Title: Lipid microdomains and complement receptor 3 communication during phagocytosis of *Histoplasma capsulatum* by macrophages

Authors: Zamith-Miranda, D. [1], Guimarães, A.J. [2], Cerqueira, M.D. [1], Lopez, P.H. [3], Rodrigues, M.L. [1,4], Pontes, B. [5], Viana, N.B. [5,6], Martinez, L.R. [7], Schnaar, R.L. [3], Nosanchuk, J.D. [7], Nimrichter, L. [1]

Institution: [1] UFRJ – Instituto de Microbiologia Paulo de Góes, [2] UFF – Instituto Biomédico, [3] John Hopkins School of Medicine – Department os Pharmacology and Molecular Sciences, [4] Fiocruz – Centro de desenvolvimento Tecnológico em Saúde, [5] UFRJ – Instituto de Ciências Biomédicas, [6] UFRJ – Instituto de Física, [7] Albert Einstein College of Medicine – Department of Microbiology and Immunology.

Abstract

Recognition and internalization of pathogens is a multifactorial process that involves transient and stable interactions. Host membrane plasticity is crucial during this event. It has been shown that *Histoplasma capsulatum* (Hc) internalization by macrophages (Mph) occurs through CR3 (complement receptor 3), a heterodimer composed by CD18/CD11b integrins. In this study we investigated the participation of glycosphingolipids (GSL) and the association of CR3 with lipid domains during Hc internalization. We used macrophage monolayers to perform adhesion and internalization assays and optical tweezers (OT) to investigate cell-cell interaction between Mph and Hc. To evaluate the lipid domain importance we depleted cholesterol from Mph using methyl-*β*-cyclodextrin (m-*β*-CD). The participation of GSL, CD18 and CD11b during Hc-Mph association was compared, as well as the distribution of these molecules on Mph surface. For these experiments we used the CerGlcT pharmacologic inhibitor P4 (1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol), and macrophages deficient in the synthesis of complex GSL (from *B4galnt1* (-/-) mice), CD11b and CD18. Our results showed that cholesterol depletion reduced the association levels only at the initial periods of incubation (15 min). OT's experiments revealed that the time required for adhesion is longer in cholesterol-depleted macrophages than in control cells. Additionally, the internalization process was reduced in m-*β*-CD-treated Mph. Mph from *B4galnt1* (-/-) mice showed a significant reduction in Hc association when compared to Mph derived from wild type mice. Contrasting with the literature CD18 seems not to participate during the initial steps of Hc-Mph interaction, which was confirmed by a partial co-localization of this integrin with GM1 and Hc at the Mph cell surface. An intense co-localization between CD11b and GM1 was visualized along the Mph surface, including at the Hc binding sites. Inhibition of GSL synthesis increased the time required for adhesion between Mph and Hc. Our data show that the complement receptor CR3 indeed has a role in the interaction between Mph and Hc. The adhesion between these cells seems to be dependent upon GSL and CD11b, while the internalization is correlated with lipid domains assemble.

Keywords: Macrophage, *Histoplasma capsulatum*, Interaction, Integrins, Glycosphingolipids

Financial Support: CNPq, CAPES e FAPERJ