López Bottentuit, Luciene; Braga Bandeira de Melo, Mariane; López Ortega, Jaime; Arroyo, Rossana; Filho Costa E Silva, Fernando

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Strategies by which some Pathogenic Trichomonads integrate Diverse Signals in the Decision-making Process

LUCIENE BOTTENTUIT LÓPEZ1, MARIANE BANDEIRA DE MELO BRAGA1, JAIME ORTEGA LÓPEZ2,3, ROSSANA ARROYO2,3 and FERNANDO COSTA E SILVA FILHO1,2∗∗

1 UFRJ-Instituto de Biofísica Carlos Chagas Filho, Ilha do Fundão – 21949-900 Rio de Janeiro, Brazil
2 CICATA (PIBIOM) del Instituto Politecnico Nacional, CP 11500, México, DF.
3 CINVESTAV del Instituto Politecnico Nacional, Apto. Postal 14-740, CP 07360, México, DF.

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ABSTRACT

The interaction between each one of *Trichomonas vaginalis* and *Tritrichomonas foetus* with their hosts is a complex process in which components associated to the cell surfaces of both parasites and host epithelial cells, and also to soluble components found in vaginal/urethral secretions, are involved. Either cytoadhesion or the cytotoxicity exerted by parasites to host cells can be dictated by virulence factors such as adhesins, cysteine proteinases, laminin-binding proteins, integrins, integrin-like molecules, a cell detachment factor, a pore-forming protein, and glycosidases among others. How trichomonads manipulate informations from the extracellular medium, transduce such informations, and respond to them by stimulating the activities of some surface molecules and/or releasing enzymes are the aspects concerning trichomonal virulence which are here briefly reviewed and discussed.

Key words: *Trichomonas vaginalis*, *Tritrichomonas foetus*, parasitic protozoa, host-cell parasite interaction relationships, trichomonal virulence.

INTRODUCTION

Trichomonads constitute a group of amithocondriate flagellate protozoa which are mostly parasite or comensal inhabiting oxygen-poor environments (Cavalier-Smith 1993). Parasitic trichomonads include *Trichomonas vaginalis* and *Tritrichomonas foetus* which are both aethiologic agents of the human urogenital and bovine trichomoniasis or trichomonosis, respectively (Honigberg 1978a,b).


The spectrum of clinical trichomoniasis in human ranges from asymptomatic carrier stage to flagrant vaginitis (Rein *et al*. 1990). The clinical significance of *T. vaginalis* infection in male urologic conditions seems to be controversial (Krieger 1981). Most of the *T. vaginalis*-harboring men are asymptomatic. However, it was observed a significant

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**Member of the Academia Brasileira de Ciências
Correspondence to: Dr. Fernando Costa e Silva Filho
E-mail: fcsf@biof.ufrj.br
morbidity caused by *T. vaginalis* in men, most commonly, urethral inflammation with non-gonococcal urethritis (Krieger 1995). As zinc is highly toxic to *T. vaginalis*, and prostatic fluids contain considerable amounts of such metal (Krieger 1995, Krieger & Rein 1982) it is possible that many healthy men could be refractory to infection with *T. vaginalis*.

It is important to claim the attention that the diagnosis of trichomoniasis in men has been a subject of discussion. Most of men aged 16-22 years which were submitted to prostatic massage were positive for trichomonas tests (Saxena & Jenkins 1991). Thus, the examination of urine sediment test routinely used for the diagnosis of trichomonas in men if not accompanied by prostatic massage may induce false results. Further, the parasite in men always search for a very poor-oxygen niche as seems to be the case of the inner regions of the male reproductive tract (Krieger 1990).

Women with acute infections usually present cervical erosion, hemorrhagic spots on the vaginal mucosa, and a mucopurulent discharge (Rein 1990). This clinical picture reveals how serious can be trichomoniasis in women. Among pregnant women *T. vaginalis* can induce preterm rupture of membranes, and also preterm birth (Cotch et al. 1991) being the parasite also able to weaken human amniochorion in *in vitro* conditions (Drapper et al. 1995).

Infected male partners of infected women can indeed lead to recurrent urogenital infections with *T. vaginalis* in women (Petrin et al. 1998). Such recurrence of *T. vaginalis* infection in women seems to be the main cause of the acute infection previously described (Guimarães Gonçalves 1992). High cure rates of infected women can be only obtained when the male partners are simultaneously treated.

