Specific and Common Antigens of *Trichomonas vaginalis* Detected by Monoclonal Antibodies

BRUCE E. TORIAN,* ROBERTA J. CONNELLY, RICHARD S. STEPHENS, AND HENRY H. STIBBS

Department of Pathobiology, University of Washington, Seattle, Washington 98195

Received 29 July 1983/Accepted 11 October 1983

Monoclonal antibodies to *Trichomonas vaginalis* were prepared by immunizing mice with a cloned isolate of *T. vaginalis*. Eight antibodies reacted with the same four isolates or strains but did not react with the other *T. vaginalis* strains or isolates tested. All eight antibodies reacted uniformly with both the body and flagella of *T. vaginalis* in the immunofluorescence assay but were unreactive by immunoblotting. The antigen(s) recognized by these antibodies was determined to be present on the surface membrane by indirect immunofluorescence assay of live organisms. The antigen(s) was found to be sensitive to periodate oxidation but resistant to pronase digestion. In addition, one monoclonal antibody was derived which reacted with all *T. vaginalis* isolates or strains tested, as well as with *Trichomonas gallinae*, *Tritrichomonas foetus*, and *Giardia lamblia*. This antibody reacted with the body but not the flagella of Formalin-fixed protozoa in the immunofluorescence assay but failed to react with live organisms. The antigen was found to be periodate resistant but pronase labile. In the immunoblot assay, this antibody detected a single *T. vaginalis* polypeptide with a molecular weight of 62,000.

*Trichomonas vaginalis* is a flagellated parasitic protozoan which is responsible for trichomoniasis, a sexually transmitted disease causing significant worldwide morbidity. Trichomoniasis is currently the most common sexually transmitted disease (23, 27). The disease in women is characterized by vaginitis with an accompanying discharge which ranges from scanty and thin to a frequently malodorous, copious green froth (7, 23). Dyshoria or increased urinary frequency are less common symptoms (7, 23). Women with trichomoniasis at child delivery may have an increased incidence of endometritis and postpartum fever (23). Infection in men has been associated with nonspecific urethritis and other urological conditions, although men harboring the parasite are frequently asymptomatic (7). The recognition of trichomoniasis as a major sexually transmitted disease, numerous reports that document the refractoriness of some *T. vaginalis* infections to treatment with metronidazole (2, 6, 24), and a recent report of a metronidazole-resistant strain (19) prompted us to investigate trichomonal antigens and their properties. Type-specific antigens of *T. vaginalis* have been demonstrated by agglutination and hemagglutination assays using cross-absorbed and nonabsorbed rabbit anti-trichomonas sera (14) and by agglutination and complement fixation assays with human sera (31). Estimates of the number of *T. vaginalis* serotypes range from two in a study involving seven strains (18) to eight in a study involving 19 strains (14). Investigators in Europe (29, 30, 31) detected four serotypes among several hundred isolates obtained in central and eastern Europe. In addition to type-specific antigens, *T. vaginalis* has been reported to possess antigens in common with other trichomonads. Kott and Adler (14), using polyvalent rabbit antisera, reported the existence of agglutinins common to *T. vaginalis* and some *Pentatrichomonas hominis* strains. Alderete (1) showed the cross-reaction of *T. vaginalis* antigens with rabbit antiserum specific for *Trichomonas foetus*. Antigens shared by *T. vaginalis* and *Trichomonas tenax* were observed by gel diffusion techniques (9), whereas antigens common to *T. vaginalis* and *Trichomonas gallinae* were demonstrated by an agglutination test with fragmented cells (26).

Previous reports of immunological evaluation of *T. vaginalis* have been performed with polyvalent anti-trichomonas sera. Using the technique of Kohler and Milstein (13), we prepared monoclonal antibodies to *T. vaginalis*. Sixteen independently derived hybrid cell lines which produce monoclonal antibodies against *T. vaginalis* were isolated. Immunological evaluation of these antibodies confirmed the presence of antigens specific to strains of *T. vaginalis* and the presence of an antigen with a determinant shared by other trichomonads. The specificity of the monoclonal antibodies and preliminary physicochemical investigation of the nature of their respective antigens are presented in this report.

