INFECTION AND IMMUNITY, July 2000, p. 4358–4360 Vol. 68, No. 7
0019-9567/00/$04.00+0

Host and Tissue Specificity of *Trichomonas vaginalis* Is Not Mediated by Its Known Adhesion Proteins

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Received 31 January 2000/Returned for modification 16 March 2000/Accepted 11 April 2000

Adhesion of *Trichomonas vaginalis* is believed to be dependent on four adhesion proteins, which are thought to bind to vaginal epithelial cells in a specific manner with a ligand-receptor type of interaction. However, the specific receptors on the host cell have not yet been identified. In this work, the ability of the *T. vaginalis* adhesins to bind to cells of different histologic derivations and from different species has been studied. HeLa, CHO, and Vero cell lines; erythrocytes from different species; and a prokaryote without a cell wall, *Mycoplasma hominis*, were employed in order to investigate the cell specificity of the *T. vaginalis* adhesins. We observed that the *T. vaginalis* adhesins are able to bind to the different cell types to the same extent, suggesting that the host and tissue specificity of *T. vaginalis* adhesion should not be due to specificity of the parasite adhesins. Our results suggest that the data published to date on the subject are probably artifactual and that the experiments reported in the literature are not appropriate for identification of protozoan adhesins.

Trichomonas vaginalis is a flagellated protozoan parasite responsible for one of the most diffused sexually transmitted diseases. The microorganism parasitizes the urogenital tract in humans, with high tissue and host specificity (19). Adhesion of the protozoan to the host cell is the primary step leading to cytopathogenicity, which is contact dependent (16). In the past, several reports have described the molecular characterization of the different steps involved in interaction of the parasite with the human vaginal epithelial cells. Adhesion of the parasite is thought to be mediated by four trichomonad surface proteins, reportedly AP65, AP51, AP33, and AP23 (5, 6), which are believed to recognize specific proteins of the host by a ligand-receptor type of interaction (3, 7, 8), although the receptors in the host cell have not yet been identified. The putative role of these proteins in adhesion has been characterized by using HeLa cells, because of their epithelial nature and the genital epithelium origin. Moreover, these cells appear more susceptible to in vitro destruction by live *T. vaginalis* than other cell types (4). Although Arroyo et al. (7, 8) were able to partially inhibit the binding of *T. vaginalis* to epithelial cells and to detect the adhesins on the protozoan surface by using polyclonal antibodies, Brugerolle et al. (10) recently demonstrated by immunogold staining with monoclonal antibodies that the putative adhesins are not localized on the trichomonad surface, but are restricted to a hydrogenosomal function.

In this report, we have investigated the ability of the four putative adhesins in binding to cells of different species and histologic derivations in an attempt to assess whether the recognition of a specific receptor in a particular cell type could be responsible for the well-known tissue specificity of the parasite. Different cell types, of human and nonhuman derivation, have been used: HeLa, CHO, and Vero cell lines; human and rabbit erythrocytes; and a bacterium without a cell wall, *Mycoplasma hominis*.

T. vaginalis cells of isolate SS-22 were cultured in Diamond's TYM at 37°C in a 5% carbon dioxide atmosphere (13). HeLa, CHO, and Vero cells were cultured in RPMI under the same conditions. The *M. hominis* strain PG21 was kindly provided by S. Razin, Hebrew University, Jerusalem, Israel, and was cultured in SP4 medium (28). Human erythrocytes of different groups were obtained from healthy human donors, and rabbit erythrocytes were obtained by bleeding of experimental animals. Identification of bound trichomonad proteins was performed by the same procedures used for identification of the putative adhesins, in order to guarantee homogeneous experimental conditions allowing for an appropriate comparison of data (7). For the ligand assay experiments, semiconfluent cell lines were detached by trypsinization, washed three times in phosphate-buffered saline (PBS), and counted. Washed cells were fixed with 2.5% glutaraldehyde in PBS for 1 h at 4°C under the conditions described in the literature (7). *M. hominis* cells from an overnight culture were washed in PBS and fixed as described for epithelial cells. In order to assess the extent of binding of the solubilized adhesins to different cell types, fixed HeLa, CHO, Vero, and *M. hominis* cells were incubated separately with total *T. vaginalis* proteins obtained by solubilization as described by Alderete et al. (7). The trichomonad proteins bound to fixed cells were detected as follows. After an overnight incubation at 4°C with the solubilized trichomonad proteins, fixed cells with the bound trichomonad proteins were collected and washed five times with TDSET buffer (10 mM Tris [pH 8.00], 0.2% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 5 mM EDTA [pH 8.00], 1% Triton X-100), resuspended in Laemmli buffer (21), and boiled, and the recovered proteins were loaded in a 10% polyacrylamide gel for visualization. The whole procedure is described in the literature as a "ligand assay" (7).

