ID-8-P-2823 Three-dimensional animated reconstruction in the study of actin cytoskeleton rearrangement in the amoebae of the genus Entamoeba (E. histolytica and E. dispar) by laser scanning microscopy

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Different species of Entamoeba perform similar functions such as adherence, cytolysis, and phagocytosis. E. histolytica and E. dispar share the same processes although the former is pathogenic while the second is non-pathogenic. Systematic comparison between both is an important research area. The ability to ingest microorganisms and target cells is associated with amoebic pathogenesis, and in this process, the binding of the parasite to the target cell is mediated by electrostatic forces and then, mechanisms that involved surface molecules begin to engage (Sateriale, A., et al., 2012). This binding leads to the activation of signaling pathways that promote reorganization of the actin cytoskeleton (Talamás-Rohana, P. and Ríos A., 2000).

The objective is to analyze the dynamics of the actin cytoskeleton between E. histolytica and E. dispar.

Entamoeba histolytica HM1-IMSS and Entamoeba dispar SAW 760 RR strains were used. Trophozoites were incubated on glass coverslips coated or uncoated with fibronectin (FN) at different times (15, 30, and 60 min). Parasites were fixed with 4% paraformaldehyde, permeabilized, and stained with rhodamine-conjugated phalloidin (Wehland, J., et al., 1977). Tracking of the restructuring of the actin cytoskeletal was done by laser scanning microscopy using a Carl Zeiss LSM 700 microscope.

Actin polymerization of Entamoeba histolytica begins at early times, both in glass and FN. Conversely, Entamoeba dispar presents a more limited and slower polymerization process. The structuring of actin in E. histolytica is induced in areas extremely close to the site of contact with the substrate, showing nucleation points, stress fibers and phagocytic structures; in comparison, E. dispar, even at longer times, shows a limited structuration of the actin cytoskeleton, as the fluorescent label is observed as amorphous clusters distributed throughout the cell volume without specific locations. Z-stack analysis, ortho-cuts, and quantification of fluorescence intensity allowed delimitation of specific sites where the re-structuring of filamentous actin is occurring in both species. To show with more detail, the actin structures formed as well as their location within the cell, we processed all optical images from “Z” cuts to achieve a three-dimensional animated reconstruction.

Manifestation of the pathogenic behavior requires cytoskeletal dynamics as this allows parasite movement and tissue penetration. Therefore, results obtained in this work let us conclude that differences in the pathogenic behavior of both species of amoeba might be due, at least in part, by the lower efficiency of the E. dispar actin cytoskeleton to reorganize in response to extracellular signals.

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Fig. 1: Actin structuring kinetics between E. histolytica and E. dispar adhered to FN. Figure 1 shows E. dispar with a cortical structure at 15 min. However, a dramatic change at 15 min in E. histolytica was seen and at 60 min, most amoebas seen in the field have well-defined new structures. E. dispar shows no significant changes even at longer times.

Fig. 2: Adherent comparison between E. histolytica (15 min) and E. dispar (60 min) using FN substrate. Figure 2A shows that structured actin of E. histolytica is totally redistributed on contact with substrate (FN), while in figure 2B, structured actin in E. dispar is redistributed through all cytoplasmic volume (Nuclei (blue) were stained with DAPI).