Identification of a Surface Antigen of *Trichomonas vaginalis*

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A major surface antigen of *Trichomonas vaginalis* was purified by using three independently derived monoclonal antibodies (two immunoglobulin M and one immunoglobulin G1) prepared against *T. vaginalis* PHS-2J. A 115,000-molecular-weight antigen and one or more components with a molecular weight of 58,000 to 64,000 were recovered when any of the three antibodies was used as an immunoadsorbent. The purified antigen reacted with all three monoclonal antibodies in an enzyme-linked immunosorbent assay, indicating that the antibodies recognized the same antigen but not necessarily the same determinant. The purified antigen was sensitive to both pronase digestion and periodate oxidation. The antigen was shown to be on the external surface of some but not all *T. vaginalis* isolates by agglutination of live organisms with the monoclonal antibodies.

A number of workers have investigated the antigenic nature of *Trichomonas vaginalis*, demonstrating antigens in common with other trichomonads, including *Pentatrichomonas hominis* (7), *Trichomonas foetus* (1, 14), *Trichomonas tenax* (6), and *Trichomonas gallinae* (11, 14). Type-specific antigens of *T. vaginalis* have also been demonstrated, but not specifically identified, by agglutination and hemagglutination assays with cross-adsorbed and nonadsorbed rabbit anti-trichomonas sera (7) and by agglutination and complement fixation assays with human sera (13). A previous study with monoclonal antibodies (14) corroborated the existence of type-specific antigens among *T. vaginalis* isolates.

Trichomonal surface molecules have been the subject of two recent investigations. Using both extrinsically and intrinsically radiolabeled trichomonads, Alderete (2) demonstrated the presence of ca. 20 polypeptides which were accessible to antibody in intact cells of a single isolate of *T. vaginalis*. The polypeptides ranged in molecular weight from 20,000 to 200,000. War ton and Honigberg demonstrated the presence of carbohydrates on the cell surfaces of *T. vaginalis* strains by lectin analysis (17, 18). All *T. vaginalis* strains tested showed significant binding to concanavalin A and wheat germ agglutinin, whereas only certain strains bound to soy bean agglutinin and castor bean agglutinin. Garden pea agglutinin did not bind to any of the strains tested. Thus, the composition of surface carbohydrates was shown to vary among *T. vaginalis* strains, although the specific surface molecules responsible for lectin binding were not identified.

Despite these efforts, very little is understood about the precise nature of antigenic surface components or their distribution among *T. vaginalis* isolates. In a previous study (14), the production of eight monoclonal antibodies which reacted with the surfaces of live trichomonads in an immunofluorescence assay was reported. The antigenic determinant(s) recognized by these antibodies was found only on certain isolates of *T. vaginalis* and was sensitive to periodate digestion but not to pronase digestion when whole cells were used as the antigen in an enzyme-linked immunosorbent assay (ELISA) (16). None of the antibodies reacted in immunoblots. Therefore, in the present study, three of these monoclonal antibodies were used to prepare immunoadsorbent columns. Antigens eluted from the columns were detected in immunoblots with polyvalent rabbit anti-*T. vaginalis* serum. The identification and partial characterization of the antigen recovered by the three antibodies are reported here.

MATERIALS AND METHODS

Organisms and cultures. *T. vaginalis* isolates PHS-2J, PHS-3, STD-1, and CDC were cultured in modified Diamond TYI-S-33 medium (3), and organisms were handled as previously described (14).

Production of antibodies. The production of monoclonal antibodies to *T. vaginalis* isolate PHS-2J has been described elsewhere (14). Ascites fluids from three independently derived cell lines, designated 2-3, 2-8, and 2-11, were used in this study. A fourth monoclonal antibody, designated 1-3, reacted with an internal antigen present in all *T. vaginalis* isolates tested (14) and served as a control. Immunoglobulin subclasses of the monoclonal antibodies were determined with an enzyme immunoassay kit (MONOAB-1D EIA; Zymed Laboratories, San Francisco, Calif.). Polyvalent antiserum to *T. vaginalis* isolate PHS-2J was prepared in New Zealand White rabbits as previously described (14). Immunogens were grown in a dialyse medium supplemented with agamma rabbit serum to avoid contamination with medium components (14). Preimmune serum, obtained from rabbits before immunization, served as a negative control.

