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# Analysis of Adhesion and Cytotoxicity of *Tritrichomonas foetus* to Mammalian Cells by Use of Monoclonal Antibodies

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**The relationship of *Tritrichomonas foetus* adhesion to mammalian cells and cytotoxicity to these targets was investigated. High-adherence and low-adherence lines of *T. foetus*, derived by repeated adhesion to HeLa cells, showed high and low cytotoxicity, respectively, to HeLa cells. When parasites were separated from targets by membranes (0.4-μm pore size), no cytotoxicity was detectable. Monoclonal antibodies elicited against *T. foetus* that lowered adhesion also lowered parasite-mediated cytotoxicity. Flow cytometry experiments revealed that the levels of an adhesion- and cytotoxicity-blocking antibody bound to the surface of high-adherence clones of *T. foetus* were higher than those in low-adherence clones. Western blots of parasite extracts separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were probed with an anti-*T. foetus* antibody. A molecule with a molecular weight of ≈190,000 composed of subunits with molecular weights of ≈140,000 to 150,000 and ≈65,000 was identified. Immunoprecipitation experiments with metabolically labeled *T. foetus* and the same antibody confirmed that similar subunits were synthesized by the parasite. These results indicate that adhesion of *T. foetus* to mammalian cells is an important step in cytotoxic damage of these targets and that a surface adhesin on the parasite is involved in the adhesion mechanism.**

The ability of pathogenic microorganisms to adhere to host cells often plays an integral role in establishing infections that lead to the disease processes in infected hosts (10, 13, 14). Parasitic protozoa that inhabit mucosal surfaces have been shown to adhere to host cells (1, 11, 13), indicating that such adhesion may play an important role in the pathogenesis of these infections.

The extracellular parasitic protozoan *Entamoeba histolytica* has been shown to adhere to mammalian cells through a galactose-specific lectin (22). Since *E. histolytica* destroys host cells by a contact-dependent cytotoxic mechanism (26), lectin-mediated adhesion appears to play an integral role in the process of host cell damage (27).

Trichomonads can display cytotoxicity to mammalian cells (2, 8), a process that appears to involve contact with the target cell in *Trichomonas vaginalis* (17). Although adhesion of *Tritrichomonas foetus* to bovine vaginal epithelial cells has been reported (11), the relationship of the adhesion process to cell or tissue damage was not examined.

In this study we describe experiments in which high- and low-adhesion lines of *T. foetus* were compared for their cytotoxicity to mammalian cells. The effects of surface-reactive monoclonal antibodies (MAbs) on parasite adhesion and cytotoxicity to mammalian cells were examined. We present evidence indicating that a 190,000-molecular-weight (MW) surface adhesin plays an important role in the adhesion of *T. foetus* to mammalian cells and cytotoxicity to these targets.

## MATERIALS AND METHODS

**Parasites.** Strains of *T. foetus* were cultured in Diamond's medium (12) containing 3% supplemented bovine serum (HyClone, Logan, Utah) as previously described (5). Cloning of *T. foetus* was done by limiting dilution on monolayers of HeLa cells (CCL 2; American Type Culture Collection, Rockville, Md.) as described in detail elsewhere (9a). Briefly, *T. foetus* cells cultured in Diamond's medium were washed in glucose-PBS (15 mM phosphate [pH 7.4] with 5.3 mM KCl, 137 mM NaCl, 2 g of D-glucose per liter, and 0.0006% phenol red) and diluted in complete RPMI (RPMI 1640–25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid containing 20 μg gentamicin per ml and 5% bovine serum) to give a concentration of 0.33 parasites per 100 μl. Diluted parasites (100 μl per well) were added to 96-well plates containing HeLa cell monolayers and cultured (37°C, high humidity, 95% air–5% $CO_2$) until parasite growth was evident (usually 2 to 3 days). Parasites were removed from positive wells, recloned, and expanded axenically in Diamond's medium.

