Parasitol Res (1998) 84: 374–381

ORIGINAL PAPER

A. Mirhaghani · A. Warton

Involvement of *Trichomonas vaginalis* surface-associated glycoconjugates in the parasite/target cell interaction. A quantitative electron microscopy study

Received: 22 June 1997 / Accepted: 10 November 1997

Abstract Cytochemical labeling with gold particle-conjugated lectins in combination with transmission and scanning electron microscopy was used to localise specific sugar residues on the *Trichomonas vaginalis* cell surface. For investigation of the role played by the surface glycoconjugates of *T. vaginalis* in the process of parasite adhesion to the target cells, selected glycan moieties of parasite surface-bound molecules were removed by treatment with α-mannosidase and β-*N*-acetylglucosaminidase. For observation of the parasite/epithelial cell interaction, human amnion membrane was employed as an in vitro model. Ultrastructure observations showed that *T. vaginalis* has distinct binding sites for concanavalin-A and wheat-germ agglutinin. This indicates the presence of mannose or mannose-like residues and *N*-acetyl-D-glucosamine-containing residues on the parasite membrane. The addition of inhibitory sugars to *T. vaginalis* incubation media diminished the subsequent labeling of the parasite cell coat with lectins. Enzyme treatment caused a significant reduction in the number of sugar residues on the cell surface of the parasite. The majority of the viable, motile, enzyme-treated *T. vaginalis* cells incubated with amnion membrane were incapable of adhering to the target cells. It was concluded that sugar residues, in particular α-D-mannose and *N*-acetyl-glucosamine, present in the parasite glycocalyx are involved in the process of *T. vaginalis* attachment to the host's epithelial cells. Removal of the *T. vaginalis* cell-surface sugars prevented the attachment to and damage of the epithelial cells.

A. Mirhaghani · A. Warton (✉)
Electron Microscopy Unit, Biological Sciences,
Macquarie University, North Ryde,
Sydney 2109, Australia
E-mail: awarton@rna.bio.mq.edu.au

Introduction

It is well established that eukaryotic cells recognise and communicate with one another through pairs of complementary structures on their surfaces; a structure on one cell carries encoded information that the structure on the other cell can decipher. Eukaryotic cells have advanced signaling systems. Cell-surface-associated molecules are among several signaling molecules that form the communication network of a cell (Singer 1992).

In the plasma membrane of all eukaryotic cells, most of the proteins exposed on the cell surface and some of the lipid molecules have saccharide chains covalently attached to them. In many cases, cell-surface glycoconjugates that carry antigenic determination for a cell are also the primary markers for cell-to-cell recognition and adhesion (Cross 1990; Ravdin and Murphy 1992; Sharon 1993; Sharon and Lis 1993; Mellors and Sutherland 1994; Knudsen et al. 1995). Therefore, the interaction between host and pathogen that results in the establishment of infection may depend on the binding of glycoconjugate molecules present on the surface coat of the parasite to their counterpart molecules on the host cell surface.

Trichomonas vaginalis is an extracellular, anaerobic, flagellated protozoan that infects squamous epithelium lining the lower part of the human urogenital tract. The mechanism by which *T. vaginalis* causes damage to the host cells is not yet well defined; however, there is strong evidence that attachment and, therefore, interaction of the parasite surface molecules with their counterpart epithelial cell receptors is critical for colonisation and establishment of infection in the host (Heath 1981; Krieger et al. 1985; Rasmussen et al. 1986; Arroyo et al. 1992, 1993; Gonzalez Robles et al. 1995).

Cytochemical labeling of the *T. vaginalis* cell coat with different lectins indicates the presence of several carbohydrate-containing components on the outer layer of the parasite body (Warton and Honigberg 1980, 1983; Brasseur and Savel 1982; Kon et al. 1988; Warton et al.

1988a; Roussel et al. 1991; Dias Filho et al. 1992; Singh 1993). We used gold particle-conjugated lectins as a marker to demonstrate the presence of several sugar-containing components on the surface coat of *T. vaginalis*. Two techniques were employed to visualise surface-sugar residues of *T. vaginalis*: (1) cytochemical labeling of thin sections of *T. vaginalis* with gold particle-conjugated lectins and (2) labeling of whole protozoa with gold particle-conjugated lectins.

To investigate the involvement of *T. vaginalis* surface-exposed sugar-containing molecules in adherence of the parasite to the epithelial cells of an in vitro system we incubated *T. vaginalis* cells with different glycosidases. This resulted in removal of carbohydrates from the outer layer of the plasma membrane of *T. vaginalis*. For investigation as to whether parasites having insufficient numbers of sugar chains on their surface could identify their target cells and adhere to them, epithelial cells of human amnion membrane were then exposed to such treated parasites.

Materials and methods

Trichomonas vaginalis culture

Trichomonas vaginalis strain WAA38, used in our experiments, was isolated from the genital tract of an Australian Aboriginal woman attending the Aboriginal Health Centre in Perth, Western Australia. The patient had severe, florid vaginitis. The processes of isolation, in vitro cultivation and cryopreservation of this strain have been described elsewhere (Warton et al. 1988a).

For various experiments, thawed *T. vaginalis* cell stock was transferred to Oxoid medium without agar (Oxoid Ltd, Basingstoke, Hampshire, England) supplemented with 5% inactivated horse serum (Commonwealth Serum Labs, Victoria, Australia) and incubated at 37 °C. After two passages (48 h each), trichomonads were counted in a Neubauer chamber and motility levels assessed. Motility was assessed as being greater than 90% before *T. vaginalis* cells were used in any experiment.

