A reduced amount of 14-3-3 protein and the implications to *Paracoccidioides brasiliensis* infection

Marcos, C.M.¹, Oliveira, H.C.¹, Assato, P.A.¹, Scorzoni, L.¹, Da Silva, J.F.¹, De Paula e Silva, A.C.A.¹, Singulani, J.L.¹, Santos, C.T.¹, Da Silva, R.A.M.¹, De Almeida, R.¹, Andrade, C.R.², Fusco-Almeida, A.M.¹, Mendes-Giannini, M.J.S.¹.

¹Faculdade de Ciências Farmacêuticas de Araraquara, UNESP – Univ. Estadual Paulista, Araraquara, SP, Brazil.

²Faculdade de Odontologia de Araraquara, UNESP – Univ. Estadual Paulista, Araraquara, SP, Brazil.

marcos_caroline@yahoo.com.br; giannini@fcar.unesp.br

INTRODUCTION

Paracoccidioides brasiliensis strain, down-expressing the adhesin Pb14-3-3, was evaluated in a murine model of infection. Mice were inoculated intratracheally with fungal transformant, Pb14-3-3 aRNA, and the pathogenicity of this strain was compared with PbWT (wild-type strain) and PbEV (empty-vector strain) assessing lung tissues evidences. Pb14-3-3 aRNA displays attenuation of the virulence triggering the formation of fewer granulomas with decreased number of yeast cells in the lungs and reduced fungal burden. Moreover, Pb14-3-3 aRNA have less ability to induce host pneumocytes apoptosis. Our results show that 14-3-3 is an important virulence factor in pulmonary infection caused by *P. brasiliensis*.

METHODOLOGY

RESULTS AND CONCLUSIONS

Attenuation of pathogenic phenotype as result of down-expressing of 14-3-3 in *P. brasiliensis* during experimental PCM

Figure 1. Mice were infected intratracheally with 3×10^5 yeast cells of each strain of *P. brasiliensis*: PbWT, PbEV and Pb14-3-3 aRNA, lungs were removed 15 days post-infection and analyzed. A) Representative histopathological findings in lungs tissues using hematoxylin-eosin (H&E) staining. Scale bars: 100µm. The arrows indicate the yeast cells. B) Score of the number of fungi and granulomas founded in the lung sections scale of 0 to 4: 0 – absence; 1 – minimum number; 2 – moderate number; 3 – important number; 4 – larger number. C) Fungal burden of lung mice. Non-infected: absence granulomas, yeasts cells and CFU. Data express the mean $\pm$ standard deviation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. PbWT and PbEV.

Pb14-3-3 is involved in the induction of pneumocyte apoptosis during infection by *P. brasiliensis*

Figure 2. TUNEL assay was used to evaluate the role of 14-3-3 in the pneumocytes apoptosis after 5 (A) and 24 (B) h of infection with each strain of *P. brasiliensis*: PbWT (Pb 18), PbEV (empty vector), Pb14-3-3 aRNA (transformant strain). Non-infected cells: negative control. Data express the mean $\pm$ standard deviation. * $p < 0.05$; *** $p < 0.001$; vs. PbWT or PbEV; ## $p < 0.01$; ### $p < 0.001$ vs. Non-infected sample.

Lower levels of Pb14-3-3 were related with decreased recovery of yeasts from mice infected with Pb14-3-3 aRNA. Maybe, the lower fungal burden observed is related with the data of apoptosis reduction. We hypothesized that Pb14-3-3 aRNA has an impaired virulence phenotype with consequences to the progression of pathogenesis, decreased apoptosis of host cells, which may be related to a more efficient immune response, since the microbe-induced apoptosis is known to play a role in the onset of infection, pathogen survival and escape from host immune response. Our data indicates the importance of Pb14-3-3 in host cell apoptosis and *P. brasiliensis* pathogenicity. This protein may be included among the virulence factors that modulate host immune responses contributing to a better understanding of *Paracoccidioides*-host interaction, elucidating information that can help in the development of new treatments or vaccines to prevent or control the infection.

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