**Antibodies and flow cytometry.** Preparation and partial characterization of the anti-*T. foetus* MAbs 32.3B3.5 (immunoglobulin G1 [IgG1]) and 32.5D2.5 (IgG1) used in this study were described previously (5, 6). The anti-bovine lymphocyte MAb 56.5C5.7 (IgG1) was derived similarly by using bovine lymphocytes from long-term mixed lymphocyte cultures as antigens (16). Briefly, bovine peripheral blood mononuclear cells (PBMC) were prepared from unrelated donors on Histopaque cushions as described previously (4), and mixed lymphocyte cultures were established (14, 24) with mitomycin C-treated PBMC as stimulator cells in 1-ml cultures. Responder PBMC were alternately treated by adding either $5 \times 10^4$ mitomycin C-treated stimulator PBMC or 25% (vol/vol) of the supernatant from concanavalin A-stimulated PBMC at intervals of 5 to 7 days. Responder PBMC were harvested after three treatments and used for immunization of female BALB/c mice for the derivation of hybridomas and MAbs (5). Each MAb was screened against responder PBMC by immunofluorescence (7) and then screened for functional inhibition of mixed lymphocyte reactions and cytotoxic T-lymphocyte responses generated in mixed lymphocyte cultures (16, 24). MAb 56.5C5.7 inhibited cytotoxic T-lymphocyte responses but did not inhibit mixed lymphocyte reactions in either isologous or heterologous bovine PBMC responder-stimulator combinations (16). In all experiments, MAbs were prepared as hybridoma

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supernatants and dialyzed against the assay medium before use. In selected experiments, MAbs were precipitated by adding an equal volume of saturated $(NH_4)_2SO_4$ (pH 7) and dialyzed against the assay medium without serum. The concentrations of these MAbs were determined by the bicinchoninic acid assay (32). Binding of MAb 32.3B3.5 to *T. foetus* was assessed by flow cytometry as described elsewhere (6), except that parasites were analyzed on a FACScan instrument (Beckton-Dickenson, Mountain View, Calif.). Fluorescein goat anti-mouse IgG (Cappel Laboratories, Malvern, Pa.) was used to detect binding of MAbs to *T. foetus*.

**Western immunoblots and immunoprecipitations.** Western blots were done as described previously (5) with hybridoma supernatants as primary antibodies and affinity-purified horseradish peroxidase-conjugated goat anti-mouse IgG (Cappel) as the secondary antibody. Immunoprecipitations were done as described previously (6), except that protein G-Sepharose (Pharmacia LKB, Piscataway, N.J.) was used as the solid-phase adsorbent. MW standards were as follows: myosin, 205,000; β-galactosidase, 118,000; phosphorylase B, 97,000; bovine serum albumin, 66,000; ovalbumin, 45,000; and carbonic anhydrase, 29,000.

**Cytotoxicity assays.** The cytotoxicity of *T. foetus* was assessed by a $^{51}$Cr release assay (8) with $^{51}$Cr-labeled HeLa cells as targets. Briefly, triplicate or quadruplicate assay wells of a 96-well plate were prepared for each experimental treatment group. Wells containing $2 \times 10^4$ $^{51}$Cr-labeled HeLa targets with either $2 \times 10^5$ or $4 \times 10^5$ *T. foetus* cells (effector/target cell ratio of 10:1 or 20:1, respectively) or controls for release without parasites present and maximum release by detergent lysis were prepared in a total volume of 200 μl and incubated at 37°C in a humidified atmosphere of 95% air–5% $CO_2$ for 20 h. After incubation, the plates were centrifuged (400 × *g*, 10 min, 20°C), and the radioactivity in 100 μl of each well's volume was determined by liquid scintillation counting. The mean counts per minute (cpm) for each treatment group were calculated, and cytotoxicity is expressed as the percent specific release as follows: [(experimental cpm − control cpm)/maximum cpm − control cpm)] × 100 (8, 25). Assay wells were briefly examined at the end of each assay with an inverted microscope to ensure that the viability of parasites was ≥90%. Spontaneous release was never greater than 35% in any experiment.

In some experiments, MAbs specific for *T. foetus* (5, 6) in the form of culture supernatants were added (1:20 final dilution) at the initiation of the cytotoxicity assay as indicated below. The percent inhibition by MAbs was calculated as follows: [(percent specific release without addition − percent specific release with MAb)/percent specific release without addition] × 100. In barrier experiments, radiolabeled HeLa cells were exposed to *T. foetus* placed in culture inserts (Transwell 3401; Costar Corp., Cambridge, Mass.) with a filter (0.4-μm pore size) barrier between the parasite and the targets.

