TITLE: SCREENING OF ANTIGENS FROM *Paracoccidioides brasiliensis* AND *Paracoccidioides lutzii* TO INDUCE MONOCLONAL ANTIBODIES PRODUCTION: A NEW THERAPY TOOL DEVELOPMENT

AUTHORS: MOURA, A.N.D.[1,2,3]; NOSANCHUK, J.D.[3,4]; SILVA, L.B.R.[1,2]; THOMAZ, L.[1]; TABORDA, C.P.[1,2]

INSTITUTION: [1]INSTITUTE OF BIOMEDICAL SCIENCES II, DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF SAO PAULO, SAO PAULO, BRAZIL**;** [2]INSTITUTE OF TROPICAL MEDICINE, DEPARTMENT OF DERMATOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF SAO PAULO, SAO PAULO, BRAZIL; [3]DEPARTMENT OF MEDICINE; DIVISION OF INFECTIOUS DISEASES, ALBERT EINSTEIN COLLEGE OF MEDICINE, BRONX, NY, USA; [4]DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY, ALBERT EINSTEIN COLLEGE OF MEDICINE, BRONX, NY, USA

ABSTRACT:

Fungi of the genus Paracoccidioides are etiological agents of paracoccidioidomycosis (PCM), one of the most prevalent systemic mycoses in Latin America. Given the costs because significant rates of recurrence, toxicities and requirement for prolonged therapy, there is an urgent need for new approaches to therapy for PCM. Previous studies developed by our group have demonstrated that passive administration of Monoclonal Antibodies (MAbs) against 43 kDa (gp43) glycoprotein from *P. brasiliensis* and of MAbs produced against the heat shock protein 60 (Hsp60) from *Histoplasma capsulatum* were protective in experimental infection with *P. brasiliensis* and *P. lutzii,* respectively. Considering that thermal shock proteins are conserved, including in humans, the determination of specific epitopes and identifying common antigens for therapeutic use is extremely promising. To screen antigenic molecules, proteins, glycoproteins, and glycolipids extractions from fungal cell mass were made. For protein extraction, mechanical lysis was used to obtain cytosolic fraction, and heating with DTT buffer was used to obtain surface fraction. Glycoproteins were extracted with Cetavlon by Lloyd method. Glycolipids were extracted with chloroform:methanol solutions followed by Folch method and were purified in size exclusion chromatography columns. To test the relative affinity and to compare specificity between the isolates, Dot Blot, Western Blot and TLC Blot were performed. Due the lower yield with glycoproteins extraction, because his nature rich in carbohydrates, and the challenge to produce antibodies with high affinity against glycolipids, proteins were selected to deeper analysis. Tests, with sera from mice infected intratracheally and different pool of sera from human patients with PCM, against proteins extracts, result in 2 common electrophoresis bands detected for all isolates. These bands are being analysed by spectrophotometry to separate and to define what proteins are contained in that. The next steps will be to figure out the importance of these molecules, and make new tests in order to choose the most promising candidate to stimulate antibodies production. Hybridoma technology for MAbs production followed by humanization are the future goals.

Keywords: *Paracoccidioides lutzii*, *Paracoccidioides brasiliensis*, Paracoccidioidomycosis, Screening of antigens, Monoclonal Antibodies

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