Experimental Parasitology 183 (2017) 69–75

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Acanthamoeba (T4) trophozoites cross the MDCK epithelium without cell damage but increase paracellular permeability and transepithelial resistance by modifying tight junction composition

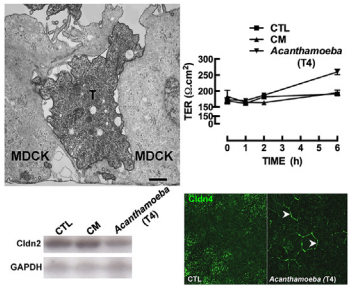
Catalina Flores-Maldonado [a, *], Arturo González-Robles [b], Lizbeth Salazar-Villatoro [b], Maritza Omaña-Molina [c], Juan Manuel Gallardo [d], Mónica González-Lázaro [b], Verónica Ivonne Hernández-Ramírez [b], Patricia Talamás-Rohana [b], Jacob Lorenzo-Morales [e], Adolfo Martínez-Palomo [b]

[a] Physiology, Biophysics and Neurosciences Department, Cinvestav-IPN, México City, Mexico
[b] Infectomic and Molecular Pathogenesis Department, Cinvestav-IPN, México City, Mexico
[c] Faculty of Superior Studies Iztacala, UNAM, México City, Mexico
[d] Medical Research in Nephrological Diseases Unit, Mexican Institute of Social Security, México City, Mexico
[e] University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, La Laguna, Tenerife, Canary Islands, Spain

HIGHLIGHTS

- *Acanthamoeba* (T4) does not damage the morphology or the actin cytoskeleton of MDCK cells.
- *Acanthamoeba* (T4) raises paracellular permeability and transepithelial resistance as it crosses MDCK monolayer.
- *Acanthamoeba* (T4) targets Cldn4 to tight junction while it sends Cldn2 to degradation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 July 2017
Received in revised form
12 September 2017
Accepted 29 October 2017
Available online 31 October 2017

Keywords:
Acanthamoeba (T4)
MDCK cells
Permeability
TER
Claudins

ABSTRACT

Free-living amoebae of the genus *Acanthamoeba* are protozoa ubiquitously found in nature. Some species of the genus are potentially pathogenic for humans provoking keratitis in healthy individuals, often in contact lens wearers and opportunistic infections such as pneumonitis, fatal granulomatous encephalitis and skin infections, particularly in immunocompromised individuals. The pathogenic mechanisms of these amoebae are poorly understood, however it had been suggested that contact dependent mechanisms are important during invasion, regardless of the epithelia type, since amoebae penetrate epithelia separating tight junction (TJ). This study was undertaken to determine whether *Acanthamoeba* sp. (T4) damages the barrier function of the TJ in MDCK epithelial monolayers. Actin cytoskeleton staining and electron microscopy analyses were performed; paracellular permeability and TJ sealing were evaluated by apicobasolateral diffusion of ruthenium red and transepithelial resistance (TER) measurements; immunofluorescence and Western blot assays were performed to locate and estimate expression of TJ protein claudins 2 (Cldn2) and 4 (Cldn4). The results show that *Acanthamoeba* sp. crosses the MDCK monolayer without altering the actin cytoskeleton or the morphology of the cells. When trophozoites or conditioned medium interact with the monolayer, paracellular diffusion of ruthenium red increases.

* Corresponding author. CINVESTAV-IPN, Av. Instituto Politecnico Nacional 2508, San Pedro Zacatenco, 07360 México City, Mexico.
E-mail address: catinef@hotmail.com (C. Flores-Maldonado).

https://doi.org/10.1016/j.exppara.2017.10.013

After 6 h, the amoebae, but not their conditioned medium, increase the TER, and Cldn2 is removed from the TJ, and its overall content in the cells diminishes, while Cldn4 is targeted to the TJ without changing its expression level.

In conclusion *Acanthamoeba* (T4) crosses MDCK monolayer without damaging the cells, increasing permeability and TER through Cldn2 degradation, and redirecting Cldn4 to TJ. These results strongly suggest that contact-dependent mechanisms are relevant during amoebae invasion.