**Adhesion assays.** Adhesion of *T. foetus* to HeLa cells was assessed as follows. The day before the assay, HeLa cells were plated in 24-well plates at a concentration of $3 \times 10^5$ cells per well in 1 ml of complete RPMI. Parasites were removed from Diamond's medium, washed twice in RPMI 1640, and resuspended to $3 \times 10^6$ *T. foetus* cells per ml of complete RPMI containing 2 to 3 μCi of [5,6-$^3$H]uracil (specific activity, 45 Ci/mmol; ICN Biomedicals, Inc., Costa Mesa, Calif.) per ml in a 25-cm$^2$ flask (Corning Glass Co., Corning, N.Y.). The suspension was incubated for 24 h at 37°C and then washed twice in complete RPMI and resuspended to $1.5 \times 10^7$ parasites per ml. On the day of the assay, $1.5 \times 10^6$ labeled *T. foetus* cells (in 100 μl) were added to each well (triplicate wells for each condition), and the plate was incubated (37°C, 95% air–5% $CO_2$) for 30 min. After incubation, the wells were gently rinsed three times with warm glucose-PBS. After the last rinse, the liquid was aspirated out of each well and 250 μl of 1% sodium dodecyl sulfate (SDS) in water was added to each well. The radioactivity in each sample was determined by liquid scintillation counting and expressed as the mean counts per minute ± the standard deviation. In some experiments, MAbs in the form of hybridoma culture supernatants were added to assay wells immediately before the parasites were added (9).

**Derivation of high- and low-adhesion *T. foetus* lines.** Lines of *T. foetus* were selected for relatively high adhesion to HeLa cells (AD$^+$ phenotype) or relatively low adhesion to HeLa cells (AD$^-$ phenotype). Uncloned *T. foetus* were removed from Diamond's medium, washed twice in complete RPMI, and resuspended $5 \times 10^5$/ml in complete RPMI. Five milliliters of the parasite suspension was added to a monolayer of HeLa cells in a 25-cm$^2$ flask, and the flask was incubated for 30 min at 37°C. After incubation, the medium containing low-adhesion *T. foetus* was decanted and the monolayer was rinsed five times with warm RPMI 1640. After the last RPMI rinse, trypsin-EDTA (GIBCO) was added and the flask was incubated for approximately 2 min. Detached parasites were then rinsed off of the HeLa cell monolayer with complete RPMI. After the initial adhesion selection, the nonadherent and adherent lines were cultured in Diamond's medium, and the selection process was repeated separately on each line four additional times. At the end of the fifth selection process, each line was cloned by limiting dilution on HeLa cells to produce 40 AD$^+$ clones and AD$^-$ clones of *T. foetus*. Preliminary adhesion experiments were performed, and representative AD$^+$ clones (tf1, tf3, tf5, tf11, and tf12) and AD$^-$ clones (tf23, tf24, tf26, and tf31) were selected and maintained in Diamond's medium.

## RESULTS

**Parasite adhesion.** The effects of surface-binding, parasite-specific MAbs on adhesion of *T. foetus* to HeLa cells were assessed in preliminary experiments with cocktails of dialyzed mixtures of MAbs added (1:5, vol/vol) at the initiation of the adhesion assay. These experiments identified two MAbs, 32.3B3.5 and 32.5D2.5, with inhibitory activity. The levels of inhibition of adhesion (33%) by the parasite-specific MAb 32.3B3.5 were approximately twice that of the irrelevant, isotype-matched control MAb, 56.5C5.7 (17%; Fig. 1). The addition of MAb 32.3B3.5 reduced adhesion of *T. foetus* to HeLa cells significantly relative to that in experiments with no added MAb or with an irrelevant MAb ($P < 0.01$; analysis of variance followed by Student Neuman-Keuls multiple comparisons). In experiments in which 100 μg of each MAb per ml was added at the initiation of the adhesion assay, exactly the same levels of inhibition were observed (data not shown). Similar results were also obtained with another parasite-specific MAb, 32.5D2.5 (data not shown). These results indicated that MAbs 32.3B3.5 and 32.5D2.5 lowered adhesion of *T. foetus* to HeLa cells.

To examine the expression of the parasite epitope to which MAb 32.3B3.5 bound, surface binding of 32.3B3.5 to live AD$^+$ and AD$^-$ clones of *T. foetus* was examined by flow cytometry (Fig. 2). All parasite samples treated with 32.3B3.5 displayed a bimodal fluorescence pattern and a fluorescence intensity higher than that of conjugate-treated

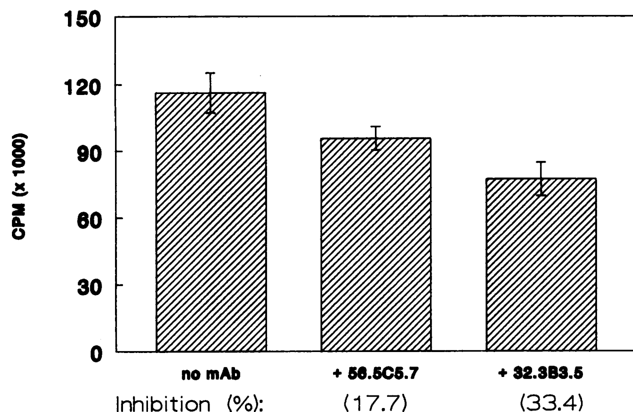


FIG. 1. Adhesion of *T. foetus* to HeLa cells is reduced with MAbs that react with the parasite surface. The adhesion assay (see Materials and Methods) was performed on AD$^+$ clone tf1 with no antibody additions or with a 1:5 final dilution of anti-bovine lymphocyte MAb 56.5C5.7 or anti-*T. foetus* MAb 32.3B3.5. Levels of adhesion are presented as means ± standard deviations. The results shown are representative of three separate experiments.

