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Original Article

Two *Trichomonas vaginalis* Surface Proteinases Bind to Host Epithelial Cells and Are Related to Levels of Cytoadherence and Cytotoxicity[1]

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Abstract

Recent reports strongly suggest that cytoadherence and cytotoxicity by *Trichomonas vaginalis* require cysteine proteinase activity. Because of the large number of cysteine proteinases synthesized by *T. vaginalis*, a ligand assay was used to identify specific proteinases which may selectively target host cells. Two cysteine proteinases from trichomonal extracts with relative molecular masses (Mr) of 65,000 daltons (65-kDa) and 30-kDa were found to avidly bind to HeLa cell and vaginal epithelial cell surfaces. The two proteinases were distinguished by differential inhibition with leupeptin and N-α-p-tosyl-L-lysine chloromethyl ketone (TLCK). Leupeptin pretreatment of live organisms inhibited the 30-kDa proteinase, which concomitantly reduced or eliminated cytoadherence. The *T. vaginalis* isolates with low levels of cytoadherence also had diminished or no detectable 30-kDa proteinase activity. On the other hand, TLCK pretreatment inhibited both the 30-kDa and 65-kDa proteinases, which resulted in decreased levels of cytoadherence and totally abolished contact-dependent cytotoxicity. Furthermore, isolates capable of attachment but with little or no cytotoxicity toward HeLa cells had no detectable host cell-bound 65-kDa proteinase. Finally, antiserum generated to each proteinase reacted by indirect immunofluorescence with live organisms, suggesting a surface location for both proteinases. This strategy and use of the ligand assay may permit for the deliniation of the role of two specific *T. vaginalis* surface proteinases in the properties of cytoadherence and cytotoxicity. (*Arch Med Res* 1995; 26:279)

KEY WORDS: Cysteine proteinases; Cytoadherence; Cytotoxicity; *T. vaginalis*.

Introduction

Trichomonas vaginalis is a sexually transmitted protozoan which parasitizes the urogenital tract of humans. Significant human suffering, especially among women, results from infection and colonization by this pathogen (1). More recently, adverse pregnancy outcome (2-4) and enhanced susceptibility to HIV seroconversion (5) have implicated *T. vaginalis* as well as other sexually transmitted diseases (STDs) (1-5) as contributing factors and etiologic agents. Although several virulence factors have been reported for this parasite (6-19), the exact contribution of these factors and properties to host cytopathology remains unknown.

Trichomonads have many proteinases, including numerous cysteine proteinases (20,21) and several metalloproteinases (22). At least twenty-three distinct

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Table 1

Proteinase Inhibitors Abolish *T. vaginalis* Cytoadherence and Cytotoxicity[a]

Expt no.	Assay time (h)	Addition	Percent activity (% inhibition)	
			Cytoadherence	Cytotoxicity
1	0.5	None	100.0 (0.0)	ND
	0.5	TLCK	50.0 (50.0)	ND
	0.5	Leupeptin	20.0 (80.0)	ND
2	4.0	None	ND	100.0 (0.0)
	4.0	TLCK	ND	0.0 (100.0)
	4.0	Leupeptin	ND	43.3 (54.7)

[a]Cytoadherence and cytotoxicity were performed at least three times as before (6,9,10,23) using *T. vaginalis* isolate NYH 286 added to HeLa cell monolayers.
[b]The length of time used for respective assay was determined before (23) to show optimal binding of parasites to cells and extent of monolayer destruction.
[c]Approximately 2 x 10^6 washed parasites were suspended in TYM medium and were pretreated with 1 mM TLCk or 0.2 mM leupeptin for 20 min at 37°C before adding to cell monolayer.
[d]The levels of attachment and host cell killing achieved by untreated parasites was taken as 100%. Numbers represent the mean of quadruplicate samples for a representative experiment. In this case the standard deviation of each sample was less than 5% of the values.

cysteine proteinase activities with relative molecular masses between 23,000 daltons (23-kDa) and 110-kDa have been detected by two-dimensional substrate gel electrophoresis (21). The cysteine proteinases were also found to undergo differential expression during *in vivo* and *in vitro* growth of isolates (21). These trichomonad proteinases were detected in vaginal secretions of patients, and antibody to proteinases were found in the patient serum and vaginal secretions (12), showing the *in vivo* relevance of this class of molecules.