**MATERIALS AND METHODS**

**Organisms and culture.** *T. vaginalis* strains were cultured in (screw-capped borosilicate glass tubes [16 by 125 mm]) by serial passage at 3- to 4-day intervals in modified Diamond TY1-S-33 medium (5) (maltose substituted for glucose) supplemented with 10% newborn calf serum or Meyer HSP-1 medium (20) (maltose substituted for glucose) supplemented with 15% horse serum. *T. vaginalis* isolates PHS-1, PHS-2, PHS-3, and PHS-4 were obtained as cervical, vaginal, or urethral specimens from patients attending the Sexually Transmitted Disease Clinic, Harborview Hospital, Seattle, Wash. Isolates STD-1 and STD-2 were obtained from John Kreiger, Department of Urology, University Hospital, University of Washington, Seattle. The CDC isolate was obtained from Henry Mathews, Centers for Disease Control, Atlanta, Ga. Isolates PHS-1, PHS-2, PHS-3, and PHS-4 were axenized in TY1-S-33 (supplemented with 1,000 IU of penicillin G and 1,000 μg of streptomycin per ml) before cloning three times by the limiting dilution method in 48-well microtiter plates (96-well plates sawed in half) in an anaerobe jar. American Type Culture Collection strains of *T. vaginalis* (ATCC 30187 and ATCC 30238), *T. gallinae* (ATCC 30229), and *T. foetus* (ATCC 30166) were cloned and maintained in either of the media described above.

Organisms for immunization were obtained by centrifuging culture tubes at 250 × g for 10 min. The cells were
washed at least three times with phosphate-buffered saline (PBS; 0.01 M, pH 7.0, with 0.15 M NaCl), resuspended in PBS, pH 7.0, to a concentration of 3.2 x 10^7 cells per ml, and immediately inoculated into mice. Greater than 90% of the cells excluded trypan blue at the time of inoculation. For enzyme-linked immunosorbant assay (ELISA) and immuno blotting, the washed, pelleted organisms were used immediately or stored at -20°C until use. For indirect immunofluorescence (IF), the final pellet was suspended in PBS (pH 7.0), with or without 0.5% Formalin, depending on the IF method used. The Formalin-fixed organisms were left overnight at 4°C, centrifuged at 250 x g for 10 min, resuspended in PBS without Formalin at an appropriate dilution, and used for IF assay the same day. *Giardia lamblia* (strain WB = ATCC 30957) was grown in both of the media previously described, and organisms were prepared for ELISA, immunoblotting, or IF by the same protocol used for trichom onads except that before the initial centrifugation, culture tubes were chilled for 10 min in an ice bath to detach the organisms from the tubes. Cultures of trichomonads and giardia were shown to be free of mycoplasma contamination by culture methods and DAPI staining (12, 25).

**Immunization protocol.** For the first fusion, BALB/c mice were immunized intraperitoneally with 8 x 10^7 live *T. vaginalis* cells (PHS-2J) suspended in 0.25 ml of PBS (pH 7.0). After 11 days, the mice were reinoculated intravenously with 8 x 10^7 T. vaginalis cells (PHS-2J) and sacrificed 3 days later. For the second fusion, BALB/c mice were immunized intraperitoneally with 8 x 10^7 live *T. vaginalis* cells (CDC), boosted intravenously with 8 x 10^7 T. vaginalis cells (PHS-2J) 30 days after the initial immunization, and sacrificed 3 days later.

**Hybridization procedure.** The myeloma cell line BALB/c MOPC 21 NS1/1 (NS1) was originally obtained from C. Milstein (Medical Research Council, Cambridge, England). The NS1/1 cell line produced light chains but no heavy chains. No immunoglobulin was therefore secreted into cell culture medium. NS/1 cells were cultivated in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 15% fetal bovine serum (Hyclone Laboratories, Inc., Davis, Calif.) at a aseptic concentration of 3.2 x 10^7/ml (1). Cultures of trichomonads were prepared for ELISA, immuno blotting, or IF by the same protocol used for trichomonads except that before the initial centrifugation, culture tubes were chilled for 10 min in an ice bath to detach the organisms from the tubes. Cultures of trichomonads and giardia were shown to be free of mycoplasma contamination by culture methods and DAPI staining (12, 25).