Indirect immunofluorescence. The indirect immunofluorescence assay of Formalin-fixed trichomonads has been previously described (14).

Agglutination of trichomonads with monoclonal antibodies. Agglutination assays of live trichomonads were conducted in the wells of Costar 96-well cell culture plates. Logarithmic-phase cells of isolate PHS-2J were harvested by centrifugation, washed three times with phosphate-buffered saline (PBS), and resuspended in PBS at a concentration of 3 × 10^5 cells per ml. The cell suspension (35 μl) was combined with 5 μl of heat-inactivated (30 min at 56°C) ascites fluid in a well and was incubated in a humidified chamber for 15 min at 37°C. After resuspension of the cells by gentle shaking, the wells were observed microscopically. Controls for autoagglutination included trichomonads incubated with PBS or...
with ascites fluid containing antibody 1-3, which is specific for an internal trichomonial component (14).

ELISA. The ELISA (16) was used to assess the binding of monoclonal antibodies to eluted antigen, to monitor antibody activity during antibody purification procedures, and to confirm previous observations (14) on the nature of trichomonial antigens in intact organisms. To determine which monoclonal antibodies bound to eluted antigens, each antigen (5 μg/ml in PBS [pH 7.6]; 50 μl per well) was adsorbed to the wells of a 96-well microtiter plate. Control wells received an equivalent concentration of Formalin-fixed cells of T. vaginalis isolate PHS-2J or isolate CDC. To monitor antibody activity during antibody purification, we conducted ELISAs with Formalin-fixed trichomonads as the antigens. The nature of trichomonial antigens was confirmed by an ELISA after subjecting Formalin-fixed trichomonads to pronase digestion or periodate oxidation as previously described (14). Assays were carried out as previously described (14), except that 0.05% Tween 20 was substituted for 1% bovine serum albumin throughout the procedure. Wells to which no antibody, antibody specific for Chlamydia trachomatis (12), or antibody 1-3 was added served as negative controls, and wells to which polyvalent rabbit anti-T. vaginalis serum was added served as positive controls.

Immunoblotting procedure. Whole solubilized trichomonads (20 to 50 μg per lane) or purified antigen (20 to 40 μg per lane) were subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (8), transferred to nitrocellulose sheets (15), and detected in an immunoblot assay with polyvalent rabbit anti-T. vaginalis serum. Immunoblotting was performed essentially as previously described (14), except that nitrocellulose sheets were not blocked with bovine serum albumin. 0.3% Tween 20 replaced 1% bovine serum albumin plus 0.05% Tween 20 in antibody-binding steps, and 0.06% hydrogen peroxide was used in the reaction. Preimmune rabbit serum served as the negative control and did not react with whole solubilized trichomonads or purified antigen in immunoblots. Prestained molecular weight standards (Bethesda Research Laboratories, Gaithersburg, Md.) included myosin (200,000), phosphorylase b (92,500), bovine serum albumin (68,000), ovalbumin (43,000), chymotrypsinogen (25,700), β-lactoglobulin (18,400), and cytochrome c (12,300).

Characterization of the eluted antigen. The nature of the eluted antigen was assessed with the immunoblot technique after pronase digestion or periodate oxidation. For pronase digestion, purified antigen (ca. 40 μg) was incubated with PBS (pH 7.6) or pronase (Calbiochem-Behring, La Jolla, Calif.) (100 μg/ml in PBS) for 2 h at 37°C. For periodate oxidation, sodium metaperiodate (0.05 M in 0.01 M sodium acetate buffer [pH 4.5]) or acetate buffer was incubated with purified antigen for 24 h at 4°C. All preparations were immunoblotted as described above.

