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Detection of *Treponema pallidum* in Lesion Exudate with a Pathogen-Specific Monoclonal Antibody

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The diagnosis of early syphilis currently requires dark-field microscopic or serologic demonstration of *Treponema pallidum* infection. Dark-field microscopy is not widely available and is complicated by the numerous saprophytic spirochetes which are present at oral and rectal mucosal surfaces. Serologic tests are positive in only 70 to 90% of patients with primary syphilis, and several days may be required for results to become available. We used a pathogen-specific, fluorescein-conjugated monoclonal antibody to examine lesion exudates from 61 patients for the presence of *T. pallidum* and compared the data with results of dark-field microscopy and serologic testing. The direct fluorescent-antibody technique revealed the presence of *T. pallidum* in 30 of 30 patients with early syphilis, and dark-field microscopy was positive for 29. Serologic tests were reactive for 27 of 30 patients with syphilis; in the 3 patients with nonreactive serologic tests, chancres had been present for 4, 6, and 21 days. Although 7 of 31 patients without syphilis had spiral organisms seen on dark-field microscopy, the direct fluorescent-antibody test was negative for all 31. The presence of nonpathogenic spirochetes was subsequently verified in 5 of 7 patients by using a second monoclonal antibody which reacts with nonpathogenic, as well as pathogenic, treponemes and related spirochetes. The demonstration of *T. pallidum* by using fluorescein-conjugated monoclonal antibodies is intrinsically specific and is as sensitive as dark-field microscopy for the diagnosis of early syphilis. This method provides a convenient, accurate means for the diagnosis of syphilis by health care providers, many of whom lack access to dark-field microscopy.

The diagnosis of early syphilis is usually based on serologic testing or dark-field microscopic demonstration of *Treponema pallidum* in lesion exudates. Reaginic serologic tests for syphilis, such as the Venereal Disease Research Laboratory (VDRL) test, are neither specific nor sensitive and may be nonreactive in up to 30% of patients with primary syphilis at the time of initial presentation (11, 12). Tests for serum antibody to *T. pallidum*, such as the fluorescent treponemal antibody absorption (FTA-ABS) test, are more specific and more sensitive but do not differentiate current infections from past infections—a major problem in the population at high risk for syphilis. The direct identification of *T. pallidum* in lesion exudates permits the rapid diagnosis of early syphilis, even in patients with a history of prior disease or in seronegative patients. Dark-field microscopy, however, requires the rapid processing of specimens, making the procedure impractical for the majority of clinical settings. Furthermore, considerable experience is necessary for the accurate differentiation of *T. pallidum* from nonpathogenic spirochetes on the basis of characteristic motility and morphology. To provide better access to rapid, direct testing for infections caused by pathogenic treponemes, we have developed a monoclonal-antibody-based test for the demonstration of *T. pallidum* in lesion exudates. This report compares data for 61 patients evaluated for primary or secondary syphilis by dark-field microscopy, serologic testing, and a new direct fluorescent-monoclonal-antibody test for the demonstration of *T. pallidum* in lesion exudates.

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MATERIALS AND METHODS

Monoclonal antibodies. Hybridomas were prepared by fusing spleen cells from mice immunized with *T. pallidum*, Nichols strain, with BALB/c MOPC 21 NS1/1 cells by modifications (13) of techniques originally described by Kohler and Milstein (10). Hybridomas were screened for the production of anti-treponemal antibodies by an enzyme-linked immunosorbent assay and immunofluorescence as previously described (10a). Antibody H9-1, used in this study, is a pathogen-specific monoclonal antibody which recognizes an antigenic determinant present on *T. pallidum* and *T. pertenue* but not on four species of human commensal treponemes (*T. vincentii*, *T. denticola*, *T. phagedenis* [biotype Reiter], and *T. refringens*), the porcine treponeme *T. hyodysenteriae*, or the related spirochetes *Borrelia recurrentis* and *Leptospira interrogans*. This antibody, which is directed against a determinant contained on a 48,000-dalton *T. pallidum* polypeptide (Fig. 1), was purified by ammonium sulfate precipitation (8) and conjugated with fluorescein isothiocyanate (7) by standard methods. Monoclonal antibody C2-1, which reacts with all the spirochetes listed above, was used for the demonstration of commensal spirochetes in an indirect immunofluorescence assay with fluorescein isothiocyanate-conjugated sheep anti-mouse immunoglobulin G (heavy and light chains) (Cappel Laboratories) as the second antibody (10a).