Human placentae

Human placentae from normal-term deliveries (Royal North Shore Hospital, Sydney) were transported to Macquarie University on ice and used immediately. Amnion membranes were separated from the chorion. Mucus and blood clots were removed. Portions of amnion that were transparent, avascular and not adjacent to the umbilical cord and decidua were selected for the assays. Amnion membranes were washed initially with phosphate-buffered saline (PBS) containing 0.02% sodium hypochlorite and then with PBS containing penicillin (100 u/ml) and streptomycin (100 μg/ml). After thorough washing, amnion membranes were transferred to RPMI 1640 medium, stretched over a plastic slide and transferred into the specially designed 96-well microchemotaxis chamber.

Preparation of *T. vaginalis* for labeling of thin sections of parasites with gold particle-conjugated lectins

Chemical fixation of T. vaginalis cells and thermal polymerisation of resin

T. vaginalis cells were harvested by centrifugation of a 48-h culture of parasites. Pellets of *T. vaginalis* cells were washed with PBS and fixed in a mixture of 2% paraformaldehyde and 0.1% glutaraldehyde in PBS (pH 7.2) for 2 h at 4 °C. Parasites were washed with PBS and clotted with 2% agar. Specimens were dehydrated in increasing concentrations of ethanol and infiltrated with 50% LR White resin and ethanol overnight at 4 °C, which was followed by 100% LR White resin infiltration for 24 h at 4 °C. Specimens were embedded in LR White resin in gelatin capsules (Agar Scientific) and polymerised at 50 °C for 24 h.

Freeze-substitution technique and UV polymerisation of LR White resin

Pellets of *T. vaginalis* cells were obtained by centrifugation of a suspension of a 48-h culture of parasites. Cells were washed twice with PBS. A drop containing cells was placed on a specimen holder and rapidly transferred to liquid propane chilled to 100 °C with liquid nitrogen. Frozen *T. vaginalis* cells were then placed into a freeze-substitution unit (Reichert CS Auto, Leica). Substitution of ice with 100% methanol was followed by fixation with 0.2% glutaraldehyde in 100% methanol. Specimens were transferred to gelatin capsules and infiltrated with LR White resin. LR White resin was polymerised under UV light.

Labeling of thin sections of *T. vaginalis* with gold particle-conjugated lectins

Ultrathin sections were cut with a diamond knife and collected on nickel grids. Sections were then incubated for 5 min in a drop of PBS. Grids were floated face down on a drop of a diluted solution of gold particle-conjugated lectin (50, 100, 200 and 400 μg/ml) for 30 min and 5 h in a humid chamber at room temperature. The lectins employed in the experiments were concanavalin A (Con A), wheat-germ agglutinin (WGA) and ulex europaeus agglutinin (UEA I), all conjugated to 10-nm gold particles (Sigma). Grids were jet-washed in PBS, rinsed in Milli-Q water and stained with uranyl acetate and lead citrate.

Prefixation of *T. vaginalis* cells with glutaraldehyde and cytochemical labeling of whole *T. vaginalis* cells with gold particle-conjugated lectins

T. vaginalis cells were prefixed with 1% glutaraldehyde for 15 min and washed twice with PBS. In each test tube, 0.4 ml of *T. vaginalis* cell suspension was incubated with 0.5 ml of diluted gold particle-conjugated Con A, WGA and UEA I lectin for 45 min at room temperature. The final concentration for each lectin was 200 μg/ml in incubation medium. For preparation of controls, *T. vaginalis* cells were incubated at room temperature with gold-conjugated lectins in the presence of the specific inhibitory sugar (α-D-mannose for Con A, *N*-acetyl-D-glucosamine for WGA and fucose for UEA I lectin). The final concentration of each inhibitory sugar in incubation medium was 0.5 mol/ml. After incubation, cells were washed twice with PBS and fixed with 2.5% glutaraldehyde overnight at 4 °C. After postfixation with OsO_4, parasites were washed twice with PBS. *T. vaginalis* cells were centrifuged, the supernatant was removed and cells were then clotted with 2% agar. Fixed *T. vaginalis* cells were dehydrated in increasing concentrations of ethanol followed by 100% acetone and then embedded in Spurr's resin. Ultrathin (50–60 nm) sections stained with uranyl acetate and lead citrate were viewed with a Jeol (JEM-100X) electron microscope at accelerating voltages of 60 and 80 kV.

Treatment of *T. vaginalis* cells with glycosidases and cytochemical labeling of enzyme-treated parasite plasma membrane with gold particle-conjugated lectins

T. vaginalis cells were washed three times with citrate buffer (pH 4.8) and then counted in a haemocytometer chamber to make a

final concentration of 10^5 cells/ml. Trichomonad cells were incubated with α-mannosidase and β-*N*-acetylglucosaminidase (Sigma) at 37 °C for 1 h (final concentrations of both enzymes were 4.6 mu/ml of incubation medium). Cells were washed twice with PBS, prefixed with 1% glutaraldehyde for 15 min, washed again and labeled with 10-nm gold particle-conjugated Con A and WGA as described above. Cells were then processed for electron microscopy. In the control tubes, trichomonad cells were incubated in citrate buffer without α-mannosidase or β-*N*-acetylglucosaminidase.

