Ixodes ricinus Spirochete and European Erythema Chronicum Migrans Disease

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From three endemic locations of erythema chronicum migrans disease in North Rhine-Westphalia, Germany, we recovered 19 isolates of a spirochete from Ixodes ricinus ticks. The infection rate in adult ticks was 16 percent. The isolated spirochete is immunologically related to the Ixodes dammini spirochete, Borrelia duttoni, and Treponema pallidum. Using indirect immunofluorescence, the sera of 90 patients with erythema chronicum migrans disease showed antibody titers against the isolated spirochete, which correlated with the clinical course. Similarly, antibodies were demonstrated in the sera of 21 patients with acrodermatitis chronic a trophicans. These results suggest an etiologic role for the Ixodes ricinus spirochete in European erythema chronicum migrans disease.

Erythema chronicum migrans, a unique skin lesion, was first recognized in 1910 [1]. During subsequent years, particularly after effective therapy with penicillin was reported in 1951 [2], the disease was generally thought to be a harmless skin disease. The skin lesion was not connected to involvement of the nervous system because the erythema was often absent or missed [3]. Involvement of joints and heart, which are rare in Europe, was not recognized at all [4,5]. Also generally not considered was the possibility that acrodermatitis chronic a trophicans might be a late manifestation of the same disease.

The observation of bites of ticks and other arthropods, seasonal occurrence, inflammatory signs, phasic course, and the successful transmission of the erythema from man to man [6] supported the concept that these were infectious diseases transmitted by arthropods. However, a number of incriminated agents could not be confirmed, as was not the microscopic finding of spirochetes by Lennoff in 1948 [7].

The reported transmission by ticks, the proven sensitivity to penicillin or tetracycline [8], and the finding of locally synthesized IgM in cerebrospinal fluid [9], as in neurosyphilis, suggested that the causative agent might be a spirochete related to Borrelia duttoni, the causative agent of tick-borne relapsing fever. We were able to confirm this hypothesis by the demonstration of rising IgM and IgG antibody titers against Borrelia duttoni in sera and cerebrospinal fluid of patients with erythema chronicum migrans (Fig. 1) [10].

In the United States, the spirochetal etiology of Lyme disease was established by
the isolation of a spirochete from *Ixodes dammini*, by the demonstration of antibodies against the new agent in the sera of patients with Lyme disease [11], and finally by the isolation of the same spirochete from the skin, blood, and spinal fluid of patients [12,13]. Burgdorfer et al. (1983) reported the isolation of a spirochete from *Ixodes ricinus* from Switzerland that was morphologically and immunologically nearly indistinguishable from the *Ixodes dammini* spirochete [14]. This finding suggested that an identical pathogenic agent might be responsible for both diseases.

The close relationship between the North American and the European disease was also shown by the demonstration of antibodies against the *Ixodes dammini* spirochete in the sera of six of nine patients with erythema chronicum migrans meningopolyneuritis from Sweden [15]. We confirmed this finding. Thirty-four of our 39 patients with antibodies (≥ 1:128) against *Borrelia duttoni* also had antibodies against the *Ixodes dammini* spirochete [16].

We report here the isolation of a spirochete from *Ixodes ricinus* in Germany, similar to the *Ixodes dammini* spirochete. Extensive antibody studies support the hypotheses of an etiologic role for this agent in erythema chronicum migrans disease [16].

**METHODS**

*Ixodes ricinus* ticks were obtained by flagging in three endemic locations for tick-borne meningopolyneuritis in North Rhine-Westphalia. Specimens of the internal organs of the ticks were cultivated in a modified Kelly's medium [12]. Indirect immunofluorescence was done by standard methods.

**RESULTS**

We recovered 19 isolates of spirochetes from *Ixodes ricinus* ticks from three separate endemic locations of erythema chronicum migrans in North Rhine-Westphalia (Table 1). The infection rate in adult ticks was 16 percent, lower than that in Switzerland (36 percent), but similar to that of endemic areas of Lyme disease in southeastern Connecticut. According to the history of our patients, two of the locations must have already been endemic 13 and 19 years ago.

By indirect immunofluorescence, sera from American and European patients revealed similar antibody titers against the isolated spirochete and the *Ixodes dam-*
TABLE 1
Isolation of Spirochetes from I. ricinus from Endemic Locations for Erythema Chronicum Migrans Disease in North Rhine-Westphalia

| Endemic Location | Adults   | Nymphs |
|------------------|----------|--------|
| Huertgen         | 3/13*    | 8/20*  |
| Koenigsforst     | 3/18     |        |
| Bad Honnef       | 1/12     | 5/5    |
| **Total**        | 7/43     | 13/25  |

*Isolates/investigated ticks
*Isolates/investigated pools [10]

TABLE 2
I. ricinus and I. dammini Spirochete: Immunologic Comparison

| Serum                          | I. ricinus | I. dammini |
|--------------------------------|------------|------------|
| Polyclonal antibody            |            |            |
| Meningopolyneuritis, patient SM| >1024      | 512        |
| Lyme disease, patient EP       | >1024      | >1024      |
| Normal human serum             | <32        | <32        |
| Monoclonal antibody            |            |            |
| Mouse monoclonal antibody      | <32        | 256        |
| Normal mouse serum             | <32        | <32        |

mini spirochete. In contrast, a monoclonal antibody against the Ixodes dammini spirochete, provided by Dr. A.G. Barbour, did not react with the Ixodes ricinus spirochete (Table 2).

