

## The Spirochetal Etiology of Erythema chronicum migrans Afzelius

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We have obtained spirochetes from *Ixodes (I.) ricinus* ticks collected in different areas in Stockholm where erythema chronicum migrans Afzelius (ECMA) cases are known to occur sporadically. Titers of antibody against spirochetal isolates, cultured in modified Kelly's medium, from Swedish *I. ricinus* and American *I. dammini* ticks were determined by indirect immunofluorescence. With the *I. ricinus* respectively the *I. dammini* spirochete as antigen the ECMA group had a significantly higher median titer (73 respectively 128) than the control group (28 respectively 31). Spirochetes have also been isolated from the periphery of one ECMA lesion by injecting skin homogenates into rabbit testicles. The findings are in agreement with spirochetal etiology in ECMA. (Received October 24, 1983.)

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Erythema chronicum migrans Afzelius (ECMA) was described for the first time in 1909 by Afzelius (1) and later on by Lipschütz (2). In 1930 Hellerström was the first to report on the association with meningitis (3). An infectious tickborne origin was suggested at an early stage. In 1948 Lennhoff, using a mercury stain, reported spirochete-like elements in skin biopsy specimens from patients with ECMA, but also in many other diseases (4). According to this and the findings of spirochetes in ticks Hollström reported in 1950 about treatment of a group of ECMA patients with spirocheticides (bismuth salts, arsenicals, penicillin) with good results (5). In 1955 Binder et al. showed that the disease could be transmitted from one human being to another (6). In 1978 we isolated spirochetes from the periphery of one ECMA lesion by injecting skin homogenates into rabbit testicles.\* The histological finding, with spirochetal structures in ECMA, has recently been confirmed by Frithz et al. with the Warthin-Starry stain (7). Barbour et al. 1983 reported isolation of spirochetes from *I. ricinus* ticks from Switzerland (8).

In 1975 Lyme disease (LD), initially called Lyme arthritis, was first recognized in the United States (9). It typically begins with one or multiple skin lesions called erythema chronicum migrans and is often followed by arthritis and sometimes by neurologic or cardiac involvement. In 1982 Burgdorfer et al. isolated a "treponema-like" spirochete from *I. dammini*, the incriminated tick vector of LD (10). In 1983 Steere et al. found a rise in specific antispirochetal antibodies in most Lyme patients. They also recovered the spirochete from the blood or skin lesion or cerebrospinal fluid from 3 out of 56 patients. Spirochetes were isolated from 1 out of 18 skin biopsy specimens from the periphery of the skin lesion but no spirochetes were found in 23 skin scrapings (11). In sections from human LD skin Berger et al. have found spirochetal structures with the Warthin-Starry silverstain (12) and Waldo et al. with the Steiner silver stain (13).

The aim of this investigation was to study the hypothesis of spirochetal etiology of ECMA.

\* Read at a meeting of the Swedish Dermatological Association, Stockholm, November 1978.

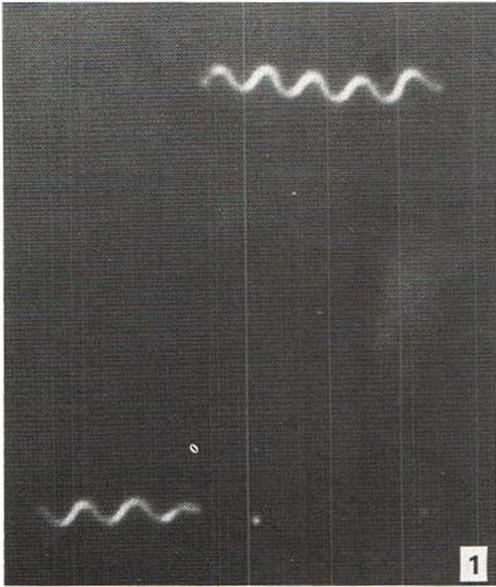


Fig. 1. Cultured *I. ricinus* spirochetes. Darkfield.

Fig. 2. Spirochete from a rabbit testicle after injection of ECMA skin homogenate. Darkfield.

## MATERIALS AND METHODS

### *Patients and controls*

The patient material was obtained from the Department of Dermatology, Södersjukhuset, Stockholm. All the ECMA patients had typical skin lesions and none of the patients suffered any complications at the time of biopsy or serum sampling. Age- and sex-correlated humans visiting our Department were chosen as controls in the serological tests. The patients and controls lived in the same area.

### *Skin biopsies*

Skin biopsy specimens (4 mm punch) were obtained from the edge of ECMA lesions from 41 patients.

