ELSEVIER

Archives of Medical Research 31 (2000) S153–S154

Archives of Medical Research

Secretory Pathway Activation by Interaction of *Entamoeba histolytica* Trophozoites with Fibronectin

Rebeca Manning-Cela, Eloisa M. Carbajal and Isaura Meza

Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del I.P.N. (Cinvestav), Mexico City, Mexico

Key Words: Entamoeba histolytica, Endoplasmic reticulum, Golgi complex, Intracellular pH, Signal transduction.

Introduction

Entamoeba histolytica has a peculiar subcellular organization. Although secretory activation is well defined and apparently necessary for cell lytic and invasive properties, typical organelles involved in the secretory pathway of eukaryotic cells, such as the endoplasmic reticulum (ER) and Golgi complex, are not easily distinguished. Instead, a lattice of fine tubules and numerous vacuoles can be observed (1,2). Very recently, protein components of both ER and Golgi apparatus were identified in some vacuoles during secretion of proteins specific for cyst wall formation (3). As trophozoites glycosylate some of their proteins, it is possible that these proteins transit through regular secretory pathways. We have used the *in vitro* model established in our laboratory, where interaction of trophozoites with fibronectin (FN) elicits active secretion of proteases to the medium (4) to monitor the presence and organization of ER and Golgi-like cellular organelles. Protease secretion induced by FN substrates is one of the trophozoite responses to the signal transduction processes known to occur during its interaction with FN. The activation of secretory functions could then, as in the case of cyst formation, trigger the organization of the secretory machinery in the trophozoites.

Materials and Methods

Trophozoites of *E. histolytica* HM-1:IMSS were grown at 37°C in axenic TYI-33 medium containing 10% bovine serum and harvested at the logarithmic phase of growth. Electron microscopy samples were prepared by standard methods, plating trophozoites on plastic dishes covered by resin and with or without a FN layer. For pHi measurements, trophozoites were incubated in medium at different extracellular pH or preincubated with 50 μM amiloride after being plated on FN-coated surfaces. Uncoated surfaces were used to plate control amebas. To measure the pHi, BCECF—a pH fluorescent indicator conjugate (Molecular Probes, Eugene, OR, USA)—was used to label the cells, detected by spectrofluorometry at 530 nm. To immunolocalize ER and Golgi structures, trophozoites were exposed to FN-coated surfaces for 30 min, then fixed, permeabilized, and immunostained using anti-ER and anti-Golgi antibodies. The effect of Brefeldin A on cell growth and perturbation of the vesicular arrangement was tested after establishing the effective concentration using 12.5, 25, 50, 75, and 100 μg/mL of the drug.

Results and Discussion

Transmission electron microscopy of trophozoites (Figure 1) indicated that binding to FN produced a large concentration of helical ribosomes at the sites of contact and numerous vacuoles at the cell edges. A notorious shape change was observed in the formed vacuoles, many of which showed residues of membranous material. This could be the result of internalization and/or endocytosis of membrane receptors and other surface components. Many phagocytic vacuoles were also observed, suggesting intensive endocytic and exocytic processes.

Changes in intracellular pH, in particular alcalinization, have been correlated with the initiation of signal transduction in many types of cells (5). As shown in Table 1, trophozoites regulate their intracellular pH (pHi) within a very small range (6.8–7.4), maintaining the pHi in the acidic side. Preincubation of trophozoites with 50 μM amiloride—an inhibitor of the Na^+/H^+ exchanger—induced acidification of the basal pHi, suggesting the presence of Na^+/H^+ exchanger. Interaction of trophozoites with FN produced cytosol alkalinization ranging from 0.3–0.5 at different pHe

Address reprint requests to: Rebeca Manning-Cela, Departamento de Biomedicina Molecular, Cinvestav, Av. Instituto Politécnico Nacional #2508, 07360 México, D.F., México. Tel.: (+525) 747-7000, ext. 5002; FAX: (+525) 747-7134; E-mail: rmanning@mail.cinvestav.mx

Presenting Author: Rebeca Manning-Cela.