Quantitative evaluation of the level of lectin binding by parasite plasma membrane

From each sample, at least 30 trichomonad cells were chosen at random and photographed. Gold particles attached to the cell surface of parasites were counted in micrographs at a magnification of 30,000. The perimeter of *T. vaginalis* was measured on a Quadra 650 Macintosh computer using the NIH Image 1.47 program. The number of gold particles attached to 1 μm of plasma membrane was calculated by division of the total number of gold particles bound to the surface of the parasite by the cell perimeter.

Exposure of amnion membrane to *T. vaginalis* cells treated with specific glycosidases

A 0.4-ml aliquot of untreated *T. vaginalis* cell suspension (10^5 cells/ml) as well as 0.4 ml of a suspension of parasites treated with α-mannosidase and with β-*N*-acetylglucosaminidase (10^5 cells/ml) were added to control and test wells, respectively. Before exposure of amnion membrane to each type of trichomonad cell suspension the viability of parasites was measured with trypan blue. In all experiments, trichomonad cell viability was higher than 90%.

Preparation of amnion membrane for scanning electron microscopy

Amnion membranes exposed for 9 h to *T. vaginalis* cell suspensions were cut into 2-mm squares and fixed for 18 h in 2.5% glutaraldehyde in PBS. Pieces of amnion membrane were transferred to a specimen holder and washed with PBS, postfixed with 1% OsO_4 in PBS, dehydrated in increasing concentrations of ethanol followed by 100% acetone and critical-point-dried using CO_2 as the transitional fluid. Pieces of tissue were attached to stubs with double-sided carbon tape, coated with 20-nm gold particles and examined with a JSM 840 scanning electron microscope operating at 6 kV.

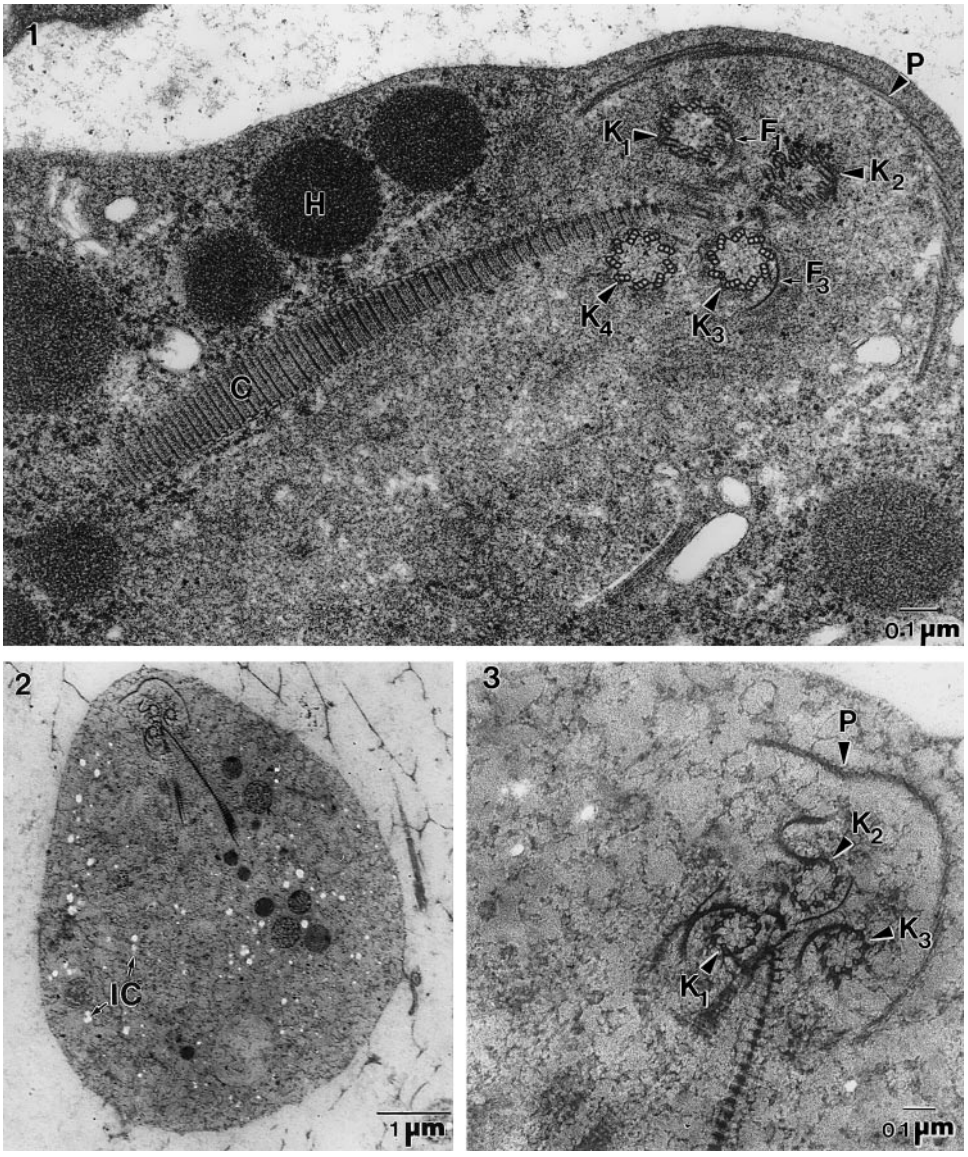

Fig. 1 Transmission electron micrograph of the anterior part of an organism, showing the pelta (*P*) and kinetosomes 1–4 (K_1–K_4) with their typical cylindrical arrangement of nine triplets of microtubules. Connecting fibrils (*F1*, *F3*) are clearly visible. The dorsal surface of the pelta is located near the cell membrane. A row of hydrogenosomes (*H*) is alligned parallel to the costa (*C*). Clear and dark disc bands of costa are clearly visible. **Figs. 2, 3** Preparation of *Trichomonas vaginalis* by the freeze-substitution technique. A *T. vaginalis* cell has been damaged by the formation of ice crystals (*IC*). Only cytoskeletal structures of the parasite are intact; other internal organelles have been either destroyed or poorly preserved (*P* Pelta, K_1–K_4 kinetosomes 1–4)

