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Discovery of the Lyme Disease Spirochete and Its Relation to Tick Vectors

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The various hypotheses concerning the etiologic agent of erythema chronicum migrans of Europe and of Lyme disease in the United States are reviewed, and an account of events that led to the discovery of the causative spirochetal agent in *Ixodes dammini* is presented. Spirochetes morphologically and antigenically similar, if not identical to, the organism detected in *I. dammini* were also found for the first time in *Ixodes pacificus* and *Ixodes ricinus,* the vectors hitherto incriminated, respectively, in western United States and Europe.

In most infected ticks, spirochetal development was found to be limited to the midgut. Ticks with generalized infections were shown to transmit spirochetes via eggs, but infections decreased in intensity and became restricted to the central ganglion as filial ticks developed to adults.

Although the mechanisms of transmission to a host are still under investigation, the spirochetes may be transmitted by saliva of ticks with generalized infections and possibly also by regurgitation of infected gut contents, or even by means of infected fecal material.

Ever since the first description of *Erythema chronicum migrans* (ECM) in Europe [1] and of Lyme disease in the United States [2], tick-transmitted toxins, viruses, rickettsiae, and spirochetes have been considered as possible causes of these ailments. Early hypotheses suggested that ECM represents an allergic reaction to a toxin associated with the tick or may be caused by a live agent transmitted during the tick's feeding process. The toxin hypothesis was abandoned when Hollström [3] demonstrated the effectiveness of penicillin in the treatment of ECM. His findings and those of Binder et al. [4], who succeeded in eliciting ECM in volunteers by transfer of lesion biopsies from a patient, strongly suggested an infectious agent as the cause of the disease.

Spirochetes became the prime candidates, because, as early as 1948, Lennhoff [5] claimed having seen "elements presenting the morphological aspects of spirochetes" in lesions of numerous dermatoses including erythema migrans. A year later, Hellerström [6] presented a paper, "Erythema chronicum migrans Afzelius with meningitis," at the 43rd Annual Meeting of the Southern Medical Association in Cincinnati. He not only referred to Hollström's treatment of patients with penicillin but also provided a lengthy discussion concerning the cause of ECM. From his paper published in the *Southern Medical Journal,* I quote ". . . it seems reasonable to raise the question of whether the ticks are carriers of spirochetes with allergizing (and im-

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munizing?) properties." Later, in his closing remarks, he stated, "Our investigations tend to demonstrate that a spirochete is the cause of the disease. Definite evidence is still lacking, but Dr. C. Lennhoff's findings of spirochetes in histologic sections prepared from lesions of erythema migrans are remarkable," and, finally, ". . . the therapeutic results with penicillin or other antispirochetal drugs indicate that the spirochete may be the causative organism." Unfortunately, Lennhoff's findings could not be confirmed and, to the best of my knowledge, no one followed up the suggestion incriminating the tick, *Ixodes ricinus,* as a vector of spirochetes.

Because of the efficiency of antibiotic treatment, viruses were ruled out as the cause of ECM. In 1962, French scientists presented serological evidence suggesting ECM to be a rickettsial disease [7,8]. Using the Girouds' slide agglutination test [9], they reported that six of seven ECM patients had antibodies in titers ≥ 1:160 against the epidemic typhus agent, *Rickettsia prowazekii,* the murine typhus rickettsia, *R. mooseri,* and/or the boutonneuse fever agent, *R. conorii.* Although these findings could not be confirmed by other investigators [10], the rickettsial etiology of ECM was kept alive by the electron microscopic demonstration of rickettsia-like microorganisms in macrophages of two ECM patients [11]. This induced Weber in Munich to have sera of 13 ECM patients evaluated against 14 rickettsial antigens by two different laboratories, namely the Institute of Virology in Bratislava, Czechoslovakia, where the complement fixation (CF) and the microagglutination (MA) tests were used, and the Rocky Mountain Laboratories in Hamilton, Montana, where, in addition to the CF test, the microimmunofluorescence (MIF) test was used. With the exception of a few nondiagnostic low titers against *R. akari,* the agent of rickettsialpox, and *Coxiella burnetii,* the cause of Q fever, all tests were negative [12].