controls. Clones of the AD$^+$ phenotype, however, displayed surface staining that was more intensive than that of AD$^-$ clones, as evidenced by the shift to the right of the higher-fluorescence population (Fig. 2B and D versus Fig. 2F and H). These data indicated that MAb 32.3B3.5 bound to the surface of live *T. foetus* regardless of adhesion phenotype but that a subpopulation of AD$^+$ cells bound more 32.3B3.5 than did a similar subpopulation of the AD$^-$ clones.

Since a parasite surface structure seemed likely to be involved in adhesion of *T. foetus* to HeLa cells, we investigated the possibility that this structure could be a lectinlike molecule by examining the effects of several sugars on the adhesion of *T. foetus*. The presence during the adhesion assay of 2.5 to 250 mM sucrose, glucose, galactose, mannose, and *N*-acetylglucosamine had no inhibitory effects on the adhesion of *T. foetus* to HeLa cells (data not shown).

**Cytotoxicity and adhesion.** AD$^+$ clones of *T. foetus* were as much as 100% more cytotoxic to HeLa cells than were AD$^-$ clones (Fig. 3, AD$^+$ tf1 versus AD$^-$ tf26). The cytotoxicity displayed by high-adhesion clones was consistently higher than that of low-adhesion clones in several assays; these differences were significant (Table 1). By using microscopic examination and the isotopic adhesion assay, we confirmed the AD$^+$ and AD$^-$ phenotypes of these *T. foetus* clones (data not shown). In filter (0.4-μm pore size) barrier experiments, no cytotoxicity of the AD$^+$ tf1 clone to HeLa cells was observed (data not shown), indicating the requirement for parasite-target contact.

The effects of the adhesion-reducing MAbs 32.3B3.5 and 32.5D2.5 on cytotoxicity of *T. foetus* to HeLa cell targets were assessed. Both MAbs substantially decreased the cytotoxicity of *T. foetus* to HeLa cells in the chromium release assay (Fig. 4). In two similar experiments, inhibition of cytotoxicity by 32.3B3.5 ranged from 61 to 45% (53% ± 11.3%, mean ± standard deviation). In these experiments and experiments with mixtures of MAbs, microscopic examination of plates immediately after assay incubation revealed highly motile parasites, indicating little or no loss in viability. These data indicated that the epitopes recognized by these MAbs were involved in the cytotoxic mechanism of *T. foetus* and that the function of the epitope was somehow reduced