Preparation of immunoadsorbent columns. Immunoglobulins of the immunoglobulin M (IgM) class (2-8 and 2-11) were partially purified from their respective ascites fluids by precipitation with 2% boric acid (4). Antibody 2-3, of the IgG1 class, was partially purified by precipitation with 45% saturated ammonium sulfate (4). Each partially purified monoclonal antibody was suspended in coupling buffer (0.2 M sodium bicarbonate [pH 9.5]), and activity was confirmed by titration in an ELISA. Immunoadsorbent columns were prepared by a modification of the method of March et al. (10). Cyanogen bromide-activated Sepharose CL-4B beads were washed and swollen on a sintered glass filter with several portions of 1 mM hydrochloric acid and then suspended in 0.2 M sodium bicarbonate (pH 9.5). Partially purified antibody was coupled to the Sepharose beads at a ratio of 1 to 3 mg of protein to 1 g of Sepharose. Coupling was carried out at 4°C for 20 to 24 h with gentle rocking. After coupling, unbound protein was removed from the beads by extensive washing with PBS. To determine coupling efficiency, we estimated the protein content of the first 20 to 40 ml of the wash. Seventy to ninety-five percent of the protein routinely bound to the beads. The remaining active sites on the beads were blocked by incubating the beads with 1 M glycine at 4°C for 3 h. After being blocked, the beads were washed with 20 volumes each of PBS (pH 7.0), 1 M acetic acid, PBS, 2.5 M sodium thiocyanate in PBS, and PBS with 0.1% azide. The immunoglobulin-conjugated beads were stored in PBS (pH 7.0), with 0.1% azide when not in use. The bed volume of individual immunoadsorbents ranged from 1 to 6 ml. To compare different T. vaginalis isolates, we constructed replicate immunoadsorbent columns from a single preparation of immunoglobulin-conjugated Sepharose beads. Each column was used exclusively for a single isolate of T. vaginalis to avoid cross-contamination of the columns or antigens. As a control for nonspecific binding, a column consisting of Sepharose not linked to any antibody was prepared and run in parallel with the immunoadsorbent columns. No antigens were detected in immunoblots of the eluate from this column.

Immunoabsorption. Washed, pelleted trichomonads were suspended in PBS (pH 7.6) containing 1.0% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride, freeze-thawed three times with a dry ice-ethanol bath, and sonicated. The suspension was centrifuged at 100,000 × g for 30 min to remove insoluble debris, and the supernatant was decanted and loaded onto an immunoadsorbent column. Before use, immunoadsorbent columns were treated with eluting buffer (2.5 M sodium thiocyanate in PBS [pH 7.6]), and then equilibrated with PBS (pH 7.6) containing 1.0% Triton X-100. Affinity chromatography was performed at room temperature. The trichomonal extract was loaded onto a column and incubated with the immunoadsorbent for 30 min. To remove unbound material, we washed the column with six times the column bed volume of PBS containing 0.5% Triton X-100 and then with six times the column bed volume of PBS. Bound material was eluted with 2.5 M sodium thiocyanate in PBS. The sodium thiocyanate eluate was collected, dialyzed against distilled water overnight at 4°C, and concentrated by lyophilization. After use, the columns were equilibrated with PBS containing 0.1% sodium azide and stored at 4°C.

Protein estimation. The protein content of antigen and antibody preparations was evaluated by the method of Lowry et al. (9). The protein content of suspensions of organisms in 1% Triton X-100 was estimated by assay of an equivalent number of organisms suspended in PBS without detergent. Greater than 95% of the cell pellet remained solubilized after detergent treatment and subsequent ultracentrifugation.