Calculation of the number of trichomonad cells attached to the amnion membrane

The total area of amnion membrane installed in each well of the chamber was calculated to be 19.625 mm^2. The total number of parasites attached to the amnion membrane in five wells was counted for each sample. The number of parasites attached to 1 mm^2 of amnion membrane was calculated by division of the total number of parasites by the total surface of exposed amnion membrane. The computer program Minitab was used for statistical analysis of the data using Student's *t*-test.

Results

Morphology of *Trichomonas vaginalis* fixed with glutaraldehyde

The mastigont area of *T. vaginalis* is presented in Fig. 1. Four anterior flagella are shown in the transverse section. Cytoplasmic organelles such as hydrogenosomes, vacuoles, costa and pelta were distributed homogeneously in the cytoplasm of the parasite.

Cytochemical labeling of thin sections

Parasites fixed with a mixture of formaldehyde and glutaraldehyde were poorly preserved. Most of the internal components of the parasite were either lost or badly damaged during the preparation of cells for transmission electron microscopy. *T. vaginalis* cells frozen in liquid propane were damaged by the formation of ice crystals. Only the *T. vaginalis* plasma membrane, cytoskeletal structures and hydrogenosome granules were intact, whereas other internal organelles were either lost or poorly preserved. No label was observed after exposure of thin sections of *T. vaginalis* to various gold particle-conjugated lectin solutions (Figs. 2, 3).