Clinical specimens. Sixty-one patients seen at the Seattle-King County-Harborview Medical Center Sexually Transmitted Disease Clinic between 27 June 1983 and 1 August 1984 for an evaluation of genital or cutaneous lesions were

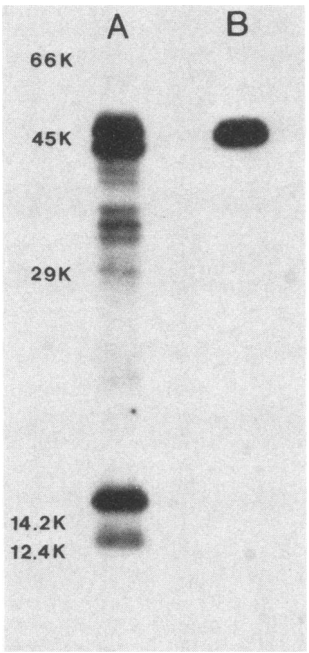

FIG. 1. Specificity of monoclonal antibody H9-1 for a 48,000-dalton molecule of *T. pallidum*, Nichols strain, as demonstrated by Western blotting (5) (lane B). A reference profile of *T. pallidum* antigens, recognized by pooled sera from patients with syphilis, is shown in lane A. Positions of molecular weight markers (in kilodaltons) are shown.

enrolled in the study. Verbal informed consent was obtained from all patients before enrollment. All patients underwent a routine clinical evaluation, including an examination for lesions or rashes, cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, and venipuncture for syphilis serologic testing. All suspicious lesions were cleaned with moist gauze, and exudate was expressed by using gentle direct pressure. One or two specimens were collected for dark-field microscopy by touching clean glass slides or cover slips to the lesion; all specimens were examined within 5 min of acquisition. For dark-field microscopy, slides were examined at a magnification of 450× (dry) for at least 5 min before being classified as negative. Spirochetes and suspicious fields were further examined at a magnification of 1,000× (oil). Additional slides were similarly obtained for monoclonal-antibody testing; they were allowed to air dry, fixed in acetone for 5 min, allowed to air dry again, and stored at −20°C until they were stained and examined.

Laboratory methods. For the direct immunofluorescence test, an acetone-fixed smear from each patient was incubated with 30 μl of fluorescein-conjugated antibody solution containing Evans blue counterstain for 30 min in a humidified chamber at 37°C. The slides were rinsed with water and air dried, and cover slips were mounted with Tris-glycerol (1:9) buffer (pH 8.5). The specimens were examined at a magnification of 630× (oil) with a Nikon Labophot epifluorescence microscope equipped with high-intensity mercury illumination and filters appropriate for the optimal detection of fluorescein.

Serologic testing (VDRL and FTA-ABS tests) was performed on sera from all subjects in accordance with standard procedures at the Seattle-King County Department of Public Health Laboratories. Cultures for herpes simplex virus were performed for 27 patients by previously described methods (3). The results of fluorescent-monoclonal-antibody staining, serology, and dark-field microscopy were determined without knowledge of data from other tests.

RESULTS

Patient characteristics. The study population consisted of 61 evaluable patients, 30 with syphilis and 31 without syphilis. Patients were considered to have syphilis when dark-field microscopy revealed *T. pallidum* displaying characteristic morphology and the typical flexing, corkscrew motility or when there was a newly reactive serum FTA-ABS test (for patients without a history of a prior reactive serum FTA-ABS test) or both. For patients with a history of prior syphilis, a fourfold or greater increase in the VDRL titer was considered to indicate reinfection. The age range was 21 to 59 (mean, 31) years for those with syphilis and 17 to 57 (mean, 34) years for those without syphilis. A total of 26 of 30 with and 27 of 31 without syphilis were male. Homosexual or bisexual men accounted for 19 of those with and 13 of those without syphilis.

A diagnosis was made in 41 of 61 (67%) patients. The 20 patients with no definitive diagnosis were all in the group without syphilis. Of 30 patients with syphilis, 22 were determined to have primary syphilis based on appearance, location, and duration of lesion; 8 had secondary syphilis. Among the patients without syphilis, nine had a genital herpes infection (five were culture positive, and four had typical lesions but negative cultures), one had condylomata acuminata, and a dental crypt was scraped in one patient to obtain commensal oral spirochetes for testing.

A total of 14 patients (47%) in the syphilis group had single lesions, and 16 patients (53%), including all 8 patients with secondary syphilis, had two or more lesions. Syphilitic lesions were located on the genitalia in 24 patients, the anus in 5 patients, and the neck in 1 patient. Among the patients without syphilis, 21 (68%) had single lesions, and 10 (32%) had two or more lesions. Nonsyphilitic lesions were located on the genitalia in 20 patients, the anus in 6 patients, and the lips, tongue, or buccal mucosa in 5 patients.