In the United States, where the first case of ECM was described in 1970 [13], some investigators believed that the syndrome was caused by an infectious nonbacterial but antibiotic-sensitive agent [14]. The subsequent investigations by Steere and associates in eastern Connecticut not only led to the description of Lyme arthritis as a new form of ECM but also to intensive efforts to establish the cause of this inflammatory disorder [2]. Among the many tests performed were serologic evaluation of acute and convalescent sera of patients for antibodies against adenoviruses, coxsackieviruses, cytomegaloviruses, hepatitis B antigen, herpes simplex, influenza types A and B, lymphocytic choriomeningitis, mumps and rubella viruses, *Mycoplasma pneumoniae,* and also against four rickettsial agents (*R. akari, R. rickettsii, R. mooseri,* and *Coxiella burnetii*). Sera of selected patients were tested also for antibodies to 216 arboviruses, 38 of which are tick-borne. All tests were essentially negative.

Epidemiological incrimination of the ixodid tick, *Ixodes dammini*—first considered to be *I. scapularis* but later described as a new species [15]—as the vector of Lyme arthritis, led to a field study of ticks in south central Connecticut [16]. All attempts to recover from ticks and their hosts viruses as possible causes of Lyme arthritis were unsuccessful.

Since 1975, my colleague Jorge Benach, from the New York State Health Department, and I have been interested in the epidemiology and ecology of Rocky Mountain spotted fever on Long Island where, during 1971–1976, a total of 124 cases with eight deaths had occurred [17]. One of our objectives concerned the demonstration of the spotted fever agent, *R. rickettsii,* in adult *Dermacentor variabilis* collected off vegetation near the homes of patients. Although almost 6

percent of ticks were found hemolymph test-positive for spotted fever group rickettsiae, isolates from 100 ticks, without exception, proved to be *R. montana,* a rickettsia nonpathogenic for man. This caused us to speculate that possibly other species of ticks, such as *I. dammini* that occurs abundantly on Long Island, and especially on offshore islands, might play an as yet unestablished role in the ecology of *R. rickettsii.* Consequently, several hundred adult *I. dammini* were collected and examined by the hemolymph test; none was infected with rickettsiae.

In late September and early October 1981, Dr. Benach provided additional collections of *I. dammini* from Shelter Island, New York, where Lyme disease was known to be endemic. Again, none of 44 males and females had rickettsial infections. The hemolymph of two females, however, contained large microfilariae that differed morphologically from *Dipetalonema (Wehrdickmansia) rugosicauda,* a microfilaria I detected in several adult *I. ricinus* in Switzerland in 1978 [18]. To determine whether these nematodes were present also in the digestive system, I dissected both ticks and prepared Giemsa-stained smears from individual midgut diverticula for microscopic examination. No microfilaria was found. Instead, I encountered poorly stained, rather long, irregularly coiled spirochetes (Fig. 1). Darkfield microscopy of additional diverticula confirmed the spirochetal nature of the organisms, which had a rather sluggish and slow movement. Additional tissues, including salivary glands, malpighian tubules, ovary, and central ganglion of either tick, were free of spirochetes.

Subsequently, 124 remaining ticks were dissected and each of their organs was examined for similar organisms. Seventy-five (60 percent) contained spirochetes that were limited to the midgut (Fig. 2). Organisms occasionally seen in preparations of hindgut and rectal ampule may have originated from midgut tissues. All other tissues were free of spirochetes.

Remembering the European literature, I could not dismiss the thought that the microfilariae did lead me to the discovery of the long-sought cause of ECM and Lyme disease.

