Sensitivity and Specificity of Monoclonal Antibodies Directed Against Antigenic Determinants of *Treponema pallidum* Nichols in the Diagnosis of Syphilis

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Murine anti-*Treponema pallidum* monoclonal antibodies were employed in studies on sensitivity and specificity of binding to examine their potential for use in the detection of low numbers of pathogenic treponemes present in various body fluids. Monoclonal antibodies were used as a primary antibody source in a solid-phase immunoblot assay system. All monoclonal antibodies assayed were capable of detecting ca. $1.0 \times 10^3$ to $2.5 \times 10^3$ treponemes. Of 13 monoclonal antibodies examined, 3 were able to detect $10^3$ virulent treponemes, and 1 of these antibodies was able to reveal the presence of as few as 500 organisms. Western blot analyses showed that all anti-*T. pallidum* monoclonal antibodies exhibiting high sensitivities for the detection of *T. pallidum* cells were directed against an abundant, 47,000-dalton surface-exposed antigen of the organism (S. A. Jones, K. S. Marchitto, J. N. Miller, and M. V. Norgard, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, B173, p. 46; K. S. Marchitto, S. A. Jones, and M. V. Norgard, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, B182, p. 48). Differences in binding properties of the various monoclonal antibodies were most likely a reflection of differential binding affinities or their specificities for different epitopes on the 47,000-dalton surface antigen. With two possible exceptions, the monoclonal antibodies tested reacted specifically with *T. pallidum*, either purified or found within a high-contaminating tissue background, and not with *Treponema phagedenis* biotype Reiter, *Haemophilus ducreyi*, *Neisseria gonorrhoeae*, herpes simplex virus type 2, or normal rabbit testicular tissue. The high sensitivity and specificity exhibited by these anti-*T. pallidum* monoclonal antibodies make them excellent candidates for employment in new syphils or other treponemal diagnostic tests designed to detect very low numbers of pathogenic treponemes in lesion exudates or other body fluids.

The prevalence of syphilis throughout the world, together with the potential debilitating nature of its clinical manifestations, points to the importance of effective measures to diagnose and control the disease. Biological properties of the etiological agent *Treponema pallidum* ssp. pallidum (*T. pallidum*), including growth requirements, specific antigenic structure, and immunological specificity, remain obscure (22). This has prevented the identification of *T. pallidum* in the early lesions of patients with acquired or congenital syphilis and in the cerebrospinal fluid (CSF) of patients with neurosyphilis by means of in vitro cultivation or by the use of rapid, simple, specific, and sensitive antigen-antibody assay systems.

At present, in the diagnosis of early syphilis, dark-field microscopy is the procedure used to identify *T. pallidum* in lesion exudates; this method is based upon the observance of characteristic spirochetal morphology and motility. The clinical diversity of early syphilitic lesions (4, 5), their similarity to those which may occur among patients with other genital ulcer diseases (25), and the lack of inconclusive nature of serological reactivity which may present during the primary stage of infection (26) point to the significance assigned to this technique in diagnosis. Further, the results of dark-field microscopy in these circumstances determine the need for treatment and epidemiological follow-up. It is unfortunate that this procedure is fraught with severe biological and technical restrictions which may result in diagnostic errors, inappropriate therapy, misdirection of epidemiological investigation, and the placement of unnecessary stigma upon the patient. The potential presence of host-indigenous, nonpathogenic treponemes in the oral cavity, genitalia, and gastrointestinal tract with morphology and motility similar to *T. pallidum* precludes an unequivocal identification by dark-field microscopy. In addition, the relatively rapid loss of motility in the presence of atmospheric oxygen necessitates rapid examination of slide preparations to ensure observance of the typical pathogen. This liability reduces the chances for accurate identification of the organism in the exudate. Furthermore, the potential presence of few treponemes in early lesions may result in a negative dark-field examination despite the discrete presence of the organism (16, 26). This lack of sensitivity is not surprising. Dark-field examination of *T. pallidum* suspensions containing $10^3$ organisms per ml of exudate is equivalent to 1 organism in 1,000 high dry fields when utilizing the calibrated microscope of one of us (J. N. M.) and would not be detectable. The limitations of the technique are further compounded by the considerable training, experience, and expertise required for the proper use of the dark-field microscope (26). As a result of these complexities, dark-field examination is either not done or often performed incorrectly (16, 26). The World Health Organization Scientific Group on Treponemal Infections has recognized this diagnostic void and has stated, "The greatest need is to improve the capability of health care units to identify treponemes" (27).

