pain	Iritis	Ceftriaxone Ceftriaxone	NE	1:64°	NE		CSF

ND, not done; NE, negative; CSF, cerebrospinal fluid.
* ELISA (positive > 200 U).
° IFT-ABS (positive is ⩾ 1:64, borderline 1:32, negative ⩽1:16)

Spirochetes isolation was successful between 2 and 16 subcultures in MKP medium. The isolates showed typical protein pattern of *B. burgdorferi* in SDS-page and were identified with monoclonal antibodies L321F11 and L221F8 as *B. burgdorferi*. The in vitro susceptibility of the isolates to antibiotics was the same as in other strains tested (13,16,17). The growth of *B. burgdorferi* on PMR agar is shown in Fig. 1.

Borreliae were isolated after antibiotic and corticosteroid therapy, from iris biopsy of one patient with chronic recurrence uveitis and acute panuveitis, as well as from skin biopsy of one patient with *E. migrans* and ocular manifestations, and also from the CSF of patients with "latent neuroborreliosis." However, the cell count in the CSF was normal and in all six reported patients the specific IgM antibody titers in serum were negative.

Serological examinations for rheumatoid factor and syphilis were negative.

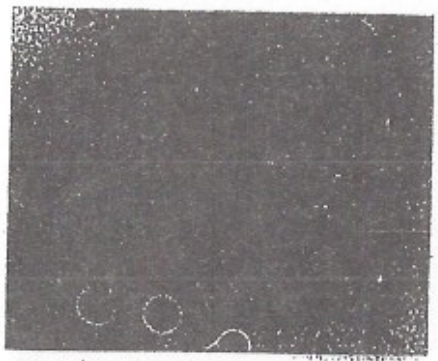

FIG. 1. *Borrelia burgdorferi* growth on PMR-agar.

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Case 1

A 24-year-old woman developed blurred vision in the right eye in 1991. She had a history of several years of chronic recurrent anterior and posterior uveitis. Since 1985 she had suffered from recurrent bilateral iridocyclitis and had been on immunosuppressive therapy. Lyme antibody titers had not been determined. Three years later in 1988, the IgG antibody titers against *B. burgdorferi* in serum were positive, while the IgM titer was negative. The patient was treated with systemic doxycycline at a dose of 200 mg/day for 4 weeks; the IgG antibody titer decreased. In 1989 the patient received doxycycline, again at a dose of 200 mg daily for 4 weeks, after the Lyme IFT-IgG had been repeatedly positive. In August 1991 the patient was admitted to hospital because of acute panuveitis with iridocyclitis, anterior chamber and vitreous cells, subtotal posterior synechiae, and a lens covered by a dense membrane. Fundoscopy revealed macular pucker, a cystoid macular edema, and an exudative inferior retinal detachment. A sector iridectomy and prepupillary membranectomy were performed to improve fundus visualization to rule out a rhegmatogenous retinal detachment. Laboratory investigations included aqueous humor for antibody titer determination against *B. burgdorferi* as well as excised iris tissue for culture isolation of borreliae. The Lyme ELISA (IgG) in serum was 593 U (norm <200 U) and in aqueous humor, 42 U.

B. burgdorferi was cultured from the iris excision and prepupillary membrane after prolonged incubation in 16 subcultures of MKP medium (13) (Fig. 2).

Borreliae could be visualized in Levaditis stained biopsy (Fig. 3). Gram stains of biopsy specimens showed no organisms and bacterial cultures (aerobic-anaerobic) showed no growth of other bacteria. *E. migrans* was not noted, although a tick bite was recalled.

Case 2

A 55-year-old woman developed *E. migrans*, 4 weeks after a tick bite. Three days later a skin biopsy and Lyme IFT and ELISA was done, and at the same time oral doxycycline 200 mg/day for 10 days was initiated. The Lyme IgM and IgG were negative; however, *B. burgdorferi* was isolated from the skin biopsy. *E. migrans* disappeared after 3 weeks.