0188-4409/00 $–see front matter. Copyright © 2000 IMSS. Published by Elsevier Science Inc.
PII S0188-4409(00)00204-6

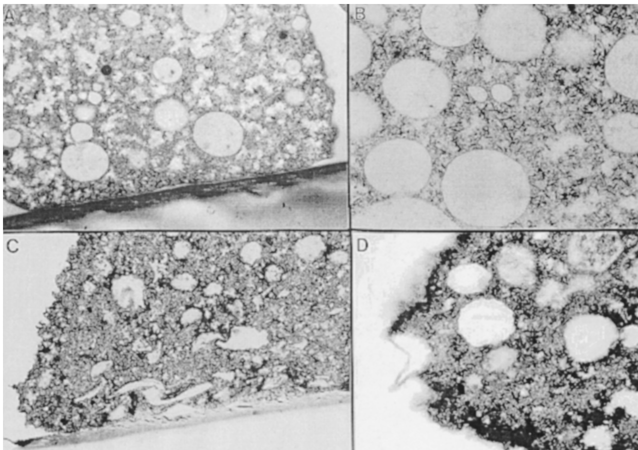

Figure 1. Transmission electron micrographs of *E. histolytica* trophozoites plated on plastic resin uncovered (A, B) or covered (C, D) with FN. Note the abundant ribosomal aggregates close to the distorted vesicles in C and D, where drastic changes of vacuole shape can also be observed.

that could be inhibited in the presence of amiloride. The activation of the Na^+/H^+ exchanger by FN interaction would explain the cytoplasm alkalinization that occurs during the activation of the secretory pathway.

Brefeldin A (BFA)—a drug that blocks protein transport through the Golgi complex—has been widely used to study the secretory pathway in many cells (6). We tested the effect of BFA on the secretory activities of trophozoites after establishing the optimal concentration of the drug as 75 μg/mL. Higher concentrations were found to be toxic for the trophozoites.

Immunofluorescence microscopy using anti-ER and anti-Golgi antibodies was utilized to determine whether the secretory apparatus of *E. histolytica* was induced to organize into a different and clear arrangement after binding to FN. Preliminary results show specific labeling of vacuoles after activation and disruption of this arrangement by BFA. As interaction of trophozoites with FN produces alkalinization of the cytosol—this phenomenon known to be part of the crosstalk between signal transduction and exocytosis—protease release via the activation of signaling pathways seems to have an effect on the organization of the secretory machinery in *E. histolytica.*

Table 1. pHi in *E. histolytica* trophozoites during binding to FN and the effect of amiloride

pHe	pHi			
	Basal	FN	Amiloride	Amiloride+FN
6.4	6.07	6.30	6.00	6.15
6.8	6.40	6.70	6.35	6.50
7.0	6.54	6.90	6.42	6.58
7.2	6.80	7.08	6.70	6.93
7.4	6.90	7.40	6.90	6.85

pHi was determined by BCECF and detected at 530 nm by spectrofluorometry; trophozoites were preincubated with 50 μM amiloride before addition of BCECF.

References

1. Mazzucuo A, Benchimol M, De Souza W. Endoplasmic reticulum and Golgi-like elements in *Entamoeba.* Micron 1997;28:241.
2. Martínez-Palomo A. The biology of *Entamoeba histolytica.* Chichester, UK. Research Studies Press/Wiley & Sons;1982.
3. Ghosh SK, Field J, Frisardi M, Rosenthal B, Mai Z, Rogers R, Samuelson J. Chitinase secretion by encysting *Entamoeba invadens* and transfected *Entamoeba histolytica* trophozoites: localization of secretory vesicles, endoplasmic reticulum, and Golgi apparatus. Infect Immun 1999;67:3073.
4. Meza I. Extracellular matrix-induced signaling in *Entamoeba histolytica*: its role in invasiveness. Parasitol Today 2000;16:26.
5. Alfonso A, Botana MA, Vieytes MR, Louzao MC, Botana LM. Effect of signal transduction pathways on the action of thapsigargin on rat mast cells. Crosstalks between cellular signalling and cytosolic pH. Biochem Pharmacol 1994;47:1813.
6. Coopers I, Andries M, Liu JL, Cesbron-Delauw MF. Intracellular trafficking of dense granule proteins in *Toxoplasma gondii* and experimental evidences for a regulated exocytosis. Eur J Cell Biol 1999;78:463.