**Rabbit-tests.** Crushed skin biopsy specimens from 6 ECMA patients were injected into the back-skin of 6 white rabbits and the rabbits were watched for 2 months. Skin specimens from 20 more patients were separately crushed in 1 ml of Nelson medium in a glass tissue homogenizer and examined under darkfield microscope. Each skin homogenate was then injected into one rabbit testicle with the other testicle left as control. Three weeks after the injection the testicles were examined under darkfield microscope for the presence of spirochetes.

**Cultures.** Skin specimens from 15 different patients were each crushed in 1 ml of modified Kelly's medium (11) in a glass tissue homogenizer and examined under darkfield microscope. The homogenate was then transferred to a glass tube containing 9 ml of modified Kelly's medium for cultivation. A 30 µg neomycin disk was added. The culture tubes were incubated at 35°C and examined by darkfield microscopy weekly for at least one month.

### *Ticks and spirochetes*

*I. ricinus* ticks were obtained by flagging in different locations in the surroundings of Stockholm where ECMA cases are known to occur sporadically. The ticks were studied under a dissecting microscope. The gut tissues were removed and examined for spirochetes by darkfield microscopy. The gut tissues from some of the ticks were placed in modified Kelly's medium, where spirochetes could be cultured. Stock cultures of these *I. ricinus* spirochetes (Fig. 1) and stock cultures of American *I. dammini* spirochetes (10) are maintained in our laboratory in modified Kelly's medium.

### *Serologic tests*

Serum samples from ECMA patients have been collected and the samples have been frozen at -70°C. The time period from the onset of the skin lesion to the time of the first visit, when serum was sampled, varied from 3 days to 11 months. About half of the patients had an ECMA duration of 2 months or more.

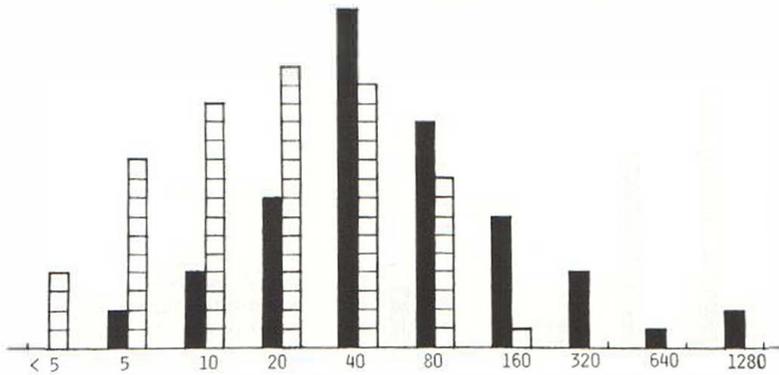


Fig. 3. Antibody titers against the *I. ricinus* spirochete in 58 ECMA patients (■) and in 66 controls (□).

Fluorescent treponemal antibody tests (14) with absorption (FTA-ABS) and without absorption (FTA) with Reiter spirochetes were performed on 20 ECMA patients and 150 controls.

Immunofluorescence (IF) tests with *Ixodes* spirochetes using a fluorescein isothiocyanate (FIT-C)-labeled goat antihuman polyvalent conjugate (National Bacteriological Laboratory, Stockholm, Sweden) were performed. Antibody titers against the *I. ricinus* spirochete were determined in sera from 58 patients and 66 controls. Against the *I. dammini* spirochete antibody titers were determined in unabsorbed sera as well as sera preabsorbed with Reiter spirochetes. Unabsorbed sera from 54 patients and 58 controls and preabsorbed sera from 31 of these patients and 39 of the controls were investigated.

The same high-titer and low-titer control serum samples were included in each test and the slides were read blindly. The end point was defined as the highest titer in which all spirochetes still fluoresced weakly.

#### Statistics

The Wilcoxon rank-sum test was used. The tests were based on the mid-rank statistic as the results are presented in class-intervals. The median has been calculated for all samples but one where the open class (titer <5) contained more than half the numbers of observations.

## RESULTS

### Skin biopsies

**Rabbit-tests.** Attempts to transmit ECMA by injecting crushed skin biopsy specimens into the back-skin of white rabbits were not successful.\* In 1 out of 20 tests spirochetes were detected in a rabbit testicle 3 weeks after the injection of skin homogenate from a patient who had had ECMA for 7 weeks. In darkfield microscopy these spirochetes moved vigorously with a quick corkscrew-like motion and, as it appeared, in a determined direction (Fig. 2). The spirochetes were then transferred to another rabbit testicle but failed to survive.\*

**Cultures.** No spirochetes were detected after culture of the skin biopsy specimens in the modified Kelly's medium.

### Ticks and spirochetes

Spirochetes were found in nymphal and adult *I. ricinus* ticks collected in the neighbourhood of Stockholm. The presence of spirochetes could be established in 6 of the 68 investigated ticks by darkfield microscopy.