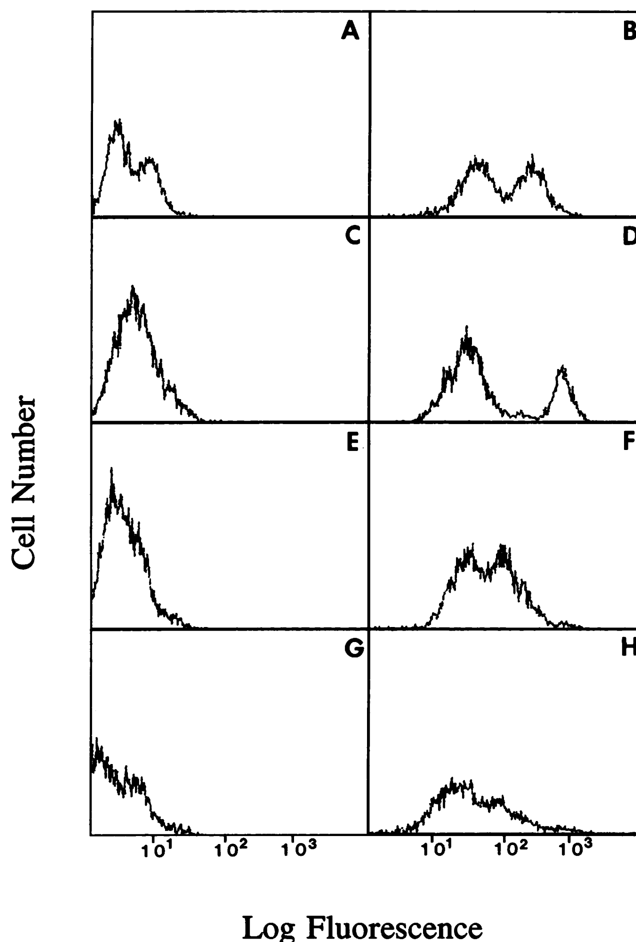


FIG. 2. Higher levels of MAb 32.3B3.5 bind to AD$^+$ clones of *T. foetus* than to AD$^-$ clones. High-adhesion clones tf1 (B) and tf9 (D) or low-adhesion clones tf23 (F) and tf24 (H) were treated with 32.3B3.5 and processed for flow cytometry (4). Fluorescence due to fluorescein-goat anti-mouse IgG is shown in panels A, C, E, and G for the cell types in panels B, D, F, and H, respectively. Curves represent determinations on 5,000 cells each.

by the presence of these MAbs during the cytotoxicity assay.

**Characterization of adhesin.** To identify the molecule(s) recognized by MAb 32.3B3.5, whole-parasite preparations were subjected to SDS-polyacrylamide gel electrophoresis (PAGE), electroblotted onto nitrocellulose, and probed with MAb 32.3B3.5. Under nonreducing conditions, a molecule of approximately 190,000 MW was recognized in five separate clones of *T. foetus* (Fig. 5, lanes 1 through 5). When extracts were electrophoresed under reducing conditions, two prominant bands at approximately 140,000 to 150,000 MW and 60,000 MW bound 32.3B3.5 (Fig. 5, lanes 6 through 10). The width of the 140,000 to 150,000-MW band could suggest partial degradation of this component or perhaps a high degree of glycosylation. A complex binding pattern was also apparent in the region of the blot just above the 140,000 to 150,000-MW band (Fig. 5, arrows), suggesting multiple minor components or partial degradation of components. Similar results were obtained when blots of *T. foetus* were probed with MAb 32.5D2.5 (data not shown).

Immunoprecipitation experiments were done with ex-

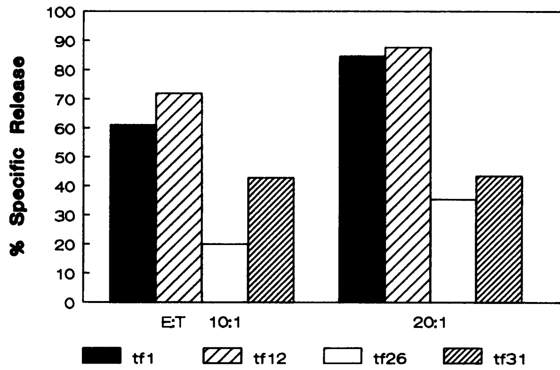


FIG. 3. AD$^+$ clones of *T. foetus* are more cytotoxic than AD$^-$ clones. The cytotoxicity of AD$^+$ clones (tf1, tf12) and AD$^-$ clones (tf26, tf31) against HeLa target cells was determined in a standard $^{51}$Cr release assay (6) at effector/target cell ratios of 10:1 and 20:1. The data shown are representative of three similar experiments.

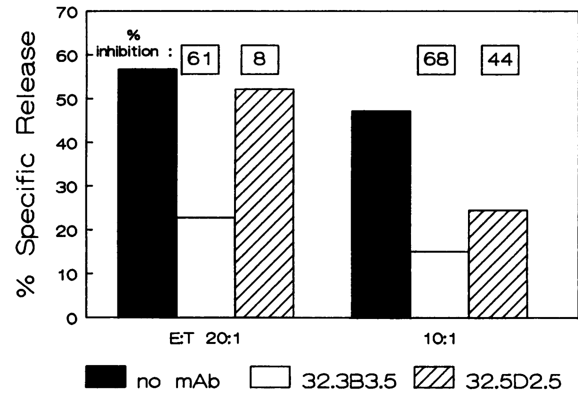


FIG. 4. Antiadhesin MAbs lower cytotoxicity of *T. foetus* to HeLa cells. Cytotoxicity of the AD$^+$ clone tf1 was determined in the standard $^{51}$Cr release assay (6) in the presence or absence of MAbs (1:20, final dilution) at effector/target cell ratios of 20:1 and 10:1. The data shown are representative of two similar experiments.