**Immunization protocol.** For the first fusion, BALB/c mice were immunized intraperitoneally with 8 x 10^7 live *T. vaginalis* cells (PHS-2J) suspended in 0.25 ml of PBS (pH 7.0). After 11 days, the mice were reinoculated intravenously with 8 x 10^7 T. vaginalis cells (PHS-2J) and sacrificed 3 days later. For the second fusion, BALB/c mice were immunized intraperitoneally with 8 x 10^7 live *T. vaginalis* cells (CDC), boosted intravenously with 8 x 10^7 T. vaginalis cells (PHS-2J) 30 days after the initial immunization, and sacrificed 3 days later.

**Hybridization procedure.** The myeloma cell line BALB/c MOPC 21 NS1/1 (NS1) was originally obtained from C. Milstein (Medical Research Council, Cambridge, England). The NS1/1 cell line produced light chains but no heavy chains. No immunoglobulin was therefore secreted into cell culture medium. NS/1 cells were cultivated in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 15% heat-inactivated fetal calf serum, 1 mM glutamine, and 1 mM pyruvate (this represents complete RPMI medium).

Spleen cells (10^7) were prepared by finely mincing spleens and passing the material through a fine nylon screen. Lymphocytes were washed three times in serum-free RPMI. NS/1 cells in logarithmic growth were washed once in serum-free RPMI. NS/1 cells and lymphocytes were fused at a ratio of 1:4 (in the first fusion) or 1:7 (in the second fusion) in 40% polyethylene glycol by centrifugation at 250 x g for 10 min at ambient temperature (25°C). The cells were washed with 10 volumes of complete RPMI and centrifuged at 160 x g for 5 min at ambient temperature. The supernatant was aspirated and the cells were gently resuspended at a concentration of 2.5 x 10^7 cells per ml in complete RPMI supplemented with 0.1 mM hypoxanthine, 4.0 x 10^-7 M aminopterin, and 1.6 x 10^-10 M thymidine (HAT medium) (16). Thymocytes from 3- to 5-week-old BALB/c mice were added to a final concentration of 2.5 x 10^6 thymocytes per ml. The suspension was plated at 190 µl per well into a 96-well microtest plate (Costar, Cambridge, Mass.). An additional feeding with HAT medium (50% substitution by volume) was given on day 6. The continued handling, feeding schedule, cloning by limiting dilution, and production of ascites were performed as described elsewhere (17).

**Preparation of polyvalent antiserum.** Organisms for immunization were grown in a dialysate (22) of TYI-S-33 supplemented with 10% ‘‘agamma’’ rabbit serum rather than calf serum. Trichomonads were harvested by centrifugation at 250 x g for 10 min, washed three times with 5 mM TES [N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], pH 7.3–0.15 M NaCl (TES-saline), resuspended in TES-saline, and frozen at -20°C until use. The protein concentration of the immunogen was 1.65 mg/ml. Antisera to *T. vaginalis* isolate PHS-2J were prepared in female New Zealand white rabbits by the method of Cooney and Kenny (4). Briefly, 1 ml of immunogen emulsified with 1 ml of incomplete Freund adjuvant was injected intramuscularly into each rabbit. After 21 days, increasing dosages (0.1, 0.2, 0.3, and 0.4 ml) of the immunogen were injected intravenously at 3- to 4-day intervals. Sera were obtained by cardiac puncture 7 days after the last immunization.

