Inhibition of *Trichomonas vaginalis* Motility by Monoclonal Antibodies Is Associated with Reduced Adherence to HeLa Cell Monolayers

JOHN N. KRIEGER,1,* BRUCE E. TORIAN,2 JOHN HOM,3 AND MILTON R. TAM,3

Departments of Urology1 and Medicine,2 University of Washington School of Medicine, and Section of Microbiology, Genetics Systems Corporation,3 Seattle, Washington 98195

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Adherence of trichomonads to host epithelial cells appears to be a critical step in the pathogenesis of trichomoniasis. We evaluated the effect of a panel of 10 monoclonal antibodies on attachment of [35S]methionine-radiolabeled *Trichomonas vaginalis* strains to HeLa cell monolayers. Of 10 monoclonal antibodies, 3 totally eliminated motility of PHS2J strain trichomonads and reduced their adherence to 48 to 60% of control values (P < 0.001). However, none of the monoclonal antibodies affected motility or adherence of STD13 strain trichomonads. Although the antibodies all reacted with PHS2J trichomonads by immunofluorescence, there was no correlation between inhibition of adherence and findings on either immunofluorescence or radioimmunoprecipitation. Direct microscopic observations showed that incubation with the monoclonal antibodies did not cause cytolysis of *T. vaginalis*. In quantitative cultures there was no difference in the number of colonies produced by parasites that had been incubated with antibodies that inhibited or had no effect on adherence. We conclude that our monoclonal antibodies reduced adherence not by cytotoxic effects or by competing for specific sites mediating adherence of the protozoa, but by inhibiting motility of *T. vaginalis*.

*Trichomonas vaginalis* is a flagellated protozoan that is transmitted by direct sexual contact (16). The protozoa infect the lower urogenital tracts of both women and men. It is estimated that there are over 2 million cases of symptomatic trichomonal vaginitis per year in the United States (16, 17, 28). *T. vaginalis* infection is also associated with common urogenital conditions in men, such as nongonococcal urethritis and prostatitis, but the precise proportion of cases is uncertain (17). Previous studies have evaluated potential mechanisms of virulence of *T. vaginalis* strains (1–12, 14, 19, 20, 26, 29, 31, 35). Colonization of mucosal surfaces of the vagina or the urethra or both appears to be critical for pathogenesis. Although cytoactive factors have been described (13, 25), cytopathology occurs primarily by contact-dependent destruction of host cells, rather than by toxins or phagocytosis of host cells by the protozoa (6, 11, 12, 21). Therefore, adherence of *T. vaginalis* to mucosal surfaces is critical for colonization of mucosal surfaces, production of cytopathology, and interaction with cellular host defenses. This report describes production of monoclonal antibodies against *T. vaginalis* and use of these antibodies to demonstrate that motility may be critical for adherence of virulent trichomonads to cultured cells.

MATERIALS AND METHODS

*T. vaginalis* strains. *T. vaginalis* PHS2J and STD13 were isolated from men with symptomatic trichomonalis in the absence of other urogenital organisms, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*. The patients' symptoms and signs resolved following treatment with metronidazole. Both strains were virulent when assayed by subcutaneous abscess production in mice (15, 24) and rapid destruction of tissue culture cell monolayers (5, 10, 14, 20, 21).

*T. vaginalis* cultivation and radiolabeling. Cultures were axenized by serial passages in Diamond TYI medium at 37°C in a 5% CO2 atmosphere (23). After isolation, the organisms were cloned in agar (22) and then either used within 2 to 3 weeks or frozen with Diamond medium containing 7.5% dimethyl sulfoxide in liquid nitrogen. *T. vaginalis* organisms were labeled with [35S]methionine, using dialyzed Biosate medium (32, 34). Labeled organisms were evaluated by phase-contrast microscopy to ensure normal morphology and ≥95% motility prior to use.

Tissue culture methods. HeLa 229 cells (ATCC CCL 2.1) were grown to confluency in RPMI medium supplemented with 10% fetal calf serum, penicillin, and gentamicin in 24-well, flat-bottomed tissue culture plates. After removal of the growth medium, the monolayers were washed twice with phosphate-buffered saline solution (PBS), pH 7.2, prior to use. In some experiments, confluent tissue culture cell monolayers were washed with 0.15 M NaCl and then fixed with either 0.25% glutaraldehyde or 0.15% NaCl or 1% Formalin for 10 min. Following fixation, cells were washed with 0.1 M glycine and then 0.15% NaCl prior to use.