The rapid direct fluorescent-antibody–dark-field microscopic technique proposed by Kellogg (10) seemed to offer

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711
promise as a more efficient method than the dark-field microscopic procedure. However, careful analysis of the data (10) indicated a lack of sensitivity, specificity, and reproducibility in comparison with dark-field examination when polyclonal antibody was utilized as conjugate.

The diagnosis of asymptomatic and symptomatic neurosyphilis also represents a serious enigma. Again, the similarity of clinical manifestations to those which occur among patients with other diseases of the central nervous system, the inability to detect *T. pallidum* in CSF of patients, and the lack of sensitive or specific nontreponemal and treponemal serological tests for detecting antibody in CSF (2, 9) contribute to the inaccuracies of differential diagnosis.

The production of murine monoclonal antibodies that react specifically with *T. pallidum* Nichols antigenic determinants and not with the host-indigenous, nonpathogenic *Treponema phagedenis* biotype Reiter or rabbit host testicular tissue antigens has been previously described (20); this work offers meaningful new avenues of approach to the development of one or more procedures which satisfy the criteria of rapidity, simplicity, cost effectiveness, high sensitivity, high specificity, and reproducibility for identification of *T. pallidum* or its constituent antigens in lesion exudates or CSF of patients with early syphilis and neurosyphilis, respectively. This report describes the capability of several anti-*T. pallidum* Nichols-specific monoclonal antibodies for detecting very low numbers of pathogenic treponemes without interacting with the etiological agents of other sexually transmitted diseases which manifest clinically as genital ulcers.

**MATERIALS AND METHODS**

**Bacterial and viral strains and antigens.** The virulent Nichols strain of *T. pallidum* (17) was used as the representative pathogen in this study. It was maintained, cultivated, and isolated from the testicles of infected rabbits as previously described (17, 20). *T. phagedenis* biotype Reiter (Reiter treponeme) was used as a representative saprophytic nonpathogenic treponeme and was cultivated and prepared as described previously (20). A suspension of *Haemophilus ducreyi* in sterile skim milk was provided by Eric Hansen. *Neisseria gonorrohoeae* isolated from a patient at the Sexually Transmitted Diseases Clinic of the Dallas City and County Public Health Department was provided by Gary Cartwright and was maintained on Thayer-Martin medium. *N. gonorrohoeae* cells were suspended in phosphate-buffered saline (PBS). Herpes simplex virus type 2 (strain 186) in skim milk was a gift of Robin Robinson. An extract of normal rabbit testicles (20) was used as a source of normal rabbit testicular antigens (containing both tissue and serum antigens) for control tests. Testicles were minced and extracted in serum-saline medium as previously described for *T. pallidum* (17, 20) but centrifuged only once for 7 min at 250 × g, yielding a turbid supernatant. Protein assays (20) indicated that 10 μl of this preparation contained ca. 120 μg of total rabbit protein and that 50% of this total protein was due to the normal rabbit serum in the serum-saline extraction medium. Preparations stored for up to 6 months at −20°C appeared to possess similar antigenic reactivities, as determined by radioimmunoassay (20).

