Syphilitic immunofluorescence in experimental relapsing fever

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Immunological cross-reactivity has been shown between related genera of the family Treponemataceae. Extracts of Borrelia cross-react with antibodies in syphilitic serum (Saurino and De Lamater, 1952) and produce positive skin tests in the later stages of syphilis (Ranké, Quilici, and Assadourian, 1967). The fluorescent treponemal antibody absorption test (FTA-ABS) is at present considered to be specific, as its name implies, for treponemal disease. However, we have found that Borrelia infection in mice also induces an antibody that reacts in the FTA-ABS test, which would suggest that Treponema and Borrelia share a common antigen.

Material and methods

ORIGIN OF TEST SERA

B. duttoni (Welcombe mouse strain), the causative organism of tick-borne relapsing fever, was used. Inocula were obtained by diluting infected mouse blood with citrated saline. Male Parkes strain mice (18–20 g.) were infected by intraperitoneal injection and blood smears were taken from each mouse immediately before death. The ratio of Borrelia to erythrocytes was determined by microscopical examination after Leishman staining. Three to four mice were killed and bled at each time of sampling and the pooled sera were stored at –20°C without preservative. Two experimental infections were produced, after the administration of 4 × 10⁸ organisms into 25 mice and 1·3 × 10⁸ into 35 mice. Six pooled samples of sera were collected in the first experiment and nine in the second experiment. Further studies were carried out on four sera reactive in the unabsorbed FTA test, with titres greater than 320, from mice with asymptomatic syphilis. Control sera were obtained from forty normal Parkes strain mice and pooled into twelve samples.

IMMUNOFLUORESCENT TESTS

Suspensions of spirochaetes were dried in advance on clear areas of slides, treated with a Teflon (Hiflon PTFE Gallenkamp) water repellent (Goldman, 1968).

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The USPHS Manual of Syphilis Serology (1969) was followed when carrying out the fluorescent treponemal antibody test on T. pallidum (Nichols strain) with the following exceptions: it was not found necessary to inactivate the serum at 56°C and the test was conducted at room temperature. When staining with serum and conjugate, the buffer did not contain 2 per cent. Tween 80.

The method for B. duttoni was that described by Coffey and Eveland (1967) and for various leptospirae, L. icterohaemorrhagiae, L. canicola, and L. pomona, that of Coffin and Maestrone (1962). Both techniques involved preliminary fixation in acetone and then through graded alcohols with final fixation in methanol. Sera were taken in 5 μl amounts, either undiluted or as reciprocal dilutions of 5, 25, 125, and 625 in phosphate buffered saline. Specific goat anti-mouse globulin conjugated with fluorescein isothiocyanate (Hyland) was used at a dilution of 1/80. Results were recorded as negative (−), equivocal (+), weak positive (wk. +), and positive (+). There was no differentiation between + + + and + + + + as used in the routine FTA-ABS test. The microscope used was a ‘Zetopan’ (Reichert) with an HB 200 high pressure mercury vapour lamp. All tests were carried out in duplicate, and read independently by two observers, concordance being required for assessment of results as positive.

ABSORPTION TESTS

The effects of absorption with the ‘sorbet’ (Burroughs Wellcome) used in the FTA absorption test and Reiter protein (Burroughs Wellcome) were tested on sera from mice experimentally infected with B. duttoni and T. pallidum. Indirect immunofluorescence was then carried out on Borrelia and T. pallidum substrates. The absorbing potency of the sorbet was assessed using human nonspecific positive control sera. These were tested at dilutions of 1 in 5, 1 in 25, and 1 in 125 in saline and in sorbet against T. pallidum and Reiter antigens.

TESTS FOR CARDIOLIPIN ANTIBODIES

The Wassermann antibody was assayed by the VDRL test (USPHS Manual, 1969) and the cardiolipin (fluorescent) antibody (Wright, Doniach, Lessoff, Turk, Grimble, and Catterall, 1970) using frozen rat, mouse, and human kidney antigen.
Results

The sera from mice infected with *B. duttoni* gave positive immunofluorescence with *T. pallidum* antigen (see Table). The appearance of the fluorescent treponemal antibody (FTA) followed the observed parasitaemia, with the maximum antibody levels on Day 4, when spirochaetes began to disappear from the blood (see Figure). A subsequent fall in antibody levels occurred and sera were FTA-negative by Day 28, indicating the transient nature of the response (Figure and Table). Titres were established against the homologous *B. duttoni* antigen. The rise and fall of the titre was as for *T. pallidum* substrates but the height of the titres was lower and the duration of positive reactions was shorter.