A role for the cysteine proteinases of *T. vaginalis* in pathogenicity has been implicated in numerous studies (6-8,14,23). More recently, the proteinases of *T. vaginalis* have been shown to participate *in vitro* in important virulence properties, such as cytoadherence (23), host cytopathogenicity (8,18), and nutrient acquisition (14). It has been hypothesized that the trichomonad proteinases may mediate reactions which promote the adverse pregnancy outcome (2-4) and the enhanced susceptibility to HIV (5).

Because of the numerous proteinases synthesized (21) and known to be released during vaginal infection (12,19), it would be important to attempt to devise a strategy by which specific proteinase(s) possibly involved in a virulence property, such as cytoadherence (10,23) and cytotoxicity (6,7), might be identified. An attempt was made to use a ligand assay as a means of enriching for trichomonad proteinases with host cell surfaces as targets. Further analysis of cell-bound proteinase activities with levels of cytoadherence and cytotoxicity might then provide insight into a role in virulence for this subset of proteinases.

Using this approach two specific trichomonad proteinases were found to avidly associate with the surfaces of host cells. A relationship was established, based on differential inhibition of the proteinases, with cytoadherence and cytotoxicity. Even more intriguing were results showing that representative isolates with or without the two proteinases correspondingly possessed high amounts or little cytoadherence or cytotoxicity of host cells, respectively. The significance of our observations is discussed in terms of the role the two specific cysteine proteinases may have as putative virulence factors and as a basis for future research.

Materials and Methods

Parasites. Organisms were grown in Diamond's Trypticase-yeast extract-maltose (TYM) medium supplemented with 10% heat-inactivated horse serum (24). Only late-logarithmic-phase organisms were used for all experiments. *Trichomonas vaginalis* NYH 286, RU 375, IR 78, and DD3 are long-term-grown isolates used in other studies (6,9,14,15,25). *T. vaginalis* T001, T002, T003, T011 and T012 are fresh clinical isolates that were recently obtained using procedures in accordance with the ethical standards established and approved by the Committee on Human Bioethics at the University of Texas Health Science Center at San Antonio.

Host Cells, Cytoadherence and Cytotoxicity. HeLa cells [American Type Culture Collection (ATCC), Rockville, Md] were maintained in Dulbecco-modified minimal essential medium (DMEM) (GIBCO Labor-

atories, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Hazleton Research Products, Denver, PA) in a 7% CO_2 environment at 37°C, as before (6,10,13). Cells were harvested at approximately 80% confluency, at which time the cells were fixed with 2.5% glutaraldehyde as previously described (10). For cytoadherence and cytotoxicity assays (6,10,23), HeLa cells were seeded at a density of 2 x 10^4 cells in 100 µl of DMEM-FBS per well into Costar 96-well microtiter plates (Bellco, New Vineland, NJ) (6). Human vaginal epithelial cells (VEC) were also obtained as before from vaginal swabs of normal women and fixed with 2.5% glutaraldehyde for cytoadherence (9). The ability of the trichomonads to parasitize HeLa cells and to cause contact-dependent host cell killing was measured by an established procedure (6,23).

Anti-Proteinase Serum and Indirect Immunofluorescence. Antibodies to the 65-kDa and 30-kDa proteinases were generated by immunization of rabbits using standard protocols with purified protein bands from polyacrylamide gels copolymerized with gelatin (10). This monospecific antiserum to each proteinase was tested for reactivity to the proteinases (21) and was used in indirect immunofluorescence experiments on live parasites (15) to determine their surface location.

