Cross-Reaction with *Borrelia burgdorferi* Antigen of Sera from
Patients with Human Immunodeficiency Virus Infection,
Syphilis, and Leptospirosis

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We have studied the cross-reaction with *Borrelia burgdorferi* of sera positive for leptospirosis, syphilis, or human immunodeficiency virus by using the microimmunofluorescence test (micro-IF). The percentage of sera reactive in the micro-IF before absorption varied from 7 to 37% and was reduced to 3 to 8% after absorption with a commercial Reiter treponemal antigen. The cross-reaction of sera positive for syphilis or human immunodeficiency virus was distinguished from the homologous reaction with sera from patients with Lyme disease in the immunoblot test results. However, the cross-reaction could not always be distinguished from the homologous reaction with sera from patients with leptospirosis whose sera scored positive in the micro-IF for *B. burgdorferi*.

*Borrelia burgdorferi* is the causative agent of Lyme disease (4, 15). The clinical manifestations of Lyme disease (16, 19) include erythema chronicum migrans and cardiac, neurologic, and articular symptoms. Because of the paucity of the organism in tissue and biologic fluids, isolation of *B. burgdorferi* is difficult (3). At present, serodiagnosis is the only practical laboratory tool. The indirect fluorescent antibody test (6, 17) and its adaptations, namely the microimmunofluorescence test (micro-IF) (8), the solid-phase fluorimmunoassay (FLAX) (12), and the enzyme-linked immunosorbent assay (13, 14), are the main tests used for the detection of antibodies to *B. burgdorferi*. While the efficiencies of all these tests are relatively high during the later stages of Lyme disease, they are much lower during the early stage. Diagnosis remains essentially clinical in the early stage of the disease.

The specificity of these serological methods has been reported to be between 84 to 93% (6, 11, 14). In this report, we have studied the cross-reaction of sera from patients with syphilis, leptospirosis, and acquired immunodeficiency syndrome with *B. burgdorferi* by using the micro-IF technique. In addition, we have confirmed a number of these micro-IF results by the immunoblot technique (5, 7, 13).

**MATERIALS AND METHODS**

**Sera.** The following sera were used in this study. (i) We obtained 10 serum specimens from 10 patients from Marseille with Lyme disease, 3 of which specimens were sampled at an early stage (erythema chronicum migrans) and 7 of which were sampled at a later stage. (ii) We also obtained 100 serum specimens from 100 patients from Marseille reactive for syphilis with a *Treponema pallidum* hemagglutination test titer of >1:160. (iii) We also tested 100 serum specimens positive for leptospirosis which were collected from patients from all of France and were stored at the Pasteur Institute. The patients with leptospirosis had positive blood cultures, seroconversion, or single high titer (≥1:200) by the microscopic agglutination test (1) and were clinically and epidemiologically compatible cases. Of the patients, 53 were considered to have *Leptospira icterohaemorrhagiae* infection, 31 were considered to have *Leptospira grippotyphosa* infection, 8 were considered to have *Leptospira australis* infection, 1 was considered to have *Leptospira ballum* infection, and 1 was considered to have *Leptospira sejroe* infection. (iv) We tested 100 serum specimens from patients positive for human immunodeficiency virus (HIV) by enzyme-linked immunosorbent assay and immunoblot and nonreactive for syphilis. Sera were scored positive for antibodies to *B. burgdorferi* if the micro-IF titer was ≥200.

Sera that scored reactive by the micro-IF were adsorbed with the Reiter treponema absorption reagent used in the fluorescent treponemal antibody absorption test for syphilis (Behringwerke AG, Marburg, Federal Republic of Germany) as described in the kit instructions. The sera were then tested by micro-IF. Briefly, the sera were diluted (1:16) in absorption medium (ultrasonicate of Reiter spirochetes) and the mixture was incubated for 60 min in a water bath at 37°C. For titration, the sera were further diluted in the absorption medium.

**Antigen preparations.** *B. burgdorferi* was grown in Barbour-Stoenner-Kelly (BSK II) medium (2) for 4 to 7 days, centrifuged, washed with phosphate-buffered saline (PBS), and then pelleted and adjusted to a protein concentration of 2 mg of protein per ml in distilled water by using a commercially available assay (Bio-Rad Laboratories, Richmond, Calif.).

**Micro-IF.** The organism was first mixed with 6% (vol/vol) egg yolk suspension in PBS (pH 7.2) and applied by dip-pen point (artist’s pen) to microscope slides, air dried, and fixed for 20 min in cold acetone. The sera were diluted 1:25 in PBS containing 3% nonfat dry milk. The sera were incubated at 37°C for 30 min in a moist chamber, washed three times in PBS, and then incubated with fluorescein-conjugated goat anti-total human immunoglobulin (Bio-Merieux, Lyon, France) containing Evans blue (1:800) as a counter stain for 30 min in a moist chamber, washed three times in PBS, dried, and mounted with glycerine. The slides were observed
with a Nikon epifluorescence microscope at ×400. The reactive sera were further tested by using fluorescein-conjugated goat anti-human immunoglobulin M (IgM) (μ chain specific) and IgG (γ chain specific) to determine anti-B. burgdorferi IgM and IgG, respectively.