RESULTS

Localization of the antigen to the parasite surface. The binding of monoclonal antibodies 2-3, 2-8, and 2-11 to the surfaces of trichomonads was previously demonstrated by an immunofluorescence assay of live parasites (14). Antibodies 2-3 (IgG1), 2-8 (IgM), and 2-11 (IgM) were each shown to agglutinate live cells of T. vaginalis PHS-2J but not those of isolate CDC. Antibody 1-3, specific for an internal
TABLE 1. Characteristics of monoclonal antibodies to T. vaginalis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Immunoglobulin class</th>
<th>Reaction with indicated T. vaginalis isolate in ELISA and immunofluorescence assays</th>
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<tbody>
<tr>
<td>2-3</td>
<td>IgG1</td>
<td>PHS-2J, PHS-3, and CDC</td>
</tr>
<tr>
<td>2-8</td>
<td>IgM</td>
<td>+</td>
</tr>
<tr>
<td>2-11</td>
<td>IgM</td>
<td>+</td>
</tr>
<tr>
<td>1-3</td>
<td>IgM</td>
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trichomonal component (14), did not agglutinate either isolate (Table 1).

Identification of the antigen recognized by the monoclonal antibodies. Solubilized antigen from T. vaginalis isolate PHS-2J was fractionated with an immunoadsorbent column containing antibody 2-8 (IgM). The purified fraction was then detected in immunoblots with polyvalent rabbit antiserum. This antiserum reacted with numerous antigens in solubilized whole-cell preparations of trichomonads, but only one or two prominent bands were detected in eluates of immunoadsorbent columns (Fig. 1). Affinity columns prepared with monoclonal antibody 2-3 (IgG1) or 2-11 (IgM) were also used. In all cases, the antigens bound had apparent molecular weights of 115,000 and 58,000 to 64,000 (Fig. 2). Preimmune rabbit serum served as a negative control in immunoblots and did not react with solubilized whole-cell preparations or purified antigens.

An ELISA was used to investigate the reactivity of monoclonal antibodies with purified antigens. Control wells were seeded with T. vaginalis cells (isolate PHS-2J or isolate CDC). Antibodies 2-3, 2-8, and 2-11 reacted with whole-cell preparations of T. vaginalis isolate PHS-2J but not with those of isolate CDC. Furthermore, the antibodies reacted with antigen purified from isolate PHS-2J with each of the three immunoadsorbent columns. Thus, antigen purified with either IgG1 or IgM antibody were detected by both IgM and IgG1 antibodies, indicating that the binding of antigen to the immunoadsorbent columns was specific and not due to nonspecific adsorption to a particular immunoglobulin class. Antibody 1-3, specific for an internal component of T. vaginalis, served as a negative control and reacted with both PHS-2J and CDC cells but not with the purified antigens. Anti-C. trachomatis monoclonal antibody (12) did not react with any of the trichomonal antigens.

Purification of antigen from heterologous T. vaginalis isolates. The three monoclonal antibodies used in this study reacted with a specific group of T. vaginalis isolates. In a previous report (14), each of these antibodies was shown to react with the same four of nine T. vaginalis isolates tested, suggesting a pattern of type specificity.

To compare different T. vaginalis isolates, we applied Triton X-100 extracts of isolates PHS-2J, PHS-3, STD-1, and CDC to replicate immunoadsorbent columns containing antibody 2-8 (IgM). Column eluates were obtained and analyzed in immunoblots with rabbit antiserum. The eluates obtained from isolates PHS-2J, STD-1, and PHS-3 contained components with molecular weights of 115,000 and 58,000 to 64,000, whereas the eluate from isolate CDC contained no...
detectable antigens (Fig. 3), indicating that the epitope, if present on the CDC isolate, is present in small amounts. However, in the solubilized whole-cell preparation of each T. vaginalis isolate, a 115,000-molecular-weight band was visualized with the polyclonal antiserum, suggesting that all isolates contain an antigen with a molecular weight of 115,000.