Incubation conditions and determination of adherence. Radiolabeled *T. vaginalis* organisms (104 to 106) were added to monolayers of human epithelial cells in plates which were incubated at 37°C. After incubation, the unattached trichomonads were aspirated and the wells were washed twice with 0.5 ml of PBS at 37°C. The adherent trichomonads were extracted by two additions of 0.5 ml of sodium dodecyl sulfate (0.25%). The two extracts were combined, and the radioactivity was determined with a liquid scintillation counter (Tri-Carb; Packard Instrument Co., Downers Grove, Ill.), using a scintillation fluid containing toluene and Triton X-100 (Sigma Chemical Co., St. Louis, Mo.). Different conditions as required for particular experiments are noted accordingly. All experiments were done in triplicate and repeated three times.

Production of monoclonal antibodies. Monoclonal antibodies were produced by using four clinical isolates of *T.*
TABLE 1. IIF, adherence, and RIP of T. vaginalis PHS2J with monoclonal antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>IIF reactiona</th>
<th>Motilityb</th>
<th>RIP bands (kDa)c</th>
<th>Adherence (% of control)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10/D2</td>
<td>1</td>
<td>115</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>1C11</td>
<td>2</td>
<td>&gt;95</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td>2C11</td>
<td>3</td>
<td>0</td>
<td>115</td>
<td>30</td>
</tr>
<tr>
<td>3C11</td>
<td>1</td>
<td>&gt;95</td>
<td>40, 36-38</td>
<td>80</td>
</tr>
<tr>
<td>C8</td>
<td>1</td>
<td>48</td>
<td>ND</td>
<td>48</td>
</tr>
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<td>D3</td>
<td>1</td>
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<tr>
<td>F3</td>
<td>2</td>
<td>&gt;95</td>
<td>40, 36-38</td>
<td>87</td>
</tr>
<tr>
<td>F8/F9</td>
<td>1</td>
<td>&gt;95</td>
<td>40, 36-38</td>
<td>90</td>
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<td>2</td>
<td>0</td>
<td>98</td>
<td>38</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>&gt;95</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

a Significance: ^P < 0.0001 versus control; ^P < 0.001 versus control; ^P < 0.03 versus control.
b Reaction pattern by indirect immunofluorescence: type 1, diffuse positive, evenly distributed but sparing the flagella (Fig. 2a); type 2, granular positive, including the flagella (Fig. 2b); 0, negative reaction.
c Molecular size of bands observed on IIF, ND, Not done.
d Adherence of radiolabeled trichomonads to HeLa cell monolayers.

T. vaginalis, including PHS2J. BALB/c mice were immunized with multiple intraperitoneal injections of living, late-log-phase T. vaginalis. Twenty-eight hybridoma cell lines secreting monoclonal antibodies were then produced by standard methods (19, 30), from which 10 monoclonal antibodies were selected for study. Six of the antibodies were of the immunoglobulin G subclass 1 and four were immunoglobulin G subclass 3.

Characterization of monoclonal antibodies by indirect immunofluorescence (IIF). T. vaginalis organisms from mid-log-phase cultures were centrifuged at 250 x g for 5 min, washed twice by centrifugation in PBS, and then fixed in 1 ml of PBS containing 10% fetal calf serum and 0.025% formaldehyde for 1 h at 4°C. A 2-μl portion of fixed organisms was then spotted onto each of 10 wells of a precleaned (7× cleaning solution; Flow Laboratories, McLean, Va.) multiwell slide (Shandon Southern Instruments, Sewickley, Pa.) and stained by methods described previously (19). Slides were examined at ×400, using a Nikon fluorescence microscope. Any reaction of ≥1+ (on a scale of 0 to 4+) was considered positive, and the staining pattern was noted.

In preliminary studies, we used IIF to define broadly reactive and narrowly reactive antibodies with 88 isolates of T. vaginalis (19). The broadly reactive antibodies (F3, 3C11, H10, D3, and F8) reacted with ≥60% of T. vaginalis isolates which were obtained from different geographic areas. The more narrowly reactive antibodies (B10, 2C11, H12, and 1C11) reacted with limited subsets of T. vaginalis isolates. T. vaginalis PHS2J reacted with all of the antibodies (Table 1). In contrast, strain STD13 only reacted with antibodies 3C11, D3, F3, and F8 by IIF.

