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Acanthamoeba castellanii: Structural basis of the cytopathic mechanisms

Arturo González-Robles [a,*], Guadalupe Castañón [a], Ana Ruth Cristóbal-Ramos [a], Amparo Lázaro-Haller [b], Maritza Omaña-Molina [c], Patricia Bonilla [c], Adolfo Martínez-Palomo [a]

[a] *Department of Experimental Pathology, Center for Research and Advanced Studies, Mexico City, Mexico*
[b] *Department of Physiology, Biophysics and Neurosciences, Center for Research and Advanced Studies, Mexico City, Mexico*
[c] *Faculty of Superior Studies, UNAM, Iztacala, Tlalnepantla State of Mexico, Mexico*

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Abstract

In this study we report observations on the structural mechanisms of the cytopathic effect of *Acanthamoeba castellanii* trophozoites on cultured MDCK cell monolayers. Co-incubations were carried out for a maximum of 24 h. The first evidence of damage to the cell monolayer was detected by measuring the transepithelial resistance of cell monolayers that interacted with the amoebae. At 6 h, transepithelial resistance diminished to 51% and amoebae required 5–6 h to produce evidence of structural injury at the light microscopy level. Following 12 h of incubation, the cell monolayer was severely damaged. After making intimate contact with the surface of target cells, trophozoites detached cells from the substrate, lysed and by means of food-cups ingested the damaged cells. There was no morphological evidence of modifications in MDCK cell membranes, membrane fusion or junction formation between the amoeba and host plasma membrane. The lytic capacity of the amoebas appears to be the result of cytotoxic factors secreted by the amoebae since, when monolayers were incubated with conditioned medium, there was also a decrease in the transepithelial resistance. Besides, mechanical injury produced by the attachment and movement of the trophozoites may contribute to the disruption of the cell monolayer. As in other pathogenic amoebae, the cytopathic action of *A. castellanii* on the cell monolayers can subjectively be separated into four stages: adhesion, cytolysis, phagocytosis, and intracellular degradation.

Index Descriptors and Abbreviations: *Acanthamoeba castellanii*; Free-living amoebae; Pathogenesis; Ultrastructure; Phagocytosis

1. Introduction

Free-living amoebae occur worldwide and are common to most soil and aquatic environments (De Jonckheere, 1991; Rodríguez-Zaragoza, 1994; Szenasi et al., 1998). They have been isolated from very diverse habitats, including soil and water from the Antarctic (Brown et al., 1982), bottled water (Rivera et al., 1981), swimming pools (Muñoz et al., 2003), dental units (Barbeau and Buhler, 2001), eye wash stations (Paszko-Kolva et al., 1990), and from the atmosphere (Rivera et al., 1987).

In this group, the genus *Acanthamoeba* is known to infect humans. *A. castellanii* is recognized as the causative agent of two clinically distinct infections namely granulomatous amoebic encephalitis, a deadly disease of the central nervous system which usually affects immunosuppressed or debilitated individuals (Ma et al., 1990; Marciano-Cabral et al., 2000; Martínez, 1980, 1991) and a sight-threatening ulceration of the cornea called amebic keratitis (Auran et al., 1987; Ma et al., 1990; Niederkorn, 1999; Sharma et al., 2004).

Despite the ubiquitous distribution of this amoeba, keratitis is relatively uncommon and is associated with the use of both soft and hard contact lenses particularly if

* Corresponding author. Present address: CINVESTAV-IPN, Av. Instituto Politécnico Nacional 2508 San Pedro Zacatenco 07360 Mexico, D.F. Mexico. Fax: +52 55 5061 3396.
E-mail address: goroa@cinvestav.mx (A. González-Robles).

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accompanied by poor hygiene (Brewitt, 1997; Butler et al., 2005; Moore et al., 1987; Schuster and Visvesvara, 2004).

The mechanisms by which *Acanthamoeba* damages target cells are poorly understood. Cytolytic processes of different protozoan parasites involve, the adhesion to target cells by recognition of host glycolipids and/or glycoproteins, lysis of host cells by mechanical means and/or secretion of proteases and phagocytosis (Arroyo et al., 1993; Khan, 2003; Martínez-Palomo et al., 1985; Yang et al., 1997). Additionally, pore-forming proteins are produced by the pathogenic protozoon *Naegleria fowleri* (Herbst et al., 2002, 2004) and *Entamoeba histolytica* (Leippe et al., 1991) which are implicated in the cytolytic activity against target cells. Studies of the pathogenicity of *Acanthamoeba* have been carried out using different cell lines or primary cornea cell cultures (Dove Pettit et al., 1996; Khan, 2001; Morton et al., 1991; Niszl et al., 1998; Shin et al., 2001; Taylor et al., 1995) to elucidate their behavior and characterize their cytopathic effect.