Fresh suspensions of *T. pallidum* cells were serially diluted (as indicated in the figures) in PBS with siliconized-glass test tubes treated with 2% dichlorodimethylsilane in toluene in an attempt to reduce the quantities of treponemes lost through glass adherence. A starting stock suspension of intact *T. pallidum* cells was quantitated by dark-field microscop y (3, 15), and a 1-μl portion of this and respective dilutions was spotted onto filter paper for use in the immunoblot assay.


**Serological tests for syphilis used to characterize anti-*T. pallidum* monoclonal antibodies.** Mouse anti-*T. pallidum* monoclonal antibodies, either affinity purified (6) or found within hybridoma clone supernatants (20), were tested for their ability to immobilize *T. pallidum* Nichols in the *T. pallidum* immobilization (TPI) test. The TPI assay was carried out, with minor modifications, as previously described (3). Where necessary, penicillinase (BBL Microbiology Systems, Cockeysville, Md.) was incorporated into the test procedure due to the possibility of residual penicillin in the hybridoma clone supernatants.

The microhemagglutination assay for *T. pallidum* antibodies (Sera-Tek; Ames Division, Miles Laboratories, Inc., Elkhart, Ind.) (23) was performed on both respective hybridoma clone supernatants (20) and protein A-Sepharose affinity-purified (6) anti-*T. pallidum* monoclonal antibodies by the methods described by the manufacturer.

**Solid-phase immunoblot assays.** A modification of the colony blot radioimmunoassay (18), originally derived from modifications of procedures described by Henning et al. (7) and Raetz (19), was used to assess the reactivity of anti-*T. pallidum* monoclonal antibodies against various antigens.

In the immunoblot assay, 1 μl of each of the following antigen preparations was spotted onto Whatman no. 42 filter paper strips; *T. pallidum* Nichols, 1 × 10⁸ to 5 × 10⁷ cells per ml (quantitated by dark-field microscopy [3, 15]) in PBS; *T. pallidum* Nichols harvested from minced primary chancre lesions of experimental rabbits, 1 × 10⁷ cells per ml; *T.

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<th>TABLE 1. <em>T. pallidum</em> monoclonal antibodies and their relevant characteristics</th>
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* Monoclonal antibodies were stored at −70°C before use.

* Microhemagglutination assay for *T. pallidum* antibodies (MHA-TP) (Sera-Tek, Ames Division, Miles Laboratories) was performed on both hybridoma clone supernatants (20) and affinity-purified (6) monoclonal antibodies according to the instructions of the manufacturer.

* TPI assay was performed on hybridoma clone supernatants (20), affinity-purified (6) monoclonal antibodies, or both as described previously (3, 20).
**phagedenis** biotype Reiter, 2 × 10^8 cells per ml in PBS; *H. ducreyi*, skim milk suspension of 5 × 10^9 CFU/ml in PBS; *N. gonorrhoeae*, 1 × 10^8 cells per ml in PBS; herpes simplex virus type 2, skim milk suspension of 1 × 10^8 PFU/ml in PBS; and normal rabbit testicular antigens, as previously described (20).

Fixation was accomplished by allowing the samples to air dry for 30 min. Filter strips with antigens were presoaked for 30 to 60 min at 4°C in PBS containing 4% (vol/vol) fetal bovine serum and 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.; radioimmunoassay grade). Strips were then exposed to the primary monoclonal antibody by moving strips to tubes containing either 8 ml of fresh presoak buffer mixed with 4 ml of a 3- to 4-day-old hybridoma clone supernatant containing anti-*T. pallidum* monoclonal antibody (20) or 10 ml of fresh presoak buffer supplemented with 20 μg of affinity-purified (6) anti-*T. pallidum* monoclonal antibody per ml. Tubes were rocked for 3 h at 4°C, and unbound monoclonal antibody was removed by washing the strips four times in successive 10-ml portions of PBS plus 4% fetal bovine serum (rocking for 30 min at 4°C for each wash). To probe for binding of monoclonal antibody to respective antigens, strips were moved to tubes containing 12 ml of PBS plus 4% fetal bovine serum supplemented with 1 × 10^6 CPM of freshly 125I-labeled (8), affinity-purified (13) rabbit anti-mouse immunoglobulin G (IgG) (heavy and light chain specific) (20) (specific activity of ca. 2.2 × 10^7 CPM/μg). Tubes were rocked gently overnight at 4°C. Excess 125I probe was removed by washing the strips four successive times in 12-ml portions of PBS (30 min at 4°C for each wash). Strips were then removed, air dried, mounted on cardboard, and exposed to Fuji X-ray film with an enhancing screen (Kodak Cronex Lightening Plus) (12) from 2 h to 4 days for autoradiographic analysis of monoclonal antibody reactivity with antigens.