**Antibody-binding assay.** The ELISA (33) was used to detect antibodies in culture fluids and ascites fluids. Briefly, 96-well round-bottomed microtiter plates were seeded with 50 µl of a suspension containing 8.6 x 10^5 cells of *T. vaginalis* isolate PHS-2J per ml (live or Formalin-fixed organisms) in PBS (pH 7.6) and incubated at 37°C overnight, allowing organisms to adhere to the wells. The PBS was then discarded, and 100 µl of 15% bovine serum albumin (BSA) in PBS (pH 7.6) was added to each well. The plates were incubated for 3 h at 37°C, and the PBS was discarded. Undiluted culture supernatant or appropriately diluted ascites fluid (50 µl) was added to each well. Plates were incubated at 37°C for 1 h, the supernatants were discarded, and the plates were washed six times with PBS (pH 7.6) containing 1% BSA. Goat anti-mouse immunoglobulin peroxidase conjugate (Cappel Laboratories, West Chester, Pa.) was added (50 µl per well). 1:1,000 dilution in PBS, pH 7.6, containing 1% BSA, the plates were incubated for 1 h at 37°C, the conjugate was discarded, and the plates were washed six times with PBS (pH 7.6). The color reagent containing 4.5 ml of PBS (pH 7.6), 0.5 ml of *ortho*-phenylenediamine (10 mg/ml in absolute methanol) and 5 µl of 30% hydrogen peroxide was then added to each well (33). The plates were incubated at ambient temperature in the dark for up to 1 h and visually inspected for positive reactions or read on a Titertek Multiskan (Flow Laboratories, Inc., Irvine, Calif.). Fluids from wells with no clones or wells to which no culture supernatant or conjugate or neither was added served as negative controls. Polyvalent rabbit anti-*T. vaginalis* serum served as a positive control with goat anti-rabbit immunoglobulin peroxidase conjugate (Antibodies Inc., Davis, Calif.) at a 1:1,000 dilution.

**Indirect IF.** Indirect IF assays of Formalin-fixed organisms were performed by the micro-IF technique of Wang and Grayston (34) with slight modification. Briefly, an appropriate dilution of each Formalin-fixed organism to be tested was spotted onto a glass slide in nine locations which were at least 1 cm from each other. Each antigen was spotted to yield a spot 1 mm in diameter. Each location (defined as an antigen group) consisted of five different antigen spots. This enabled the simultaneous staining of five different antigens with a single drop of appropriately diluted antibody. The slides were allowed to air dry for 1 h, fixed in acetone for 15 min, and then air dried. Each antigen group was then incubated with one bacteriological loopful of appropriately diluted culture supernatant or ascites fluid which entirely covered one antigen group. The slides were incubated for 45 min at 37°C in a moist chamber, washed vigorously three times in PBS and three times in distilled water, and then dried at 37°C. Goat anti-mouse immunoglobulin fluorescence-labeled conjugate at a 1:50 dilution (Antibodies Inc.) was added to each antigen group with a bacteriological loop. The
slides were then incubated, washed, and dried as above. Cover slips were mounted with a drop of solution containing para-phenylenediamine (10 mg/ml in 90% glycerol and 10% PBS) (11) and slides were observed for fluorescence with a Wild M-20 (Wild, Switzerland) microscope. Anti-Chlamydia trachomatis ascites fluid was used as a negative control. To ensure that antibodies were specific for T. vaginalis and not other medium components, organisms were assayed in the micro-IF after being grown in HSP-1 medium with 15% horse serum. This medium shares no components with TYI-S-33.

To determine whether the antigens detected by micro-IF were on the surface membrane of the parasites, we performed an IF assay on live organisms. Briefly, 50 µl of heat-inactivated ascites fluid was added to 100 µl of a suspension of washed T. vaginalis (2 × 10^6 cells per ml in PBS, pH 7.0). The mixture was incubated at 37°C for 30 min. The cells were washed three times in PBS and resuspended in 100 µl of PBS. Fluorescein-conjugated goat anti-mouse immunoglobulin (50 µl, 1:50 dilution in PBS) was then added to the cells. After incubation at 37°C for 30 min, centrifugation at 250 × g for 10 min, and three washes in PBS, the cells were resuspended in PBS. Trichomonads remained motile throughout this procedure. Three-microliter portions of the suspension were allowed to air dry on glass slides, and a cover slip was mounted as described for the micro-IF.