Serum from patients with syphilis, Lyme disease, and erythema chronicum migrans meningopolyneuritis reacted with the Ixodes ricinus and Ixodes dammini spirochetes as well as with Borrelia duttoni and Treponema pallidum. Absorption with Treponema phagedenis antigen diminished the reactions, especially those of the syphilis serum (Fig. 2).

Electron microscopy of the spirochete isolated from Ixodes ricinus revealed a

FIG. 2. I. ricinus spirochete: Antibody titers before and after absorption with T. phagedenis antigen.
FIG. 3. Electron micrographs of the *I. ricinus* spirochete. The spirochetes had a transverse diameter of about 0.2 μm, a length of 8.8 to 33 μm, and 12 to 14 flagellae. The organism is seen in longitudinal section (right panel) and in cross section (left panel).

diameter of 0.2 μm and a length of 8.8 to 33 μm. The spirochete showed 12 to 14 flagellae, apparently more than the *Ixodes dammini* spirochete and the *Ixodes ricinus* spirochete from Switzerland (Fig. 3) [14].

The antibody response of patients with erythema chronicum migrans disease correlated with the clinical course. One patient with erythema alone who was treated with penicillin showed a fourfold decrease in the IgG antibody titer between acute disease and convalescence. Another patient who did not receive penicillin therapy developed meningopolyneuritis. This patient had a fourfold rise in titer between the fifth and tenth weeks followed by a slow decrease after three months. Concomitant cerebrospinal fluid showed an IgG antibody titer as high as 1:64 followed by a fourfold decrease. Both patients had only minimal specific IgM antibody (Fig. 4).

Comparison of antibody titers in patients and controls revealed high levels in patients. Of 39 patients with erythema chronicum migrans alone, 50 percent of those

FIG. 4. Erythema chronicum migrans alone (A) and tick-borne meningopolyneuritis (B): Clinical course and corresponding antibody titers against the *I. ricinus* spirochete.
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FIG. 5. Antibody titers against the *I. ricinus* spirochete in serum samples from 90 patients with erythema chronicum migrans (ECM) disease.

treated with antibiotics showed antibody titers of 1:64 or higher against the *Ixodes ricinus* spirochete. In contrast, 90 percent of 51 patients with meningopolyneuritis who did not receive antibiotic therapy had elevated titers. In these groups, 20 percent and 68 percent, respectively, had IgM antibody titers of 1:32 or higher (Fig. 5). Cerebrospinal fluids showed IgG antibody titers as high as 1:64 (Fig. 6).

Fourfold rises or decreases of IgG or IgM antibodies could be demonstrated in 50 cases. Rises generally occurred between the third and the sixth week after the onset of the disease. Fourfold changes of specific IgG antibody in cerebrospinal fluid were seen in 22 cases (Fig. 7).

The first example of a late complication is the demonstration of antibodies against the *Ixodes ricinus* spirochete in patients with acrodermatitis chronica atrophicans.

FIG. 6. Titers against the *I. ricinus* spirochete in CSF from 27 patients with tick-borne meningopolyneuritis.
FIG. 7. Antibody titers against the *I. ricinus* spirochete in paired serum samples from patients with erythema chronicum migrans (ECM) disease.

This chronic skin disease, which occurs in Europe [17], is presumed to be transmitted by *Ixodes ricinus* and is thought to be related to erythema chronicum migrans. In the first phase of the disease, the skin lesion becomes red, blue, and edematous. During the second phase, it atrophies and becomes extremely thin, like cigarette paper. The disease usually begins in the peripheral part of one extremity, but later

FIG. 8. IgG (●) and IgM (○) antibody titers against the *I. ricinus* spirochete in 21 patients with acrodermatitis chronica atrophicans (*y* = years after onset).
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can involve all four extremities. All 21 of our patients with acrodermatitis chronica atrophicans had elevated IgG antibody titers against the *Ixodes ricinus* spirochete (Fig. 8). Although acrodermatitis chronica atrophicans can be treated with penicillin, a fourfold decrease in antibody titers could as yet be demonstrated in only two patients.

DISCUSSION

The spirochete isolated from *Ixodes ricinus* in three endemic areas in North Rhine-Westphalia seems to be the etiologic agent of European erythema chronicum migrans disease. Immunologically the agent is closely related to *Borrelia duttoni* and the etiologic agent of Lyme disease. Further studies will show whether there are different strains of *Ixodes ricinus* spirochetes. Nevertheless, the diseases on the two continents differ in some respects. In general, European erythema chronicum migrans disease is less severe than Lyme disease. Multiple erythemas, joint and heart involvement, and recurrences of meningopolyneuritis are rare or unknown in Europe.

The antibody response, which correlated with the clinical course of 90 patients with erythema chronicum migrans disease, also indicates an etiologic role for the *Ixodes ricinus* spirochete, although for definitive proof the spirochete must be isolated from man. Even so, antibody tests have already enabled us to clarify the diagnosis in questionable cases of erythema chronicum migrans disease. Although they are currently unknown, it is possible that late clinical manifestaitons of the spirochetosis may come to light, similar to the late stages of syphilis.

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