Needless to say, I shared these observations with several of my colleagues, including Dr. Benach, who not only saw to it that I was well supplied with field-collected ticks from Shelter Island, but also provided sera from patients with clinically diagnosed Lyme disease for preliminary serological identification of the organism, and also Dr. Barbour, who at the time was engaged in a study of the

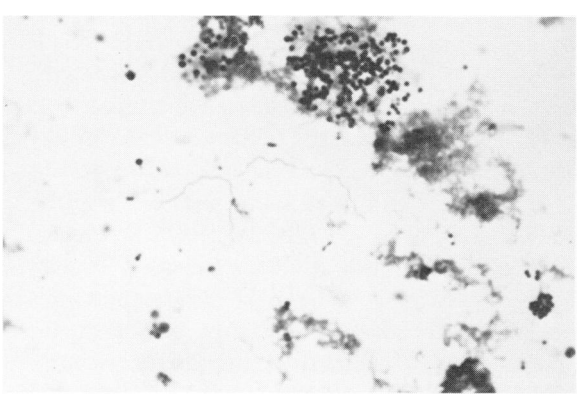

FIG. 1. Faintly stained Lyme disease spirochetes in midgut smear of *Ixodes dammini* from Shelter Island, New York (Giemsa stain, 1,750 ×).

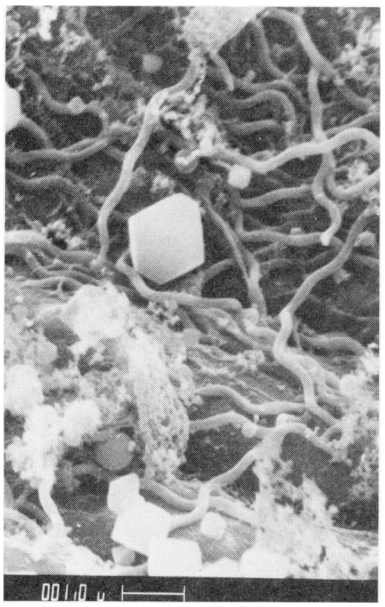

FIG. 2. Lyme disease spirochetes in midgut of *Ixodes dammini* from Shelter Island, New York. Hemoglobin crystals in the center and on bottom of picture (scanning electron micrograph).

variable major proteins of cultured tick-borne relapsing fever spirochetes and who offered his expertise to culture and immunochemically characterize the organism.

The antigenic relatedness of the *I. dammini* spirochete to the etiologic agent of Lyme disease was established by indirect immunofluorescence as well as by western blot analysis of sera from both Lyme disease and ECM patients [19,20]. Our initial indirect evidence that this organism might be the cause of this disease was subsequently confirmed by isolating from patients spirochetes indistinguishable from those detected in *I. dammini* and by the microscopic demonstration of spirochetes in skin biopsies of cutaneous lesions of several Lyme disease patients [21–24].

One of our subsequent research objectives was to determine whether *I. ricinus,* the incriminated vector of ECM in Europe, was also a carrier of spirochetes. Evidence that this was the case was obtained from smears prepared in 1978 from nymphal ticks at the University of Neuchâtel in Switzerland, where I spent several months conducting a tick/rickettsial survey in various parts of that country. Of 135 smears of ticks collected in the Seewald forest on the Swiss Plateau, where according to medical authorities ECM had occurred in the past, 23 (17 percent) contained spirochetes that tinctorially and morphologically appeared similar to those detected in *I. dammini.*