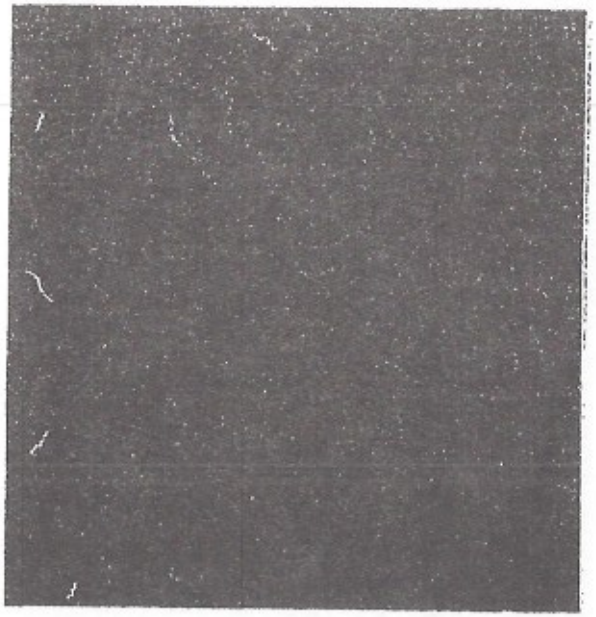

FIG. 2. *Borrelia burgdorferi* from iris biopsy (darkfield ×600).

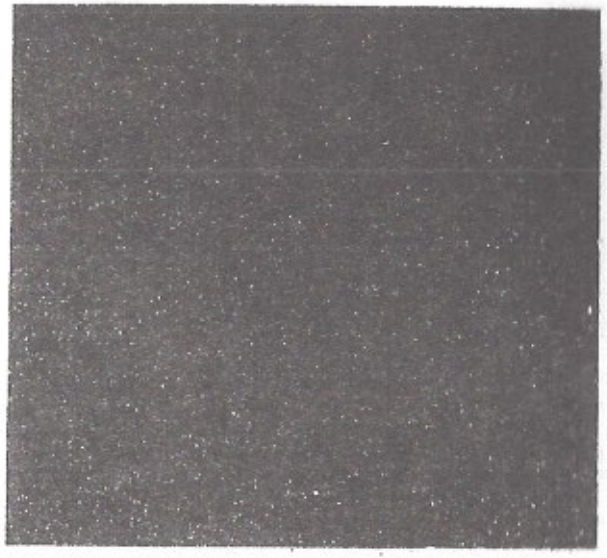

FIG. 3. *Borrelia burgdorferi* (Levaditi stained iris biopsy).

At the follow-up examination, 4 weeks after the antibiotic therapy, a subsequent biopsy (taken in the immediate vicinity of the prior biopsy) for culture of *B. burgdorferi* was negative, as was the Lyme serology.

The patient responded well for 1 year but then developed iritis and uveitis of the left eye. She also noted episodes of vertigo and tinnitus. Cultures and stains for bacteria and fungi were negative, the Lyme IgM and IgG were normal. For the following 6 weeks the inflammation was treated with high doses of topical and systemic corticosteroids with moderate effect. After 1 month of therapy she developed iritis and uveitis of the right eye. Intravenous ceftriaxone 2 g/day were administered for 3 weeks due to her Lyme history and symptomatology in both left and right eyes. The patient has remained well since, without further recurrence.

Case 3

A 17-year-old man had noted several tick bites during the months of August to December after having jogged in the woods. In December (within 2 weeks) he developed a bilateral tinnitus. A complete EENT examination as well as neurologic examination was normal. Upon physical examination, the patient was afebrile and meningeal signs were not present. Bilateral tinnitus was the only clinical symptom. Lumbar puncture revealed normal cell counts (3/3), and total protein (21 mg/dl). Oligoclonal IgG bands were not detected and no intrathecal specific antibodies against *B. burgdorferi* could be demonstrated.

Serum Lyme IFT IgG was positive (1:64). IgM was negative (<1:32). *B. burgdorferi* was isolated from CSF after 2 weeks incubation in MKP medium. The patient was treated with cefotaxime 3 × 2 g per day i.v. over 5 days followed with cephalexin for 8 days. Control cultures for *B. burgdorferi* 3 months later were negative. The same Lyme serological test results were obtained as those prior to therapy.

Case 4

A 60-year-old otherwise healthy man developed recurrent episodes of red, painful eyes in 1990 and was treated with topical corticosteroids. In early 1991 he developed short-lived vertigo with headaches.