Electrophoresis and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed (10, 18). The B. burgdorferi was solubilized in a solution containing 2% sodium dodecyl sulfate and 5% 2-mercaptoethanol. The samples were applied to a 3.9% stacking gel, and the antigens were electrophoresed on a 10% separating gel at a constant current of 20 mA at 20°C. The proteins were transferred to nitrocellulose sheets (18) for 4 h with a current of 500 mA at +4°C. After transfer, the sheet was blocked in Tris-buffered saline, pH 8.0 (TBS), containing 5% nonfat dry milk overnight. The sheet was then washed with TBS, dried, cut into 0.5-cm lanes, and stored at 5°C until further use. Each serum specimen was diluted to 1:100 with TBS containing 3% nonfat dry milk and incubated with a lane containing the electrophoresed and electroblotted antigens overnight at 20°C with rocking. Each lane was then washed three times for 10 min in TBS and incubated for 2 h in peroxidase-conjugated goat anti-human immunoglobulin diluted 1:400 in TBS–3% milk. The lane was washed three times in TBS, and the antigen-antibody reaction was revealed with horseradish peroxidase-color development reagent containing 4-chloro-1-naphthol (Bio-Rad).

RESULTS

Eight serum specimens from Lyme disease patients were micro-IF positive before and after absorptions. Three of these serum specimens had both IgG and IgM to B. burgdorferi. The eight positive cases reacted in the immunoblot (>5 bands). The immunoblot test result for Lyme disease patient 5 is shown in Fig. 1. Among the 100 serum specimens reactive in tests for syphilis, 37 were micro-IF positive before absorption and 4 were micro-IF positive after absorption. The antibodies in these sera were of the IgG class. The immunoblot test results for syphilis patients 6, 7, and 8 are shown in Fig. 1. Antibodies in these sera were mostly against an antigen band with an approximate molecular mass of 38 kilodaltons.

Among the 100 serum specimens from patients with leptospirosis, 23 were weakly micro-IF positive (IgG, <200; IgM, <50) and 7 were positive before and after absorption. Of the latter specimens, six were from L. icterohaemor rhagiae infections and one was from an L. grippotyphosa infection; six had IgM (ranging from 1:50 to 1:800), and six had IgG (from 1:100 to 1:800). The immunoblot test results for four of these seven patients are shown in Fig. 2. Two serum specimens reacted with ≥5 antigen bands, and five serum specimens reacted with ≥2 bands. In the two former cases (Fig. 2, patients 1 and 2), leptospiroa organisms were isolated during the disease and no history of Lyme disease was reported.

Among the 100 serum specimens from patients with HIV infection, 20 scored positive by micro-IF before absorption. The micro-IF-positive serum specimens reacted with ≥2 antigen bands in the immunoblot test. The immunoblot test results for four of these serum specimens are shown in Fig. 3. Most serum specimens contained antibodies to an antigen band with an approximate molecular mass of 35 kilodaltons, and some serum specimens (for example, that from patient 11) had antibodies to an antigen band with a molecular mass of 45 kilodaltons. Three serum specimens remained micro-IF positive after absorption.

FIG. 1. Immunoblot test results for patients diagnosed with Lyme disease (patient 5) and with syphilis (patients 6 to 8).
DISCUSSION

The specificity of the IFA for Lyme disease has been reported to be 84 to 93%, depending on the inclusion of treponemal infections (6, 9, 14). To our knowledge, HIV-positive sera were not tested previously, and leptospirosis sera were tested in small numbers (14). In 1986, the Marseille Laboratory received 5,000 serum specimens for HIV testing; 1,796 were positive for HIV. Seventy-nine percent of the patients were drug addicts. If we project our results from all the serum specimens which were positive for HIV (1,794) in the Marseille region, 54 (3%) of the serum specimens after absorption would be micro-IF reactive for Lyme disease. This cross-reaction is not related to syphilis, because we selected sera nonreactive in tests for syphilis.

At the Marseille Laboratory, we test per year approximately 20,000 serum specimens for syphilis and we have found that approximately 5,000 serum specimens score positive for syphilis. We have randomly tested 100 sera reactive in tests for syphilis and found that 4% were still positive in the micro-IF for Lyme disease after absorption. Hunter et al. (9) reported that the cross-reaction observed with syphilis sera was absorbed by using Reiter treponema. In our study, using a commercial Reiter treponemal preparation, four serum specimens remained positive. This may be related to the procedure of absorption, which was different in our study. These results imply that 200 serum specimens positive for syphilis would also be positive for Lyme disease. Thus, the serodiagnosis of these sera would be based on the RPR card test (14), which detects antibody to cardiolipin produced during the course of syphilitic infection but not during Lyme disease. If the cardiolipin test is reactive because of a double infection, specific tests for syphilis, e.g., the fluorescent treponemal antibody absorption test, should be performed.

Because leptospirosis is not a frequently encountered disease, previous studies have reported the testing of only a few serum specimens. We tested 100 seropositive serum specimens that had been submitted to the National Center for Leptospirosis at the Pasteur Institute to ascertain that cross-reaction did not exist. Out of 100 serum specimens, 7 were positive, and the cross-reacting antibodies were not removed by Reiter antigen. This indicates that the cross-reacting antibodies in patients with leptospirosis were not to antigens present in Reiter treponemes.

In our study, the specificity of the micro-IF for two of the three diseases studied was significantly increased after the sera were absorbed with the Reiter antigen. Where Lyme disease is not common, such as in southern France, micro-IF results and probably test results from other serologic techniques, even after serum absorption, may yield an unacceptably high number of false-positives. Thus, in such areas, strong clinical and epidemiological data should be required for the diagnosis of Lyme disease.

Our immunoblot test results, with the exception of the test results on sera from patients with leptospirosis, concur with the suggestion of Grodzicki and Steere (7) that a serum specimen scores positive for Lyme disease when more than three bands are detected in the immunoblot test. We conclude that the immunoblot test should be used to confirm test results obtained by the more conventional technique in geographic areas where Lyme disease is not endemic or has a low incidence.

LITERATURE CITED