**Western blots of treponemal proteins.** *T. pallidum* was purified by the Percoll density gradient method of Hanff and colleagues (P. A. Hanff, S. J. Norris, M. A. Lovett, and J. N. Miller, Sex. Trans. Dis., in press). After density gradient centrifugation, treponemes were washed free of Percoll by suspending the cells in PBS, followed by centrifugation at 13,500 × g for 5 min. This step was repeated several times to ensure that the organisms were free of Percoll.

Treponemes were suspended in 1 ml of PBS and sonicated on ice for 1 to 5 min with a microtip at a 50% pulse with a Branson model 350 sonicator at a setting of 4 to 5. The 1-ml sonicate was diluted with 0.5 ml of digestion buffer composed of 0.1875 M Tris-hydrochloride (pH 6.8), 30% (vol/vol) glycerol, 6% (wt/vol) sodium dodecyl sulfate, and 0.25% (wt/vol) bromophenol blue as tracking dye. As a modified Laemmli procedure, the sonicate suspension was solubilized and reduced by boiling for 5 min in the presence of 5% (vol/vol) 2-mercaptoethanol before loading onto sodium dodecyl sulfate-polyacrylamide gels (11) and subsequent electrophoresis. Gels with molecular weight markers were fixed and stained with 0.1% Coomassie brilliant blue.

Electrophoretic transfer of proteins to nitrocellulose paper was effected by a modification of the Western blot methods of Towbin et al. (24) and Burnette (1), using a Trans-blot apparatus (Bio-Rad Laboratories, Richmond, Calif.). After a 5- to 10-min equilibration period in a 20-mM Tris base, 150 mM glycine, and 20% methanol), polyacrylamide gels were placed on wet filter paper (Whatman no. 1), and strips of nitrocellulose (Bio-Rad; 0.45 μm) were layered over the individual lanes. A second piece of filter paper was

layered over the nitrocellulose, and the sandwich was placed between supports and loaded into the Trans-blot apparatus with nitrocellulose facing the anode. The chamber was filled with blotting buffer, and a voltage gradient of 8 to 10 V/cm was applied for 16 h at 4°C.

Specific immunological detection of antigens was employed. Nitrocellulose strips were incubated in 20 μg of primary antibody (murine anti-*T. pallidum* monoclonal antibody) per ml in PBS plus 0.05% Tween 20 for 1 to 3 h. The strips were then washed twice in PBS plus Tween 20 for 10 min. For detection of bound monoclonal antibody, the strips were incubated for 1 h in a 10^-3 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.). Excess antibody was removed from the strips by washing four times thoroughly in PBS plus Tween 20. The strips were rinsed in distilled water and developed with one part of a 3-mg/ml solution of 4-chloro-1-naphthol (Sigma) in methanol mixed with five parts of 200 mM sodium chloride plus 10 mM Tris-hydrochloride (pH 7.2). Hydrogen peroxide was added to a final concentration of 0.01%, and the strips were immersed in this solution for 10 min. As purple bands appeared, the strips were placed in a solution of 0.1% sodium azide to inhibit the peroxidase reaction. The strips were then dried and mounted.

