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Research brief

Entamoeba histolytica: Cyst-like structures *in vitro* induction

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Abstract

The cyst of *Entamoeba histolytica* is responsible for amebiasis infection. However, no axenic *in vitro* system exists that promotes mass encystation for studying this process of this human-infecting parasite. Cyst-like structures of *E. histolytica* obtained in this work were induced using TYI-S-33 media in combination with enterobacterias *Escherichia coli* and *Enterococcus faecalis* conditioned media, high CO_2 tension and histamine. Cyst-like structures showed the same characteristics of a typical *E. histolytica* cyst: aggregation, resistance to 0.15% sarcosyl for 10 min, high signal of fluorescence under UV light when stained with 10% calcofluor M2r and the surface topology showed a wrinkled wall. In addition these structures are multinucleated with condensed chromatin attached to nuclear membrane, contain big vacuoles and ribonucleoproteic helices in the cytoplasm and also present a thin cell wall. Last all characteristics are all the same as a typical of *E. histolytica* cyst.

Index Descriptors and Abbreviations: TYI, tripticase, yeast, and iron; IU, international units; LPE, liver pancreatic extract; SCFAs, short chains fatty acids; PBS, phosphate buffer saline

Keywords: Protozoa; *Entamoeba histolytica*; Cyst; Encystation; Amoeba

Entamoeba histolytica is a protozoan parasite of humans and the causative agent of intestinal amebiasis. This disease is the third most common parasitic cause of death in the world and it is a major health problem in developing countries (Stanley, 2003). Amebiasis is acquired by ingestion of the *E. histolytica* cyst in contaminated food or water. Two stages can be recognized in the life cycle of *E. histolytica*: the trophozoite and the cyst. Trophozoites, which are ameboid motile cells, measuring 10–40 μm in diameter, which multiply by binary fission; they have a simple cytoplasm with abundant vacuoles. The cyst is spherical, non-motile, tetranucleated, measuring 8–20 μm in diameter and encased in a rigid, refringent wall constituted by chitin that confers resistance of physical and chemical agents to mature cyst (López-Romero and Villagómez-Castro, 1993; Das and Guillin, 1991). Unfortunately, very little is known about the molecular and cell biology of *E. histolytica* encystation due to the absence of a medium or method that supports *in vitro* mass encystations of *E. histolytica*. Elucidation of encystation will lead to a better understanding of this process and therefore to the possibility of a better control of amebiasis. Encystment of *E. histolytica* has been observed only in xenic cultures (Dobell, 1928; Cleveland and Sanders, 1930; Stone, 1935; Kessel et al., 1944; Zuckrman and Meleney, 1945 and Chang, 1946), and although has been also reported encystation in axenic cultures, not all of the characteristics of the mature cyst were shown (Rivera and Correa, 1986; Nayeen et al., 1993; Mata-Cárdenas and Saíd-Fernández, 1986; Saíd et al., 1993; and Campos-Góngora et al., 2004). In this study,

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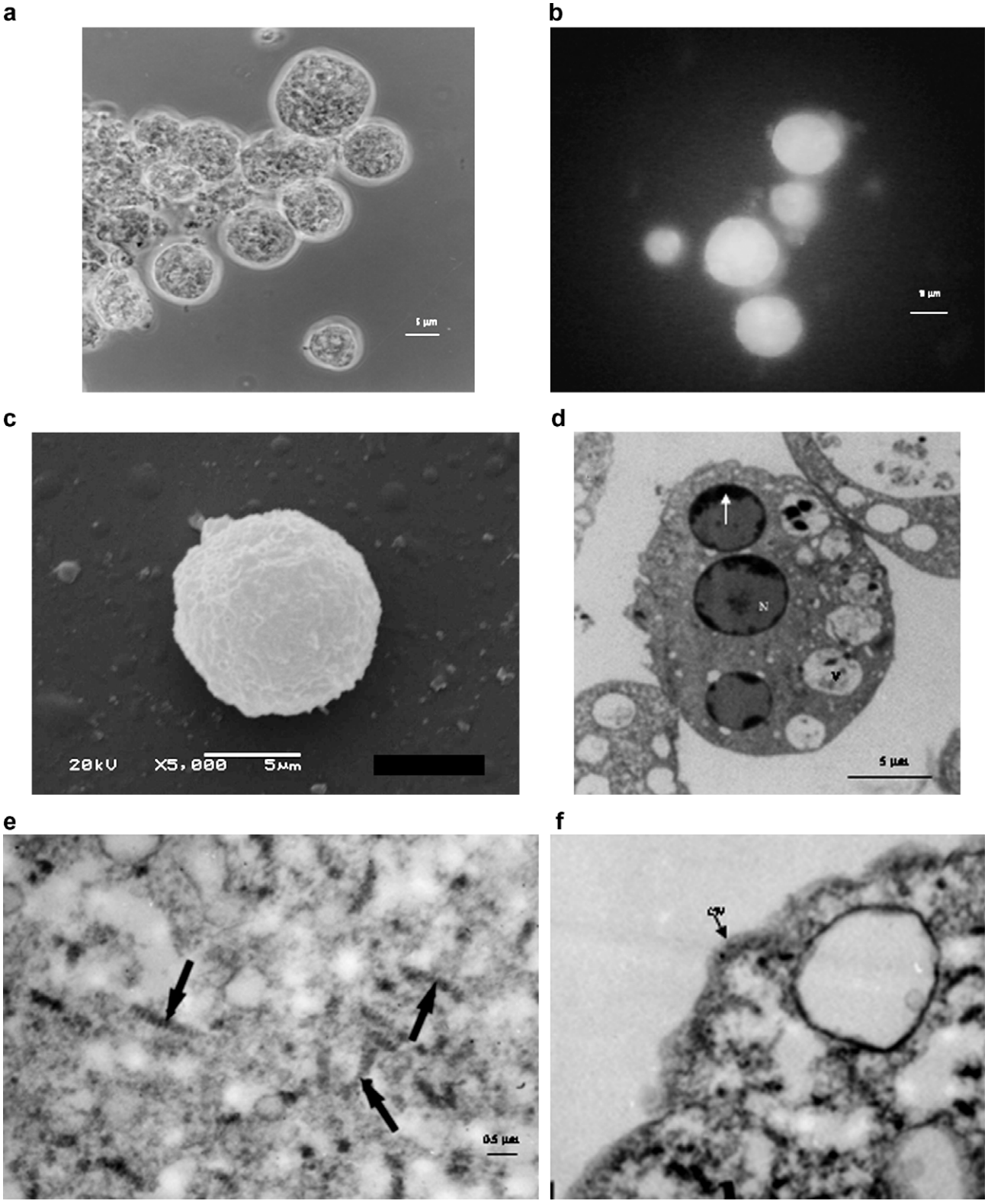

Fig. 1. Cyst-like structures obtained *in vitro* showing characteristics as a typical *E. histolytica* cyst. Cyst-like structures showed: (a) aggregate resistant to 0.15% sarcosyl for 10 min (100×) and (b) high signal of fluorescence under UV light when stained with 0.1% calcofluor M2r (40×), (c) observed by scanning electron microscopy, surface is not smooth but is corrugated or lighter wrinkled (5000×), (d) semifine sections showed multinucleated cyst (N, nuclei), condensed chromatin associated to nuclear membrane (arrow) and big vacuoles (V) presented in cytoplasm (7200×). (e) Transmission electron micrography showed cytoplasmatic ribonucleoproteic helices (arrows) (30,000×) and (f) a thin cell wall (CW) is observed above of the cell membrane (12,000×).