**Physicochemical characterization of antigens.** The physicochemical nature of the trichomonal antigens was assessed by ELISA after subjecting T. vaginalis isolate PHS-2J preparations to pronase digestion or periodate oxidation (28). Live or Formalin-fixed organisms were adsorbed to the wells of microtiter plates before treatment. For the pronase treatment, 50 µl of pronase (100 µg/ml in PBS, pH 7.6; Calbiochem, LaJolla, Calif.) or PBS was added to each well. The plates were incubated at 37°C for 2 h and washed three times with PBS (pH 7.6) before assay. Periodate oxidation was performed at 4°C for 24 h with 0.05 M sodium metaperiodate (Sigma Chemical Co., St. Louis, Mo.) in 0.01 M sodium acetate buffer, pH 4.5. The plates were washed three times with PBS and tested by ELISA. Organisms were observed adhering to the wells of the microtiter plates before the addition of the color reagent.

**Immunoblotting procedure.** T. vaginalis antigen preparations were solubilized with an equal volume of 2x sample buffer (4% sodium dodecyl sulfate, 10% β-2-mercaptoethanol, 0.02% bromophenol blue, 20% glycerol, 137 mM Tris-hydrochloride, pH 6.8) for 4 min at 100°C. Antigen samples containing 45 µg of protein were electrophoresed through 10% polyacrylamide slab gels in the discontinuous buffer system described by Laemmli (15). Electrophoresis was performed at 20 mA in the stacking gel and 40 mA in the separating gel and was continued until the solvent front reached the bottom of the gel. 14C-methylated protein standards visualized after autoradiography included myosin (200,000), phosphorylase B (97,400), ovalbumin (46,000), carbonic anhydrase (30,000), and cytochrome c (12,300) (New England Nuclear Corp., Boston, Mass.). Immunoblotting of the gels was performed by the method of Towbin et al. (32). Briefly, the proteins were electrophoretically transferred to nitrocellulose paper (Bio-Rad Laboratories, Richmond, Calif.) at 10 V for 16 h. The nitrocellulose sheets were then incubated with 5% BSA in PBS, pH 7.6, for 3 h at 37°C. The sheets were subsequently incubated for 45 min at ambient temperature with appropriate dilutions of ascites fluids (in PBS, pH 7.6, containing 1% BSA and 0.05% Tween 20), washed for 1 h with six changes of TES-saline containing 0.05% Tween 20, and incubated for 1 h with peroxidase-conjugated goat anti-mouse immunoglobulin antiserum (1:1,000 in PBS, pH 7.6, containing 1% BSA and 0.05% Tween 20). After washing for 1 h with six changes of TES-saline containing Tween 20, the sheets were washed once in TES-saline without Tween 20 and then stained for approximately 5 min with a solution containing 0.50 mg of 4-chloro-1-naphthol per ml and 0.001% hydrogen peroxide in TES-saline.

**Protein estimation.** Protein estimations of antigen preparations were determined by the method of Bradford (3).

**RESULTS**

**Isolation of hybrid cell lines producing anti-trichomonal antibodies.** Hybridization was performed by fusion of NS/1 myeloma cells and lymphocytes from BALB/c mice immunized with isolate PHS-2J only or a combination of isolates PHS-2J and CDC. A total of 1,536 microtest wells were seeded from two hybridizations (768 wells per hybridization). Twelve days after each fusion, the culture fluid from each well was tested by ELISA for antibodies against T. vaginalis (PHS-2J). Of the 22 culture supernatants demonstrating antibody against T. vaginalis in the first fusion, 11 gave particularly strong reactions. These cultures were serially passaged at low density, reassayed, cloned twice by the limiting dilution method, and assayed for antibody after each cloning. This process resulted in the isolation of five phenotypically stable, cloned cell lines that produced monoclonal antibody against T. vaginalis. In the second fusion, 18 culture supernatants reacted strongly with T. vaginalis. These wells were handled as described above to yield 11 phenotypically stable, cloned cell lines. Antibodies produced by the first fusion were designated 1-1 through 1-5 and those from the second fusion were designated 2-1 through 2-11. Of the 16 monoclonal antibodies, 9 were chosen for analysis.