**RESULTS**

Sensitivity of *T. pallidum* detection with monoclonal antibodies in the immunoblot assay. Figures 1 and 2 show the results of *T. pallidum* detection with monoclonal antibodies in the immunoblot assay during initial screening experiments. A total of 11 different monoclonal antibodies were examined for their ability to detect decreasing levels of *T. pallidum* cells (Fig. 1). All antibodies tested detected spots containing 100,000 and 10,000 treponemes. Five of these clones, 3G5, 4H7, 13C6, 13G10, and 8G2, reacted with as few as 1,000 *T. pallidum* cells. In this particular experiment, however, monoclonal antibody 8G2 produced a somewhat higher background binding level of reactivity than the other antibodies tested. Figure 2 shows the results of sensitivity testing with monoclonal antibody 11E3 and four of the five most sensitive antibodies (8G2, 1F4, 13C6, and 3G5) previously examined in Fig. 1. The starting level of *T. pallidum* cells per spot was increased fivefold (Fig. 2). All five antibodies tested by immunoblot assay (Fig. 2) reacted well with 500,000 and 50,000 treponemes, and the PBS negative control was clearly negative. Among these clones, only antibody 13C6 reacted with fewer than 50,000 *T. pallidum* cells; as few as 500 treponemes could be reproducibly detected (Fig. 3) by antibody 13C6. The reactivity of

![Graph](http://jcm.asm.org.com/ by guest)
antibody 13C6 with only 50 treponemes was not reproducible and was only observed occasionally (Fig. 2). Due to the apparently increased ability of monoclonal antibody 13C6 to detect low levels of treponemes as compared with other monoclonal antibodies tested, antibody 13C6 was mixed in combination with other selected monoclonal antibodies. The antibody mixtures were reacted with T. pallidum cells in an attempt to assess whether selected combinations of monoclonal antibodies could significantly enhance the detection of even fewer treponemes. The reactivity of monoclonal antibody 13C6 was not significantly enhanced when reacted against T. pallidum in combination with antibodies 8G2, 3G5, and 9B12 (Fig. 3). Antibody 13C6 was essentially as reactive with T. pallidum when used alone or in combination. Similarly, monoclonal antibody 9B12 yielded no increase above its own signal when used in combination with 3G5. As in other cases, the PBS control was negative. As supported by results shown in Fig. 2, the detection of as few as 500 T. pallidum cells by antibody 13C6 (Fig. 3) was reproducible.

Figure 4 shows the results of an additional immunoblot assay with monoclonal antibodies selected on the basis of reactivity in Fig. 1 to 3 but tested against a somewhat more selective dilution series of treponemes. Upon retesting in this immunoblot assay, treponemes were carefully diluted to accurately determine the limits of sensitivity for the monoclonal antibodies. Monoclonal antibody 11E3 was capable of detecting between 1,000 and 2,500 T. pallidum cells, although background binding to filters by monoclonal antibody 11E3 has been a hindrance in these assays (Fig. 4). The nature of this background binding to cellulose or nitrocellulose has been unclear but reproducible, even though others (Jones et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984) have shown monoclonal antibody 11E3 to be specifically directed against a 47,000-dalton immunodominant antigen of T. pallidum. Monoclonal antibody 8G2, as in previous experiments, yielded a discrete signal above background when reacting with only 1,000 treponemes. The detection characteristics for monoclonal antibody 13C6 were similar to those observed for monoclonal antibody 8G2. As found in previous experiments, prolonged exposure of the autoradiograms shown in Fig. 4 revealed that 13C6 was capable of detecting as few as 500 treponemes in this assay (data not shown). Such prolonged incubation for autoradiography did not significantly increase the background, thereby allowing T. pallidum detection at this low level. Thus, provided that background in the test assay system could be kept to a minimum, monoclonal antibodies 11E3, 13C6, and 8G2 show extreme sensitivity in the ability to detect as few as 500 to 1,000 treponemes. The remaining antibodies tested, 3G5, 4H7, 13C8, 13G10, and 9B12, all revealed the presence of 2,500 T. pallidum cells; this was slightly reduced from those sensitivities exhibited by clones 11E3, 13C6, and 8G2. Figure 4 shows background binding of the rabbit anti-mouse probe to a similar test in which PBS was used in place of monoclonal antibody. Although some minor background binding was observed at the lowest dilutions (i.e., when
FIG. 5. Western blot of *T. pallidum* antigens with anti-*T. pallidum* monoclonal antibodies. Solubilized antigens of *T. pallidum* were detected after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and protein transfer by incubating nitrocellulose strips with various anti-*T. pallidum* monoclonal antibodies or with PBS as a negative control before immunological detection with horseradish peroxidase-conjugated goat anti-mouse IgG. K, Kilo daltons.