In the spring of 1982, the U.S. Department of Agriculture gave us permission to import about 600 adult *I. ricinus* from the Seewald forest [20]. Of 201 individually examined ticks, 73 (36.3 percent) were infected with spirochetes. The organisms were limited to the midgut in 69 ticks but were found in all the tissues of the other four ticks. Of an additional 180 females that were fed on rabbits, 39 (21.9 percent) were infected. Two of them had a generalized infection whereas the other 37 had spirochetes in their midgut only. Both females with generalized infections transmitted spirochetes via eggs to 100 percent larval ticks in one and 60 percent in the other. However, as the larvae developed to nymphs and adults, the degree of spirochetal infection gradually decreased to a level of few organisms in tissues of the central ganglion only. This spirochetal behavior is in sharp contrast to the massive and

prolonged development of tick-borne spirochetes and suggests that the growth conditions in the hemocoele of *I. ricinus* are inferior to those in the midgut. Morphologically, the *I. ricinus* spirochete appeared indistinguishable from the *I. dammini* organisms, and antigenic similarities between the two spirochetes were apparent by direct immunofluorescence and SDS-PAGE protein profiles as well as by indirect immunofluorescence and western blot analysis of sera from ECM and Lyme disease patients [20].

Convinced that ECM of Europe and Lyme disease in the United States are expressions of one and the same etiologic agent, we directed our attention toward the west coast of the United States where the first case of ECM was contracted in Sonoma County, California, in 1975 and where the black-legged deer tick, *I. pacificus,* had been incriminated as the vector. In collaboration with Dr. Robert Lane from the University of California at Berkeley and Dr. Robert Gresbrink from the Oregon State Health Department in Portland, we initiated a tick/spirochete survey in southwestern Oregon and in north central California—areas where *I. pacificus* is abundant. The test procedures were similar to those applied to *I. dammini* and *I. ricinus.* Adult ticks collected by flagging vegetation in the spring and early summer were dissected individually, and the midgut diverticula were smeared on a microscope slide. After air-drying and after ten-minute fixation in acetone, the smears were treated with FITC conjugates prepared from sera of New Zealand white rabbits that had been immunized with the Lyme disease (Shelter Island isolate) spirochete. Ticks with spirochetes in their midgut were further dissected to determine the presence of organisms in other tissues.

Of 645 *I. pacificus* from Oregon and of 550 ticks from California, 13 (2 percent) and 5 (0.9 percent), respectively, contained spirochetes that morphologically and by fluorescence microscopy appeared indistinguishable from the spirochetes found in *I. dammini* and *I. ricinus.* Five of the 13 infected ticks from Oregon and two of the five infected ticks from California had a generalized infection; the remaining positive ticks had spirochetes only in the midgut. As yet, we have not succeeded in establishing in modified Kelly's medium an isolate for immunochemical analysis.

The low percentage of spirochete-infected ticks in the West certainly is reflected by the small number of Lyme disease cases reported so far. It is quite possible, however, that there exist, within the distributional areas of *I. pacificus,* foci with higher infection rates providing a greater potential for human disease.

In concluding my presentation, I would like to emphasize that studies pertaining to the relationship of Lyme disease and ECM spirochetes to their respective tick vectors are still in the initial phases. As yet, little is known about the development of these organisms in ticks and about the mechanics by which they are transmitted to host animals and man. Nevertheless, the observations and findings discussed above permit the following conclusions:

1. Spirochetes antigenically similar, if not identical, to each other have been found associated with *Ixodes ricinus, I. dammini,* and *I. pacificus,* the currently known tick vectors of ECM in Europe and of Lyme disease in North America.
2. Spirochetal development in most ticks is limited to the midgut. In a few ticks, the organisms penetrate the gut epithelium and invade the hemocoele and various tick tissues. Their presence in the ovary may lead to transovarian transmission. Nevertheless, there is evidence that spirochetal infection in filial ticks decreases and becomes restricted to the central ganglion as the ticks develop to nymphs and adults.

3. It is speculated that transmission of spirochetes during the feeding process may occur via saliva by ticks with generalized infection as well as by regurgitation of infected gut content. Contamination by spirochete-infected fecal material cannot be excluded.
4. Finally, in view of the unusual behavior (limited distribution, loss of infection intensity) of the spirochetes in ticks, the possibility that other hematophagous arthropods, such as biting flies, gnats, and mosquitoes, may play a role as mechanical vectors of ECM and Lyme disease cannot be ruled out.