**Reaction patterns of monoclonal antibodies in the micro-IF assay and IF assay of live cells.** Serial dilutions of each antibody tested were reacted against seven isolates and two strains of T. vaginalis and one strain each of T. gallinae, T. foetus, and G. lamblia. The antibody titers were greater than 1:3,000 with ascites fluids. At titration endpoints, two distinct IF patterns were observed (Fig. 1). Eight monoclonal antibodies stained both the body and flagella, whereas antibody 1-3 stained only the body of the trichomonads. The body/flagella antibodies stained the body and flagella of the cells uniformly. The intensity of the stain on different strains of trichomonads varied slightly, depending on the strain employed. Antibody 1-3 stained only the body of the trichomonads and G. lamblia. This antibody appeared to be excluded from the nucleus of all cells tested. Organisms grown in HSP-1 gave a fluorescence pattern identical to that of those grown in TYI-S-33. Monoclonal antibody specific for C. trachomatis (28) served as negative control and did not react with any of the organisms tested. IF assay of live organisms suggested that eight antibodies were specific for determinants located on the surface membrane, and one (antibody 1-3) which failed to react with live organisms was likely to be specific for a cytoplasmic component.

**Physicochemical nature of trichomonal antigens.** The sensitivity of trichomonal antigens to various physicochemical treatments was examined by the ELISA test. Pronase digestion of T. vaginalis isolate PHS-2J abolished the binding of monoclonal antibody 1-3 but did not alter the binding of any other antibodies. All monoclonal antibodies, with the excep-
tion of 1-3, did not react with antigen preparations which had been treated with periodate. Results were identical for Formalin-fixed and live organisms.

Immunological specificity of anti-trichomonal monoclonal antibodies in the ELISA. In the ELISA, titration endpoints of ascites fluids were greater than 1:10,000. At endpoint dilutions, two different specificity profiles were observed (Table 1). Eight antibodies reacted with antigenic determinants found only on specific strains or isolates of *T. vaginalis*. Antibody 1-3 reacted with all strains and isolates of *T. vaginalis* tested as well as with *T. gallinae*, *T. foetus*, and *G. lamblia*. Wells to which no antibody, no conjugate, or monoclonal antibody specific for *C. trachomatis* were added served as controls and were consistently negative.

Identification and characterization of trichomonal antigens by the immunoblot technique. Trichomonads were subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and β-mercaptoethanol. The separated polypeptides were transferred to nitrocellulose paper for immunological analysis. Antibodies reactive in the micro-IF to both the body and flagella of the organisms did not react by immunoblotting. Antibody 1-3, reactive with only the body of *T. vaginalis*, detected a single polypeptide band of approximately 62,000 molecular weight in all strains and isolates of *T. vaginalis* tested (Fig. 2). This antibody detected antigens in *T. gallinae* (molecular weight, 62,000) and *T. foetus* (molecular weight, 60,000) (data not shown). Interestingly, antibody 1-3 also reacted with a 68,000-molecular-weight antigen of *G. lamblia* (Fig. 3).

**DISCUSSION**

Trichomoniasis is currently the most frequently diagnosed sexually transmitted disease (23, 27). Surprisingly, little information has been presented concerning the antigenic nature of the parasite and the relationship of antigens to virulence or disease pathogenesis. Alderete (1) has demonstrated overall similarities in protein profiles between various strains of *T. vaginalis* based on comigration and intensity of individual protein bands in Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gels and radioimmunoassays. In spite of overall similarities in protein profiles, type-specific antigens of *T. vaginalis* have been reported (14, 31). In addition, trichomonads have been demonstrated to have significant differences in virulence as assessed by lesion development in mice (10). These differences may be mediated by internal parasite functions and may be reflected by subtle antigenic differences in either protein or carbohydrate antigens on the surface membrane.