large numbers of treponemes and tissue contaminants were present), no binding in this test was observed near the limits of detection obtained when samples were treated with the respective monoclonal antibodies. The rabbit anti-mouse IgG probe used in this study, although purified by affinity chromatography, was never preabsorbed against rabbit testicular tissue antigens to reduce minor antibody components that cross-react with rabbit tissue antigens which may be present in the probe preparation. In any event, all the monoclonal antibodies shown in Fig. 4B and tested in the immunoblot assay were capable of detecting ca. 1,000 to 2,500 treponemes per spot test.

**Western blot analyses.** Several monoclonal antibodies pre-selected upon the basis of high sensitivity for *T. pallidum* detection were employed in Western blot assays to determine their respective binding to *T. pallidum* antigens. Antibodies 4H7, 8G2, 11E3, 3G5, 13C6, 13C9, 9B12, and 13G10 bound to similar antigens possessing apparent molecular weights of 47,000 (major band) to 48,000 (minor band) (Fig. 5). When tested in the Western blot or other immunoblot assays with equivalent amounts of *T. phagedenis* biotype Reiter cells, none of these monoclonal antibodies bound to Reiter antigens (14, 20; Marchitto et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984; Jones et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984). Anti-*T. pallidum* monoclonal antibodies directed against the 47,000-dalton surface immunogen of *T. pallidum* have proved to possess the highest degree of sensitivity for the detection of *T. pallidum* cells and antigens; this is consistent with previous conclusions that the 47,000-dalton immunogen of *T. pallidum* may be one of the most abundant immunodominant antigens on the surface of virulent organism(s) (14; Jones et al., Abstr. Annu. Meet.

lesions of rabbits (row 2) and suspended in a heavy background of the tissue exudate also yielded positive signals. All antibodies tested failed to react with the nonpathogenic Reiter treponeme, a clinical isolate of *H. ducreyi*, as well as with an extract of normal rabbit testicular material. With the possible exception of monoclonal antibody 13C8, each of the antibodies also failed to react with herpes simplex virus type 2 and *N. gonorrhoeae*. These results indicated a high degree of specificity of the monoclonal antibodies for *T. pallidum*.

When monoclonal antibody 13C6 was tested in a similar assay for reactivity with these sexually transmitted pathogens (data not shown), minor cross-reactivity with the *N. gonorrhoeae* and normal rabbit tissue preparations was observed.

**DISCUSSION**

Results presented in this study employing monoclonal antibodies to detect *T. pallidum* cells extracted from rabbit tissue and *T. pallidum* cells present in experimental syphilitic lesion exudate provide promise for exploiting the application of monoclonal antibody detection systems in the diagnosis of treponemal infections of humans. Most of the monoclonal antibodies described in this report were shown to be directed primarily against a *T. pallidum* antigen with an apparent molecular weight of 47,000. Previous studies have shown that the 47,000-dalton antigen of *T. pallidum* is an abundant, surface-exposed immunogen of the organism (Jones et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984) which is found in several pathogenic treponemal subspecies but is absent in a representative nonpathogen, *T. phagedenis* biotype Reiter (14; Marchitto et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984). Anti-*T. pallidum* monoclonal antibodies directed against the 47,000-dalton surface immunogen of *T. pallidum* have proved to possess the highest degree of sensitivity for the detection of *T. pallidum* cells and antigens; this is consistent with previous conclusions that the 47,000-dalton immunogen of *T. pallidum* may be one of the most abundant immunodominant antigens on the surface of virulent organism(s) (14; Jones et al., Abstr. Annu. Meet.