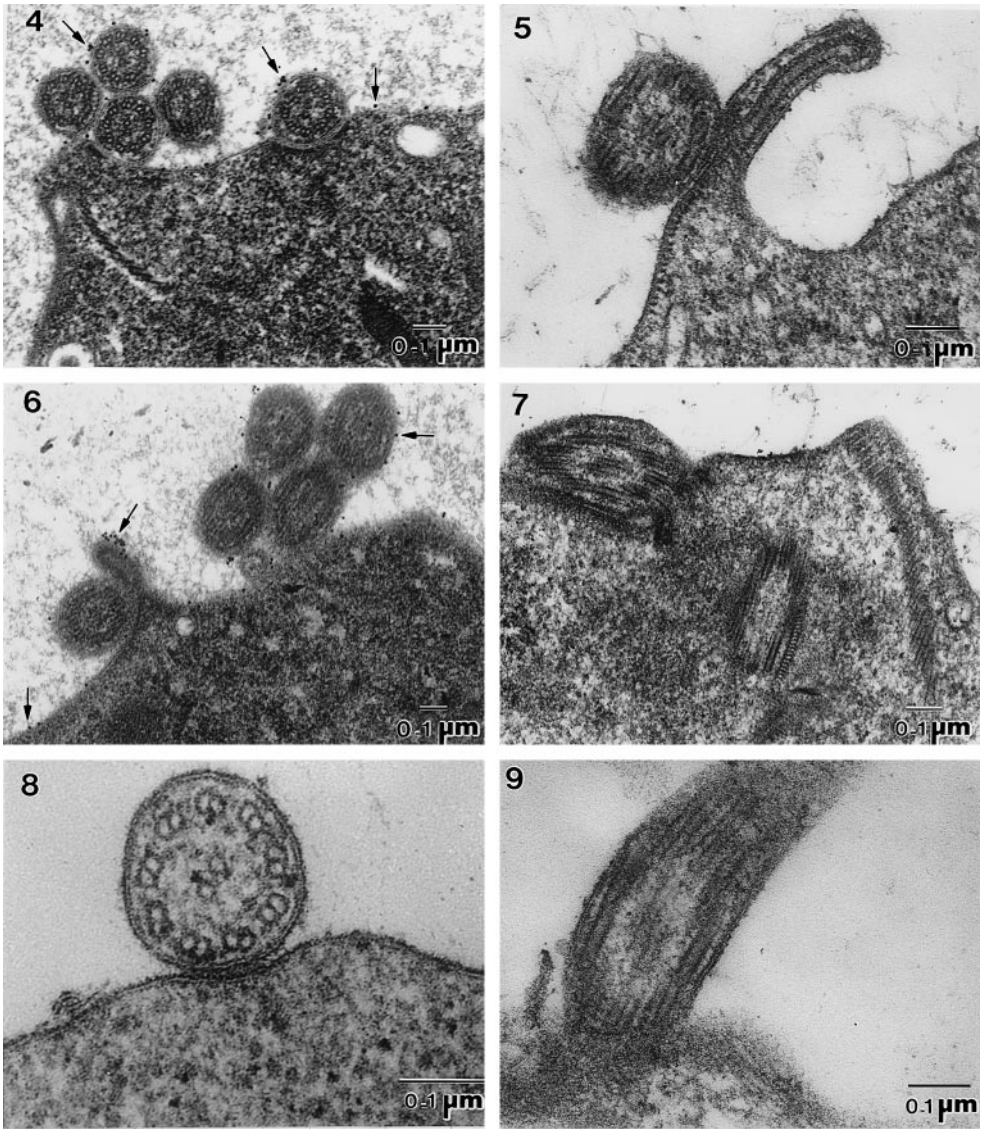

Fig. 4 Transverse section of a *T. vaginalis* prefixed with glutaraldehyde and labeled with gold particle-conjugated Con A. Gold particles (*arrows*) are adjacent to the *T. vaginalis* cell periphery and flagella. Labeling is restricted to some defined areas of the parasite cell surface. The labeling pattern shows non-homogeneous distribution of sugar residues on the external layer of the parasite cell coat. **Fig. 5** Control: *T. vaginalis* cells were incubated with gold particle-conjugated Con A in the presence of α-D-mannose. No gold label was observed in the presence of inhibitory sugar. **Fig. 6** *T. vaginalis* cells prefixed with glutaraldehyde and labeled with gold particle-conjugated WGA. The gold particles (*arrows*) show the location of the *N*-acetyl-D-glucosamine-like residues in the *T. vaginalis* glycocalyx. **Fig. 7** Control: cytochemical labeling of a *T. vaginalis* cell with gold particle-conjugated WGA in the presence of *N*-acetyl-D-glucosamine. Addition of *N*-acetyl-D-glucosamine to the incubation media inhibits the formation of lectin-sugar complex on the parasite cell surface. **Fig. 8** *T. vaginalis* cells were prefixed with glutaraldehyde and labeled with gold particle-conjugated UEA I lectin. The parasite cell surface is devoid of gold label. The absence of gold particles on the parasite cell coat indicates that fucose is not present on the surface of *T. vaginalis*. **Fig. 9** Control: *T. vaginalis* cells were incubated with gold particle-conjugated UEA I in the presence of fucose. The *T. vaginalis* cell surface is devoid of gold label

Cytochemical labeling of whole *T. vaginalis* cells

After cytochemical labeling with Con A and WGA, electron-dense gold particles attached nonhomogeneously to the surface membrane of *T. vaginalis*. This suggests that the parasite has lectin-binding sites on its surface. Cytochemical labeling of a *T. vaginalis* cell with gold particle-conjugated Con A indicates the presence of mannose or mannose-like components on the parasite surface membrane (Fig. 4). *N*-Acetyl-D-glucosamine or *N*-acetyl-D-glucosamine-like residues on *T. vaginalis* membrane were visualised by labeling of parasites with gold particle-conjugated WGA (Fig. 6). We did not observe any binding of gold particle-conjugated UEA I lectin to the plasma membrane of the parasite (Fig. 8). This indicates that fucose is not among the surface-bound carbohydrate molecules of *T. vaginalis*.

No label was observed with Con A or WGA when α-D-mannose and *N*-acetyl-D-glucosamine, respectively, were added to the incubation media, indicating that the lectin carbohydrate-binding reaction on the surface of the parasite can be blocked by the competitive action of these sugars (Figs. 5, 7).

Binding of gold particle-conjugated Con A to *T. vaginalis* cells treated with α-mannosidase

Quantitative data on the Con A-gold particle distribution on the surface of untreated *T. vaginalis* cells and α-mannosidase-treated parasites are presented in Table 1. The average number of gold particles attached to 1 μm of plasmalemma was 6.33 for untreated *T. vaginalis* cells and 1.00 for parasites incubated with α-mannosidase before cytochemical labeling with gold particle-conjugated Con A. The difference in the average number of gold particles attached to the surface of enzyme-treated *T. vaginalis* cells versus untreated parasites was statistically significant ($P < 0.0001$).

Binding of gold particle-conjugated WGA to *T. vaginalis* cells treated with β-*N*-acetylglucosaminidase

Results of the quantitative analysis of WGA-gold particle distribution on the surface of untreated parasite cells and β-*N*-acetylglucosaminidase-treated trichomonad cells are presented in Table 1. The average number of gold particles attached to 1 μm of parasite plasma membrane was 2.45 for untreated cells and 0.52 for cells incubated with β-*N*-acetylglucosaminidase. The difference observed between these two groups of *T. vaginalis* cells was statistically significant ($P < 0.0001$).

Exposure of amnion membrane to *T. vaginalis* cells treated with α-mannosidase

Results of the quantitative analysis of parasite/epithelial cell interaction after α-mannosidase treatment are presented in Table 2. The average number of parasites attached to amnion membrane in each well was 35,044 and 1,592 for untreated and enzyme-treated trichomonad cells, respectively. This indicates that about 87% of untreated trichomonad cells added to control wells were attached to the epithelial cell monolayer. This ratio was about 4% for *T. vaginalis* cells treated with α-mannosidase.

Exposure of amnion membrane to *T. vaginalis* cells treated with β-*N*-acetylglucosaminidase

Data on the parasite/epithelial cell interaction after β-*N*-acetylglucosaminidase treatment are presented in Table 2. Only 3.2% of enzyme-treated *T. vaginalis* cells were attached to the epithelial cells of amnion membrane. This ratio was 87% for untreated trichomonad cells. The average number of parasites attached to amnion membrane was 35,044 and 1,314 for untreated and treated parasite cells, respectively.