tracts of *T. foetus* after labeling with [$^{35}$S]methionine-cysteine (Trans Label) and MAbs 32.3B3.5 and 56.5C5.7. When SDS-PAGE was performed under reducing conditions, several bands were resolved in the precipitate of MAb 32.3B3.5 (Fig. 6, lane 2), with strong signals at approximately 50,000, 140,000, and 178,000 MW (large arrows) and a faint signal at approximately 65,000 MW (double arrows). The distorted bands at approximately 50,000 and 24,000 MW likely resulted from the gel being overloaded with MAb 32.3B3.5, as the bands are in the positions of the heavy and light chains, respectively (based on the Coomassie blue staining pattern). Although other bands above 200,000 MW were evident in the 32.3B3.5 precipitate (Fig. 6, top of lane 2), they also appeared as faint bands in the control 56.5C5.7 precipitate (Fig. 6, top of lane 1), suggesting that some nonspecific binding may have occurred. No material appeared to bind directly to Sepharose-protein G adsorbant without antibody (Fig. 6, lane 3).

These results indicated that a 190,000-MW parasite molecule identified by *T. foetus*-specific MAbs on blots was likely to be an adhesin involved in adhesion of the parasite to HeLa cells (Fig. 1). The immunoprecipitation results with 32.3B3.5 indicated that at least two subunits in the 140,000 to 150,000-MW and 60,000- to 65,000 MW ranges, identified on blots, were synthesized by the parasite along with additional polypeptides (e.g., 178,000 MW).

TABLE 1. Cytotoxicity of clones of *T. foetus* to HeLa targets[a]

| Parasite clone | Mean % specific release ± SD (*n*) |
|---|---|
| Tf1 | 61.3 ± 4.5 (3) |
| Tf3 | 52.6 ± 8.3 (3) |
| Tf5 | 55.6 ± 2.3 (3) |
| Tf23 | 32.6 ± 8.7[b] (3) |
| Tf26 | 26.3 ± 3.5[b] (3) |
| Tf26 | 23 ± 4.2 (2) |
| Tf31 | 42 ± 0 (2) |

[a] Results are given for an effector/target cell ratio of 10:1.
[b] Statistically significantly different from results with clones Tf1, Tf3, and Tf5 by Student's *t* test ($P < 0.05$).

## DISCUSSION

Adhesion was previously shown to occur between *T. foetus* and mammalian cells (30), and antibody has been shown to reduce the levels of such adhesion (11, 15). Corbeil and colleagues (11, 15) showed that adhesion to bovine vaginal epithelial cells could be inhibited by immune bovine serum and the IgG1 fraction of this serum. Antibody binding results on blots of reduced samples indicated that antigens similar in size to those binding MAb 32.3B3.5 (Fig. 5) bound bovine antibodies and MAbs. In both instances a material with an approximate MW of 60,000 on Western blots was recognized by the antibody. Bovine antibodies reacted with antigens in a broad band ranging from approximately 116,000 to 190,000 MW (11). These sizes encompass the size range of the 140,000 to 150,000-MW antigens recognized by MAb 32.3B3.5 in five clones of *T. foetus* (Fig. 5, lanes 6 through

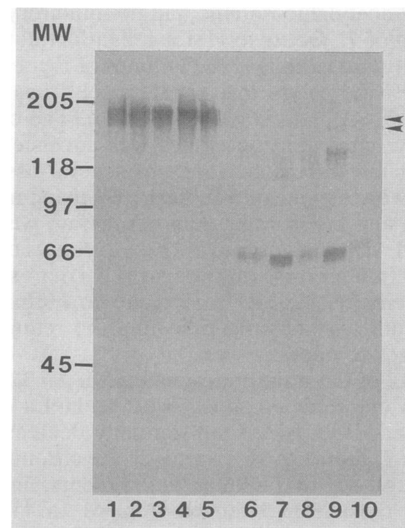


FIG. 5. Western blot reactivities of MAb 32.3B3.5 with five AD$^+$ clones of *T. foetus*. Approximately $8 \times 10^4$ cells of clones tf1, tf5, tf3, tf11, and tf12 per lane were subjected to SDS-PAGE (7.5% T) under nonreducing (lanes 1 through 5) or reducing (lanes 6 through 10) conditions. The gel was then electroblotted and probed with MAb 32.3B3.5 as previously described (3). The MWs of standards (in thousands) are indicated at the left.

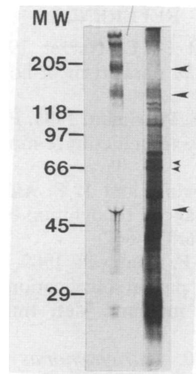


FIG. 6. Immunoprecipitation of metabolically [$^{35}$S]methionine-cysteine-labeled *T. foetus* polypeptides by antiadhesin antibody. Precipitates were subjected to SDS-PAGE (7.5% T) and fluorography (72 h). Lanes: 1, precipitate of 56.5C5.7; 2, precipitate of 32.3B3.5; 3, no MAb; 4, whole labeled extract. The MWs of standards (in thousands) are indicated at the left.