FIG. 6. Solid-phase immunoblot assay of anti-*T. pallidum* monoclonal antibodies against various sexually transmitted pathogens. Row 1, 1×10⁶ purified *T. pallidum* cells; row 2, a primary lesion, produced by intradermal inoculation of a rabbit, was excised and minced in 100 µl of PBS, and the number of organisms (1×10⁷) per ml of exudate was quantitated by dark-field microscopy (3, 15) before the application of 1 µl to the filter; row 3, 2×10⁶ *T. phagedenis* biotype Reiter cells; row 4, *H. ducreyi*; row 5, *N. gonorrhoeae*; row 6, herpes simplex virus type 2; row 7, normal rabbit testicular tissue extract.
Am. Soc. Microbiol. 1984; Marchitto et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1984). Preliminary evidence derived from competitive binding assays suggests that although the monoclonal antibodies described (Fig. 5) are apparently directed against the same surface antigen(s) of *T. pallidum*, it is probable that these antibodies recognize different epitopes of the antigen or exhibit different binding affinities. Monoclonal antibodies against certain epitopes of this immunodominant antigen of *T. pallidum* characteristically are reactive in the TPI test, may be reactive in the microhemagglutination assay for *T. pallidum* antibodies, and even possess complement-dependent neutralizing activity against virulent organisms. The fact that monoclonal antibody 13C6 and other monoclonal antibodies react with a dominant surface antigen of *T. pallidum* is consistent with their relatively high sensitivity level of detection in the immunoblot assay.

It is noteworthy to emphasize the high degree of sensitivity for the detection of *T. pallidum* cells that was achievable with monoclonal antibodies against *T. pallidum*. Virtually all of the anti-*T. pallidum* monoclonal antibodies surveyed in this study were capable of efficiently detecting 5,000 to 10,000 treponemes (Fig. 1, 3, and 4). These antibodies could also detect 10,000 organisms present within a high-tissue background (Fig. 6). Also of significance was the observation that several of the anti-*T. pallidum* monoclonal antibodies were capable of revealing the presence of fewer than 5,000 organisms without the application of sophisticated immunoenhancing procedures. For example, the eight monoclonal antibodies employed in experiments shown in Fig. 4B efficiently detected as few as 2,500 treponemes upon prolonged exposure (4 days) of the X-ray film during autoradiography. With regard to these sensitivity studies, however, the most encouraging results were obtained when monoclonal antibodies from hybridoma clones 8G2, 11E3, and 13C6 were examined for their ability to detect fewer than 1,000 *T. pallidum* cells. The combined results of Fig. 2 to 4 showed that these three monoclonal antibodies could detect as few as 500 to 1,000 *T. pallidum* cells. The technical problem associated with the background binding of monoclonal antibody 11E3 to cellulose filter paper has hindered its utility for detecting very low numbers (i.e., less than 1,000 organisms). Alternative conditions to eliminate this background binding are currently being examined. The most promising sensitivity results were achieved with monoclonal antibody 13C6. Monoclonal antibody 13C6 is routinely capable of detecting as few as 500 *T. pallidum* organisms in the immunoblot detection assay system (Fig. 2, 3, and 4B). The fact that a monoclonal antibody may be capable of detecting so few bacterial cells in the absence of additional immunoenhancement is one of our most significant findings. Indeed, the results further substantiate the feasibility of employing one or more monoclonal antibodies directed against *T. pallidum* for the detection of low levels of organisms in human lesion exudates and other body fluids.