Characterization of the trichomonal antigen. The nature of the antigen recognized by monoclonal antibodies 2-3, 2-8, and 2-11 was investigated by purification of the antigen from T. vaginalis isolate PHS-2J with an immunoadsorbent column containing antibody 2-8, followed by treatment of the purified antigen with pronase or periodate and immunoblot analysis with polyclonal rabbit anti-T. vaginalis serum. Periodate oxidation abolished virtually all the antigen-antibody reactivity of the 115,000-molecular-weight antigen and some but not all of the reactivity of the lower-molecular-weight components, whereas pronase digestion abolished the reactivity of all the components (data not shown).

DISCUSSION

In this report, we identified a surface antigen possessing a type-specific determinant(s) present on only certain T. vaginalis isolates. Three monoclonal antibodies representing both IgM and IgGl class antibodies reacted with whole organisms in a type-specific manner. More importantly, the three monoclonal antibodies were shown to be specific for a single antigen, since immunoadsorbent columns containing any of the three monoclonal antibodies removed trichomonal components of molecular weights 115,000 and 58,000 to 64,000 which reacted with each of the three antibodies in an ELISA. In contrast, antibody I-3, which is specific for an internal parasite component, did not react with any fractions eluted from the immunoadsorbent columns. The antigenic determinant(s) recognized by the three antibodies possessed identical susceptibility to pronase and periodate, demonstrating identical profiles in indirect immunofluorescence assays, and had the same distribution among the T. vaginalis isolates studied.

Although it might be argued that the components recovered were nonspecifically adsorbed, the recovery of the same components by both IgM and IgGl class antibodies argues strongly against this possibility, since the components would have had to adsorb nonspecifically to two different immunoglobulins. A column of Sepharose beads which were not linked to any antibody served as a control for nonspecific binding of trichomonal components to Sepharose. When the Sepharose column was run, no antigen was recovered from the eluate, demonstrating that nonspecific interaction of trichomonal molecules with the Sepharose beads did not occur.

An antigen of molecular weight 115,000 was always recovered from solubilized trichomonal preparations by immunoadsorption with columns containing monoclonal antibody 2-3, 2-8, or 2-11. One or a series of bands with a molecular weight of ca. 58,000 to 64,000 also was observed in most purified preparations. The relationship of the lower-molecular-weight bands to the 115,000-molecular-weight antigen has not been ascertained. They may be subunits, precursors to, or breakdown products of the 115,000-molecular-weight antigen.

Alderete (2) used polyvalent rabbit anti-T. vaginalis sera to precipitate radiolabeled surface polypeptides from solubilized trichomonads. Among the precipitated polypeptides were two with molecular weights near 115,000, one of which may correspond to the antigen which we have identified. Kott and Adler (7) demonstrated the antigenicity of trichomonal carbohydrates by performing a hemagglutination assay with erythrocytes coated with a carbohydrate-containing extract of whole trichomonads. The lectin-binding studies conducted by Warton and Honigberg (17, 18) showed that carbohydrates are present on the surface of T. vaginalis and that the composition of exposed trichomonal carbohydrates varies among isolates.

Pronase treatment of purified antigens resulted in the disappearance of the antigen in immunoblots. However, pronase treatment of live (14) or Formalin-fixed organisms in the ELISA caused no decrease in the binding of monoclonal antibodies 2-3, 2-8, or 2-11. The periodate sensitivity of the determinant(s) recognized by these antibodies in the intact organism was reported previously (14) and was confirmed in this study with purified antigens, suggesting that the antigen may contain a carbohydrate structure. Although periodate can react with polypeptides (5), the susceptibility of the antigenic determinant(s) in intact T. vaginalis cells to periodate oxidation but not to pronase digestion indicates that periodate is probably acting on a carbohydrate structure in this case. As a control, the internal antigen recognized by monoclonal antibody 1-3 was, under the same conditions, sensitive to pronase but not to periodate, indicating its probable proteinaceous nature. Taken together, the data suggest that the surface antigen contains a polypeptide moiety which is inaccessible to pronase in the intact organism under the conditions tested and that the determinant(s) recognized by our monoclonal antibodies may be a carbohydrate moiety.

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LITERATURE CITED