

## Review

# Cytoadherence and pathogenesis of *Trichomonas vaginalis*

Adegbaju, A. and Morenikeji, O. A.\*

Parasitology Unit, Department of Zoology, University of Ibadan, Nigeria.

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***Trichomonas vaginalis*, a flagellate protozoan parasite is responsible for trichomoniasis, the number one non-virally sexually transmitted disease. Although both men and women are infected, it causes disease almost exclusively in women. There are up to 250 to 350 million new cases of trichomonad vaginitis annually worldwide. *T. vaginalis* has the ability to attach to host cells (cytoadherence) thereby establishing infection as the organism overcomes the constant secretion of the vagina. Two classes of proteins are involved in cytoadherence of *T. vaginalis*, these are the four adhesion proteins AP65, AP51, AP33 and AP23 and Cysteine proteinase. Although, the exact mechanism of its pathogenesis has not been clearly elucidated, many mechanisms are thought to be involved. These include cell-to-cell adhesion, hemolysis, cell detaching factors, excretion of soluble factors and evasion of the host immune system. Trichomoniasis is strongly associated with several complications in pregnancy and with an increase in the transmission of human immunodeficiency virus.**

**Key words:** *Trichomonas vaginalis*, cytoadherence, pathogenesis.

## INTRODUCTION

*Trichomonas vaginalis* (Donne, 1836) is a flagellated protozoan parasite found in the human vagina and urethra. *T. vaginalis* is responsible for trichomoniasis, the number one non-viral sexually transmitted disease known worldwide (Kucknoor, 2005). It is also the cause of Trichomonad vaginitis in women (Marquardt et al., 2003) and non-gonococcal, non chlamydial urethritis in men (Kreiger et al., 1995). Trichomonad vaginitis causes considerable discomfort and mental distress (Marquardt et al., 2003).

Although both men and women are infected, it causes disease almost exclusively in women, while men remain asymptomatic (Marquardt et al., 2003); however some women have been found to be asymptomatic (Valandkhani, 2004). Among women, there are up to 250 to 350 million new cases of trichomonad vaginitis annually, world-wide (WHO, 1995).

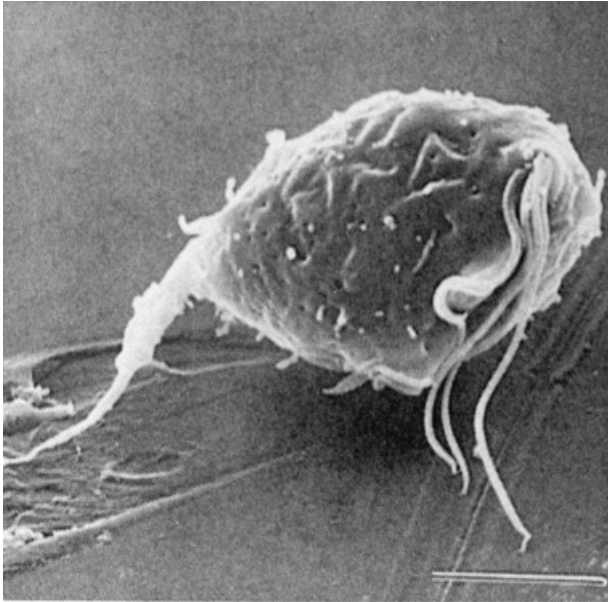
## General morphology

*T. vaginalis* only exist as trophozoite and does not form cyst (Figure 1) (Ichhpujani and Bhatia 1998; Marquardt et al., 2003). Although there are reported cases by Petrin et al. (1998) that under unfavourable growth conditions, *T. vaginalis* can round up and internalize the flagella, in which it is believed that these forms are pseudocysts, because they have not been reported to give rise to normal motile forms (Honiberg and Brugerolle, 1990).

