LS-7-P-1769 Behavior of the Lysosome related organelle during differentiation of Giardia intestinalis

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Giardia intestinalis is an unicellular parasite that commonly causes diarrheal disease all over the world. Giardia is an eukaryotic cell that presents two nuclei with nuclear membranes, an endomembrane system consisting of the endoplasmic reticulum (ER) and peripheral vesicles (PVs) and cytoskeleton structures such as the adhesive disk, median body and the funis. During its life cycle the protozoan presents two developmental stages: the flagellated trophozoite which attaches to the microvillus border of the small intestine and is responsible for the disease symptoms, and the cyst which is the resistant infective form. The transformation of trophozoites into cysts is known as the encystation process and characterized by the appearance of large vesicles named encystation secretory vesicles (ESVs). Although typical lysosomes are also not found, this parasite presents a large number of peripheral vesicles (PVs) that show acid phosphatase activity, and accumulate macromolecules ingested by the protozoan. Enzyme cytochemistry, showed that acid phosphatase, SH-containing proteins, and glucose-6-phosphatase, are localized in the PVs. Furthermore, PVs also contains cysteine endoproteases that are orthologous to the cathepsin L and cathepsin B found in lysosomes of higher organisms and are therefore useful markers of cell compartments where protein degradation takes place. These data suggest that the PVs fulfill all criteria to be identified as early and late endosomes, as well as lysosomes, representing an ancient structure that later on, during evolution, was separated into distinct compartments. In the present study we decided to analyze further the endomembrane system of G. duodenalis during the process of encystation. In order to analyze the behavior of PVs during the differentiation process of the parasite, G. intestinalis were induced to encyst in vitro. Lucifer yellow and Acridine orange markers were used to track the PVs during encystment. Moreover, acid phosphatase cytochemistry technique was performed. The results were observed using fluorescence microscopy and transmission electron microscopy (TEM), respectively. Biochemistry analysis of phosphatase activities was performed, measuring the rate of p-nitrophenol (p-NP) production. Our data show a fluorescence decrease during encystment process when Lucifer yellow and Acridine orange dyers were used. The same results were observed during cytochemical localization of acid phosphatase activity: a reduction in the electron dense stain was noted in parasites after 21h pos-encystment induction.

References

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Fig. 1: Cytochemistry for acid phosphatase in giardia trophozoite (a) and 21h-encysted (b) analyzed by TEM. The encysted cell is identified by ESV; the peripheral vesicles (PV) are indicated with arrows. The decrease of acid phosphatase staining is noted (b). Moreover, plasma membrane phosphatase detection (arrowhead) is only observed in encysted cell.