Despite these encouraging sensitivity data, a disappointing result was obtained with monoclonal antibody 13C6 upon further testing. Repeated testing with our solid-phase immunoblot method revealed that monoclonal antibody 13C6 possessed minor cross-reactivity with both *N. gonorrhoeae* and normal rabbit testicular tissue preparations. The nature of this cross-reactivity remains obscure, but experiments are in progress to assess more accurately the characteristics and degree of this cross-reactivity.

With respect to the overall specificity of anti-*T. pallidum* monoclonal antibodies for pathogenic treponemes, test methods employed in this study showed that 9 of the 12 anti-*T. pallidum* monoclonal antibodies assayed exhibited good specificity for *T. pallidum*, even in the presence of a high-tissue background (Fig. 6). These nine monoclonal antibodies failed to react with *T. phagedenis* biotype Reiter, *H. ducreyi*, *N. gonorrhoeae*, or a high titer of herpes simplex virus type 2.

The overall detection levels for *T. pallidum* achieved in this study employing monoclonal antibody detection technology merits comparison to the dark-field microscopic procedure. The routine slide preparation involved in performing the dark-field microscopic procedure includes the collection of lesion exudate with a volume of ca. 5 to 10 μl. This volume is placed on a microscope slide, covered, and viewed under the dark-field microscope. For an experienced technician to visualize at least 1 identifiable *T. pallidum* cell in several observed fields, the initial concentration of treponemes in the lesion exudate must approach at least 10⁵ organisms per ml as determined by individual dark-field microscopic procedures (15). Consequently, a 10-μl volume would possess a total of 10,000 treponemes. However, to be successful in identifying at least 1 organism per high dry field that is clearly characteristic of the organism and which is not misconstrued as cellular debris or spirochete fragments, a more practical concentration for successful detection of *T. pallidum* in this technique would approach a total of 10³ treponemes per preparation (i.e., 10⁵ organisms per ml). These estimates also do not take into account the severe masking that takes place as the result of *T. pallidum* organisms attaching closely and tightly to host cellular tissue debris in the preparation. Only free-floating, motile treponemes in the preparation can easily be visualized. Consequently, the use of current monoclonal antibody technology for the detection of *T. pallidum* may be as much as 10- to 100-fold more sensitive than that achieved through the use of the direct fluorescent-antibody–dark-field (10) microscopic examination. Further, the substitution of monoclonal antibody for polyclonal antibody in the dark-field microscopic technique may result in a procedure with improved sensitivity, specificity, and reproducibility. When one adds to this the possibility of further increasing sensitivity and detection levels through the use of immunoenhancement procedures (i.e., sandwich-type assays employing multiple antibody steps), it is clear that the use of anti-*T. pallidum* monoclonal antibodies for the detection of *T. pallidum* cells in human body fluids remains both feasible and promising.

It is concluded from our results that anti-*T. pallidum* monoclonal antibodies can exhibit good specificity and high sensitivity for the detection of *T. pallidum* organisms. Indeed, monoclonal antibody 8G2 has provided the highest degree of sensitivity for detection (ca. 1,000 treponemes) and no cross-reactivity with other sexually transmitted pathogens tested (Fig. 6). On the basis of these positive results, two additional avenues of investigation are warranted. First, the immunoblot assay described in this report, employing 125I-labeled antibody probes, does not reflect the method of choice for routine diagnostic procedures to be carried out in various clinical settings. Problems associated with radioactive probes in general, including the health and safety of personnel and the environment, preclude their overall usefulness. However, many other immunoenhancing detection systems, analogous to those currently in use (21), are being explored. Second, similar studies to those presented here should employ clinical samples from humans to demonstrate correlations between the experimental findings presented in this report and those that may be ascertained when using...
human clinical materials. Thus, this study has provided a foundation for further progress in the development of a new diagnostic test for primary, early congenital, and central nervous system syphilis that may have the characteristics of high specificity, high sensitivity, rapidity, simplicity, and cost effectiveness.

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