This urinogenital pathogen varies in size and shape (Petrin et al., 1998); the average length and width being 10 and 7µm, respectively (Marquardt et al., 2003). *T. vaginalis* has four anterior flagella and a recurrent flagellum attached to the body by an undulating membrane. The fifth recurrent flagellum not trailing beyond the undulating membrane reaches up to the middle of the body (Smyth, 1996; Ichhpujani and Bhatia, 1998; Marquardt et al., 2003). The flagella and the undulating membrane give the parasite a characteristic quivering motility (Ichhpujani and Bhatia, 1998). A round nucleus is located at its anterior portion (Ichhpujani and Bhatia, 1998; Petrin et al., 1998).

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\*Corresponding author. E-mail:  
jumokemorenikeji@yahoo.co.uk. or  
jumoke.morenikeji@mail.ui.edu.ng. Tel: +234 8055275915.



**Figure 1.** *T. vaginalis* parasite as seen in broth culture (Petrin et al., 1998).

A slender hyaline rod-like structure called axostyle, commences at the nucleus and bisects the protozoan longitudinally. It protrudes through the posterior end of the parasite terminating in a sharp point. This structure is thought to anchor the parasite to the vaginal epithelial cells (Petrin et al., 1998). The parabasal body is single and V-shaped and has a filament associated with it (Marquardt et al., 2003). The order Trichomonadida to which *T. vaginalis* belongs lack mitochondrion (Marquardt et al., 2003). Granules can be seen associated with the costa and axostyle. There are two sets of granules: paracoastal and paraxostylar. The latter set is arranged along the axostyle in three parallel rows which is a distinguishing feature of *T. vaginalis* (Petrin et al., 1998). Biochemical studies have shown that these bodies serve roughly the same function as mitochondria since they contain enzymes of the Krebs cycle (Marquardt et al., 2003). These granules are termed hydrogenosomes (Petrin et al., 1998; Marquardt et al., 2003) because the end product of carbohydrate metabolism is molecular hydrogen rather than water as in other eukaryotes (Marquardt et al., 2003).

### Reproduction and life cycle of *T. vaginalis*

*T. vaginalis* exist only as trophozoite and lack a cystic stage. The small ovoid flagellates reproduce by longitudinal binary fission, without the disappearance of the nuclear membrane (Brugerolle, 1975). Although several oversized round forms of the trichomonad are known to exist in dividing growth phase culture: those without flagella, with flagella and dividing nucleus and those

with flagella and multiple nuclei (Petrin et al., 1998). It was thought that these forms are not stages in the life cycle but rather, they arise during certain unfavourable conditions (Honiberg and Brugerolle, 1990).

### Epidemiology and modes of transmission

Trichomonal infection has been encountered in every continent and climate and with no seasonal variability (Petrin et al., 1998). The estimated incidence is over 250 million cases worldwide, making the most prevalent non-virally sexually transmitted disease agent (Quinn and Krieger 1990; WHO, 1995). The incidence rate depends on many factors including age, sexual activity, number of sexual partners, other STDs, sexual customs, phase of menstrual cycle, techniques of examination, specimen collection and laboratory technique (Petrin et al., 1998).

Humans are the only natural hosts for the transmission of *T. vaginalis* (Petrin et al., 1998). The trophozoite is transmitted from one person to another, usually by sexual intercourse (Ichhupujani and Bhatia, 1998; Smyth, 1996; Marquardt et al., 2003). An important evidence to support this fact is the high rate of infection of the urethra and/or prostrate in male partners of infected females (Petrin et al., 1998).

Non-sexual transmission includes sharing of soiled clothing like towels (Smyth, 1996; Marquardt et al., 2003). There are no recent data to support a non-sexual transmission of this parasite (Alderete et al., 1995). However, these cases are rare because this flagellate die outside the human body unless they are protected from drying (Catteral, 1972; Krieger, 1981).