Identification of Host Cell-Binding Proteinases. The ligand assay has been used for the identification of trichomonad adhesins (10,25) and employed here to identify cysteine proteinases which bind to the surfaces of HeLa cells and VECs. Briefly, 2 x 10^7 organisms washed three times were resuspended in 500 µl of phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 4 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.0) and lysed with 50 µl of 10% sodium deoxycholate (Sigma Chemical Co., St. Louis, MO) (10,25). After gentle homogenization and incubation for 20 min at 4°C, this detergent extract was diluted to 1 ml volume, layered on a 10% sucrose cushion and centrifuged at 16,000 x g for 30 min at 4°C (10,25). Clarified protein extract was then added directly to 10^6 pelleted fixed HeLa cells or VECs and incubated for 1 h at 37°C with gentle stirring in siliconized microfuge tubes. Cells were then centrifuged at 800 x g for 5 min, washed three times with PBS containing 0.05% sodium deoxycholate, and suspended in electrophoresis dissolving buffer containing 2% β-mercaptoethanol (26). After heating for 20 min at 60°C, cells were removed by centrifugation and eluted proteins analyzed by 10% sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) containing 0.2 % co-polymerized gelatin (Bio-Rad Laboratories, Richmond, CA) as a substrate (20,21,27). After electrophoresis, SDS was removed by washing the gel for 2 h in 2.5% Triton X-100. To activate proteinases, the gel was immersed in 100 mM sodium acetate buffer, pH 4.0, containing 1 mM dithiothreitol for 2 h at 37°C with gentle stirring, followed by staining the gels with 0.25% Coomassie blue R-250 for 1 h. After destaining, clear bands indicative of proteolytic activity were visualized against a stained background. Inhibition of proteinases was accomplished by pretreatment of live trichomonads in PBS containing TLCK or leupeptin (Sigma) for 20 min at 37°C (23).

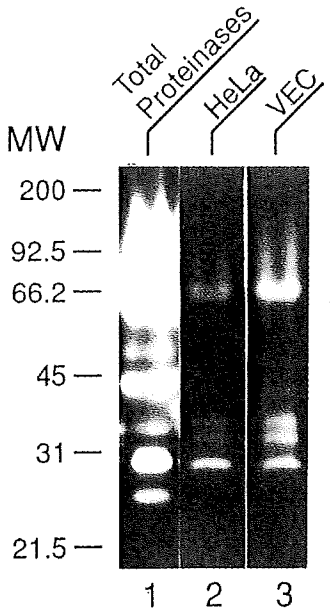

Figure 1. Identification of epithelial cell-binding proteinases present in detergent extracts of *T. vaginalis* NYH 286 using substrate-gel electrophoresis, as described in Materials and Methods. VEC refers to vaginal epithelial cells.

Results

Selective Binding by T. vaginalis *Proteinases to HeLa Cells*

We combined the use of the ligand assay (10,25) with electrophoretic analysis for proteinase activity (20,21,27) to determine whether any of the numerous proteinases of *T. vaginalis* (21) might bind to host cell surfaces. In this way a selective enrichment of a subset of proteinases might provide a means for identifying any specific proteinases involved in certain properties, i.e., cytoadherence and cytotoxicity. Figure 1 shows the representative pattern of total trichomonad proteinases (lane 1) incubated with fixed HeLa cells or vaginal epithelial cells (VECs). Two prominent proteinase activities with relative molecular weights of 65-kDa and 30-kDa were always detected in contrast to other minor bands. In >30 experiments performed to date with a total of 30 fresh and long-term-grown isolates, only the 65-kDa and 30-kDa proteinase activities always bound to HeLa cells

(lane 2) and VECs (lane 3) when compared to the numerous proteinases of the total extract (lane 1) and as reported before (21). These epithelial cell-binding proteinases could only be recovered after heating fixed cells in electrophoresis dissolving buffer (24), suggesting a high-affinity binding to host-cell surfaces. Proteinase activities were never detected from fixed HeLa cells or VECs handled by themselves (not shown).

Selective Inhibition of the 65-kDa and 30-kDa Proteinases

We demonstrated that proteinase inhibitors, like TLCK and leupeptin, were effective in decreasing *T. vaginalis* cytoadherence and contact-dependent cytotoxicity (Table 1). For example, Table 1 shows representative results from numerous experiments. Leupeptin was effective in reducing by 80 to 100% the levels of cytoadherence. In contrast, TLCK, while affecting levels of adherence by ~50%, always drastically diminished or abolished cytotoxicity (Table 1).