Table 1 Number of gold particles attached to 1 μm of *Trichomonas vaginalis* plasmalemma[a]

	Number of gold particles per 1 μm *T. vaginalis* plasmalemma
T. vaginalis cells treated with α-mannosidase and labeled with gold particle-conjugated Con A	1.003 ± 0.351
Untreated *T. vaginalis* cells labeled with gold particle-conjugated Con A	6.333 ± 1.353
P	0.0001
Trichomonad cells treated with β-*N*-acetylglucosaminidase and labeled with gold particle-conjugated WGA	0.529 ± 0.196
Untreated trichomonad cells labeled with gold particle-conjugated WGA	2.454 ± 0.419
P	0.0001

[a] Data represent mean values ± SD ($n = 30$)

Table 2 Results of exposure of amnion membrane to untreated *T. vaginalis* cells, cells treated with α-mannosidase and cells treated with β-*N*-acetylglucosaminidase[a]

	Exposure of amnion membrane to *T. vaginalis* cells treated with α-mannosidase	Exposure of amnion membrane to *T. vaginalis* cells treated with β-*N*-acetylglucosaminidase	Exposure of amnion membrane to untreated *T. vaginalis*
Total number of *T. vaginalis* cells attached to amnion membrane per well	1,592 ± 253.7	1,314 ± 250.4	35,044 ± 735.1
Percentage of *T. vaginalis* attached to epithelial cells	4%	3.2%	87%

[a] Data represent mean values ± SD

Discussion

There is strong evidence that an adhesion-dependent cytotoxicity mechanism is responsible for the destruction of the epithelial cells of a culture system used in vitro or of stratified squamous epithelium lining human urogenital tract (in vivo conditions) by *Trichomonas vaginalis* (Nielsen and Nielsen 1975; Krieger et al. 1985; Rasmussen et al. 1986; Silva Filho and De Souza 1988; Warton et al. 1988b; Arroyo et al. 1992, 1993; Gonzalez Robles et al. 1995; Mirhaghani and Warton 1996). This indicates that parasite and target epithelial cells recognise one another through sets of complementary structures on their surfaces. Therefore, adhesion of *T. vaginalis* to the epithelial cells is probably initiated by binding of cell-surface-associated ligands of one cell to the specific transmembrane receptors on the other.

Controversy exists regarding the nature of *T. vaginalis* surface-bound molecules involved in cytoadherence. *T. vaginalis* cell-surface-associated proteins and sugar-containing components have been implicated in host cell recognition by the parasites. Alderete and Garza et al. (1985) have reported that proteins of the surface coat of *T. vaginalis* are involved in adherence of the parasite to the target cell of an in vitro system. Alderete and Garza (1988) identified four high-molecular-weight proteins on the surface of a highly virulent strain of *T. vaginalis*. *T. vaginalis* cells pretreated with antibodies against these proteins lost their ability to bind to the target cells. The authors concluded that these proteins located on the surface coat of *T. vaginalis* are parasite virulence factors and are involved in the adherence of protozoa to their target cells.

On the other hand, it has been postulated that activity of the *T. vaginalis* surface-exposed glycoconjugates is essential for adhesion of the parasite to the target epithelial cells (Cappuccinelli et al. 1975; Brasseur and Savel 1982; Warton and Honigberg 1983; Roussel et al. 1991; De Azevedo et al. 1991).

Pretreatment of *T. vaginalis* with Con A (blocking mannose or mannose-like residues on the surface coat of the parasite) inhibited the adhesion of the parasite to the substrate. This indicates the involvement of sugar-containing molecules in parasite cytoadherence (Cappuccinelli et al. 1975). Brasseur and Savel (1982) have demonstrated that sialoglycolipids present on the *T. vaginalis* surface play a vital role in adhesion of the protozoan to the host cells. This adhesion constitutes an important step in the expression of parasite virulence. These authors have suggested that glycoconjugate components located on the surface coat of trichomonad cells are parasite membrane receptors involved in cytoadherence. Roussel et al. (1991) reported that a highly pathogenic strain of *T. vaginalis* could not cause cytopathic damage to the McCoy cell line in the presence of mannose and *N*-acetylglucosamine. Therefore, these authors concluded that *T. vaginalis* cells bear membrane saccharides that are responsible for the parasite's pathogenicity. The interaction between *T. vaginalis* and macrophages was analysed by De Azevedo and collaborators (1991). It was demonstrated that the first step of such interaction was the attachment of macrophages to the parasite surface. According to these authors, mannose-containing and glucose-containing receptors on the surface of *T. vaginalis* are directly involved in the adhesion of the two interacting surfaces (parasite/macrophages). Since pretreatment of *T. vaginalis* with Con A significantly increased parasite ingestion by the macrophages, the authors suggested that Con A altered the physicochemical properties of the parasite's surface molecules involved in the adhesion of the two cells.

We employed different lectins labeled with colloidal gold for the identification, localisation and quantitative evaluation of *T. vaginalis* surface sugar residues at the ultrastructural level.

According to several studies (Horisberger 1984; Benhamou and Ouellette 1986; Morioka et al. 1987; Rudin et al. 1989), thin sections of various pathogenic agents can be labeled with gold particle-conjugated lectins. Mannose was found in the cell walls and in some vacuoles after labeling of thin sections of *Candida utilis* with gold particle-conjugated Con A (Horisberger 1984). Benhamou and Ouellette (1986) employed various lectin-gold complexes to label different glycoconjugates in the cell wall of the fungus *Ascocalyx abietina* on thin sections. Morioka et al. (1987) reported the labeling of thin sections of *Escherichia coli* with WGA-gold complex. Rudin et al. (1989) labeled thin sections of two

strains of *Trypanosoma rangeli* with Con A, *Griffonia simplicifolia* agglutinin, *Pisum sativum* agglutinin, *Ricinus communis* agglutinin, *Vicia villosa* agglutinin and *Triticum vulgare* agglutinin.