Figure 1A shows the *T. vaginalis* proteins that bind to fixed HeLa (lane 1), CHO (lane 2), and Vero (lane 3) cells as a result of the ligand assays. As expected, the proteins that adhered to HeLa cells (lane 1) have the same molecular masses as the adhesion proteins described by Arroyo et al.—AP65, AP51, AP33, and AP23 (7). As can be observed by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns, the four adhesins also bind to CHO and Vero cells. These cells were obtained from hamsters and monkeys and were derived

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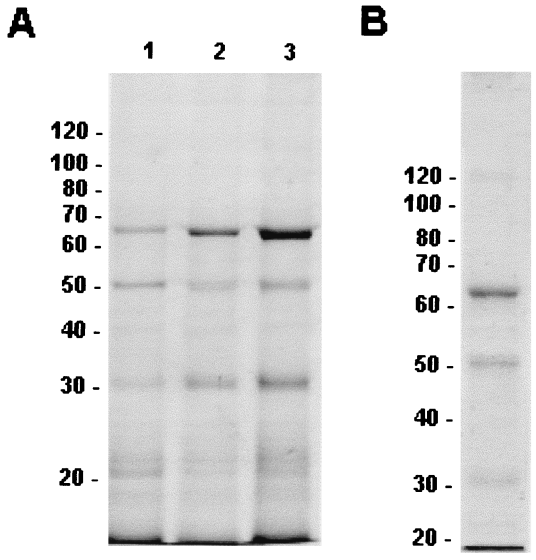

FIG. 1. (A) Representative ligand assays of *T. vaginalis* proteins obtained with fixed HeLa (lane 1), CHO (lane 2), and Vero (lane 3) cells. (B) Representative ligand assay of *T. vaginalis* proteins obtained with fixed *M. hominis* cells. Cells were detached by trypsinization or collected by centrifugation, washed, fixed with glutaraldehyde, and incubated with total *T. vaginalis* proteins. The adhered trichomonad proteins were detached by boiling fixed cells in Laemmli buffer, subjected to electrophoresis on 10% polyacrylamide gels, and revealed with Coomassie blue. Molecular mass markers (kilodaltons) are indicated on the left.

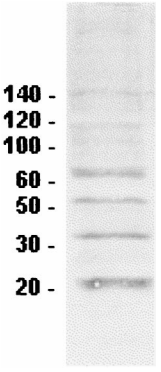

FIG. 2. Representative immunoblot showing the *T. vaginalis* proteins that bind to trypsinized human group 0 erythrocytes. The same proteins could be observed with trypsinized and nontrypsinized human and rabbit erythrocytes. Washed erythroctes were incubated with total *T. vaginalis* proteins, and membranes were collected, washed, subjected to electrophoresis, and transferred to nitrocellulose. Bound parasite proteins were then revealed by using total anti-*T. vaginalis* antibodies. Molecular mass markers (kilodaltons) are indicated on the left.

from tissues of different types. Moreover, CHO cells are fibroblasts. Therefore, a tissue specificity of the adhesin binding seems unlikely. More surprisingly, the four proteins are able to bind to the same extent to fixed mycoplasma cells (Fig. 1B), which, being prokaryotic, possess extremely different surface structures compared to vaginal epithelial cells.

In order to assess the extent of binding of the four proteins to nonfixed membranes, human and rabbit erythrocytes were used. Moreover, experiments were also performed after trypsinization of erythrocytes for digestion of the external protein domains (17). Erythrocytes were washed and prepared as described previously (18). The trichomonad proteins bound to both trypsinized and nontrypsinized erythrocytes were detected after coincubation of cells with total trichomonad proteins for 2 h, three washes in PBS, electrophoresis, and transfer onto nitrocellulose filters. The bound trichomonad proteins were revealed by incubation of the nitrocellulose filters with total anti-*T. vaginalis* antibodies (17).

When trypsinized and nontrypsinized human and rabbit erythrocytes were used, the results obtained were the same as with the other cell types. Figure 2 shows a representative immunoblot pattern obtained with human trypsinized group 0 erythrocytes. Moreover, as reported previously by us (1, 17), two other adhesive proteins could be observed to bind to the erythrocyte membrane, having molecular masses of 120 and 140 kDa (Fig. 2).