10) and immunoprecipitation experiments with metabolically labeled *T. foetus* (Fig. 6). In other work, MAbs directed against surface molecules of *T. foetus* have been shown to inhibit adhesion of the parasite to bovine vaginal epithelial cells (15). These antibodies react with a complex group of parasite molecules ranging from 50,000 to 66,000 MW on blots from gels run under reducing conditions. Two other MAbs that were specific for *T. foetus* but not surface reactive did not inhibit adhesion to vaginal epithelial cells. In contrast to the previously observed antibody reaction patterns (11, 15), in our study the lower-molecular-weight band that bound MAb 32.3B3.5 appeared to be a single band on Western blots (Fig. 5), suggesting little or no proteolytic degradation of this molecule. We also observed additional minor reactions above the larger component, suggesting the presence of several antigenic molecules in this area of the gel. This complex pattern could be due to multiple native antigenic components or to degradation products of the larger (unreduced) material. The high level of proteinase activity reported for *T. foetus* (18) suggests that antigens could easily be rapidly degraded upon cell disruption, resulting in complex patterns of antibody binding. Differences in the susceptibilities of two major subunits of the adhesin to proteinase activity could therefore account for the distinct antibody binding patterns (Fig. 5, lanes 6 through 10). Antibody binding patterns with unreduced samples indicate that the adhesion-inhibiting MAb 32.3B3.5 recognized one major component in five clones of *T. foetus* at approximately 190,000 MW (Fig. 5). Collectively, the blot results indicate that the adhesin of *T. foetus* that binds 32.3B3.5 is multimeric (possibly a heterodimer) with disulfide bonding between the subunits.

Immunoprecipitation experiments with MAb 32.3B3.5 and metabolically labeled *T. foetus* were performed to further investigate the antigens bearing epitopes of this MAb. The results of these studies indicated that at least three polypeptides (65,000, 141,000, and 178,000 MW) were specifically precipitated under reducing conditions (Fig. 6, lane 2). Two of these are likely to be the same as those identified on Western blots by 32.3B3.5. The two additional signals at approximately 50,000 and 24,000 MW that were exactly superimposable over the heavy and light chains of 32.3B3.5 and the fact that these regions of the gel were overloaded (revealed by Coomassie blue staining) suggested that these signals were artifactual. The proximity of the signal at 50,000 MW to the faint band at 65,000 MW suggested that the large amount of heavy chain present altered the gel position of the small subunit of the adhesin compared with the pattern in Western blots (Fig. 5). We are uncertain about the significance of the third band at 178,000 MW. Possibilities include a third subunit of one large molecule, a component of a complex made up of several molecules, or simply a coprecipitate. Although faint bands in the control lane (Fig. 6, lane 1) corresponding to the two strong signals above 200,000 MW in the 32.3B3.5 precipitate (lane 2) may have been due to nonspecific binding, the signals were much stronger in the antiparasite precipitate. Additional work is under way to resolve these issues and gain more structural and functional information on this adhesin.

The biological significance of adhesion of *T. foetus* was demonstrated by showing that increased adhesion capacity for target cells correlated with increased parasite-mediated cytotoxicity. Cytotoxicity experiments with AD$^+$ and AD$^-$ lines of *T. foetus* indicated that AD$^+$ parasites were more cytotoxic to HeLa target cells than were AD$^-$ clones (Fig. 3). Additional experiments in which surface-reactive MAbs elicited against *T. foetus* were tested in an adhesion assay identified MAbs that lowered adhesion of *T. foetus* to HeLa (Fig. 1). Two MAbs, 32.3B3.5 and 32.5D2.5, were investigated for their inhibitory effects on cytotoxicity of *T. foetus* against HeLa cells and shown to lower parasite-mediated cytotoxicity (Fig. 4). Thus, adhesion via epitopes defined by these MAbs appears to be an important component in the mechanism of cytotoxicity to host cells by *T. foetus*. The capability to adhere to mammalian cells (11) (Fig. 1 and 3) and display cytotoxicity to host cells (8) could allow *T. foetus* to damage tissues such as bovine placental tissue and fetal tissues. Invasion of these tissues by *T. foetus* has been shown (7, 20, 28), and abortions as late as 8 months of gestation have been reported in cows infected with *T. foetus* (7, 28). These reports suggest that host cell damage could lead to sufficient tissue damage to contribute to abortion in infected cows. Many aspects of the mechanism of abortion in bovine trichomoniasis remain unknown, however, and other parasite molecules such as proteinases could be involved (18). A cysteine proteinase of *E. histolytica*, termed histolysin, has been shown to mediate rounding and detachment of fibroblasts in vitro (19), and *T. foetus* has been shown to produce a cysteine proteinase that is released extracellularly (33). Host responses to the infection, such as inflammatory responses during tissue damage, could also play a significant role in the abortion process.