REFERENCES

1. Afzelius A: Erythema chronicum migrans. Acta Derm Venereol (Stockh) 2:120–125, 1921
2. Steere AC, Malawista SE, Snydman DR, et al: Lyme arthritis. An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 20:7–17, 1977
3. Hollström E: Penicillin treatment of erythema chronicum migrans Afzelius. Acta Derm Venereol (Stockh) 31:235–243, 1951
4. Binder E, Doepfmer R, Hornstein O: Experimentelle Übertragung des Erythema chronicum migrans von Mensch zu Mensch. Hautarzt 6:494–496, 1955
5. Lennhoff C: Spirochetes in aetiologically obscure diseases. Acta Derm Venereol (Stockh) 28:295–324, 1948
6. Hellerström S: Erythema chronicum migrans Afzelius with meningitis. South Med J 43:330–334, 1950
7. Degos R, Tourraine R, Arouete J: L'erythema chronicum migrans. Syph 89:247–260, 1962
8. Giroud P, Capponi M, Dumas N: Rickettsioses et lésions cutanées en dehors de syndromes fébriles. Bull Soc Path Exot 55:958–961, 1962
9. Giroud P, Giroud ML: Agglutination des rickettsies, test de séroprotection et réaction d'hypersensitivité. Bull Soc Path Exot 37:84–93, 1944
10. Weber K: Erkrankungen nach Zeckenbiss und ihre Behandlung. Therapie Gegenw 112:1402–1409, 1973
11. Sandbank M, Feuerman EF: Ultrastructural observation of rickettsia-like bodies in erythema chronicum migrans. J Cutaneous Pathol 6:253–264, 1979
12. Weber K: Serological study with rickettsial antigen in erythema chronicum migrans. Dermatol 163:460–467, 1981
13. Scrimenti RJ: Erythema chronicum migrans. Arch Dermatol 102:104–105, 1970
14. Mast WE, Burrows WM: Erythema chronicum migrans and "Lyme arthritis." JAMA 236:2392, 1976
15. Spielman A, Clifford CM, Piesman J, et al: Human babesiosis on Nantucket Island, USA: Description of the vector, *Ixodes (Ixodes) dammini,* N.Sp. (Acarina: *Ixodidae*). J Med Entomol 15:218–234, 1979
16. Wallis RC, Brown SE, Kloter KO, et al: Erythema chronicum migrans and Lyme arthritis: Field study of ticks. Am J Epidemiol 108:322–327, 1978
17. Benach JL, White DJ, Burgdorfer W, et al: Changing patterns in the incidence of Rocky Mountain spotted fever on Long Island (1971–1976). Am J Epidemiol 106:380–387, 1977
18. Aeschlimann A, Burgdorfer W, Matile H, et al: Aspects nouveaux du rôle de vecteur joué par *Ixodes ricinus* L. en Suisse. Acta Tropica 36:181–191, 1979
19. Burgdorfer W, Barbour AG, Hayes SF, et al: Lyme disease—a tick-borne spirochetosis? Science 216:1317–1319, 1982
20. Burgdorfer W, Barbour AG, Hayes SF, et al: Erythema chronicum migrans—a tickborne spirochetosis. Acta Tropica 40:79–83, 1983
21. Steere AC, Grodzicki RL, Kornblatt AN, et al: The spirochetal etiology of Lyme disease. New Eng J Med 308:733–740, 1983
22. Benach JL, Bosler EM, Hanrahan JP, et al: Spirochetes isolated from the blood of two patients with Lyme disease. New Eng J Med 308:740–742, 1983
23. Berger BW, Clemmensen OJ, Ackerman AB: Lyme disease is a spirochetosis. Am J Dermatopathol 5:115–124, 1983
24. Waldo ED, Sidhu GS: The spirochete in erythema chronicum migrans. Am J Dermatopathol 5:125–127, 1983