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Tritrichomonas foetus: Budding from Multinucleated Pseudocysts

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Tritrichomonas foetus **is a flagellated protozoan parasite that causes trichomoniasis, a major sexually transmitted disease in cattle.** ***T. foetus*** **presents a simple life cycle, exhibiting only the trophozoitic form. However, under unfavorable growth conditions, the trophozoites, which are polar and flagellated, can round up and internalize their flagella forming pseudocysts. In this form no cyst wall surrounds the cell and it also displays a distinct mitosis when compared with the trophozoite form. In pseudocyst mitosis, the cell proceeds with duplication of cytoskeletal and mastigont structures; nuclear division occurs but without the corresponding cytoplasm division. Thus, giant multinucleated cells which present many mastigont structures are formed (approximately 62% of the population). These polymastigont/multinucleated cells are maintained when the cells are under stress conditions. When environmental conditions become favorable, the flagella are externalized and new flagellated trophozoites one by one, gradually bud from the multinucleated cell. Thus, in order to better understand the pseudocyst mitosis, the polymastigont formation and the generation of new cells by this budding process, video microscopy and other complementary techniques, such as immunofluorescence and transmission electron microscopy were used.**

Key words: electron microscopy; mitosis; multinucleated cells; parabasalids; pseudocyst; *Tritrichomonas foetus*.

Introduction

Trichomonadea, commonly called trichomonads, comprise a class of amitochondriate protists belonging to the phylum Parabasalia (Hampl et al. 2004). Among these microorganisms, *Tritrichomonas foetus* is the causative agent of bovine trichomoniasis, one of the most widespread sexually transmitted diseases in cattle. Bovine trichomoniasis is a major cause of infertility and abortion leading to considerable economic losses in beef-producing areas of the world (Rae and Crews 2006). Nowadays *T. foetus* is recognized as being able to infect other animals. In domestic cats, *T. foetus* colonizes the colon, resulting in chronic, large-bowel diarrhea (Stockdale et al. 2008). The organism has also been described as an inhabitant of the porcine gastrointestinal and nasal mucosa. Related to this point

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T. foetus and *Tritrichomonas suis* (a gastrointestinal commensal of pigs) are now considered to be strains of the same species on the basis of structural and molecular data (Lun et al. 2005; Tachezy et al. 2002). Additionally, studies have also recognized that canine trichomoniasis (an intestinal disease) may be caused by infection with *T. foetus* (Gookin et al. 2005).

A non-dividing *T. foetus* is characterized by a pear-shaped body, one anterior nucleus, a ribbon of microtubules forming the pelta-axostyle complex, which runs from the basal bodies to the cell tip and hydrogenosomes lining the axostyle. *T. foetus* presents three anterior flagella and a recurrent one that runs toward the posterior region of the cell adhered to the cell body, forming an undulating membrane (Honigberg and Brugerolle 1990). The mastigont system also comprises several skeletal structures including the costa that underlies the recurrent flagella and two parabasal filaments that support a single Golgi complex (Benchimol et al. 2001).

Like other trichomonads, *T. foetus* presents only the trophozoite form. However, under unfavorable environmental conditions, such as a decrease in nutrients, drugs or abrupt changes in temperature, this organism rounds up forming pseudocysts. In this form, the flagella are internalized, but no cyst wall surrounds the cell (Pereira-Neves et al. 2003). Although pseudocysts can be observed in several species of trichomonads, including *T. foetus* and the related human pathogen *Trichomonas vaginalis*, their biological aspects are under-appreciated by most protozoologists. In addition, little is known about the behavior and role of pseudocysts in the trichomonad cell cycle.

Transformation of such polarized pear-shaped cells into rounded cells with internalized flagella led to several questions concerning pseudocyst behavior and metabolic activities. For several years, pseudocysts were considered to be an irreversible and degenerative form (Honigberg and Brugerolle 1990; Mattern et al. 1973; Samuels 1959). However, currently, it is believed that this form is reversible and that its formation represents a defense mechanism to unfavorable environmental conditions (Lipman et al. 1999; Mattern and Daniel 1980; Pereira-Neves et al. 2003). The infectivity of fecal pseudocysts has been described for amphibians (Pereira and Almeida 1940), rodents (Lipman et al. 1999; Mattern and Daniel 1980) and birds′ trichomonads (Friedhoff et al. 1991; Pereira and Almeida 1940). Studies performed by our group demonstrated that *T. foetus* pseudocysts are able to adhere to host cells and that the pseudocyst adhesion rate is even higher than for the pear-shaped parasites (Mariante et al. 2004). Thus, studies to elucidate the role of pseudocysts in the life cycle of *T. foetus*, as well as their relationship with trichomoniasis, are gaining more importance.

All trichomonad genera studied to date, including *T. foetus*, exhibit a special type of mitosis called cryptopleuromitosis (Brugerolle 1975) where the nuclear envelope persists and the mitotic spindle is extranuclear. The microtubules of the mitotic spindle are polarized by two rod-shaped structures named atractophores that are appended to the base of the basal bodies and play the role of centrosomes. Previously, we have characterized the main phases of cell division in pear-shaped trichomonads (Ribeiro et al. 2000, 2002a, 2002b) and we have shown that it is markedly distinct from pseudocyst mitosis (Pereira-Neves et al. 2003). However, there are several open questions concerning pseudocyst mitosis, such as the involvement of the axostyle and flagella during the mitotic process and to clarify whether cytokinesis occurs. Thus, the present study intends to gain a better understanding of these aspects of *T. foetus* pseudocyst behavior. To carry out this study we used video microscopy, fluorescence and electron microscopy. Here we show that cytokinesis is arrested during pseudocyst mitosis while the nucleus proceeds with sequential karyokineses. Thus, pseudocysts become multinucleated originating a polymastigont form. Interestingly, new individuals, as pear-shaped cells, separate from these multinucleated cells by a budding process, when under specific conditions.

Results and Discussion

Pseudocyst Induction

Standard cultures of *T. foetus* present a low frequency of pseudocysts (Pereira-Neves et al. 2003), thus efficient methods to induce the transformation of the pear-shaped parasites into pseudocysts are essential for the in vitro studies of these forms. We have shown that *T. foetus* pseudocyst formation can be successfully induced if the parasites are kept at temperatures below 16 °C (Granger et al. 2000). In addition, pseudocyst formation and reversibility can be obtained by rapid cooling and warming schemes (Granger et al. 2000). Thus, in order to obtain a large number of pseudocysts, cultures of *T. foetus*