A tick bite or *Erythema migrans* had never been seen. The neurological examination was completely normal.

Serum Lyme IgG was 1:64 and IgM was negative. Because of positive serum Lyme IFT IgG, CSF

was investigated (see Table 2). Lumbar puncture revealed normal values for cell counts and total protein. Antibody to *B. burgdorferi* in CSF were not detected, however, when CSF was cultivated in MKP medium, *B. burgdorferi* could be isolated.

There was no serological evidence for a bacterial or viral infection, except Lyme borreliosis. The ceftriaxone was administered at 2 g/day for 14 days. At the follow-up examination 4 months after the antibiotic therapy, cultures for *B. burgdorferi* were negative and the complaints disappeared.

Case 5

A 62-year-old woman had a tick bite in February 1990. Within 10 days she developed fever and cervical lymphadenopathy followed by conjunctivitis, arthralgias, numbness on toes and fingers, and extreme fatigue. The patient received oral penicillin for 12 days in February and again in May. In June 1991 the patient was referred to a neurological clinic due to persistent arthralgias and numbness. The neurological examination showed hypaesthesia and hypalgesia on toes and fingers of both sides as well as pallhypesthesia on malleoli left 4/8 and right 5/8. The rest of the neurological findings were normal. Lumbar puncture released 64 mg/dl protein and 6/3 cells. Antibody titers to *B. burgdorferi* in serum and CSF were negative, but *B. burgdorferi* was isolated from CSF after 4 weeks incubation in MKP medium. Now ceftriaxone was given 2 g/day i.v. for 14 days. Antibiotic treatment resulted in marked reduction of arthralgias and numbness. Cultures from CSF were negative.

Case 6

A 25-year-old man was admitted to hospital because of intensive radicular pain and blurred vision. He also had minor headaches, but he denied having fever or chills. The neurologic finding was a discrete hypaesthesia on the left forearm and bilateral iritis was present. One year earlier he had a 2-week episode of blurred vision, which cleared with oral prednisone therapy.

The patient had no history of tick bite and none of *E. migrans*. The IFT IgG antibody titers against *B. burgdorferi* in serum were positive (1:64); antibody titers in CSF were negative. Lumbar puncture revealed normal values for cell counts and total protein; nevertheless, *B. burgdorferi* was isolated from CSF. The isolation was successful on the second subculture in MKP medium. Ceftriaxone was administered 2 g daily intravenously for 14 days.

Five months after the therapy he remained well and a culture for *B. burgdorferi* from CSF was negative.

DISCUSSION

The diagnosis of Lyme disease is based on clinical symptoms, epidemiology, specific IgG and IgM antibody to *B. burgdorferi* in serum and CSF and isolation of borreliae. The diagnosis may be difficult in the late phase of the disease, particularly for ophthalmogists and rheumatologists. Characteristic of Lyme borreliosis is that its clinical picture is rarely complete and symptoms are overlapping, which makes diagnosis more difficult. A borrelial infection is usually confirmed by determining *B. burgdorferi* antibodies. However, interpretation of serological tests and results may not be straightforward. False-positive and false-negative results occur. Negative serologic results do not necessarily exclude *Borrelia* infection (18–20). As shown here and previously reported, antibiotic therapy may abrogate the antibody response to the infection, but *B. burgdorferi* may persist. In clinically unclear cases, much greater significance is therefore attached to the isolation of *B. burgdorferi*.

We were able to isolate *B. burgdorferi* from CSF and skin biopsies months to years after the antibiotic therapy and disappearance of *Erythema migrans*. The lack of repeated insect bite and *Erythema migrans*, negative AB-titers against *B. burgdorferi* and negative CSF examination suggest persistence of *B. burgdorferi* rather than reinfection.

How often *B. burgdorferi* may persist in the CSF, skin, or other tissues after therapy or its effect in producing atypical manifestations of disease is not known. The reason for the persistence of *B. burgdorferi* in patients after the treatment with antibiotics is not completely understood. A number of factors may play a role, e.g., virulence of *B. burgdorferi*, tissue penetration of antibiotics, insufficient antibiotic therapy (either duration or dose), intracellular localization of borreliae (21), possibility of *B. burgdorferi* survival in tissue and certain types of cells, and not at least the immunity of patients. The capacity of *B. burgdorferi* to hide in various human tissue (heart muscle, spleen, brain) (22–24) and an insufficient antibiotic tissue level are critical for the therapy.