*T. foetus* is observed in the urogenital cavity of cows being that it is able to migrate upward through the cervix and invade uterus (Honigberg 1978b). In bulls the parasite can be found from the preputial cavity until urethra, and also in deeper parts of the animal’s urogenital tract. The parasite is usually found harbored in the animal’s preputial cavity, and once infected bulls may harbor *T. foetus* throughout their lives (Yule et al. 1989).

Among infected calves it is frequent the occurrence of endometritis, also accompanied by uterine, cervical, and vaginal catarrh. Since endometritis and uterine catarrh prevent fertilization affecting the regularity of the estrous cycle, most of the infected calves can remain permanently steriles.

The interaction of each one of *T. vaginalis* and *T. foetus* with the epithelium lining urogenital cavities is the initial and the crucial step to establishment of both human and bovine trichomoniasis.

The examination of both vaginal and cervical smears of women or calves may reveal cytomorphological alterations induced by trichomonads. The smears are typically rich in polymorphonuclear elements, and a high number of isolated epithelial cells or whole epithelium fragments (Petrin et al. 1998). A carefully done pathological study carried out among a population of sexually promiscuous women which presented recurrent trichomoniasis have also revealed that the severity of trichomoniasis can induce cytopathological stages of dysplasia/metaplasia (Guimarães Gonçalves, 1992). These data seem to support some others which indicate the possibility of *T. vaginalis* to be directly associated to uterine cancer (Berggren 1969, Yap et al. 1995, Zhang & Begg 1994).

Altogether these data emphasize the importance of studies dealing with the molecular mechanisms by which pathogenic trichomonads interact with host cells.

Since the 40’s attempts have been done to bring at light detailed data concerning interaction between each one of the here referred trichomonads with cultured epithelial cells. As far as can ascertained, since the original descriptions of *Trichomonas vaginalis* and *Tritrichomonas foetus*, Hogue (1943) and the Honigberg’s research group provided much of the groundwork on trichomonadal pathogenesis. They were the pioneers in the investigation of the *in vitro* interaction between trichomonads and sheets formed by epithelial cells.
The data obtained from studies carried out in
in vivo as in in vitro conditions support the general
idea that both T. vaginalis and T. foetus need to ad-
here to host cells in order to exert their cytopathic
effects. However, the possibility of both pathogenic
trichomonads to exert their cytotoxicity activities on
cultured epithelial cells without cytoadherence de-
serves to be considered since secreted and released
products by the parasites, mainly glycosidases (Silva
Filho & de Souza 1988, Silva Filho et al. 1986) and
a cell-detaching factor (Garber et al. 1989) into both
acellular and cellular culture media have been shown
to be highly toxic to some epithelial cells.

Even carefully done, much of the studies foc-
using the host cell-pathogenic trichomonads inter-
action relationships carried out during the last 25
years at different laboratories produced data which
seem to be conflictive, and in some cases diametri-
cally opposite. This apparent discrepancy may be
related much more to the strains of the parasites as
well as the epithelial cells used during the experi-
ments than to remarkable different points of view
from the authors.

Fresh isolates of T. vaginalis that are mantained
just after axenization as stabaltes, and subsequently
cloned comprise very different populations of the
parasite (Fattel Facenda 1997). A highly virulent
Mexican strain of T. vaginalis named CNCD 147 is
represented by subpopulations ranged from very low
to high degrees of virulence. Two of such subpopu-
lations -MXL-411, and MXL-5213- are completely
different in morphological, cytoadherence, and cy-
totoxicity terms (Fattel Facenda 1997) and may be
also different in terms of the occurence of cytoplasmic
RNA-viruses (Wang et al. 1987). These data
strongly suggest (a) the possibility of the selection of
a T. vaginalis population during a particular experi-
ment, and that (b) the concept of strain in T. vaginalis
needs to be carefully considered or still it should be
further revised.

Epithelial and also non-epithelial cells have
been used in studies concerning pathogenic
trichomonads-host cell interaction relationships.
The epithelial cell lineages used in such studies in-
clude WISH (Martinotti et al. 1986), HeLa
(Alderete & Pearlman 1984), MDCK (Silva Filho
& de Souza 1988), and sheets formed by each one
of human (Arroyo et al. 1993) and bovine (Singh
et al. 1999) vaginal epithelial cells among others.
However, excepting MDCK cells, no studies con-
cerning determination of some basic biological and
biophysical parameters are available on most of the
in vitro epithelial models used for interaction with
pathogenic trichomonads. Thus, even apparently
forming monolayers or spots of cell sheets on inert
surfaces we still do not know if these cells present,
for instance, electrical resistance comparable to that
observed in in vivo conditions. From such inference
it is reasonable to consider that experiments carried
out with different strains or clones of the trichomon-
ads concomitantly with different cultured epithelial
cells might result in the previously referred discrep-
ancies.