In order to assess that the proteins observed to bind to the different cell types were the same and that they were the four putative adhesins described for HeLa cells, *T. vaginalis* proteins bound to fixed HeLa cells as a result of a ligand assay were detached by boiling in Laemmli buffer, electrophoresed, and transferred onto nitrocellulose. The membrane filter containing the four putative adhesins was then incubated with total anti-*T. vaginalis* antibodies for 2 h and washed, and the bound antibodies were then eluted by incubation for 5 min with glycine buffer (200 mM glycine [pH 2.8]) and neutralized with 1 M imidazole (pH 7.8), as described previously (17). Ligand assays of *T. vaginalis* proteins performed with CHO, Vero, and mycoplasma cells and erythrocytes were then electrophoresed and transferred onto a nitrocellulose filter, which was probed with the eluted antiadhesin antibodies. The eluted antiadhesin antibodies recognized four *T. vaginalis* proteins bound to all of these cell types, with molecular masses of 65, 51, 33, and 23 kDa.

The results obtained in this work suggest that binding of the four *T. vaginalis* putative adhesins should not involve a protein receptor on the host cell, but rather an affinity for other structures. An affinity of the putative adhesins for membranes could be suggested. In fact, these proteins are able to bind to trypsinized cells. Moreover, two of the four adhesins, AP65 and AP33, have been characterized, and their sequences are known (6, 14, 25). As can be inferred from their protein sequences, which have a striking homology with the hydrogenosomal enzymes malic dehydrogenase (decarboxylating) (2, 6, 15, 22, 25) and succinyl coenzyme A synthetase (14, 23), respectively, these proteins could possess an intrinsic affinity for membranes. On the basis of these data, we can suppose that the use of solubilized proteins, instead of live intact parasites, as well as the use of trypsinized and glutaraldehyde-fixed epithelial cells, is not a more appropriate technique to detect adhesins. Therefore, the data suggest that the host and tissue specificity of *T. vaginalis* adhesion should not be dependent on the specificity of the four adhesins. It then seems likely that there must be other factors responsible for this specificity that could be researched in terms of other mechanisms of adhesion (9, 11, 12, 23), requirements for other recognition mechanisms (24, 27), or the need for factors such as hormones (26) or other molecules present in the vaginal environment (18). As a matter of fact, we cannot exclude that the tissue specificity is merely related to the fact that, following introduction into the genital tract, trichomonads can parasitize only vaginal cells.

This work was supported by grants of The University of Sassari (Progetto di Ricerca sul 60%) and by the MURST (Cofinanziamento 1997).

The technical assistance of Edmondo Manca was greatly appreciated.