Further results on adhesin expression in clones of *T. foetus* with different cytotoxic abilities indicated that the adhesin of *T. foetus* plays an important role in the cytotoxic mechanism of *T. foetus*. Flow cytometry results showed that the levels of MAb 32.3B3.5 bound to the more cytotoxic AD$^+$ clones were higher than those bound to the less cytotoxic AD$^-$ clones of *T. foetus* (Fig. 3). These results imply that AD$^+$ *T. foetus* expresses more of the adhesion molecule epitope than does AD$^-$ *T. foetus*. Since AD$^+$ *T. foetus* clones were more cytotoxic than AD$^-$ clones, the level of expression of this putative adhesion molecule would appear to be a correlate of an important virulence determinant with regard to the potential of the parasite to damage mammalian cells as has been shown for *E. histolytica*. Although steric hindrance of adhesin function caused by antibody 32.3B3.5 binding to a molecule close to but distinct from the actual adhesin could result in lowered adhesion or cytotoxicity, we believe that the flow cytometry and cyto-

toxicity assay results with $AD^+$ and $AD^-$ clones argue against this possibility. Experiments in which purified adhesin is evaluated for inhibition of parasite adhesion may address this issue.

Adhesion to host cells has been shown to be important in the ability of *E. histolytica* to damage host cells (26) and to be largely dependent on a galactose-specific lectin located on the surface of the parasite (22). Cytotoxicity of *E. histolytica* towards CHO cells (26) and adherence (27) were greatly reduced by *N*-acetyl-D-glucosamine or galactose but not by other monosaccharides. We did not observe lowered adhesion of *T. foetus* to HeLa cells in the presence of several concentrations of monosaccharides (see Results). In similar experiments, no effects of sugars on adhesion of *T. vaginalis* to HeLa could be demonstrated (1). Although these negative results do not rule out the possible participation of lectinlike molecules in adhesion of trichomonads to mammalian cells, they indicate that lectins similar to the galactose-specific lectin of *E. histolytica* are unlikely to be the prominant adhesins of *T. foetus*.

Recently Arroyo et al. (3) reported four surface proteins of *T. vaginalis* with MWs ranging from 23,000 to 65,000 were involved in adhesion and cytotoxicity to HeLa cells. Polyvalent rabbit antibodies directed against the 65,000-, 51,000-, and 33,000-MW molecules reduced adhesion by approximately 50%, whereas an antibody against the 65,000-MW molecule lowered cytotoxicity by approximately 50%. Whereas the reduction of adhesion by the anti-*T. foetus* MAb was less than with *T. vaginalis* (approximately 30% reduction), the reduction of cytotoxicity was comparable (approximately 50%). Participation of additional, unidentified molecules in adhesion of *T. foetus* to mammalian cells, however, is suggested by the fact that blocking of adhesion by MAb 32.3B3.5 (and 32.5D2.5) was incomplete (Fig. 1), although MAbs to distinct epitopes of the galactose-specific lectin of *E. histolytica* have been shown to enhance or inhibit parasite adhesion (23). Additional adhesins are likely to participate in adhesion of *T. foetus* to mammalian cells, since other adhesins, such as the laminin receptor reported in *T. vaginalis* (31), could easily play a role in a complex process such as adhesion. These results, together with the inability of *T. foetus* to mediate significant cytotoxicity through a filter barrier (see Results), emphasize that cell-cell contact is necessary for cytotoxicity in trichomonads (17).

Although the precise molecular composition of the adhesin of *T. foetus* defined by MAb 32.3B3.5 is not completely known, we have shown in metabolic labeling experiments with [$^{35}$S]methionine that there are polypeptide components recognized by 32.3B3.5 (Fig. 6) and that the adhesin can be purified in sufficient quantities to obtain structural information (6a). Since systemic immunization with other pathogens of the bovine reproductive tract has been shown to be efficacious in protection against infection (29), the immunogenicity in cattle of the adhesin defined by MAb 32.3B3.5 is currently being evaluated. Recent results with *E. histolytica* suggest that although several mechanisms may be involved in host tissue damage (13), immunization with an adhesion molecule can result in protection against tissue invasion by this parasitic protozoan (21).

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