However, all the authors have shown that either
T. vaginalis as T. foetus are able to damage sheets or
monolayers formed by all the assayed epithelial cells
(Alderete & Pearlman 1984, Krieger et al. 1985,
Rasmussen et al. 1986, Silva Filho & de Souza
1988).

After a basic characterization of the in vitro be-
havior of human or bovine vaginal epithelial cells
they will obviously be the appropriate cellular mod-
els to study the host cell-T. vaginalis or T. foetus
interactions, since the chemical composition of the
apical surface-facing parasites in epithelial cells
greatly varies according to the organs or tracts from
which they have been obtained.

It has been pointed out that the cytoadhesion is
a key property to the colonization and infection
of T. vaginalis (Alderete & Garza 1985), and prob-
ably also T. foetus (Silva Filho & de Souza 1988).
Trichomonal cytoadhesion is a highly specific event
where molecules associated to both parasites and
host cells, and also to some host soluble molecules
found in vaginal/urethral secretions, are involved.
The components displayed on the cell surface of the
parasites plays important roles in cytoadhesion.

Nonetheless, the knowledgement of the parasite and the host epithelial cell surface molecular repertoires is of relevance.

RECOGNIZING THE EPITHELIAL CELL SURFACE

Trichomonal cytoadhesion like other cell-cell interaction processes is a very complex phenomenon which is always preceded by cellular recognition. The last is in turn, governed by electrostatic, van der Waals, and Lewis acid-base interactions (see van Oss 1994). It means that short and long-range forces generated into the interaction media may reduce/prevent or impulse the mutual interaction between the surfaces of pathogenic trichomonads and the epithelial cells.

Either pathogenic trichomonads or the apical domain of epithelial cells possess negatively charged surfaces, being that much of such surface negativeness comes from the ionization of carboxyl groups from sialic acids residues, and phosphates from phospholipids susceptible to treatment with phospholipase C (Silva Filho et al. 1986). In addition, the cell surface of pathogenic trichomonads is hydrophobic (Bonilha et al. 1992). Therefore, either modulation of the exposition of sialic acid residues as well as expulsion of water of hydration (van Oss 1997) are necessary to the parasites to approach the epithelial cell surface. The presence of both endogenous neuraminidase and phospholipase activities (Silva Filho et al. 1989) in trichomonads may be related to the ability of the parasites to contact the epithelial cell surface to distances below 30 nm (van Oss 1994).

Both T. vaginalis and T. foetus when found in axenic cultures tend to be uniform in shape. A pear or oval shape is predominantly observed among microorganisms swimming into culture medium. By contrast, rounded and ameboid forms of T. vaginalis are found among parasites attached to the culture tubes walls, and mainly among those found adhered on sheets formed by vaginal epithelial cells (VEC) (Arroyo et al. 1993).

Treatment of the parasites with depolymerizing agents of microfilaments such as the cytochalasins B or D induces a high inhibition of the attachment of T. vaginalis to inert surfaces (Silva Filho et al. 1987). These cytoplasmic expansions performed by the parasites are indeed essential to the parasites to remain attached to glass or plastic surfaces (Bonilha et al. 1992) as well as to adhere onto epithelial monolayers formed by MDCK cells (Silva Filho & de Souza 1988). Taking into account the hydrophilicity of the media where the mutual interaction of trichomonads with host cells takes place, and the electrostatic barrier generated between the two interacting surfaces, the presence of pseudopods and/or fillopodia in T. vaginalis should represent a very efficient mechanism displayed by the parasite to contact the epithelial surface. Through the emission of thin pseudopods much of the interstitial water surrounding the parasite could become expelled.