In this report, we demonstrated the presence of an antigen with a determinant shared by *T. gallinae*, *T. foetus*, and *G. lamblia* as well as by all strains and isolates of *T. vaginalis* tested. The antigens in the various protozoa had similar molecular weights, ranging from 60,000 to 68,000. This close association in molecular weight between the antigens in the protozoa tested is suggestive of a common function. Reactivity of antibody 1-3 to the antigen was abolished by treatment of the antigen with pronase, suggesting that the antigen may be protein. Furthermore, we have shown that the antigen is unlikely to be a surface membrane component, as no antibody is bound by live organisms and the entire body of the organism (with the exception of the nucleus) is homogeneously stained in IF assays of Formalin-fixed organisms. The exclusion of the stain by the nucleus suggests that the antigen may be cytoplasmic.

The remaining eight monoclonal antibodies suggested a pattern of type specificity. Four out of nine isolates or strains of *T. vaginalis* tested in ELISA and IF assays reacted with the eight antibodies in a similar manner. The remaining five strains or isolates did not react with these antibodies. The results demonstrate a minimum of two antigenic types of *T. vaginalis*, as defined by the monoclonal antibodies in this study. It is likely that more than two immunotypes exist but we were unable to detect them since the initial antibody screening of the hybrids was performed with only the isolate used as the immunogen for the fusions. Not all isolates from the Seattle area were reactive with these antibodies, although the immunogen used for the fusions was isolated in Seattle. The presence of at least two immunotypes in the Seattle area is consistent with previous observations of several immunotypes occurring within a limited geographic area (8). Although several Seattle isolates failed to react with these antibodies, one isolate from a different geographic area

**TABLE 1. Immunological specificity of anti-trichomonal monoclonal antibodies in the ELISA**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Specificity of antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-1, 1-4, 2-3, 2-4, 2-6, 2-7, 2-8, and 2-11</td>
</tr>
<tr>
<td><em>T. vaginalis</em></td>
<td></td>
</tr>
<tr>
<td>PHS-2J</td>
<td>+</td>
</tr>
<tr>
<td>PHS-11</td>
<td>-</td>
</tr>
<tr>
<td>PHS-3A</td>
<td>+</td>
</tr>
<tr>
<td>PHS-4A</td>
<td>-</td>
</tr>
<tr>
<td>STD-1</td>
<td>+</td>
</tr>
<tr>
<td>STD-2</td>
<td>-</td>
</tr>
<tr>
<td>30187</td>
<td>-</td>
</tr>
<tr>
<td>30238</td>
<td>+</td>
</tr>
<tr>
<td><em>T. gallinae</em></td>
<td></td>
</tr>
<tr>
<td><em>T. foetus</em></td>
<td>-</td>
</tr>
<tr>
<td><em>G. lamblia</em></td>
<td></td>
</tr>
</tbody>
</table>
suggestions that the determinant(s) recognized by these antibodies may be carbohydrate. Kott and Adler (14) have previously demonstrated the immunogenicity of the polysaccharide fraction of T. vaginalis, although specific antigens were not characterized. Together, these observations suggest significant carbohydrate involvement in the antigenicity of T. vaginalis. The identical pattern of immunological specificity demonstrated with these antibodies suggests that the antibodies may react with the same determinant.

One antibody (1–3), developed but not included in this report, was apparently directed toward a media component. The antibody reacted with numerous polypeptides in trichomonad antigen preparations when assayed by immunoblotting. Furthermore, this antibody recognized numerous peptide bands in immunoblots of serum. These observations emphasize the importance of extensive controls, and are consistent with a report demonstrating the ability of T. vaginalis to avidly bind specific serum proteins (21).

Monoclonal antibodies should prove to be extremely useful for antigenically distinguishing between T. vaginalis strains, provided that a limited number of immunotypes exist and that a broad spectrum of monoclonal antibodies capable of detecting them are developed. Additionally, these monoclonal antibodies will provide the means for purification and detailed analyses of their respective antigens.

ACKNOWLEDGMENTS

We thank San-Pin Wang and C. C. Kuo for the use of their facilities, George Kenny for advice and critical comments, and Shao C. Hung for technical assistance.

LITERATURE CITED