On previous interaction studies carried out in our laboratory using the protozoan *E. histolytica* and MDCK cell monolayers as target cells, we noted a rapid destruction of target cells through the cytolytic activity of this parasite (Martínez-Palomo et al., 1985). Similar results were obtained when equivalent concentrations of *Trichomonas vaginalis* strains with differing degrees of virulence were interacted with MDCK cells, although the results were not as pronounced and it took longer to destroy the monolayers (González-Robles et al., 1995).

Using the same model, in the present study we report the early interactions produced by *A. castellanii* trophozoites and conditioned culture medium on the integrity of the cell monolayers. The damage was evaluated by measuring the transepithelial electrical resistance (TER) of monolayers mounted in Ussing chambers. By light and electron microscopy, structural analysis was also performed to evaluate the cytopathic effect produced by the amoebae. The interaction between the amoebae and target cells resulted in a decrease in the TER to 51% compared with control values after 6 h of co-culture. In contrast, when monolayers were maintained in the presence of amoebae conditioned medium the TER was only lowered to 86% over the same time period.

2. Materials and methods

2.1. Amoebae

Acanthamoeba castellanii trophozoites, isolated from the contact lens associated with a human case of keratitis in Hospital Luis Sánchez Bulnes, Mexico City, were grown and maintained in axenic culture in 2% Bacto Casitone (Pancreatic digest of casein, Becton Dickinson, Sparks, MD) supplemented with 10% fetal bovine serum (Gibco, Grand Islands NY). Cultures were incubated at 26 °C in borosilicate tubes (Pyrex, Mexico). Trophozoites were harvested after 3 days during the logarithmic phase of growth by chilling the culture tubes in an ice-water bath for 15 min. After centrifugation at 2000 rpm for 5 min, the pellets were resuspended in culture medium.

Acanthamoeba castellanii strain was identified by morphological characteristics according to Page (1988).

2.2. Amoeba conditioned medium

Conditioned medium was based on cell number grown in a specific volume of culture medium. After 72 h there were approximately 4×10^6 amoebae in 7 ml of medium.

2.3. Cell cultures

Monolayers of epithelial cells of the established MDCK line of canine kidney origin (Madin Darby Canine Kidney) were grown on 25 cm^2 cell culture flasks (Corning, Corning Incorporated, NY) in Dulbecco's modified Eagle's medium (Microlab, Mexico) supplemented with 10% fetal bovine serum (Gibco, Grand Islands, NY) and antibiotics in a 5% CO_2 atmosphere at 37 °C.

Confluent monolayers were incubated in the same conditions in a mixture of Bactocasitone and Dulbecco's modified Eagle's medium in equal proportions and *A. castellanii* trophozoites were added in a 1:2 target cell:amoeba ratio. In a parallel assay freshly trypsinized MDCK cells were incubated for 1–2 h with the amoebae in 1:1 ratio.

2.4. Light microscopy

Observations of the co-cultures were done every hour for 6 h using an Olympus inverted research microscope IMT equipped with an incubator chamber (Olympus Optical, Tokyo, Japan). Some co-incubations with MDCK cell monolayers were carried out for 12 and 24 h.

Photographs were obtained using a Zeiss AxioCam MRc digital camera (Carl Zeiss Vision GmbH, Germany).

2.5. Electrophysiology

Experiments with amoebae were performed as previously reported (González-Robles et al., 2004, 1995). Briefly, collagen covered nylon discs with confluent MDCK cell monolayers were mounted in Ussing chambers filled with Eagle's medium (Cereijido et al., 1986). The difference of potential between the chambers was measured with Ag/AgCl electrodes. The resting potential was measured; short (2 s) and low intensity (100 $\mu A/cm^2$) current pulses were delivered. The current deflection elicited was detected with a second electrode set placed 2 mm apart from the membrane. The contributions of the collagen support and bathing solution were taken into account. All values correspond exclusively to the cell monolayer. Transepithelial resistance was calculated according to Ohm's law. Values expressed as Ω cm^2 of MDCK cell monolayers represent the average of six measurements carried out in two sets of experiments.

Target cells cultured in the presence of amoebae or in the presence of ameba conditioned medium alone were