Although this mechanism could be not easy to imagine intuitively by some parasitologists and microbiologists, one may picture the process as a squeezing action, exerted by surrounding, partly hydrogen-bounded water molecules, on the hydrophobic molecules or particles in the midst. Similar to what occurs during the bacteria-phagocyte interaction (van Oss et al. 1975), by means of its neuraminidase and/or phospholipase activities T. vaginalis could reduce its surface negativeness, and by means of the emission of pseudopods it could improve an efficient way to ensure its contact with host cells or with other microorganisms, as seems to be the case of the interaction between erythrocytes and bacteria and T. vaginalis (Rendón-Maldonado et al. 1998).

Further, the movement of the five flagella easily found in pear-shaped trophozoites of T. vaginalis and the four ones found in T. foetus trophozoites are of importance not only to the locomotion of the parasite into liquid media but also to the induction of liquid currents that become parasites able to easily uptake nutrients from the surround-
ing medium. It is important to claim the attention that, as clearly demonstrated to the function of cilia in some prokaryotic cells, flagellar movement of pathogenic trichomonads seems to be not enough to ensure the approaching of the parasites to cell monolayers. The interfacial forces residing in the bulk solution must be indeed considered in order to provide knowledge on how to manipulate host environment to hamper the recognition of the host epithelial cells by pathogenic trichomonads.

Little is still known on the surface components of epithelial cells involved in their recognition by the here cited trichomonads. Some glycoconjugates of the apical domain of MDCK-I cell surfaces are the candidates to be recognized by both trichomonads, meanly by *T. foetus* (Bonilha et al. 1995). On the other hand, lectin molecules found at the cell surface of trichomonads appear to be important to the epithelial cell recognition by these trichomonads (Babál & Russel 1999). It means that the recognition of the host epithelial cells by both *T. vaginalis* and *T. foetus* may be partially related to some parasitic surface lectins.

**ADHERING TO THE EPITHELIAL CELLS**

It was clearly demonstrated by the Alderete’s research group that two classes of molecules at least are directly implicated in the adhesion of *T. vaginalis* to both VEC and HeLa cells. One of these molecules class was named adhesin (Alderete et al. 1995), and the other one is represented by cysteine proteinases (Arroyo & Alderete 1989). The following four adhesins (APs) have been identified in *T. vaginalis*: AP65, AP51, AP33 and AP23 (Arroyo et al. 1992). Recently, the Arroyo’s group identified a 120kDa surface protein in *T. vaginalis* that may be a new adhesin (Yañez-Gómez 1999).

The *ap* 65-1, *ap* 65-2, and the *ap* 65-3 genes are present within *T. vaginalis* genome in multiple copies. Interestingly, the isoforms 1, 2, and 3 of the AP65 exhibit significant homology to the hydrogenosomal malic enzyme (Hrdý & Müller 1995) either at amino acid as nucleotide levels (Alderete et al. 1995, O’Brien et al. 1996). As previously pointed out (O’Brien et al. 1996) there is the possibility of gene sharing (Platigorsky & Wistow 1989) in *T. vaginalis*. The possibility of the occurrence of gene sharing as well as multifunctional surface proteins in pathogenic trichomonads and other parasitic protozoa constitute very exciting areas to be explored.

The expression of APs on the cell surface of *T. vaginalis* is alternate with the expression of P 270 which is a highly immunogenic protein (Alderete 1988). This alternance in the expression of these known surface markers of *T. vaginalis* seems to be related to a mechanism displayed by the parasite to its evasion from the immune system.

At least in *T. vaginalis* both adhesins and surface proteinases activities may be functionally associated since CP activity is required to parasite adhesion to HeLa cell monolayers. Multiple activities of cysteine proteinases have been found among parasitic protozoa (North et al. 1990) including *T. foetus* (Mallinson et al. 1995). However the CPs in *T. foetus* are much associated to the cytotoxicity exerted by the parasite to cultured epithelial cells rather than cytoadhesion (Silva Filho & de Souza 1988, Melo Braga 2000). Recently it was observed in *T. vaginalis* a relationship between a 65kDa proteinase whose activity could be inhibited by E-64, and the ability of the parasite to damage HeLa cell monolayers (Alvarez-Sánchez et al. 2000).

Trichomonal adhesins are much more than “biological glues”. Their syntheses are greatly enhanced as soon as *T. vaginalis* contacts HeLa cell monolayers VEC (Arroyo et al. 1993). It means that there is a specific signalling for ameboid transformation in the parasite that is started during the binding of adhesins to their partners residing at the epithelial cell surface. The possibility of CP activities to be initially implicated in the modulation of the exposition of cell surface components in *T. vaginalis*, and the resulting exposition of new surface glycoconjugates residues to be triggers for the signalling of adhesin synthesis seems to be a very interesting subject.
Based on some cytochemical (Benchimol et al. 1986) and physico-chemical (Silva Filho & de Souza 1986, Silva Filho et al. 1986) findings on the trichomonal cell surface it is possible to infer that the plasma membrane of *T. vaginalis* and mainly of *T. foetus* possesses a considerable level of fluidity.