### CLINICAL MANIFESTATION AND DISEASE

Human trichomoniasis is now widely recognized as a prevalent sexually transmitted disease, capable of causing considerable morbidity (Valandkhani, 2004). The vaginal epithelium is the most important site for the initial contact of the parasite infection in humans (Valandkhani, 2004). *T. vaginalis* infects the squamous epithelium in the genital tract and the clinical manifestation could be:

1. Acute:
  - Diffuse vulvitis
  - Yellow or green discharge
  - Hemorrhagic spots in the vaginal mucosa
2. Chronic:
  - Mild symptoms with pruritus
  - Vaginal discharge mixed with mucus

The infection can result in varying degrees of vagini-

tis, and also infertility, premature labour, premature rupture of the placental membranes, and low birth weight infants (Hardy et al., 1984; Cotch, 1990). Laga et al. (1991) reported a relative risk of developing invasive cervical cancer and six fold higher probability of infection by human immunodeficiency virus (HIV).

It is not clear why infection in some women is symptomatic and in others is asymptomatic (Garber et al., 1989). Alderete et al. (1986) suggested the existence of two strains of virulent and less virulent *T. vaginalis* which differed in their morphological characteristics and intrinsic virulence that cause variable symptoms.

### Host parasite interaction

Vaginal infection with *T. vaginalis* may take place prior to puberty, but they are only transient since they clear up spontaneously. At puberty, the bacterial flora of the vagina change and pH become 4.5 (Marquardt et al., 2003). Oestrogen increases vaginal secretion and makes it acidic by promoting the break down of glycogen to lactic acid. *Lactobacillus acidophilus* a normal flora in the vaginal environment is also known to provide vaginal acidity with pH of 4.0 - 4.5 due to the break down of glycogen into high level of lactic acid.

Despite the changes which take place in the vagina at sexual maturity, it remains a hostile environment for *T. vaginalis* and other potential organism such as *Mycoplasma hominis* and *Gardnerella vaginalis* that live there (Marquardt et al., 2003). There is continual turnover of epithelial cells and they probably vary physiologically with the stage of menstrual cycle (Marquardt et al., 2003). Under the influence of estrogen and progesterone, the vaginal epithelium becomes cornified, a thick mucus is secreted and the epithelium proliferates and becomes infiltrated with leukocytes.

The establishment of *T. vaginalis* in the vagina is puzzling since the normal pH of the vagina is very acidic 4.5, while the organism thrives in less acidic pH > 5 (Petrin et al., 1998). The relationship between protective lactobacilli and *T. vaginalis* is not completely understood. Valandkhani (2004), demonstrated that *Lactobacillus acidophilus* has enhancing effect on the adhesive ability of *T. vaginalis* to vaginal epithelial cells which is probably due to the reduction in vagina pH (4.5), peaking only during the initial phase of the attachment. *T. vaginalis* was unable to grow well when a higher concentration of *L. acidophilus* is present (Abraham et al., 1996). There was increase in the number of *T. vaginalis* after the reduction of *L.*

*acidophilus*, at the end of menstrual cycle and during menopause, thus increasing symptoms of trichomoniasis. The parasite also seems to have a deleterious effect on *L. acidophilus* (Petrin et al., 1998).

Several mechanisms have been proposed; *T. vaginalis* has been observed to phagocytize bacteria and this may occur with lactobacillus as well (Petrin et al., 1998). Another hypothesis is that products such as proteinases secreted by *T. vaginalis*, may destroy the lactobacilli (Petrin et al., 1998).

### CYTOADHERENCE

Cytoadherence is one of the early steps essential for colonization and persistence of a pathogen in the infectious process (Beachey, 1988). Trichomonads attachment to host cells is a prerequisite for the establishment of infection as the organism must overcome the constant secretions of the vagina (Alderete et al., 1995). In addition, the parasite must survive in an adverse host environment, which is nutrient limiting for optimal growth and multiplication (Lehker and Alderete, 1990; Gorrell, 1985) and which contains specific anti-trichomonal immunoglobulin as well as numerous soluble trichomonad proteinases (Alderete et al., 1991). Also, the site of infection of *T. vaginalis* is under constant hormonal influence during the progression of the menstrual cycle (Lopez, 1950).

Cytoadherence by trichomonads is dependent on time, temperature and pH (Arroyo and Alderete, 1989). Adherence, however, does not correlate directly with virulence, since virulent strains isolated from symptomatic patients exhibited wide differences in their ability to adhere to host cells (Krieger et al., 1990).