Characterization of monoclonal antibodies by radioimmunoprecipitation (RIP). [35S]methionine-labeled T. vaginalis at a concentration of 1 to 2 mg of protein per ml were solubilized by vortexing vigorously for 2 min at 4°C in a buffer containing PBS (pH 7.6), 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 1% Aprotinin (Sigma). The antigen was then centrifuged at 40,000 x g for 1 h at 4°C. The supernatant, containing >95% of the total label, was added to siliconized, screw-capped tubes (13 by 100 mm; 200 to 400 μl per tube). Standard procedures were then used for the assay (32-34).

Effect of monoclonal antibodies on morphology and motility of PHS2J and STD13. Mid-logarithmic-phase cultures of T. vaginalis exhibiting normal morphology and >95% motility were washed twice by centrifugation in PBS and then diluted to 10^5 organisms per ml of PBS containing diluted monoclonal antibodies. After incubation at 37°C in 5% CO_2-95% room air, the numbers of organisms, their morphology, and the proportion of organisms exhibiting characteristic motility were determined by using a hemacytometer and phase-contrast microscopy. Each experiment was performed in triplicate and repeated on three occasions.

Effect of monoclonal antibodies on adherence of PHS2J and STD13 to monolayers of tissue culture cells. Radiolabeled T. vaginalis exhibiting normal morphology and >95% motility were washed twice by centrifugation in PBS and then suspended at a concentration of 2 x 10^7/ml in Diamond medium containing 1:100 dilutions of individual monoclonal antibody ascites fluids for 1 h at 37°C in 5% CO_2-95% air. The organisms were then added to fixed HeLa cell monolayers for measurement of adherence, using standard assay conditions. Each experiment was repeated in triplicate on three to four occasions.

Effect of monoclonal antibodies on viability of T. vaginalis. Quantitative pour plate cultures were used to determine the viability of T. vaginalis after exposure to monoclonal antibodies (18). A 0.25-ml sample containing approximately 10^7 organisms that had been incubated with individual monoclonal antibody-containing ascites fluids for 1 h at 37°C in 5% CO_2 was removed and inoculated onto a polystyrene petri plate (60 by 15 mm; Falcon, Becton Dickinson Labware, Cockeysville, Md.). Diamond medium (4.75 ml) containing 0.5% purified agar was added to each plate. After solidifying, the plates were incubated for 5 days at 37°C in brewer jars (Gas-Pak; BBL Microbiology Systems, Cockeysville, Md.). Colonies were then counted with a dissecting microscope. Each experiment was repeated three to six times.

Statistical analysis. Repeated-measures analysis of variance was used to compare adherence of T. vaginalis strains to tissue culture cell monolayers when experiments were conducted over various time periods. The paired t test was used to compare adherence of T. vaginalis to tissue culture cell monolayers for experiments with a fixed period of incubation.

RESULTS

Effects of fixation of T. vaginalis or epithelial cells on adherence. Radiolabeled T. vaginalis adhered to and damaged monolayers of HeLa cells in identical fashion to unlabeled T. vaginalis. To focus only on the adhesion process, we developed an assay which tested adherence of T. vaginalis to fixed HeLa cell monolayers. In contrast to the lack of disruption of glutaraldehyde- or Formalin-fixed monolayers, obvious disruption of the unfixed monolayers was apparent by phase-contrast microscopy after 30 min of incubation with T. vaginalis. Adherence of PHS2J trichomonads was higher than adherence of STD13 trichomonads to both fixed and unfixed monolayers (Fig. 1; P < 0.05). The mean adherence of both T. vaginalis strains was higher to glutaraldehyde- or Formalin-fixed monolayers than to unfixed monolayers (P < 0.05 at 30 min for each strain), probably reflecting disruption of unfixed monolayers.

Fixation of T. vaginalis PHS2J with glutaraldehyde (0.25% for 15 min) reduced adherence to HeLa cell monolayers,
measured after 15 min from 58.8 ± 9.5% (mean ± standard deviation) to 5.3 ± 2.4% (P < 0.001). Similar results were obtained with Formalin (1% for 15 min) as the fixative. These findings suggest that the primary glutaraldehyde- or Formalin-sensitive component responsible for binding was present on the parasite cell surface, rather than on the epithelial cell surface, or that dead trichomonads do not bind to the tissue culture cell monolayers.