Although we employed several methods (chemical fixation of *T. vaginalis* cells, thermal polymerisation of resin and the freeze-substitution technique, UV polymerisation of resin) to prepare *T. vaginalis* cells for electron microscopy, no label was observed after exposure of thin sections of *T. vaginalis* to various gold particle-conjugated lectins (Figs. 2, 3). Prefixation of whole *T. vaginalis* cells with glutaraldehyde followed by cytochemical labeling with gold particle-conjugated lectins showed that *T. vaginalis* has distinct binding sites for Con A (Fig. 4) and WGA (Fig. 6). This indicates the presence of mannose or mannose-like residues and of *N*-acetyl-D-glucosamine-containing residues on the parasite surface. The *T. vaginalis* cell surface was devoid of gold particles after cytochemical labeling of parasites with UEA I lectin (Fig. 8). This indicates that fucose is not among the *T. vaginalis* surface-associated carbohydrate-containing molecules.

The distribution of gold particles attached to parasite plasma membrane was irregular, indicating the nonhomogeneous distribution of carbohydrates on the outer layer of the parasite. The binding of gold particle-conjugated lectins to the surface coat of *T. vaginalis* cells was inhibited by the addition of specific competitive sugars (α-D-mannose and *N*-acetyl-D-glucosamine) to incubation media (Figs. 5, 7). This confirms that labeling is mediated specifically by an interaction between sugar-binding sites of lectins and carbohydrate residues on the surface coat of the parasite.

T. vaginalis cells were incubated with α-mannosidase and β-*N*-acetylglucosaminidase. Treatment of parasites with glycosidases did not cause any perturbation of *T. vaginalis* viability and motility. Employment of trypan blue, a dye that is taken up by dead cells, confirmed the integrity of the parasite population after treatment with enzymes.

Significant quantitative changes were observed in the number of gold particles that attached to the outer surface of parasites treated with α-mannosidase and β-*N*-acetylglucosaminidase. The average number of gold particles found attached to 1 μm of untreated *T. vaginalis* plasmalemma was almost 6-fold that seen bound to the α-mannosidase-treated parasite cell coat (Table 1). After treatment with β-*N*-acetylglucosaminidase, *T. vaginalis* cells lost almost 80% of their surface-exposed *N*-acetyl-D-glucosamine-containing molecules (Table 1). This indicates that some of the sugar chains located on the *T. vaginalis* surface were cut off by the action of enzymes.

For investigation as to whether the presence of *T. vaginalis* surface-exposed glycoconjugates was essential for adhesion of the parasite to the target cell, amnion membrane was exposed to enzyme-treated *T. vaginalis* cells. According to our results, 87% of untreated trichomonad cells added to control wells attached to the epithelial monolayer. This ratio was 4% for *T. vaginalis* cells treated with α-mannosidase and 3.2% for parasites treated with β-*N*-acetylglucosaminidase (Table 2). The majority of viable, motile, enzyme-treated *T. vaginalis* cells could not identify the target cells or adhere to them.

Our results suggest that sugar residues present in the *T. vaginalis* glycocalyx are involved in recognition of the host cells and in subsequent adhesion of the parasite to the epithelial cells.

It has been reported that proteins of the surface coat of trichomonads are responsible for the attachment of parasites to the epithelial cells of an in vitro system. *T. vaginalis* cells pretreated with antibody against cell-surface proteins lost the ability to bind to target cells (Alderete and Garza 1985, 1988). In a eukaryotic cell, most of the membrane-bound proteins are linked covalently to sugar chains. Therefore, pretreatment of *T. vaginalis* cells with antibodies against cell-surface proteins can cause perturbation of the glycan moieties of *T. vaginalis* surface-associated molecules or impair the integrity of the sugar chains of the membrane-associated glycoproteins of parasites. Although we do not exclude the possibility of the involvement of *T. vaginalis* proteins in cytoadherence, failure in recognition of the target cells by antibody-treated parasites could be due to insufficient or improper function of the sugar chains impaired by the action of antibodies against *T. vaginalis* surface proteins.

In conclusion, our observations support the hypothesis that surface-exposed sugar-containing components of *T. vaginalis* play an important role in the recognition of and adherence to the target cells by the parasite. *T. vaginalis* with inadequate numbers of sugar-chain receptors fail to attach to the epithelial cells and damage the target cells.

References

Alderete JF, Garza GE (1985) Specific nature of *Trichomonas vaginalis* parasitism of host cell surfaces. Infect Immun 50: 701–708

Alderete JF, Garza GE (1988) Identification and properties of *Trichomonas vaginalis* proteins involved in cytoadherence. Infect Immun 56: 28–33

Arroyo R, Engbring J, Alderete JF (1992) Molecular basis of host epithelial cell recognition by *Trichomonas vaginalis*. Mol Microbiol 6: 853–862

Arroyo R, Gonzalez Robles A, Martinez-Palomo A, Alderete JF (1993) Signalling of *Trichomonas vaginalis* for amoeboid transformation and adhesin synthesis follows cytoadherence. Mol Microbiol 7: 299–309

Benhamou N, Ouellette GB (1986) Ultrastuctural localization of glycoconjugates in the fungus *Ascocalyx abietina*, the scleroderris canker agent of conifers, using lectin-gold complex. J Histochem Cytochem 34: 855–867