### Molecules involved in cytoadherence of *T. vaginalis*

The cell surface of the trichomonad plays a major role in adhesion, host-parasite interaction, nutrient acquisition, and the proteins and glycoproteins displayed on the surface have function in this regard (Petrin et al., 1998). Alderete et al. (1995) demonstrated that at least two classes of molecules are directly implicated in the adhesion of *T. vaginalis* to vaginal epithelial cells (VECs): Adhesins and cysteine proteinase.

### Adhesins

Adhesins are surface proteins synthesized by *T. vaginalis* (Garcia et al., 2003). There are four types of these adhesin proteins (APs). These are AP65, AP51, AP33 and AP23. These adhesins appear to interact with host cells via ligand-receptor-type interactions and fulfill criteria of adhesins (Beachey et al., 1988). Multigene families encode these proteins (Alderete et al., 1995, 1998). These adhesins are co-localized on the trichomonad membranes (Garcia et al., 2003) and

the amounts of the adhesins were increased under high iron conditions (Lehker et al., 1991).

### **Cysteine proteinase**

The presence of surface expressed adhesins is not sufficient for cytoadherence (Alderete et al., 1995). The adhesins on the parasite surface are protected by proteins, from the family of cysteine proteinases being elaborated during normal growth. Expression of adhesin functionality, therefore, requires unmasking of the adhesins by degrading the protective proteins (Alderete et al., 1995).

*T. vaginalis* has many cysteine proteinase (CPs) (Bozner and Demes, 1990; Neale and Alderete, 1990). At least 23 different cysteine proteinases were identified by two dimensional (2-D) substrate gel electrophoresis (Neale and Alderete, 1990). The cyteine proteinase of *T. vaginalis* is known to participate in a variety of important virulence properties (Alderete et al., 1991) such as cytotoxicity (Alvarez-sanchez et al., 2000), hemolysis (Dailey et al., 1990), immune response evasion (Prvenzano and Alderete, 1995) and cytoadherence (Arroyo and Alderete, 1989). This indicates the possibility of multiple roles of the proteinase during infection (Neale and Alderete, 1990).

Trichomonad cysteine proteinases degrade a prominent surface immunogen of *Trichomonas vaginalis* (Arroyo and Alderete, 1989). Therefore it is conceivable that unmasking of adhesins by proteinases residing on the parasite surface is required for host cell recognition and binding (Arroyo and Alderete, 1989).

### **Mechanism of cytoadherence of *T. vaginalis***

Upon enzymatic removal of the specific protein covering the adhesins by proteinase (cysteine proteinase) (Marquardt et al., 2003), the four trichomonad adhesin proteins then mediate the attachments to vaginal epithelial cells (VECs) (Arroyo et al., 1992).

Initially after binding to VECs, the cell body of *T. vaginalis* becomes elongated (Figure 2A), with pseudopods forming at site of contact throughout the periphery of the organism. Multiple focal contact points, seen as short filopodia or lamellopodia (Figure 2B), are also evident. The host-parasite, a membrane-membrane association becomes more prominent with numerous contact points and thin lamellopodia (Figure 2C). Interestingly, within 20 min, single organisms attached to the VECs were followed by other trichomonads, which were always found adjacent to each other with multiple membrane interdigitations (Figure 2D). This is a case of apparent recruitment of other trichomonads to the VECs surface following parasitism by one or few organisms. This is supported by works of previous investigators who have described cytotoxicity of cells in mono-

layer cultures as resulting from congregation of parasites at defined foci, rather than a uniform killing of the cells in monolayer culture (Alderete and Pearlman 1984; Pindak et al., 1986; Rasmussen et al., 1986).

The change in shape from ellipsoid to amoeboid possibly confers an enhanced binding of the parasite to the VEC (Arroyo et al., 1993). A possible reason to adopt the amoeboid morphology may be a need to maximize.