It seemed logical to attempt next to determine whether the HeLa cell-binding proteinases were selectively inhibited by TLCK and leupeptin. Live organisms were pretreated with TLCK or leupeptin prior to the ligand assay, and conditions used did not affect parasite viability or motility. Figure 2 (lane 2) shows complete inhibition of both the 65-kDa and 30-kDa proteinases by TLCK. Leupeptin treatment, on the other hand, selectively inactivated the 30-kDa proteinase (lane 3). Since live organisms were treated prior to solubilization for the ligand assay, these results also possibly indicate that the inhibitors acted on proteinases localized on the parasite surface.

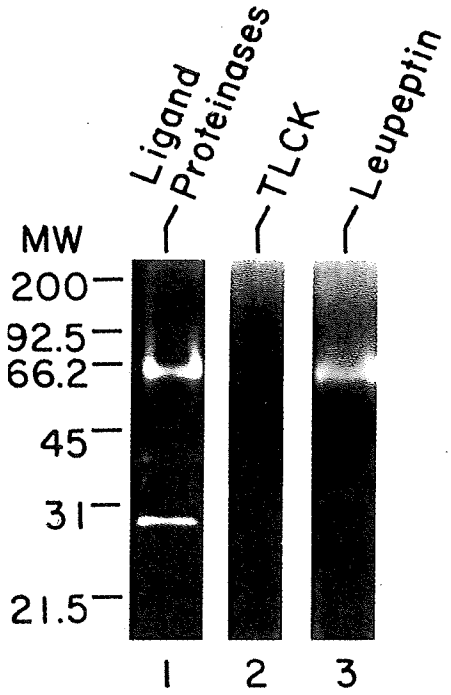

Figure 2. Effects of TLCK and leupeptin on the activity of epithelial cell-binding cysteine proteinases from *T. vaginalis* NYH 286 using substrate-gel electrophoresis as in Figure 1. The cell-binding trichomonads proteinases were eluted from fixed HeLa cells after incubation with detergent extract as described in Materials and Methods. Inhibition of proteinases was accomplished by pretreatment of live trichomonads with 1 mM TLCK, (lane 2) or 0.2 mM leupeptin (lane 3) for 20 min at 37°C before detergent extraction, and compared to untreated organisms (lane 1). MW refers to molecular weight standards in kDa.

Relationship Between the T. vaginalis *65-kDa and 30-kDa Proteinases with Host Cytotoxicity and Cytoadherence, Respectively*

Many isolates were tested further for the presence of host cell-binding proteinase activities and evaluated for levels of cytoadherence and cytotoxicity as shown in Table 1. All long-term-grown isolates, known to have depressed levels of host cytoadherence, possessed decreased amounts of cell-bound 30-kDa proteinase activity (Figure 3A). Fresh isolates, on the other hand, had both higher amounts of 30-kDa proteinase bound to cells and elevated levels of adherence. Furthermore, long-term-grown isolates with diminished cytoadherence had no detectable 30-kDa proteinase (Figure 3A), as evidenced by the representative isolates IR 78, DD3 and AL8W. These data strongly suggest a relationship between the 30-kDa proteinase with the property of cytoadherence.

Figure 3B also shows that isolates without detectable 65-kDa proteinase, such as isolate T003, had correspondingly less cytotoxicity. Alternatively, isolates with higher amounts of 65-kDa proteinase, like T001, T011 and T012, gave enhanced host cell killing. All long-term-grown isolates with reduced 65-kDa proteinase activity were unable to efficiently kill host cells under the assay conditions used. No less than 50 different isolates were examined, and results were obtained which were identical to those of the representative isolates shown here.

Antiserum to the 65-kDa and 30-kDa Proteinases Detects the Surface of Live Trichomonads

Indirect immunofluorescence was performed on live parasites with monospecific antiserum generated to the 30-kDa and 65-kDa proteinases. The presence of antibody to the respective proteinases was established using an immunoprecipitation assay recently described (21). Both antisera, but not control, prebleed serum, readily detected the surface of live organisms, indicating a surface location for the host cell-binding proteinases (data not shown). These data are consistent with the experiments performed above on the selective inhibition of the proteinases upon treatment of live organisms with TLCK and leupeptin.

