TITLE: ACTIVITY OF MONOCLONAL ANTIBODY F1.4 GENERATED AGAINST *Paracoccidioides brasiliensis* IN EXPERIMENTAL INFECTION

AUTHORS: BONICHE, C[1]. THOMAZ, L[1]. TABORDA, CP[1].

INSTITUTION: [1]Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo*.* Avenida Professor Lineu Prestes, 1374. CEP 05508-000. Butantã, SP, Brazil.

ABSTRACT:

Paracoccidioidomycosis (PCM) is a systemic mycosis, endemic in Latin America and caused by fungi from genus *Paracoccidioides.* The usual therapy consists in antifungal drugs as azoles, polyenes and sulphonamides administered for long periods, causing a low adherence of patients to the treatment after the first signs of clinical improvement appear, leading to relapses, that are more difficult to treat. Therapeutic alternatives for PCM has leaded to the use of monoclonal antibodies, which has proven their ability to modify the course of disease in mice infected with yeasts of *Paracoccidioides* spp. Passive transference of monoclonal antibodies (MAbs) could potentiate the immune response, which in addition to chemotherapy, could help reducing treatment periods, reduce the consequences of PCM, and result in a treatment option for anergic patients. This study aims to analyze the activity of MAb F1.4 against *P. brasiliensis*; produced by hybridoma technology with antigen obtained by alkaline extraction from Pb18 strain yeasts. According to our results the MAb F1.4 is an IgG1 and also recognizes *P brasiliensis* and *P. lutzii*. A phagocytosis assay with J774.1 macrophages like and *P. brasiliensis* or *P. lutzii* yeast cells incubated for 24 hours revealed that yeasts that were opsonized with 100 µg of MAb F1.4 were three times more phagocytized and killed than opsonized yeasts with 100 µg of an irrelevant MAb, or non-opsonized yeasts, by increasing the nitric oxide production by the macrophages. An invertebrate model was used to evaluate the protection and the toxicity of MAb F1.4 in *Galleria mellonella* larvae infected with Pb18 and treated with this MAb. The survival rate of the infected group and the group not infected, that received MAb F1.4 at the end of the test did not present statistical difference with the control group. We performed direct immunofluorescence to evaluate the cellular interaction between the yeasts and hemocytes, and the MAb F1.4 does not interfere in the phagocytosis by the hemocytes; indicating that MAbs are probably not recognized by the innate immune system of this invertebrate, even because larvae do not have an adaptive immune system, with production of immunoglobulin. Although MAb F1.4 does not interfere with phagocytosis, it induces another process related to the protection of larvae known as phenoloxidase activity and induced oxygen derivates, like oxide nitric.

KEYWORDS*:* Paracoccidioidomycosis, immunotherapy, *Paracoccidioides brasiliensis*, monoclonal antibodies, *Galleria mellonella*.

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