Cytochemical analysis on the formation of the cyst wall during trophozoite-cyst transformation in *Giardia lamblia*

V. Midlej¹,², W. de Souza³,⁴,⁵, and M. Benchimol¹,⁴,⁵

¹Universidade Santa Úrsula, Rio de Janeiro, Brazil; ²Instituto de Ciências Biológicas, UFRJ, Rio de Janeiro, Brazil; ³Instituto de Biofísica Carlos Chagas Filho-UFRJ; ⁴Instituto Nacional de Metrologia e Qualidade Industrial – Inmetro; ⁵Instituto Nacional de Ciência e Tecnologia em Biologia Estrutural e Biomagens, Rio de Janeiro, Brazil

*Giardia lamblia*, the causative agent of giardiasis, is a parasitic protist placed in the earliest divergence and deepest branch in eukaryote evolution. Despite its medical importance some basic aspects of the biology of the protozoan is not well understood. The parasite cycle includes two developmental and morphological forms, the trophozoite and the cyst. Trophozoites colonize in the upper small intestine by attaching to the epithelial cells. While the trophozoites pass through the small intestine, they produce a rigid extracellular matrix or cyst wall (CW) that protects them from the external environment. The CW is composed by proteins and carbohydrates. During formation of the CW, the cyst wall proteins (CWP) 1, 2 and 3 are synthesized by the protozoan, sorted to a new structure designated as encystation-specific secretory vesicles (ESVs) and, subsequently, released at the site of CW assembly on the protozoan surface. Although the presence of carbohydrates in cyst wall has been reported, the site where these molecules are assembled has not been established. The lack of specialized vesicles that concentrate large amounts of carbohydrate-containing molecules raises the question concerning how this material is exported to the CW. In this study the distribution of the different sugar residues and the origin of the carbohydrate components of the cyst wall were studied using a cytochemical approach. Immunocytochemistry was also used for localization of the cyst wall protein 1 (CWP1) and two techniques were used for localization of carbohydrates: (1) incubation of thin sections in the presence of periodic acid-thiosemicarbazide-silver proteinate (PA-TS-SP), and (2) in the presence of fluorescein- or gold particles-labeled lectins (WGA and DBA). The labeled cells were observed by Confocal Laser Scanning Microscopy (CLSM) and Transmission Electron Microscopy (TEM). Interestingly, a set of vesicles labeled with DBA, which reveals the presence of N-acetyl-galactosamine, was observed by CLSM in the encysting cells. These vesicles were distinct from the ESVs since they were not labeled with antibodies recognizing the CWP1. Therefore, they constitute vesicles that were not previously detected and then designated as Encystation Carbohydrate-Containing Vesicles (ECVs). Additional data support the view that ECVs are distinct from the ESVs. These include (1) they are electron lucent whereas ESVs are electron dense; (2) they do not react with antibodies against cyst wall proteins; (3) their contents are positive for carbohydrates, as revealed using the PA-TSC-SP technique, whereas ESVs display a negative reaction; and (4) this cell compartment exhibits a positive labeling for DBA lectin indicating the presence of N-acetyl-galactosamine, whereas ESVs are negative. To evaluate if ECVs could be vesicles involved in the endocytic pathway, endocytic markers such as Lucifer Yellow and Acridine Orange were used. No co-localization of these markers with ECVs was observed. Based on these observations we suggest that the ECVs may represent a new structure involved in the cyst wall formation of *Giardia lamblia*.

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