**Standard conditions for the adherence assay.** Adherence of *T. vaginalis* PHS2J to fixed tissue culture cell monolayers was studied at times up to 30 min (Fig. 1). At concentrations of ≥5 × 10^4/ml, an average of 62% of PHS2J trichomonads and 53% of STD13 trichomonads were attached to the monolayer after 30 min of incubation. A linear correlation was found between the degree of attachment and the concentration of trichomonads, up to a ratio of one parasite per HeLa cell (data not shown).

Based on our preliminary experiments, standard conditions for the assay were as follows: radiolabeled *T. vaginalis* organisms (10^5 per well) were added to each glutaraldehydefixed monolayer containing approximately 10^6 HeLa cells. After incubation for 15 min at 37°C in 5% CO2–95% air, the free and attached parasites were extracted for scintillation counting. This period of incubation assured that the linear portion of the adherence curve was assayed.

**Characterization of monoclonal antibodies by IIF.** Two distinct patterns of IIF were noted with the monoclonal antibodies (Fig. 2): a diffuse IIF pattern, excluding the flagella (antibodies B10/D2, 3C11, C8, F8/F9, and H10); and a more granular IIF pattern, including a flagellar stain (antibodies 1C11, 2C11, F3, and H12). There was no difference in the staining pattern of the four monoclonal antibodies that reacted with both strains PHS2J and STD13.

**Characterization of monoclonal antibodies by RIP.** Because the monoclonal antibodies failed to immunoblot under a variety of conditions, we used RIP to identify the antigens reactive with monoclonal antibodies and to demonstrate the parasite origin of these antigens. Three patterns were observed when nine of the monoclonal antibodies were immunoprecipitated by using [35S]methionine-labeled strain PHS2J (Fig. 3). Six monoclonal antibodies (B10, 1C11, 2C11, D3, F3, and H12) reacted with single protein bands, at either 115 or 98 kilodaltons (kDa). Three other antibodies (3C11, F3, and F8) reacted with four protein bands, a tightly packed doublet of approximately 40 kDa and a second doublet of approximately 36 to 38 kDa. We were unsuccessful in immunoprecipitating antibody C8 for reasons that are uncertain. All monoclonal antibodies that reacted with STD13 by IIF produced bands on RIP.

**Effects of monoclonal antibodies on morphology and motility of PHS2J and STD13.** When motility of PHS2J trichomonads was evaluated after incubation with serial fivefold dilutions of monoclonal antibodies, the 50% inhibitory titers were 1:500 for antibodies 2C11, H10, and H12 and 1:100 for antibody C8 (Table 1). The six remaining monoclonal anti-
Effect of monoclonal antibodies on viability of *T. vaginalis*. To determine whether inhibition of parasite motility or adherence or both by toxic monoclonal antibodies reflected killing of the protozoa, quantitative cultures were performed following incubation of strain PHS2J trichomonads with monoclonal antibodies 2C11, C8, H10, and H12 for 30 min at 37°C in 5% CO2 and were compared with the results of two monoclonal antibodies, F3 and F8, that did not inhibit either motility or adherence. The mean CFU per plate was 495 ± 89 for trichomonads incubated with monoclonal antibodies that inhibited adherence or motility or both, 638 ± 161 for trichomonads incubated with monoclonal antibodies that had no effect on adherence and motility, and 564 ± 78 for antibodies that were incubated with PBS. Since there was no significant difference in the number of *T. vaginalis* colonies, these results suggest that inhibition of parasite motility and adherence properties by monoclonal antibodies resulted in no permanent damage to the trichomonads.

**DISCUSSION**

Although trichomoniasis is a common urogenital tract infection, little is known about events occurring during colonization of the host and production of disease (16, 17). Because attachment of the parasites appears to be critical for colonization and pathogenesis (5, 6, 14, 21), we defined factors that influence adherence of radiolabeled *T. vaginalis* to HeLa cell monolayers and determined the effect of monoclonal antibodies on adherence.

Fixation of the HeLa cell monolayers with either glutaraldehyde or Formalin did not reduce adherence of the trichomonads, whereas fixation of the protozoa reduced adherence by >90%. This implies that the critical glutaraldehyde- or Formalin-sensitive components mediating adherence were present on the parasite cell surface and not on the epithelial cell surface. Another possibility is that dead trichomonads lose the ability to adhere. This finding also allowed us to investigate only the initial adherence of trichomonads to HeLa cells in a controlled system, without the interference of monolayer destruction. Previous workers have also emphasized the importance of surface characteristics of the protozoa, particularly plasticity in expression of cell surface antigens in vitro (2, 3, 6-8, 26, 29, 35).