Direct immunofluorescence staining of clinical samples. All slides scored as positive for *T. pallidum* contained five or more distinct, sharply outlined, apple-green fluorescein-stained treponemes with the typical corkscrew morphology (Fig. 2). Although the numbers of organisms per slide varied from patient to patient, treponemes demonstrated by this method were always seen within 1 min of observation. Slides were scanned for 3 min before they were considered negative. In no case did background fluorescence impair the recognition of treponemes. Little staining of nontreponemal debris was noted, although faint green reticulate matter, which was easily differentiated from *T. pallidum*, was seen in 13 specimens. Cellular debris, which was counterstained by Evans blue, appeared orange-red.

Comparison of dark-field microscopic and serologic diagnosis of syphilis with the direct immunofluorescence test. Table 1 summarizes the results of direct monoclonal-antibody immunofluorescence tests, dark-field microscopy, and serologic tests for all patients. In all 30 patients with early syphilis, monoclonal-antibody staining of lesion exudates revealed brightly fluorescent spiral organisms (Fig. 2). *T. pallidum* was not revealed by direct immunofluorescence tests in any patient without syphilis.

Dark-field microscopy revealed *T. pallidum* in 29 of 30 patients with syphilis and commensal spirochetes in 7 of 31 (23%) patients without syphilis. Dark-field microscopic demonstration of typical spiral organisms in exudates from

patients with syphilis was occasionally hampered by refractile debris in the specimens and, in some cases, required more than 5 min of careful observation before a single typical organism could be identified.

Serologic testing revealed a newly reactive FTA-ABS test in 25 of 30 patients with syphilis. One of two patients with a history of prior treated syphilis had a rise in VDRL titer from 4 to 16 dilutions, and in the other patient, although no earlier VDRL titer was available, typical spirochetes were revealed by dark-field microscopy at the time of diagnosis, and the VDRL test was reactive at 16 dilutions. The VDRL and FTA-ABS tests were nonreactive in three patients with positive dark-field microscopy; each of these patients had penile chancres which had been present for 4, 6, and 21 days and was classified as having primary syphilis. Of 31 patients without syphilis, 29 had nonreactive VDRL and FTA-ABS tests. Two patients without active syphilis had histories of previous treatment for syphilis; in one, the VDRL test had been nonreactive for more than 1 year but the FTA-ABS test remained reactive, and the second had a clinical diagnosis of rectal herpes simplex infection and was serofast with a VDRL test reactive at 1 dilution (see below).

Two patients without syphilis were initially referred to us with a tentative diagnosis of syphilis based upon a misidentification of spirochetes in dark-field microscopic preparations. They were determined not to have syphilis after further evaluation by one of the authors. One of these patients had a history of prior treated syphilis and a rectal ulceration clinically resembling a rectal herpes simplex infection; spiral organisms were seen on dark-field miroscopy, but the patient was serofast at 1 dilution. After the intramuscular administration of 2.4×10^6 U of benzathine penicillin, the lesion resolved, but the VDRL test remained positive at 1 dilution. Although cultures for herpes simplex virus obtained from the lesion at the time of enrollment were negative, the patient experienced recurrent rectal discomfort during the month after the initial evaluation and was noted to have a small, tender rectal papule. The other patient had large vulvar condylomata acuminata; spiral organisms were seen on dark-field microscopy, and the patient was referred to the investigators with a tentative diagnosis of secondary syphilis (condylomata lata). This patient was seronegative, and the condylomata acuminata resolved with topical podophyllum resin therapy. In this patient and in four of the six other patients without syphilis for whom commensal spiral organisms were seen on dark-field microscopy, no *T. pallidum* was detected by specific immunofluorescence staining. The presence of non-*T. pallidum* spirochetes was confirmed, however, by indirect immunofluorescence staining with the pan-spirochete-reactive monoclonal antibody C2-1.

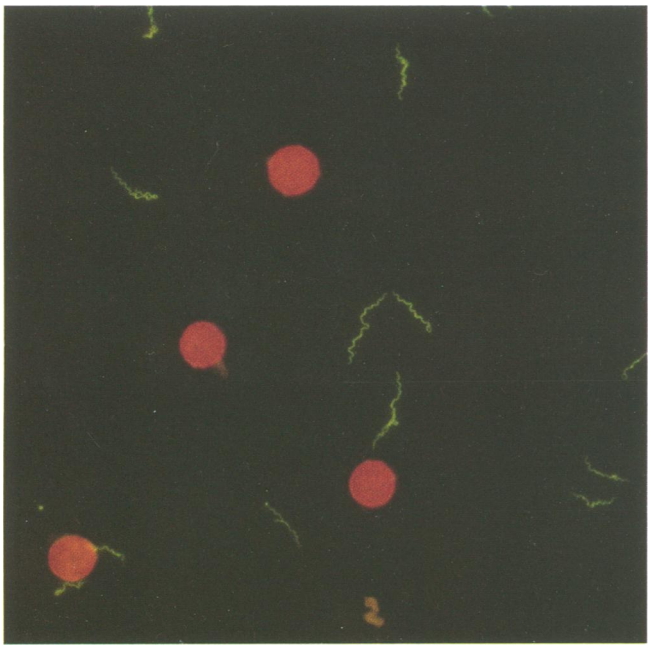

FIG. 2. Direct immunofluorescence staining of *T. pallidum* in lesion exudate from a patient with primary syphilis by use of monoclonal antibody H9-1 (original magnification, 630×).