### **Signal transduction of *Trichomonas vaginalis***

Arroyo et al. (1993) presented evidence for the specificity in signal transduction as a function of *T. vaginalis* with certain host cells. Arroyo and his group identified three signals transduced during or immediately following recognition and binding of *T. vaginalis* with host cells.

- The reproducible and dramatic morphological transformation (Figure 2) is a function of host cell (Alderete et al., 1995).
- A signal distinct from that mediating a change in shape involve the stimulation of parasites after attachment to synthesize, to a greater extent the adhesin proteins.
- The VEC initially parasitized by a single or few organisms, ultimately have numerous adjacent trichomonads. A form of recruitment of other parasite to site of infection may imply that a signal, perhaps a chemoattractant generated by the organism, is responsible (Alderete et al., 1995).

### **PATHOGENESIS**

Although *T. vaginalis* is the most intensely studied trichomonad and is the world's most common cause of nonviral STDs, the exact mechanism of its pathogenesis has not been clearly elucidated (Petrin et al., 1998). This is due to lack of good animal model which has limited the ability to conduct standardized, controlled research on transmission, pathogenesis, immune response, and drug and vaccine development (Petrin et al., 1998).

The interaction of *T. vaginalis* with the members of the resident flora of the vagina may be an important factor (McGrory et al., 1994), and, like many other protozoans, *T. vaginalis* has demonstrated many mechanisms which are used to evade the host immune system (Alderete et al., 1992; Alderete et al., 1995). This includes:

### **Adherence and adhesion**

The first step in pathogenesis of *T. vaginalis* is adherence. It has been observed that the side opposite the



*nal*, CDF, causes cytophthic effects in cell culture (Pindak et al., 1986). CDF is probably a factor in pathogenesis, since *Pentatrichomonas hominis*, a nonpathogenic species does not show CDF activity (Garber et al., 1989). Garber et al. (1989) found that purified CDF was active within pH 5.0 - 8.5 with the optimum activity at pH 6.5 and inactivity below 4.5. This is of clinical relevance since the normal pH of the vagina is 4.5 but is greater than 5.0 during trichomoniasis. The rise in vagina pH during trichomoniasis may therefore be crucial in the pathogenesis of this disease (Petrin et al., 1998).

### Immune system evasion

In a hostile changing environment, *T. vaginalis* can survive and flourish. Its ability to evade the host immune system is an important aspect of pathogenesis. Avoidance of complement is a strategic tactic, which is used by *T. vaginalis* to overcome the human immune system (Petrin et al., 1998). Resistance to complement is dependent upon a high concentration of iron (Alderete et al., 1995), a nutrient which indeed abundant during menses. It seems that iron upregulate the expression of CPs which has been found to degrade the C3 portion of the complement on the surface of the organism; this allows the organism to evade complement-mediated destruction (Alderete et al., 1995).

This parasite also secretes copious amounts of highly immunogenic soluble antigens (Alderete and Garza, 1984). A continuous release of these antigens may neutralize antibody or cytotoxic T lymphocytes, thus short-circuiting specific anti-*T. vaginalis* defense mechanisms (Alderete and Garza, 1984). As well, *T. vaginalis* can coat itself with host plasma proteins. This coating does not allow the hosts immune system to recognize the parasite as foreign (Peterson and Alderete, 1982).

### CONCLUSION

Trichomoniasis is not merely a nuisance disease of women. It is an unpleasant, irritating, and potentially dangerous disease that can go undiagnosed for years and is often passed on by an asymptomatic carrier.

*T. vaginalis* is a very complex organism, from its biochemistry to the mechanisms of pathogenesis. Areas of pathogenesis that should be pursued include defining soluble factors, further elucidating the contact-dependent relationship between the vaginal epithelium and *T. vaginalis*, and defining how the organism can establish itself in a normally inhospitable and changing environment. It will also be important to further define the role of the human immune system in trichomoniasis in order to develop targeted intervention strategies.

Certainly, treatment of all infected individuals, whether symptomatic or asymptomatic, as well as public educa-

tion and prevention programs will help in curbing the spread of the disease.

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