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Recovery of Lyme Disease Spirochetes from Patients

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Since the summer of 1982, we have cultured patient specimens for Lyme disease spirochetes. Of 118 patients cultured, four specimens yielded spirochetes: two from blood, one from a skin biopsy specimen of erythema chronicum migrans (ECM), and one from cerebrospinal fluid. All four isolates appeared identical when examined with a monoclonal antibody. However, attempts to recover the spirochete from synovium or synovial fluid were unsuccessful. In addition, the organism could not be visualized in skin or synovial biopsy specimens using the avidin-biotin peroxidase complex detection system. Thus, the current yield in culturing spirochetes from patients is quite low, and it is not yet known whether the organism is still alive later in the disease when arthritis is present.

In 1981, spirochetes were first isolated from the tick vector of Lyme disease, *Ixodes dammini* [1], and the following year were recovered from patient specimens [2,3]. We report here the current status of culture efforts on patients and the results of attempts to visualize the organism in affected tissues.

METHODS

Blood, skin biopsy specimens of erythema chronicum migrans (ECM), cerebrospinal fluid, lymph-node aspirates, and urine cultures were obtained from adults with the recent onset of Lyme disease, and, when possible, joint fluid, synovium, or cartilage were obtained from patients with Lyme arthritis. For blood cultures, 30 ml of citrated blood were processed in three ways. A tenth of a milliliter of whole blood was transferred directly to one tube containing culture medium (see below). The remaining blood was sedimented (1,000 *g* for ten minutes), and 0.1 ml of plasma near the buffy coat was transferred to a second tube of medium. Finally, the plasma was centrifuged (12,000 *g* for 20 minutes), and the resulting plasma pellet was resuspended and transferred to a third tube of medium. Cerebrospinal fluid and joint fluid were cultured in a similar manner. A tenth of a milliliter of these specimens was cultured directly. Then, the remaining fluid was centrifuged and the resulting pellet cultured in a second tube of medium. Lymph-node aspirates from enlarged tender inguinal nodes, and clean-catch urine specimens (0.1 ml) were put directly into medium. Skin biopsy specimens were obtained at either the center or leading edge of

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ECM lesions. Synovium and cartilage were attained during synovectomies. These specimens were ground in 1 ml of medium with a mortar and pestle, and 0.1 ml of the emulsion was transferred to one tube of medium.

Specimens were cultured in eight-milliliter Pyrex screw-cap tubes that contained 7 ml of a modified Kelly's medium [4]. We used a combination of ingredients [2] from previous modifications by Stoenner et al. [5] and Barbour et al. [6]. The cultures were incubated at 33°C and examined by darkfield microscopy (40× objective) weekly for one month. Each culture (0.1 ml) was subjected to one blind passage at one week. Isolates were examined by immunofluorescence, with use of a monoclonal antibody made against the original isolate [7].

We examined skin and synovium from Lyme disease patients using the avidin-biotin peroxidase complex (ABC) detection system (Vector Laboratories, Burlingame, CA) [8]. After deparaffinizing, trypsinizing, and quenching with normal goat sera, the tissue was incubated with high-titered serum (1:100) from a patient with Lyme disease followed by 20 μl of biotinylated goat anti-human IgG, and then 20 μl of avidin-biotinylated peroxidase complex. The substrate was 5 mg of diaminobenzidine tetrahydrochloride (DAB) in 10 ml of 0.5M Tris/HCl buffer (pH 7.6) with 4 μl of 30 percent H_2O_2.

RESULTS

During the 1982 summer, of 56 patients cultured, three specimens yielded spirochetes (Table 1) [2]. The isolates were recovered from different patients and from different parts of the body: one from blood, one from a skin biopsy specimen of ECM, and one from cerebrospinal fluid. Of the 25 patients on whom blood cultures were done, 14 had evidence of disseminated infection, often with multiple annular skin lesions. These patients were seen from two to 30 days after the onset of symptoms (median, eight days). The remaining 11 patients had a single skin lesion and minor constitutional symptoms. The blood isolate was recovered from the resuspended plasma pellet of the patient with disseminated infection seen earliest after the onset of symptoms. The patient had had ECM, headache, fever, chills,

TABLE 1
Isolation of Lyme Disease Spirochetes from Patients

Material	No. of Cultures	No. Positive
1982 Summer		
Blood (resuspended plasma pellet)	25	1
Skin biopsy (ECM)	18	1
Cerebrospinal fluid	5	1
Lymph-node aspirate	10	0
Urine	6	0
1983 Summer		
Blood (resuspended plasma pellet)	40	1
Cerebrospinal fluid	2	0
1982 and 1983		
Joint fluid	17	0
Synovium	4	0
Cartilage	2	0

myalgias, arthralgias, malaise, and fatigue for two days prior to culture. The culture was subjected to blind passage after one week, and spirochetes were found only in the subculture after an additional two weeks. Simultaneous cultures of whole blood and plasma from the same patient were negative.

