Shape variations of Trichomonas vaginalis in presence of different substrates

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ABSTRACT

Shape variations of strains of Trichomonas vaginalis were demonstrated by scanning electron microscopy (SEM) following inoculation on McCoy cell monolayers and after interaction with human erythrocytes. The parasites adhered to both cells and presented amoeboid forms, with pseudopodia-like extensions. Several amoeboid organisms swam freely over the cell monolayers and produced aggregates. These unusual forms of the urogenital flagellate may be a response to the composition of the culture medium and may play an important role in the pathogenic process of T. vaginalis.

Key words: Trichomonas vaginalis, shape variations, scanning electron microscopy.

INTRODUCTION

Trichomonas vaginalis is a very common cause of infection of the female genito-urinary tract, and trichomonosis is recognized as a major sexually transmitted disease. Its clinical presentation ranges from a totally asymptomatic infection to a severe vaginitis. Women require drug intervention to eliminate this parasite1, illustrating an adaptation to survival in the constantly changing environment of the human vagina.2 Adherence to host vaginal epithelial cells is essential for initiation and maintenance of infection by this mucosal parasite, and four trichomonad surface proteins that mediate parasite cytoadherence to host cells have been identified.3,4 The adherence is accompanied by shape variations of the trophozoites. Abnormal forms of T. vaginalis, with variation in size and shape, have previously been reported.5-10 Giant forms measuring between 30 and 50 µm in diameter appeared in liquid medium 12 h after incubation.7 Previous studies with Trichomonas

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tenax also reported giant forms of a strain maintained in culture for two years. Actively swimming forms are ellipsoidal or ovoidal, sometimes spherical. All strains are able to form pseudopodia-like extensions, which are used in feeding, for attachment to stationary objects, but not for amoeboid movement. In the presence of higher concentrations of agar, of certain food particles, and of various substrate layers (cells or tissues in vivo and in vitro) trichomonads tend to be amoeboid. Here we report amoeboid forms of *T. vaginalis* observed using scanning electron microscopy (SEM) after inoculation on McCoy cell monolayers and after interaction with human erythrocytes.

**MATERIALS AND METHODS**

**Parasites** – Two strains of *T. vaginalis* were used in this investigation. The VG strain was isolated from a woman with symptomatic vaginitis attending at the Venereal Disease Department of Charles Nicolle Hospital, Rouen, France. The 30,238 *T. vaginalis* strain is metronidazole-resistant, from the American Type Culture Collection (ATCC). The organisms were cultured axenically in vitro at 37 ºC in Diamond’s medium tryptase-yeast extract-maltose (TYM), without agar, pH 6.0, supplemented with 10% heat inactivated bovine serum, penicillin (1000 UI ml⁻¹) and streptomycin sulphate (1 mg ml⁻¹). Isolates were subcultured every 48 h in TYM medium.

**McCoy cell tissue culture** - McCoy cell tissue was performed in a 24-well, flat bottom, cell tissue essential medium (MEM) (Eurobio, Paris) containing essential amino acid solution (10 µl ml⁻¹) (Flobio, France), foetal calf serum (100 µl ml⁻¹, Bio-Merieux, France) and netilmicin (10 µl ml⁻¹) (Netromicine-Unicet, France). The cells were incubated at 37 ºC in the humid atmosphere of a CO₂ incubator (5% CO₂ in air). The cells were used at confluence, washed three times with the same medium. Each monolayer of McCoy cells was inoculated with *T. vaginalis* at 2 x 10⁶ organisms ml⁻¹.

**Human erythrocytes** – Peripheral blood was drawn from healthy human volunteers of O group, with equal volume of Alsever’s solution. The plasma was discarded by centrifugation (250 x g for 5 min) and the erythrocytes were washed three times in sterile PBS buffer. The pellet containing the erythrocytes was subsequently used to investigate the interaction with *T. vaginalis* using a erythrocytes: protozoa ratio of 100:1.