Taking into account the membrane fluidity in *T. vaginalis* it is reasonable to think the occurrence of patching and capping of adhesin molecules during the cytoadhesion of the parasite to cultured epithelial cells. It is possible that when the cytoadhesion just begins the adhesins might be found distributed over the entire cell surface of *T. vaginalis*, and when the process is entirely completed the adhesins might be preferentially located at pseudopods/filopodia of parasites spreading onto epithelial cell monolayers.

**SOME EXTRACELLULAR MATRIX COMPONENTS MAY TELL TRICHOMONADS WHAT THEY HAVE TO DO**

Extracellular matrix (ECM) components are secreted by cells in order to model the intercellular space. The networks formed by extracellular matrix components exert profound effects over cells. Thus, tissue homeostasis is intimaly related to the extracellular matrix organization.

Both *laminins* and *fibronectin* are ECM glycoproteins important to the maintenance of the integrity of the urogenital walls. *Laminins* (LMN) are trimeric molecules with eleven genetically distinct chains (Engvall & Wewer, 1996), some of them forming the basement membrane (Tryggvsson 1993). *Fibronectin* (FN) is a dimeric glycoprotein (Hynes 1992a) composed by three types of multiple repeats or modules (FN 1, FN 2, and FN 3), and it is known as a prototype of adhesive glycoproteins.

The abilities of these ECM molecules to serve as substrates for cell adhesion, spreading, and migration are widely known. Moreover, putative cell adhesion sequences have been mapped to domain III of the α1, and α1 chains of laminin-1. The sequences include RGD in mouse α1, and YIGSR in mouse α1 (Yamada & Kleinman, 1992). The RGD sequence is also found within the cell-binding domain III (region repeats 8-11) of fibronectin (Hynes 1992a).

These discrete aminoacid sequences may represent instructional informations for most of the studied prokaryotes and eukaryotes. Among pathogenic microorganisms it has been shown that some of them are able to recognize each one of the cell adhesion-binding sequences (Furtado et al. 1992, Lopes et al. 1988, Silva et al. 1999, Talamás-Rohana & Meza 1988).

Either FN as LMN as well as other RGD or YIGSR containing molecules are potentially able to form molecular bridges between pathogens and host cells. Such cellular intercourse can convey a wide range of different messages to both cells. A simple touch of some cells including parasitic protozoa to each one of immobilized FN or LMN can result in drastic alterations in cellular morphology which is almost always followed by proteolytic activation (Avila & Calderón 1993, Li et al. 1995, Vásquez et al. 1995).

By analogy to what occurs to the individuals in a society, we can state that FN or LMN can be responsible for the affection or hostility between cells. Hostility seems to be what occurs during FN or LMN-mediating cell-parasite interaction while affection can be driven by FN or LMN between healthy neighboring cells having the same embryonic origin.

A 118kDa surface molecule enables *T. vaginalis* and *T. foetus* to recognize laminin-1, and also to adhere to LMN-revested substrates (Silva Filho et al. 1988). Specific recognition of laminin-1 by *T. vaginalis* is preferentially located at YIGSR aminoacid sequence (Silva Filho et al. 1998) while FN recognition by *T. foetus* is carried out through some manose residues-containing glycoconjugates (Silva Filho & de Souza 1987).

It is very important to point out that to like that observed in most of the investigated cells (Wewer & Engvall 1994) the binding of laminin to the surface components of trichomonads is improved in the
presence of Mn$^{++}$ (Wewer & Engvall 1994) contrariwise to both Ca$^{++}$ and Mg$^{++}$ which decrease the affinity of the laminin-1 receptor to its ligand. By contrast, Ca$^{++}$ and Mg$^{++}$ are essential to increase the affinity of FN receptors to the human plasma fibronectin (Hynes 1992 b).

Therefore, results obtained from experiments focusing the ECM molecules-pathogens interaction which are not well designed with respect to ion requirements could induce misinterpretations on the occurrence of LMN or FN binding molecules on pathogens surfaces.