Brasseur P, Savel J (1982) Evaluation de la virulence des souches de *Trichomonas vaginalis* par l'etude de l'effet cytopathogene sur culture des cellules. C R Soc Biol (Paris) 176: 849–860

Cappuccinelli P, Cagliani I, Cavallo G (1975) Involvement of a surface concanavalin A binding glycoprotein in the adhesion of *Trichomonas vaginalis* to substrates. Experientia 31: 1157–1159

Cross GAM (1990) Glycolipid anchoring of plasma membrane proteins. Annu Rev Cell Biol 6: 1–39

De Azevedo NL, Silva Filho FC, De Souza W (1991) The interaction of *Trichomonas foetus* and *Trichomonas vaginalis* with macrophages. J Submicrosc Cytol Pathol 2: 319–326

Dias Filho BP, Andrade AFB, De Souza W, Esteves JG, Angluster J (1992) Cell surface saccharide differences in drug-susceptible and drug-resistant strains of *Trichomonas vaginalis*. Microbios 71: 55–64

Gonzalez Robles A, Lazaro-Haller A, Espinosa-Cantellano M, Anaya-Velazquez F, Martinez-Palomo A (1995) *Trichomonas vaginalis*: ultrastructural bases of the cytopathic effect. J Euk Microbiol 42: 641–651

Heath JP (1981) Behaviour and pathogenicity of *Trichomonas vaginalis* in epithelial cell cultures. Br J Vener Dis 57: 106–117

Horisberger M (1984) Electron-opaque markers: a review. In: Polak JM, Varndell IM (eds) Immunolabelling for electron microscopy. Elsevier, Amsterdam, pp 17–26

Knudsen KA, Soler AP, Johnson KR, Wheelock MJ (1995) Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. J Cell Biol 130: 67–77

Kon VB, Papadimitriou JM, Robertson TA, Warton A (1988) Quantitation of concanavalin A and wheat germ agglutinin binding by two strains of *Trichomonas vaginalis* of differing pathogenicity using gold particle-conjugated lectins. Parasitol Res 75: 7–13

Krieger JN, Ravdin JI, Rein MF (1985) Contact-dependent cytopathogenic mechanisms of *Trichomonas vaginalis*. Infect Immun 50: 778–786

Mellors A, Sutherland R (1994) Tools to cleave glycoproteins. Tibtech 12: 15–18

Mirhaghani A, Warton A (1996) An electron microscope study of the interaction between *Trichomonas vaginalis* and epithelial cells of the human amnion membrane. Parasitol Res 82: 43–47

Morioka H, Tachibana M, Suganuma A (1987) Ultrastructural localization of wheat germ agglutinin-binding sites in *Escherichia coli* with wheat germ agglutinin-gold complex. J Electron Microsc (Tokyo) 36: 36–39

Nielsen MH, Nielsen R (1975) Electron microscopy of *Trichomonas vaginalis* Donne': interaction with vaginal epithelium in human trichomoniasis. Acta Pathol Microbiol Scand 83: 305–320

Rasmussen SE, Nielsen MH, Lind I, Rhodes JM (1986) Morphological studies of the cytotoxicity of *Trichomonas vaginalis* to normal human vaginal epithelial cells in vitro. Genitourin Med 62: 240–246

Ravdin JI, Murphy CF (1992) Characterization of the galactose-specific binding activity of a purified soluble *Entamoeba histolytica* adherence lectin. J Parasitol 2: 319–323

Roussel F, De Carli G, Brasseur PH (1991) A cytopathic effect of *Trichomonas vaginalis* probably mediated by a mannose/*N*-acetyl-glucosamine binding lectin. Int J Parasitol 21: 941–944

Rudin W, Schwarzenbach M, Hecker H (1989) Binding of lectins to culture and vector forms of *Trypanosoma rangeli* Tejera, 1920 (Protozoa, Kinetoplastida) and to structure of the vector gut. J Protozool 36: 532–538

Sharon N (1993) Lectin-carbohydrate complexes of plants and animals: an atomic view. TIBS 18: 221–226

Sharon N, Lis H (1993) Carbohydrates in cell recognition. Sci Am 268: 74–81

Silva Filho FC, De Souza W (1988) The interaction of *Trichomonas vaginalis* and *Trichomonas foetus* with epithelial cells in vitro. Cell Struct Funct 13: 301–310

Singer SJ (1992) Intercellular communication and cell-cell adhesion. Science 255: 1671–1677

Singh BN (1993) Lipophosphoglycan-like glycoconjugate of *Trichomonas foetus* and *Trichomonas vaginalis*. Mol Biochem Parasitol 57: 281–294

Warton A, Honigberg BM (1980) Lectin analysis of surface saccharides in two *Trichomonas vaginalis* strains differing in pathogenicity. J Protozool 27: 410–419

Warton A, Honigberg BM (1983) Analysis of surface saccharides in *Trichomonas vaginalis* strains with various pathogenicity levels by fluorescein-conjugated plant lectins. Z Parasitenkd 69: 149–159

Warton A, Kon VB, Papadimitriou JM (1988a) Demonstration of concanavalin A and wheat germ agglutinin-binding sites on the *Trichomonas vaginalis* surface coat using lectin-gold particle conjugates. J Electron Microsc (Tokyo) 37: 134–140

Warton A, Papadimitriou JM, Venaille TJ, Mendis AHW, Robinson BWS (1988b) Human amnion membrane as a model for studying the host-parasite relationship in trichomoniasis. Int J Parasitol 18: 1003–1005