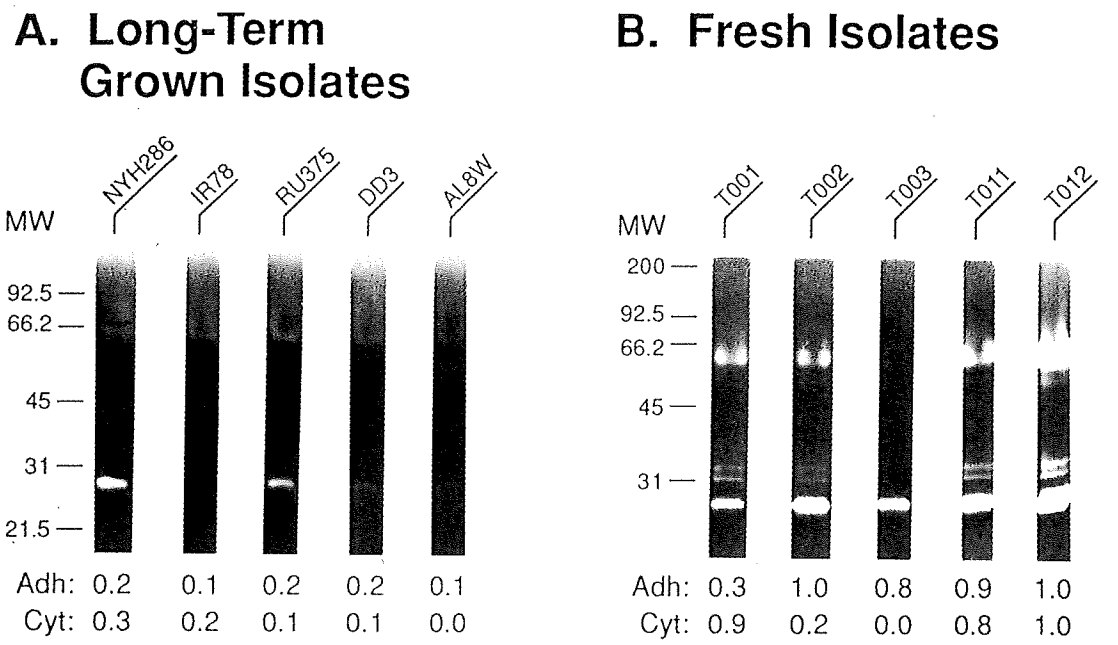

Figure 3. Relationship between HeLa cell-binding proteinases of long-term-grown isolates (A) vs. fresh clinical isolates (B), using substrate-gel electrophoresis as in Figure 1, with levels of host cytoadherence and cytotoxicity. Cytoadherence (Adh) and cytotoxicity (Cyt) for each isolate were determined with organisms from the same culture used for preparing extract for the ligand assay as indicated in Materials and Methods section. The values of cytoadherence and cytotoxicity were compared with isolate NYH 286 as in Table 1. MW refers to molecular weight standards in kDa.

Discussion

Given the high number of total proteinases in *T. vaginalis* (20,21,27), an approach which identifies certain proteinases with possible roles in virulence would be important. This report strongly suggests that two cysteine proteinases of *T. vaginalis* are capable of binding to host cell surfaces. As such, these two proteinases may be studied further for any contribution to virulence properties.

The relationships between the two proteinase activities with cytotoxicity and cytoadherence is especially noteworthy. A reduction by ≥80% in the levels of cytoadherence (Table 1) by leupeptin inhibition of the 30-kDa proteinase (Figure 2), in addition to the greatly reduced or absent cytoadherence by parasites without the 30-kDa proteinase (Figure 3), strongly suggested a role for this molecule in the attachment to host cells (23). Moreover, the 30-kDa proteinase activity and correspondingly higher levels of cytoadherence were always elevated in fresh-isolates when compared with long-term-grown isolates, as reported before (10). Whether this proteinase is involved in the property of cytoadherence, as has been proposed before by us (23), remains to be determined.