The adhesion of *T. vaginalis* to ECM molecules-covered substrates is a transient adhesion process since fibronectin at least is fully digested by trichomonal proteases (Becerril Garcia 1998, Crouch & Alderete 1999) including the previously reported CP 65 (Alvarez-Sánchez et al. 2000). This might reflect that *T. vaginalis* is able to perform an adhesion/de-adhesion process like some phagocytes and tumor cells. Further, a highly regulated and specific protease activity which is released by trichomonds during their contacts with ECM components (Bózner & Demes 1991) enables the parasites to invade tissues. Having *T. vaginalis* the necessary tools to digest ECM networks it can be predictable that the parasite can indeed perform tissue invasion. Tissue invasion by *T. vaginalis* could help to explain the presence of the parasite associated to human endocervix (Guimarães Gonçalves 1992). In addition, it would be also of interest to know whether the isoform 5 of laminin (laminin-5) that is observed at high amounts in urogenital ulcers (Klainulainen et al. 1997) plays any role in the association of *T. vaginalis* to both ecto and endocervix.

The binding of LMN to the trichomonad surface is a trigger to or it impairs the intracellular signalling yet described (Silva Filho 1998). The LMN binding to the *T. vaginalis* surface seems to be mediated much more by a laminin-binding protein (LBP) (Gee et al. 1997) rather than by an integrin (Hynes 1992b) since as previously here referred, YIGSR instead of RGD is the sequence preferentially recognized by the parasite to the ligand. This signalling triggering or impairing by the binding of LMN to the *T. vaginalis* cell surface always results in the formation of tight association sites between the parasite and LMN-covered glass slides. Such adhesion sites resemble focal adhesion processes (Otey 1996). The phosphorylation of pp125 FAK as well as activation of some components of the cell signalling cascade in *T. vaginalis* (Silva Filho 1999) strongly suggest that (a) the YIGSR-binding surface component in the parasite might be a transmembrane protein or (b) the LMN recognition by the parasite might be carried out by YIGSR-binding surface molecules while signalling could be triggered by an integrin or an integrin-like molecule which recognize the RGD sequence on the ligand. Since the RGD sequence of LMN can be also recognized by the parasite even at very low extent, the last hypothesis deserve to be considered.

Several eukaryotes facing LMN or FN-covered biological and non-biological substrates exhibit focal adhesion processes (Otey 1996). Such very organized cytoplasmic expansions in these cell models represents sites where proteases are released.

By an analogy to that occurs in most of the investigated tumor cells it is reasonable to infer that cell signalling triggering or impairing by the binding of each one of LMN or FN on *T. vaginalis* cell surface could result in the formation of specific focus to proteases releasing.

**IRON IS AN ESSENTIAL NUTRIENT FOR TRICHOMONADS**

Much of the data yet available on trichomonal virulence seems to point out that parasitism in trichomonads is an iron-dependent phenomenon. The role played by iron in infections has been extensively revised (Weinberg & Weinberg, 1995). In the case of microorganisms which are found inhabiting environments of low redox potentials, iron has a pivotal role not only to the surveillance of these microorganisms as well as to the molecular expression of their virulence markers. In addition,
the defense mechanisms displayed by trichomonads against oxidative stress generated by superoxide radicals are centered on superoxide dismutase (SOD) activities (Kitchner et al. 1994, Lindmark & Müller 1974). All the SOD found in trichomonads have iron as cofactor (Viscogliosi et al. 1996).

Of clinical significance is the competition established between microorganisms and also between microorganisms and the epithelium lining cavities for iron. It was observed many years ago that the iron content of the skin may enhance the susceptibility of humans to some fungal infections (King et al. 1965). Thus, the ability of cavitary microorganisms to acquire iron as well as the intracellular fate of its uptake still are highly relevant opened questions.

At first sight the iron sources available for T. vaginalis and T. foetus into hosts are restricted to some proteins (transferrin and lactoferrin, mainly) (Peterson & Alderete 1984, Tachezy et al. 1996) and red blood cells (Dailey & Alderete 1990, Krieger 1995). However, the squamous epithelial wall of the human vaginal cavity has an iron content which varies throughout the hormonal cycle of the host. Upon treatment with estradiol, epithelial cells became much more adherent to T. vaginalis, and also they became a very attractive target to parasite phagocytosis (Silva Filho & Bonilha 1992). These data suggest that epithelial cells may be another source of iron for T. vaginalis.