We confirmed the observation that *T. vaginalis* strains differ in their adherence characteristics to tissue culture cell monolayers (5, 9). However adherence of the protozoa to tissue culture cell monolayers did not correlate directly with virulence. *T. vaginalis* PHS2J exhibited significantly more adherence to HeLa cell monolayers than strain STD13, but both strains were isolated from symptomatic patients and both were virulent in model systems. Thus, the relationship between adherence and virulence may be complex. Adherence of protozoa to host mucosal surfaces may allow expression of contact-dependent cytotoxic characteristics. Highly virulent organisms may not require a high degree of adherence to cause cytopathology, while high-level adherence may be essential for organisms with lower inherent levels of virulence-associated properties.

Monoclonal antibodies produced against *T. vaginalis* were used to demonstrate that adherence of the protozoa to HeLa cell monolayers depended on parasite motility. The antibodies, each reactive with different trichomonad antigens (19), were characterized by IIF with fixed organisms, by RIP with solubilized organisms, and by their toxic effects with living organisms. All antibodies reacted by IIF with the immunizing PHS2J strain, but only four antibodies reacted with an unrelated STD13 strain.
Quantitative microscopic observations showed that the monoclonal antibodies did not cause direct cytolyis of *T. vaginalis*. However, exposure to four antibodies significantly reduced or totally eliminated motility of PHS2J trichomonads, even though three of these antibodies did not bind to flagella in IIF. The three antibodies with the greatest inhibitory effects on motility (2C11, H10, and H12) also reduced adherence of radiolabeled PHS2J organisms to monolayers of HeLa cells to 25 to 38% of control values (*P* < 0.001). Although all antibodies tested reacted with PHS2J by IIF, there was no correlation between inhibition of adherence and either IIF or RIP patterns, suggesting that a number of molecules may be involved in parasite motility and target cell adherence. In control experiments, none of the antibodies inhibited either motility or adherence of the STD13 trichomonads, although four antibodies were positive by IIF.

A key finding was that the monoclonal antibodies that inhibited motility and adherence of PHS2J trichomonads were not directly cytotoxic to the parasites. There was no significant difference in the number of colonies produced by parasites that had been incubated with antibodies that inhibited adherence, antibodies that had no effect on adherence, or controls containing no antibodies. This suggests that reduced adherence of the parasites reflected decreased motility, rather than competition by the antibodies for specific sites mediating attachment of the parasites to epithelial cell surfaces. Another possibility is that binding of monoclonal antibodies that inhibited motility led to perturbations of the parasite cell surface with nonspecific inhibition of *T. vaginalis* adherence to the tissue culture cells. In this respect our antibodies differ from the monoclonal antibody described by Alderete and Kasmala that caused complement-independent lysis of certain *T. vaginalis* strains (8). The monoclonal antibodies that inhibited motility and adherence of PHS2J trichomonads reacted with 98- and 115-kDa bands on RIP, but other antibodies that produced similar bands had no effect on either motility or adherence. Thus, another possibility is that only certain epitopes on the 98- and 115-kDa proteins are involved with motility and adherence of *T. vaginalis*.

Antibodies generated against *T. vaginalis* during natural infections may also inhibit motility of the protozoa. Our results suggest that such antibodies could interfere with binding of the protozoa to host epithelial surfaces. In addition, decreased parasite motility might facilitate resolution of infection by limiting the ability of the parasites to evade host defenses, such as the cellular defense mechanisms described by Rein and others (27). It may be possible to use this, or a similar, assay system to select monoclonal antibodies that bind to the specific molecules mediating adherence of the protozoa to host cells. It might also be possible to use this approach to develop specific antibodies or drugs that interfere with adherence of the protozoa to host cells.

In summary, adherence to host cell surfaces is important for colonization and pathogenesis of *T. vaginalis*. Critical components mediating adherence of radiolabeled trichomonads to monolayers of HeLa cells were present on the parasite cell surface. Monoclonal antibodies that abolished *T. vaginalis* motility also reduced adherence, but these antibodies were not cytotoxic for the protozoa. Understanding trichomonad motility and adherence to host mucosal surfaces may provide new avenues for therapy and insights into host-parasite interactions.

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**LITERATURE CITED**