We also showed here (Table 1) and before (23) that, under certain conditions, TLCK but not leupeptin was able to inhibit killing of host cells. The presence of the 65-kDa cell-binding cysteine proteinase was related to the ability of the parasites to destroy cells in monolayer cultures. TLCK inhibition of both cell-binding proteinases prevented the parasite killing of host cell monolayers *in vitro*. Although further experiments will be necessary to prove that the 65-kDa proteinase mediates cytotoxicity, these data do suggest that this proteinase may predispose host cells to trichomonal-mediated damage.

The synthesis of many proteinases by the parasite during infection has been established (12,21). Many patients have a large array of soluble trichomonad proteinases in vaginal secretions in addition to vaginal antibody to the proteinases (12), providing evidence of relevance during trichomoniasis. In addition, the release by trichomonads, during *in vitro* growth and multiplication, of numerous proteinases (19) illustrates the active process by which these molecules are present during the dynamics of the infection.

The binding of both proteinases with host cell surfaces may be indicative of either specific recognition of substrate by the proteinases or the existence of receptors on host cells for the proteinases. Either possibility awaits further experimentation. It is interesting to speculate on the mechanism by which the 30-kDa proteinase may be involved in cytoadherence. Previous work has shown the requirement of proteinase action on the trichomonal surface prior to attachment to host cells (23). A hypothesis was proposed whereby the *T. vaginalis* adhesins (10,25),

which are sensitive to the cysteine proteinases released by the parasite (10) and possibly protected from trichomonad proteinases being released during growth (19), would require unmasking prior to receptor recognition on host cells. It is possible that deposition of proteinases on the host cell surface represents a step in the preparation of adhesins for function. It is also conceivable that the proteinase(s), once on the host surface, have the adhesin proteins as substrates. In this scenario a receptor-ligand type of interaction necessary for cytoadherence may require first the deposition of the two proteinases.

It is noteworthy that no proteinase activity was ever detected from purified adhesins tested similarly(10). Even more recently (unpublished observations), recombinant adhesins were found to have no detectable proteinase activity under these experimental conditions. This is reaffirmed by the absence of any immunoreactivity of the anti-proteinase serum with any of the four purified adhesins (10). Thus, these epithelial cell-binding parasite proteinases represent molecules distinct from the bona fide adhesins (10).

Attempts were also made to demonstrate whether anti-proteinase serum inhibited the properties of cyto-adherence and/or cytotoxicity. Unfortunately, possibly due to the low-titered specific antibody, efficient inhibition was not observed. Alternatively, it is possible that the antibody is ineffective at inhibiting the enzyme activity or is removed from the parasite surface during the co-incubation periods between parasites and host cells. The extensive membrane perturbations that occur upon *T. vaginalis* recognition of epithelial cells has been established (13). This, too, may account for mobilization of the antibody-neutralized proteinase away from the site of action (13). At any rate, future work with possibly affinity-purified, high-titered antibody and/or purified proteinases should yield insight into the role of these molecules in the host-parasite interaction.

An earlier paper by us also provided evidence for a surface proteinase (21), albeit a proteinase with a different electrophoretic mobility than that seen here for the 30-kDa and 65-kDa proteinases. It is equally noteworthy that the host cell-binding proteinases reside on the surface. This may be the first report on the binding of two surface proteins from *T. vaginalis* to host cells. This presence of surface proteinases by a parasite that synthesizes many cysteine (20,21) and metalloproteinases (22) may be significant and requires more experimentation.