Lysis of red blood cells has been suggested by some authors as a mechanism of iron uptake by T. vaginalis (Lehker et al. 1990). Human red blood cells can be phagocyted by pathogenic trichomonads (Rendón-Maldonado et al. 1998) probably as an attempt of the parasite to acquire iron from haemoglobin. Haemolysis by T. vaginalis may also be mediated by the insertion of pores on the red blood cell plasma membrane, being that such porin activity is inhibited by EDTA (Fiori et al. 1996).

One of the mechanisms for iron uptake used by both T. vaginalis and T. foetus involves respectively binding of the iron-carrying proteins lactoferrin and transferrin to high affinity cell surface receptors (Lehker & Alderete 1992, Tachezy et al. 1996).

However, little is still known on the transferrin and lactoferrin receptors of trichomonads. It seems obvious that transferrin receptors in T. foetus are the same to that fully studied in metazoa: a type II membrane protein, with an N-terminal cytoplasmic domain of 61 aminoacids, a 28 aminoacids transmembrane region and a 671 aminoacids extracellular region which is glycosilated at Asn727 (da Silva et al. 1996). Interestingly, the αβ3 integrin involved in the recognition and binding to laminin-1 is a transferrin receptor which is usually found in prostatic-carcinoma cells (Coppolino et al. 1995). Further, the conserved RGD aminoacid sequence found within FN and LMN polypeptide chains has also been found in transferrin receptors from human, mouse, rat, Chinese hamster, and chicken (Dubljevic et al. 1999).

These data reinforce what we have stressed on the possibility of a trichomonad surface molecule to exert more than one function. Evidences have been accumulated on the multifunctionalitity of various cell surface molecules. What we have named as LBP, integrin-like molecule, adhesin, and also surface CP of trichomonads, might be only two or three chemical entities presenting different functional sites. Depending of what signal is received by pathogenic trichomonads from the host environment the parasites might reveal one functional site within a surface molecule which might be in turn, unrevealed in the absence of the appropriate signal.

As clearly demonstrated by the Arroyo’s group the 120kDa surface protein of T. vaginalis is only detected in parasites that had been obtained from cultivation in acellular medium supplemented with high iron amounts (Yañez-Gómez, 1999). Whether iron is important to gene activation of this protein or the protein pre-exists at the cell surface being iron a cofactor to reveal its functionality needs to be answered.

Iron transporters are found in mammalian cells (Eide 1997) and in Saccharomyces cerevisae (Eide 1998), and they are often important for the home-
ostasis of such eukaryotes. These iron transporters surface proteins are easily detected in cells collected from cultures permanently exposed to high iron amounts. These data seem to indicate that a surface glycoprotein with iron-transporter function is revealed when the ion to be dislocated from outside to inside is in excess in the extracellular medium. If this argument has consistency it would be expected that the 120kDa surface protein of *T. vaginalis* could also be related to iron transport beside to be an adhesin.

Iron uptake by *T. vaginalis* (Peterson & Alderete 1984) and by *T. foetus* (Melo Braga 2000) modulates the functionalities of both adhesins and surface CPs, interfering with the ability of these trichomonads to adhere and to exert their cytopathic effects. However, as previously observed by Arroyo & Alderete (1995) iron has a positive regulation for adhesins while it has a negative regulation for surface CPs (Fattel-Facenda 1999). It means that the same extracellular signal can induce different detectable responses at the *T. vaginalis* surface.

CONCLUSIONS

Even investigated since the 40's we are just beginning to understand the basic mechanisms underlying trichomonal virulence. A great deal has been learned about the molecular composition of the cell surface of pathogenic trichomonads including details of functional aspects of some surface molecules. This appears to correlate with the abilities of both *T. vaginalis* and *T. foetus* to cytoadhere and also to exert their cytotoxicities to cultured epithelial cells. Currently, less is known about the epithelial surface molecules which upon or not intervenience of some soluble factors found into host urogenital cavities are recognized by the parasites. Fortunately, many of the necessary tools are at hand, and it is expected that rapid progress will be made in answering these remaining questions.

A detailed characterization of the interactions between extracellular matrix components and pathogenic trichomonads including a chemical study of the receptors, the role played by iron on the functionality of such surface molecules, and the intracellular pathways associated with the trichomonal responses to laminins, fibronectin, and some collagen species, should provide an additional exciting area of research over the next few years.

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