**Scanning electron microscopy** - The McCoy cell tissue cultures inoculated with *T. vaginalis* were fixed for SEM 6 h after infection. The medium was carefully decanted and replaced with 2.5% (v/v) glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at room temperature for 2 h 30 min. The cultures were then rinsed in PBS for 30 min, and then adhered to glass coverslips previously coated with 0.1% poly-L-lysine (Sigma Chemical Company, St Louis), and post-fixed in 1% osmium tetroxide in 0.1 M at room temperature for 2 h. Trichomonads were maintained with erythrocytes during 30, 60 and 90 min and the SEM fixation was the same as performed for infected McCoy cell tissue cultures. Fixed samples were dehydrated in ethanol and critically point dried with CO₂. The coverslips were mounted on stubs and lightly coated with gold particles and examined with a Philips XL30 scanning electron microscope.

**RESULTS AND DISCUSSION**

The presence of a large number of adherent *T. vaginalis* in the McCoy cells presented an opportunity for a SEM study of morphological adaptations of trichomonads to the surface of a cell. Trichomonads presented amoeboid forms and in some instances, several amoeboid organisms were applied to one another by their pseudopods, forming groups of organisms. The parasites swam freely over the cell monolayers and formed aggregates consisting of numerous cells (“swarming”) (Figure 1).

Figure 2 shows the pseudopodia-like extension appearing from the surface of the flagellate. The parasite typically applied itself to the cells by its ventral surface, that is, the surface opposite to that invested with the undulating membrane.

The amoeboid forms started to appear in the first or second passage, and were then seen persistently in the subsequent subcultures, but
in variable number and always intermixed with pyriform trophozoites.

After 30 and 60 min of incubation of *T. vaginalis* and erythrocytes, SEM showed amoeboid forms of the parasite applied to the erythrocytes (Figures 3 and 4).

Some authors claim that the shape of the organism varies according to the composition of the growth medium. Studies carried out in several cell cultures to investigate the pathologic process caused by *T. vaginalis* show that the more virulent strains tend to settle on and adhere to the culture cells; the less inherently virulent strains (perhaps as a result of prolonged cultivation) have less tendency for cytadherence. Moreover, the typically amoeboid virulent trichomonads apply themselves very closely to the cell culture elements.\(^\text{16}\)

The urogenital trichomonads vary in size and shape. Physicochemical conditions (e.g., pH, temperature, oxygen tension, and ionic strength) affect the shape of trichomonads; however, shape tends to be more uniform among cells grown in nonliving culture media than among those observed in vaginal secretions and urine. In general, in axenic cultures grown in liquid media, for example, trypticase-yeast extract-maltose (TYM), with or without low concentrations of agar and without solid food particles, the organisms are usually ellipsoidal, ovoidal, or spheroidal.\(^\text{17}\)

Mechanical action, or at least injury resulting

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**Figure 1.** Several amoeboid trophozoites of *Trichomonas vaginalis* are attached to one another by their pseudopods, forming a group of organisms. (M McCoy cell; T trichomonad). **Figure 2.** Pseudopodia-like extensions exhibited by *Trichomonas vaginalis*. Note the parasite attached to the McCoy cell by its ventral surface. (AF anterior flagella; M McCoy cell; P pseudopodia-like extensions; UM undulating membrane). **Figure 3.** Amoeboid trophozoite of *Trichomonas vaginalis* applied to an erythrocyte after 30 min of interaction between parasites and erythrocytes. (E erythrocyte; T trichomonad). **Figure 4.** Amoeboid trophozoite of *Trichomonas vaginalis* after 60 min of interaction between parasites and erythrocytes. (E erythrocyte; T trichomonad).
from direct contact between the parasites and the culture cells, plays a very important role in pathogenesis associated with urogenital trichomonois. The significance of these shape variations is still to be assessed, and the influence of the constituents of the culture medium on their development should also be studied.

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