References

1. Krieger JN. Urologic aspects of trichomoniasis. Invest Urol 1981; 18:411.
2. Minkoff H, Grunebaum AN, Schwarz RH, Feldman J, Cummings M, Crombleholme W, Clark L, Pringle G, McCormack WM. Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. Am J Obstet Gynecol 1984; 150:965.
3. Cotch MF, Pastrek JG III, Nugent RP, Yeng DE, Martin DF, Eschenbash DA. Demographic and behavioral predictors of *Trichomonas vaginalis* infection among pregnant women. Obstet Gynecol 1991; 78:1087.
4. Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RS. A review of premature birth and subclinical infection. Am J Obstet Gynecol 1992; 166:1515.
5. Laga M, Manoka A, Kivuvu M, Malele B, Tuliza M, Nzila N, Goeman J, Behets F, Batter V, Alary M, Heyward WL, Ryder RW, Piot P. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. AIDS 1993; 7:95.
6. Alderete JF, Pearlman E. Pathogenic *Trichomonas vaginalis* cytotoxicity to cell culture monolayers. Br J Vener Dis 1984; 60:99.
7. Krieger JN, Ravdin JI, Rein MF. Contact-dependent cytopathogenic mechanisms of *Trichomonas vaginalis*. Infect Immun 1985; 50:778.
8. Krieger JN, Posson MA, Rein MF. Beta-hemolytic activity of *Trichomonas vaginalis* correlates with virulence. Infect Immun 1983; 41:1291.
9. Alderete JF, Demeš P, Gombošova A, Valent M, Fabušova M, Janoška A, Stefanovic, Arroyo R. Specific parasitism of purified vaginal epithelial cells by *Trichomonas vaginalis*. Infect Immun 1988; 56:2558.
10. Arroyo R, Engbring J, Alderete JF. Molecular basis of host epithelial cell recognition by *Trichomonas vaginalis*. Mol Microbiol 1992; 6:853.
11. Lehker MN, Chang TH, Dailey DC, Alderete JF. Specific erythrocyte binding is an additional nutrient acquisition system for *Trichomonas vaginalis*. J Exp Med 1990; 171:2165.
12. Alderete JF, Newton E, Dennis C, Neale KA. The vagina of women infected with *Trichomonas vaginalis* has numerous proteinases and antibody to trichomonad proteinases. Genitourin Med 1991; 67:469.
13. Arroyo R, González-Robles A, Martínez-Palomo A, Alderete JF. Signalling of *Trichomonas vaginalis* for amoeboid transformation and adhesin synthesis follows cytoadherence. Mol Microbiol 1993; 7:299.
14. Dailey DC, Chang T, Alderete JF. Characterization of a hemolysin of *Trichomonas vaginalis*. Parasitology 1990; 101:171.
15. Alderete JF, Kasmala L, Metcalfe EC, Garza GE. Phenotypic variation and diversity among *Trichomonas vaginalis* and correlation of phenotype with contact-dependent host cell cytotoxicity. Infect Immun 1986; 53:285.
16. Hogue MJ. The effect of *Trichomonas vaginalis* on tissue-culture cells. Am J Hyg 1943; 37:142.
17. Health JP. Behaviour and pathogenicity of *Trichomonas vaginalis* in epithelial cell cultures. A study by light and scanning electron microscopy. Br J Vener Dis 1981; 57:106.
18. Garber GE, Lemchuk-Favel LT, Bowie WR. Isolation of a cell detaching factor of *T. vaginalis*. J Clin Microbiol 1989; 1548.
19. Lockwood BC, North MJ, Coombs GH. The release of hydrolases from *Trichomonas vaginalis* and *Tritrichomonas foetus*. Mol Biochem Parasitol 1988; 30:135.
20. Coombs GH, North MJ. An analysis of the proteinases of *Trichomonas vaginalis* by acrylamide gel electrophoresis. Parasitology 1983; 86:1.

21. Neale KA, Alderete JF. Analysis of the proteinases of representative *Trichomonas vaginalis* isolates. Infect Immun 1990; 58:157.
22. Bozner P, Demes P. Proteinases of *Trichomonas vaginalis* and *Trichomonas mobilensis* are not exclusively of cysteine type. Parasitology 1990; 102:113.
23. Arroyo R, Alderete JF. *Trichomonas vaginalis* surface proteinase activity is necessary for parasite adherence to epithelial cells. Infect Immun 1989; 57:2991.
24. Diamond LS. The establishment of various trichomonads of animals and man in axenic cultures. J Parasitol 1957; 43:488.
25. Alderete JF, Garza GE. Identification and properties of *Trichomonas vaginalis* proteins involved in cytoadherence. Infect Immun 1988; 56:28.
26. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond) 1970; 227:680.
27. Lockwood BC, North MJ, Scott KI, Bremner AF, Coombs GH. The use of a highly sensitive electrophoretic method to compare the proteinases of trichomonads. Mol Biochem Parasitol 1987; 24:89.