Antibiotic treatment with amoxicillin or doxycycline has been recommended for *E. migrans*, penicillin G, and cephalosporins, ceftriaxone, and cefotaxime for central nervous system infection and late stages. It is known that the therapy of late stages of the Lyme borreliosis can be complicated. Not seldom, there are known recurrences of the disease and persistence of *B. burgdorferi*, also after adequate antibiotic therapy (18,25).

The persistence of *B. burgdorferi* and clinical recurrence in *E. migrans* stage are rarely noted (18,20), as the therapy seems to be mostly effective and sufficient. In certain circumstances the clinical and laboratory investigations (3–4 months after the completion of antibiotics therapy), are probably too short for the enddiagnosis. Furthermore, in most *E. migrans* patients a control of the therapy effect is never done. However, we must take into consideration that changes in clinical symptomatology (after months) can lead the patient to change doctors.

The current antibiotic therapy (antibiotic, dose, duration) is very different, so we have very different clinical and laboratory findings. However, the randomized trials comparing various antibiotics in their clinical response, minimal inhibitory concentration (MIC) and the serum and CSF concentration support the selection for stage-specific treatment. According to the data of recent clinical studies, the cephalosporins are more efficient than penicillin G in late (26,27) but not in early Lyme borreliosis (28). Dattwyler et al. (27), Diringer et al. (29) and Pal et al. (30) reported that ceftriaxone and cefotaxime were effective in treating patients with meningoencephalitis and late borreliosis who did not respond to penicilin G therapy.

The CSF concentrations of penicillin G, cefotaxime, and ceftriaxone in our studies demonstrate that both cephalosporins penetrate to a greater extent than penicillin. The CSF levels are evidently above the MIC 90 values for *B. burgdorferi*. The concentration of penicillin G did not reach the MIC 90 in any of our patients (31,32). Data from controlled clinical studies are still scanty, and the observation period after the therapy is often too short. Furthermore, proof of a successful therapy is based not only on the disappearance of clinical symptoms but also on the elimination of *B. burgdorferi*; this is difficult to achieve and seldom performed. However, the recurrence of the disease, longtime persistence of *B. burgdorferi* in untreated as well as in treated patients, unpredictable progression of the disease and the isolation of *B. burgdorferi* from CSF (without inflammatory signs) of patients with *E. migrans* (n.p.), it seems appropriate to treat patients in Stage I as effectively as possible. The isolation of *B. burgdorferi* from CSF in *E. migrans* without inflammatory signs support an early dissemination of the borreliae.

The results of randomized prospective therapy studies and case reports show that also with adequate antibiotic therapy cure is often impossible with one treatment course. An interval therapy with substantially larger doses of antibiotics—2 × 200 mg doxycycline, 2 × 800 mg amoxicillin for 7 days/2 times with an antibiotic-free interval of 7 days—is advisable. The patients with an active central nervous infection, carditis, or eye manifestations should be treated intravenously with ceftriaxone or cefotaxime (1 × 4 g or 2 × 3 g/day, 7 days/2 times with an antibiotic-free interval of 7 days).

This treatment regimen, which takes into consideration the long generation time of *B. burgdorferi* and the antibiotic mechanism of action can probably be more effective than the regimen used. The higher doses of antibiotics reach correspondingly effective higher serum, CSF, and tissue antibiotic concentrations and the repeated doses of antimicrobials killed the survivor borreliae. Likewise a combination of two antibiotics must be taken in consideration. The interval therapy and/or a combination therapy are often used, are obligatory in the treatment of complicated or chronical bacterial infection. However, currently recommended treatment regimens are inadequate for some patients; the therapy ought to be realized and controlled more individually.

In conclusion, our first isolation of *B. burgdorferi* from eye tissue confirms invasion of the ocular space by *B. burgdorferi*. Furthermore, this isolate demonstrates that in eye conditions we have to think of Lyme infection—on *B. burgdorferi* persistence and on an adequate therapy with antibiotics. Further cases, with varied clinical symptoms and courses, show that negative serological tests do not exclude *B. burgdorferi* infection.

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