REFERENCES

1. **Addis, M. F., P. Rappelli, G. Delogu, F. Carta, P. Cappuccinelli, and P. L. Fiori.** 1998. Cloning and molecular characterization of a cDNA clone coding for *Trichomonas vaginalis* alpha-actinin and intracellular localization of the protein. Infect. Immun. **66:**4924–4931.
2. **Addis, M. F., P. Rappelli, P. Cappuccinelli, and P. L. Fiori.** 1997. Extracellular release by *Trichomonas vaginalis* of a NADP+ dependent malic enzyme involved in pathogenicity. Microb. Pathog. **23:**55–61.
3. **Alderete, J. F., P. Demés, A. Gombošova, M. Valent, M. Fabušova, A. Janoška, J. Štefanovič, and R. Arroyo.** 1988. Specific parasitism of purified vaginal epithelial cells by *Trichomonas vaginalis*. Infect. Immun. **56:**2558–2562.
4. **Alderete, J. F., and G. E. Garza.** 1985. Specific nature of *Trichomonas vaginalis* parasitism of host cell surfaces. Infect. Immun. **50:**701–708.
5. **Alderete, J. F., and G. E. Garza.** 1985. Identification and properties of *Trichomonas vaginalis* proteins involved in cytadherence. Infect. Immun. **56:**28–33.
6. **Alderete, J. F., J. L. O'Brien, R. Arroyo, J. A. Engbring, O. Musatovova, O. Lopez, C. Lauriano, and J. Nguyen.** 1995. Cloning and molecular characterization of two genes encoding adhesion proteins involved in *Trichomonas vaginalis* cytoadherence. Mol. Microbiol. **17:**69–83.
7. **Arroyo, R., J. Engbring, and J. F. Alderete.** 1992. Molecular basis of host epithelial cell recognition by *Trichomonas vaginalis*. Mol. Microbiol. **6:**853–862.
8. **Arroyo, R., A. Gonzàlez-Robles, A. Martìnez-Palomo, and J. F. Alderete.** 1993. Signalling of *Trichomonas vaginalis* for amoeboid transformation and adhesin synthesis follows cytoadherence. Mol. Microbiol. **7:**299–309.
9. **Bonilha, V. L., M. do C. Ciavaglia, W. De Souza, and F. Costa e Silva Filho.** 1995. The involvement of terminal carbohydrates of the mammalian cell surface in the cytoadhesion of trichomonads. Parasitol. Res. **81:**121–126.
10. **Brugerolle, G., G. Bricheux, and G. Coffe.** 2000. Immunolocalization of two hydrogenosomal enzymes of *Trichomonas vaginalis*. Parasitol. Res. **86:**30–35.
11. **Cappuccinelli, P., I. Cagliani, and G. Cavallo.** 1975. Involvement of a surface concanavalin-A binding glycoprotein in the adhesion of *Trichomonas vaginalis* to substrates. Experientia **15:**1157–1159.
12. **Casta e Silva Filho, F., W. de Souza, and J. D. Lopes.** 1988. Presence of laminin-binding proteins in trichomonads and their role in adhesion. Proc. Natl. Acad. Sci. USA **85:**8042–8046.
13. **Diamond, L. S.** 1957. The establishment of various trichomonads of animals and man in axenic cultures. J. Parasitol. **43:**488–490.
14. **Engbring, J. A., and J. F. Alderete.** 1998. Three genes encode distinct AP33 proteins involved in *Trichomonas vaginalis* cytoadherence. Mol. Microbiol. **28:**305–313.
15. **Engbring, J. A., J. L. O'Brien, and J. F. Alderete.** 1996. Trichomonas vaginalis adhesin proteins display molecular mimicry to metabolic enzymes. Adv. Exp. Med. Biol. **408:**207–223.
16. **Fiori, P. L., P. Rappelli, and M. F. Addis.** 1999. The flagellated parasite *Trichomonas vaginalis*: new insights into cytopathogenicity mechanisms. Microbes Infect. **1:**149–156.
17. **Fiori, P. L., P. Rappelli, A. M. Rocchigiani, and P. Cappuccinelli.** 1993. *Trichomonas vaginalis* haemolysis: evidence of functional pore formation on red cell membranes. FEMS Microbiol. Lett. **109:**13–18.
18. **Gold, D., and I. Ofek.** 1992. Adhesion of *Trichomonas vaginalis* to plastic surfaces: requirement for energy and serum constituents. Parasitology **105:**55–62.
19. **Honigberg, B. M.** 1978. Trichomonads of importance in human medicine, p. 275. *In* J. P. Kreier (ed.), Parasitic protozoa, vol. II. Academic Press Inc., New York, N.Y.
20. **Hrdy, I., and M. Muller.** 1995. Primary structure of the hydrogenosomal malic enzyme of *Trichomonas vaginalis* and its relationship to homologous enzymes. J. Eukaryot. Microbiol. **42:**593–603.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
22. **Lahti, C. J., P. J. Bradley, and P. J. Johnson.** 1994. Molecular characterization of the alpha-subunit of *Trichomonas vaginalis* hydrogenosomal succinyl CoA synthetase. Mol. Biochem. Parasitol. **66:**309–318.
23. **Lehker, M. W., and D. Sweeney.** 1999. Trichomonad invasion of the mucous layer requires adhesins, mucinases, and motility. Sex. Transm. Infect. **75:**231–238.
24. **Miraghani, A., and A. Warton.** 1998. Involvement of *Trichomonas vaginalis* surface-associated glycoconjugates in the parasite/target cell interaction. A quantitative electron microscopy study. Parasitol. Res. **84:**374–381.
25. **Rappelli, P., A. M. Rocchigiani, G. Erre, M. M. Colombo, P. Cappuccinelli, and P. L. Fiori.** 1995. Sequence of cDNA coding for a 65 kD adhesive protein for the specific detection of *Trichomonas vaginalis* by PCR. FEMS Microbiol. Lett. **129:**21–26.
26. **Silva Filho, F. C., and V. L. Bonilha.** 1992. Effect of estrogens on the adhesion of *Trichomonas vaginalis* to epithelial cells in vitro. Braz. J. Med. Biol. Res. **25:**9–18.
27. **Silva Filho, F. C., J. Ortega-Lopez, and R. Arroyo.** 1998. YIGSR is the preferential laminin-1 residing adhesion sequence for *Trichomonas vaginalis*. Exp. Parasitol. **88:**240–242.
28. **Waites, K. B., and D. Taylor-Robinson.** 1999. *Mycoplasma* and *Ureaplasma*, p. 782–794. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.

Editor: W. A. Petri, Jr.