TABLE 1. Comparison of monoclonal-antibody demonstration of *T. pallidum* with dark-field microscopy and serologic tests

Test	Result for indicated no. of patients						
	Syphilitic			Nonsyphilitic			
	26	3	1[a]	28	1[a]	1	1[a]
Monoclonal antibody staining	+	+	+	−	−	−	−
Dark-field microscopy	+	+	−	−	−	+	+
FTA-ABS	+	−	+	−	+	−	+

[a] Patients with history of prior treated syphilis.

DISCUSSION

The diagnosis of early syphilis in most offices and clinics is a difficult clinical problem. In a specialty clinic in which the prevalence of syphilis was high, the positive predictive value of a clinical diagnosis based on rash or lesion morphology alone was only 78% (2). In other clinical settings in which syphilis is uncommon and in which clinicians are less experienced, the positive predictive value of a clinical diagnosis of syphilis is doubtless lower. If serologic criteria alone are used, differentiation of a past infection from a current infection may be impossible. Furthermore, serologic tests may be negative in early syphilis. Although dark-field microscopy is of great value for rapid diagnosis, microscopes equipped with dark-field condensers are rarely available except in veneral disease clinics. In addition, dark-field microscopy is not reliable for patients with oral lesions, in which commensal treponemes may closely resemble *T. pallidum*. Efforts to demonstrate *T. pallidum* in direct smears and tissues with silver stains have not been entirely satisfactory because of nonspecificity; connective tissue may be stained and misidentified as *T. pallidum* (1). In the early 1960s, a number of investigators (4–6, 9, 14) developed direct and indirect immunofluorescence tests which accurately demonstrated *T. pallidum* in tissues and lesion exudates from patients and in animals with experimental syphilis. The specificity of polyvalent human or rabbit antisera for these tests was enhanced by dilution (4) or by absorption with the Reiter treponeme (*T. phagedenis*) (6, 9). The direct fluorescent-antibody *T. pallidum* test resulting from these studies is accurate and reliable but has not come into wide use either in the United States or in other nations. The specificity of the direct fluorescent-antibody *T. pallidum* test is not intrinsic, however, and is dependent upon extensive absorption of polyvalent antisera.

Our direct monoclonal-antibody test for the demonstration of *T. pallidum* in lesion exudates is highly sensitive for the diagnosis of early syphilis and is intrinsically specific. In this

study, all 30 patients with early syphilis had a positive direct monoclonal-antibody test, whereas only 28 had diagnostic serologic tests, and 29 had typical spirochetes visualized by dark-field microscopy. None of the 31 patients without syphilis had positive direct immunofluorescence tests. The FTA-ABS test was positive in 2 of the 31 patients without syphilis because of prior treated syphilis. Perhaps more importantly, in seven patients without syphilis, dark-field microscopy revealed motile spiral organisms which, in two instances, were confused with *T. pallidum* by experienced venereologists. Thus, direct immunofluorescence was more specific than dark-field microscopy. Cellular debris, which is highly refractile when viewed in dark-field microscopy and may hinder the visualization of treponemes, was stained with Evans blue counterstain, which neither hindered nor confused the visualization of the brightly fluorescent treponemes stained with the direct monoclonal-antibody reagent.

Although the direct monoclonal-antibody test is unlikely to replace dark-field microscopy in many sexually transmitted disease clinics because of the low cost of the latter, the direct immunofluorescence test represents a potentially valuable diagnostic tool for use in private practice offices and clinics, in which genital ulcer disease is relatively uncommon and in which dark-field microscopy is rarely performed. Even in sexually transmitted disease clinics, this method could be used for the evaluation of anal, rectal, or oral lesions, for which dark-field microscopy is difficult. After preparation of acetone-fixed direct smears, slides could be readily transported to a location at which staining and immunofluorescence microscopy could be performed. The monoclonal-antibody reagent may also have applications for the detection of pathogenic treponemes in tissue sections from patients with cutaneous manifestations of secondary syphilis or the occasional patient with syphilitic involvement of other tissues (cardiovascular system, central nervous system, lymph nodes, etc.).

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