The isolate from the skin biopsy was obtained from the periphery of the lesion of a patient who had had symptoms for three and one-half weeks. At disease onset, in addition to ECM, the patient had had headache, photophobia, arthralgias, fever, and malaise. At the time of culture, the skin lesion was large, but the patient had only low back pain. This culture was also positive only in the subculture after three weeks.

The isolate from the cerebrospinal fluid was recovered in primary culture after two weeks of incubation; it was from a patient who had chronic meningitis. Two and a half months previously, ECM had developed in this patient and had been followed by intermittent headache, stiff neck, fever, right-sided facial palsy, arthritis of the elbows, hips, and knees, malaise, and fatigue. Two weeks before the culture, she had begun taking 60 mg of prednisone a day. At the time of culture, the spinal fluid contained 27 white cells per cubic millimeter, with 76 percent lymphocytes and 24 percent granulocytes; the protein concentration was 30 mg per deciliter, and the glucose 73 mg per deciliter.

During the 1983 summer, blood was cultured from 40 patients and cerebrospinal fluid from two patients (Table 1), but only one culture of blood yielded spirochetes. Of the 40 patients on whom blood cultures were done, 26 had evidence of disseminated infection. They were seen from three to 31 days after the onset of symptoms (median, nine days). The blood isolate was recovered from the resuspended plasma pellet of the patient with disseminated infection seen earliest after the onset of symptoms. This patient had had ECM, multiple secondary annular lesions, headache, neck stiffness, fever, left knee pain, malaise, and fatigue for three days prior to culture. The organism was recovered in primary culture from the plasma pellet, but not from simultaneous cultures of whole blood and plasma.

The four patient isolates appeared identical when examined with a monoclonal antibody made against the original isolate [7]. In each instance, the titer was 1:1,024; control slides of *Leptospira grippotyphosa, Borrelia hermsii,* or *Treponema pallidum* showed no fluorescence.

During the past two years, 23 specimens of joint fluid, synovium, and cartilage were cultured, but all were negative (Table 1). Similarly, using the avidin-biotin peroxidase complex detection system, no spirochetes were visualized in synovial samples from ten patients nor in skin biopsy specimens of ECM from eight patients.

DISCUSSION

The current yield in culturing spirochetes from patients is quite low. Three of our four patients were quite ill at the time of culture, which may have been a factor in recovery of the organisms. In addition, the timing of blood cultures may be an important variable since the organism has been recovered from blood only after the first several days of symptoms [2,3]. Nevertheless, the difficulty in recovery and the possible need to concentrate specimens and to passage cultures suggest that the number of organisms in affected tissues is small. Direct visualization of organisms in these tissues seems to be even more problematic. Drs. Berger, Clemmensen, and Ackerman saw spirochetes in skin biopsy specimens of four of 14 patients with ECM [9]. However, using an immunoperoxidase stain, we did not see them in either skin or synovial biopsy specimens. Although culture or visualization of spirochetes per-

mits definitive diagnosis, neither procedure is currently practical for routine diagnosis of this infection.

Because of the results of cultures from both ticks and patients [1,2], we postulate the following pathogenetic sequence. We believe that the tick injects the spirochete into the skin or bloodstream of the patient [1,2]. After an incubation period of three to 32 days, we think that the organism migrates outward in the skin (ECM). This hypothesis is supported by recovery of the spirochete from skin biopsy specimens of ECM [2] and by direct visualization of the organism in skin by immunoperoxidase and Warthin-Starry [9] stains. In some patients, the spirochete seems to remain localized in the skin or regional lymph nodes. In others, we suspect that it is disseminated in blood to organs, particularly the brain, liver, or spleen, or to other skin sites resulting in secondary annular lesions. We doubt that secondary lesions result from several tick bites because such lesions may be numerous and lack indurated centers.

What is the pathogenesis of the later manifestations of Lyme disease? Since the organism has been recovered from the CSF of one patient with neurologic abnormalities two and a half months after the onset of ECM [2], we believe that the meningitis results from direct invasion of the cerebrospinal fluid by the spirochete. Thereafter, the fate of the organism is unknown. Unlike other spirochetal infections, the most common late manifestation of Lyme disease is arthritis. We do not yet know whether the Lyme disease spirochete is still present at that time—and if so, where it is—or whether it simply initiates a self-propagating inflammatory host response. The issue of whether a persistent infectious agent is necessary for continued disease activity or whether such an agent triggers disease, which is then followed by autoimmunity, is of central importance in immune-mediated diseases.

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