### Serologic tests

Positive FTA-tests were found both in patients and controls and no statistical difference could be observed. FTA-ABS tests were negative in all patients and controls.\*

\* Read at a meeting of the Swedish Dermatological Association, Stockholm, November 1978.

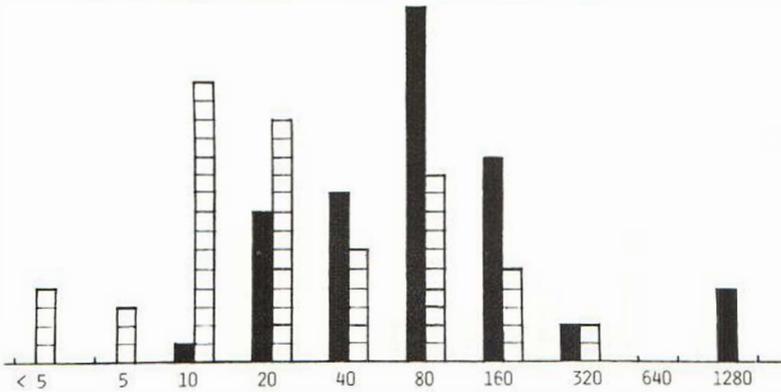


Fig. 4. Antibody titers against the *I. dammini* spirochete in 54 ECMA patients (■) and in 58 controls (□).

By indirect *IF-tests* with the *I. ricinus* spirochete (Fig. 3) the patient group had a significantly higher median titer (73) than the control group (28) ( $p < 0.001$ ). The same difference could also be observed with the *I. dammini* spirochete (Fig. 4) with a median titer for the patient group of 128 and for the control group of 31 ( $p < 0.001$ ). Twenty-one patients were tested at the same time both with *I. ricinus* and *I. dammini* spirochetes as antigens and in these patients the test results were the same  $\pm$  one titer step. After absorption with Reiter spirochetes and with the *I. dammini* spirochete as antigen (Fig. 5) the patient group had a median titer of 25 and the control group of  $< 5$  ( $p < 0.001$ ). There was no statistically titer difference in patients with an ECMA duration of 2 months or more compared to patients with an ECMA duration of less than 2 months.

## DISCUSSION

Since long ECMA is known as a tickborne disease and many findings, such as the histological picture with plasma cells in the skin infiltrates, the mercury stain with spirochete-like elements and the therapeutical success with different spirocheticides, have pointed in the direction of spirochetal etiology. In 1978 we therefore performed FTA tests to investigate a possible cross-reactivity to treponema antigens and we also injected skin homogenates into rabbit testicles in order to try to multiply possible spirochetes analogously to *treponema pallidum* propagation.

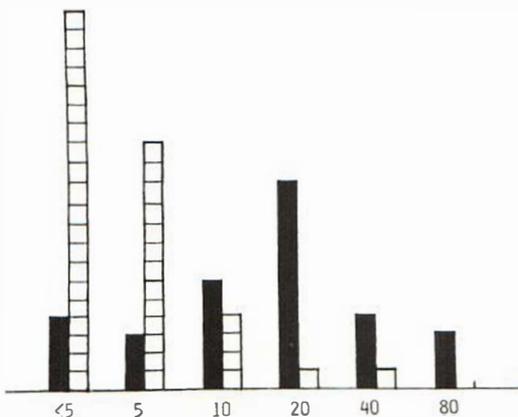


Fig. 5. Antibody titers against the *I. dammini* spirochete after absorption with Reiter spirochetes in 31 ECMA patients (■) and in 39 controls (□).

The finding of spirochetes in a rabbit testicle after the injection of skin homogenate from one of our ECMA patients and the present results of the serologic studies with both Swedish *I. ricinus* and American *I. dammini* spirochetes are in agreement with spirochetal etiology in ECMA. The indirect IF test with whole Ixodes spirochetes as antigen is, however, not a diagnostic method for the individual ECMA patient because of the overlapping between patients and controls. In the hope of minimizing this we also tried absorption with Reiter spirochetes to remove crossreacting antibodies. In these tests there was less overlapping between patients and controls, but the absorption procedure has been difficult to standardize and a better serologic method is desirable for diagnostic test. Our findings are also in agreement with the results of Burgdorfer et al. (15) who reported a close similarity immunologically between *I. dammini* spirochetes from the United States and *I. ricinus* spirochetes from Switzerland. There are, however, differences between the clinical picture of ECMA and the clinical picture of early Lyme disease which may indicate that the spirochetes are not identical.

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