Forty control sera from uninfected mice, with phosphate buffered saline as diluent, gave one definite positive and one doubtful positive result at a dilution of 1/5. Sorbent abolished both these positives. No pooled specimens of the control sera were positive using phosphate-buffered saline diluent.

The treponemal antibody titre in the mice infected with *Borrelia* was not reduced using sorbent, despite the ability of sorbent to abolish treponemal fluorescence in non-specific control sera. The highest titres of these sera were 1/25 (two sera) and 1/125 (one serum). Undiluted Reiter protein antigen abolished the fluorescence in both the control and test sera (see Table).

**TABLE Development of fluorescent antibodies during Borrelia parasitaemia, using different diluents**

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Days after Borrelia infection</th>
<th>Borrelia antibodies</th>
<th>Treponemal antibodies</th>
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<tr>
<td>1</td>
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*B. duttoni* antigens gave a positive immunofluorescent reaction with the four sera from asymptomatic syphilitic mice. This reactivity was likewise abolished by the undiluted Reiter protein antigen.

The VDRL and cardiolipin fluorescence tests were negative and no antinuclear factors were seen at any stage.

Discussion

The FTA-ABS test is a standard confirmatory test for treponemal infection, but there is no doubt that the common group antigen which occurs in a variety of spirochaetes (Deacon and Hunter, 1962) can produce antibody in experimental animals which is detected by the FTA test (Tringali and Cox, 1970). Therefore, antibody to commensal spirochaetes could account for some anomalous false positive results in human sera.

In our experiments, *Borrelia* induced a positive FTA-ABS reaction which appeared at the height of the *B. duttoni* parasitaemia and then rapidly declined. *Borrelia agglutinating* (Balteanu, Russ, and Voiculescu 1948) and adhering antibodies (Schuhardt, 1942) behave in an identical way, quite unlike *Borrelia* immobilizing antibodies whose persistence and strain specificity is analogous to the treponeme immobilins (Coffey and Eveland, 1967). Sorbent and Reiter protein antigen represent different extracts of *T. reiteri*. It has been recently shown that sorbent is a less efficient absorbent (Wilkinson and Wiseman, 1971). This finding is confirmed here by the failure of sorbent diluent to remove the antibody detected in the FTA test. This treponemal fluorescence was abolished by using the more effective
Reiter protein. Effective absorption implies that there is a group antigen shared between *T. pallidum* and *T. reiter* and *B. duttoni*. Conversely, the cross-reacting fluorescence of mouse syphilitic sera with *Borrelia* antigen, which resisted sorbent but was abolished by Reiter protein could well be an expression of the common group antibody. However, the failure of *Borrelia*-infected mice to produce higher titres against *B. duttoni* was possibly due to denaturation of the *B. duttoni* antigen. This cross-reactivity does not extend to the *Leptospirae* since these organisms were negative in this test.

The FTA-ABS test detects an antibody unrelated to cardiolipin antibodies because both the VDRL and the syphilis tissue fluorescence tests were negative. Mice rarely develop cardiolipin antibodies in treponemal disease (Wright and Doniach, 1971), hence the negative finding on this occasion.

Patients with syphilis, when subjected to fever therapy using relapsing fever as the pyrogen (Plaut and Steiner, 1944), showed no cross-protective immunity. The *Borrelia* agglutination tests (Saurino and De Lamater, 1952) and complement-fixing tests (Wolstenholme and Gear, 1948) were also negative in syphilis. Nevertheless, high blood counts of *Borrelia* are recorded with relapsing fever in man (Bryceson, Parry, Perine, Warrell, Vukotich, and Leithhead, 1970), and it is possible that the FTA-ABS test with sorbent may be found positive in such patients.

**Summary**

The development of a transient positive FTA test was found in an experimental *B. duttoni* infection in mice. This antibody could be removed by undiluted Reiter protein antigen but not by sorbent. The possibility that a positive FTA-ABS test could arise in human relapsing fever is discussed.

We should like to thank Miss A. Parr (Interdepartmental Laboratory, Guy’s Hospital) and Miss S. Denton (Mill Hill) for their technical help, Dr. A. Grimble and Prof. M. H. Lessof (Guy’s Hospital) for their valuable advice and encouragement and Dr. R. Broughton (Department of Aerobic Bacteriology, Burroughs Wellcome) for the strains of *Leptospirae*.

**References**


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**Immuno-fluorescence syphilitique dans la fièvre récurrente expérimentale**

**SOMMAIRE**

On a observé l’apparition d’une positivité transitoire du test FTA-ABS dans l’infection expérimentale de la souris par *B. duttoni*. L’anticorps peut être éliminé par l’anti-gène protéique de Reiter non dilué mais non par le sorbant. La possibilité que l’épreuve FTA-ABS soit positive chez l’homme dans la fièvre récurrente est discutée.
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