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Tritrichomonas foetus Displays Classical Detergent-resistant Membrane Microdomains on its Cell Surface

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Tritrichomonas foetus **is a serious veterinary parasite that causes bovine trichomoniasis, a sexually transmitted disease that results in reproductive failure and considerable economic losses in areas that practice natural breeding.** ***T. foetus*** **is an extracellular parasite, which initially adheres to and infects the urogenital tract using a diverse array of surface glycoconjugates, including adhesins and extracellular matrix-binding molecules. However, the cellular mechanisms by which** ***T. foetus*** **colonizes mucosal surfaces and causes tissue damage are not well defined. Several studies have demonstrated the involvement of pathogen or host lipid rafts in cellular events that occur during pathogenesis, including adhesion, invasion and evasion of the immune response. In this study, we demonstrate that detergent-resistant membranes are present in the plasma membrane of** ***T. foetus*****. We further demonstrate that microdomains are cholesterol-enriched and contain ganglioside GM1-like molecules. In addition, we demonstrate that lipid microdomains do not participate in** ***T. foetus*** **adhesion to host cells. However, the use of agents that disrupt and disorganize the plasma membrane indicated the involvement of the** ***T. foetus*** **lipid microdomains, in cell division and in the formation of endoflagellar forms. Our results suggest that trophozoites and endoflagellar forms present a different plasma membrane organization.**

Key words: *Tritrichomonas foetus*; detergent-resistant membrane microdomains; lipid rafts; methyl-β-cyclodextrin; filipin; ganglioside GM1.

Introduction

Tritrichomonas foetus is a serious veterinary pathogen that causes bovine trichomoniasis, a sexually transmitted disease that results in reproductive failure and considerable economic losses in areas that practice natural breeding (Alstad et al. 1984; BonDurant 1985; Clark et al. 1983). This disease has distinct clinical presentations in bulls and cows (BonDurant 1997). In bulls, the parasite is detected in the preputial cavity, the urethra and deeper parts of the urogenital tract (Parsonson et al. 1974). Infected bulls can harbor *T. foetus* throughout their lives without exhibiting clinical symptoms. In contrast, in cows, the effects of the

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disease vary from asymptomatic infection to severe clinical manifestations that include vaginitis, cervicitis, endometritis and pyometra which can result in transient infertility or fetal loss (Anderson et al. 1996; López et al. 2000; Parsonson et al. 1976).

T. foetus is an extracellular parasite, which adheres to and infects the urogenital tract (Honigberg 1978). Previous studies have suggested that a diverse array of surface glycoconjugates, including adhesins (Corbeil et al. 1989; Singh et al. 1999; Woudwyk et al. 2013) and extracellular matrix (ECM)-binding molecules (Petrópolis et al. 2008; Silva-Filho and de Souza 1988), play important roles during the interaction of the parasite with its host. However, the cellular mechanisms by which *T. foetus* colonizes mucosal surfaces and causes tissue damage are not well understood.

Evidence suggests that plasma membrane lipids are not distributed homogeneously and that microdomains with specialized functions exist in the plasma membrane (Simons and Toomre 2000). One such domain, the lipid raft, is a highly ordered and tightly packaged membrane domain with low fluidity. Lipid rafts are enriched in cholesterol or other sterols, glycosphingolipids, and phospholipids with a higher degree of saturated fatty acyl chains than the rest of the membrane (Maxfield 2002; Simons and Toomre 2000). The presence of membrane microdomains allows for the inclusion and exclusion of specific membrane proteins based on their attachment to the membrane via lipid anchors or specific protein-lipid interactions. Glycosylphosphatidylinositol (GPI)-anchor, double acylation and transmembrane proteins with the capacity to interact with cholesterol are examples of proteins modified with a hydrophobic attachment that are often found in lipid microdomains (Laughlin et al. 2004). A type of these microdomains is termed detergent-resistant membranes (DRMs) because they are resistant to solubilization in cold nonionic detergents, especially Triton X-100 at 4 °C (Brown and Rose 1992; Goldston et al. 2012).

Recent studies concerning the physiological role of lipid rafts have demonstrated that these membrane regions play important roles in a variety of cellular functions, including polarization, signal transduction, endocytosis, secretion and cell-cell and cell-pathogen adhesion (Antal and Newton 2013; Bal et al. 2013; Grimmer et al. 2002; Ha et al. 2003; Harris et al. 2001; Koumangoye et al. 2011; Mañes et al. 1999; Pierini et al. 2003; Resnik et al. 2011; Sharma et al. 2012). The presence of these microdomains has also been demonstrated in parasitic protozoa, such as *Trypanosoma brucei*, *Leishmania* spp., *Toxoplasma gondii*, *Plasmodium* spp., *Giardia intestinalis* and *Entamoeba histolytica* (Goldston et al. 2012). In addition, some studies have demonstrated the involvement of raft-like membrane domains in the interaction between host cells and pathogenic protozoa (*Leishmania* spp*., Plasmodium falciparum* and *Giardia lamblia*) (Dermine et al. 2005; Goldston et al. 2012; Humen et al. 2011; Karmakar et al. 2011; Koshino and Takakuwa 2009; Murphy et al. 2007; Sen et al. 2011; Yoneyama et al. 2006). However, there are no reports concerning lipid microdomains in *T. foetus*. Because trichomonad membranes contain cholesterol and sphingolipids (Beach et al. 1990), it is conceivable that lipid domains exist in the plasma membrane of this organism. The aims of this study were to identify and characterize lipid microdomains in *T. foetus* and determine whether these domains are involved in the interaction of this parasite with host-cells in vitro.

Results

Detection of Cholesterol and Lipid Microdomains in *T. foetus*

T. foetus cells that were fixed and stained with filipin exhibited an intense fluorescence in their plasma membranes, in their flagellar membranes and in some organelles (Fig. 1), indicating the presence of cholesterol in these structures. To determine whether the cholesterol molecules were localized in microdomains, fluorescent lipid analogs were used to distinguish raft regions from other membrane domains. *T. foetus* were disrupted by methyl-β-cyclodextrin (MBCD), a derivate of the family of cyclic oligomers of glucose which have a polar surface and a hydrophobic cavity that can accommodate small hydrophobic molecules (Beseničar et al. 2008), and were stained with DilC16, an order-preferring lipid analog, and FAST-Dil, a non-order-preferring lipid analog (Fig. 2A-D). Control cells that were stained with DilC16 exhibited fluorescence only in the plasma membrane, whereas control cells stained with FAST-Dil also exhibited fluorescence in intracellular structures. In contrast, DILC16 labeling of MBCD-treated cells was abolished or concentrated in a single region of the plasma membrane (Fig. 2E-H). Similar results were observed for cells treated with filipin (Fig. 2I-L). In addition, in treated cells that were labeled with fluorescent cholera β-toxin (CTxβ-Alexa-488), which detects the glycosphingolipid lipid raft marker GM1, fluorescence was absent in the plasma membrane