1. Introduction

Free-living amoebae of the genus *Acanthamoeba* are cosmopolitan organisms, which can be isolated from diverse terrestrial and aquatic environments. Some species of this genus are etiological agents of several pathologies, such as amoebic keratitis, a sight-threatening corneal infection often among contact lens wearers (Niederkorn et al., 1999; Sharma et al., 2004), pneumonitis (Martinez and Janitschke, 1985), skin infections (Morrison et al., 2016) and granulomatous amoebic encephalitis, particularly in immunocompromised individuals (Marciano-Cabral et al., 2000; Martinez, 1980, 1991). The pathogenic mechanisms of these amoebae are poorly understood. A recent *in vitro* assay demonstrated that these organisms are able to damage epithelial cells in culture and produce focal injuries (González-Robles et al., 2013). Omaña-Molina et al. (2013, 2017) described the early morphological events during the invasion, including the penetration of trophozoites into different epithelia (olfactory, respiratory, alveolar space and renal tubule). In the nasal epithelium, trophozoites separate and lift off the most superficial cells, then migrate and penetrate between the cells without causing cytolytic effect on adjacent cells, reaffirming the idea that contact-dependent mechanisms are important during the invasion of *Acanthamoeba* trophozoites, regardless of the epithelia type. The epithelia are the first barrier that microorganisms have to overcome during invasion (Lu et al., 2013). Through this tissue active and selective exchanges of both nutritious and waste substances are carried out, generating compositionally distinct fluid compartments in the organisms. In addition to cell polarity, the function of epithelia depends on TJ which that is located right on the border between the apical and lateral membrane domains, binding the cells together and obliterating the intercellular space, forming a barrier that prevents free diffusion of substances through intercellular spaces and evidenced by using electron dense tracers such as ruthenium red (Farquhar and Palade, 1963; Martínez-Palomo et al., 1971). Khan and Siddiqui (2009) reported that *Acanthamoeba* affects the microvascular endothelial cells of the human brain and changes the integrity of the monolayer by targeting TJ, which is a complex of multiple elements that include transmembrane, cytoplasmic plaque, signaling and adapter proteins that link TJ to the actin cytoskeleton (Günzel and Yu, 2013). It is well known that the binding of pathogens or their toxins, while endangering the integrity of the epithelium, can alter both structure and function of the TJ. Claudins are directly involved in the barrier function of these junctions, these proteins are a family that have four transmembrane domains, both NH2 and COOH termini are cytoplasmic, in the extracellular side the two loops bind with the corresponding loops of the claudins of the neighboring cells (Furuse et al., 1998a, 1998b) thus binding the cells to each other.

The aim of this work was to determine whether *Acanthamoeba* (T4) induces changes in TJ structure that affect the barrier function in MDCK cells. Likewise, the sealing efficiency of TJ mediated by claudin remodeling was evaluated.

2. Materials and methods

2.1. Amoebae

Amoebae used in this study were isolated from an amoebic keratitis case from the Hospital "Asociación para evitar la ceguera en México, Luis Sánchez Búlnes", México City. Based on morphological taxonomic criteria (Page, 1988) the isolated amoeba corresponds to *Acanthamoeba royreba*. The molecular identification of the amoebic strain was carried out by sequencing the Diagnostic Fragment 3 (DF3) of the 18S rDNA gene of *Acanthamoeba* (Booton et al., 2002; Lorenzo-Morales et al., 2006). The sequence of the amoeba exhibited a 98% homology with the *A. royreba* Oak Ridge strain (Genbank entry: U07417; González-Robles et al., 2013).

In the laboratory, the amoeba was cultured in borosilicate tubes (Pyrex, Mexico) in axenic conditions in 2% bactocasitone (pancreatic digest of casein, Becton-Dickinson, Sparks, MD) supplemented with 10% fetal bovine serum (PAA laboratories GMBH, Austria), and a mix of 60.2 mg/l of Penicillin G sodium salt with 100 mg/l of Streptomycin sulfate antibiotics. Cultures were incubated at 30 °C. All assays were performed with trophozoites harvested at the end of the logarithmic phase of growth.

2.2. MDCK cells

MDCK cells were obtained from the American Type Culture Collection and cloned. The sub-clone 7.11 was selected for its capacity to develop blisters and for its low basal transepithelial resistance (approximately 150–250 Ωcm^2, Flores-Benitez et al., 2007). The cells were grown on 25 cm^2 cell culture flasks (Costar Corning, NY) in Dulbecco's modified Eagle's medium (DMEM, Gibco-Life Technologies, Carlsbad, CA) supplemented with 10% bovine serum (Gibco, Grand Islands, NY), 100 U/ml of penicillin and 100 U/ml of streptomycin (In Vitro, Mexico City, Mexico) in a 5% CO_2 atmosphere at 37 °C.

2.2.1. Co-incubation of trophozoites with MDCK cell monolayers

MDCK cell monolayers were trypsinized and grown in Petri dishes (Costar Corning, NY). Cultures were mantained at 37 °C in a 5% CO_2 atmosphere, and 24 h later, confluent monolayers were obtained. Trophozoites were added in a 1:2 MDCK: *Acanthamoeba* (T4) ratio in DMEM-Bacto Casitone 1:1 ratio. Incubations were carried out for different times (0, 1, 2 and 6 h) at 37 °C.

2.2.2. Incubation of MDCK cell monolayers with conditioned medium

Conditioned medium was obtained as follows: culture flasks containing 5 ml of DMEM with 2% fetal bovine serum and 6×10^6 trophozoites in exponential growth phase were incubated at 30 °C for 24 h. Viability of trophozoites was determined using the trypan blue exclusion method before collecting the conditioned medium. The supernatant was removed, centrifuged and filtered through a 0.22 μm Durapore membrane (Millipore, Bedford, Massachusetts).