

TITLE: THE *Paracoccidioides brasiliensis* 43-kDA GLYCOPROTEIN (GP43) IS ABLE TO INDUCE NEUTROPHIL EXTRACELLULAR TRAPS (NETS) BY HUMAN NEUTROPHILS.

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ABSTRACT

Paracoccidioides brasiliensis (*P. brasiliensis*), a dimorphic fungus, is one of the causative agent of Paracoccidioidomycosis (PCM). The main and most well-characterized antigenic component of *P. brasiliensis* is a 43-kDa glycoprotein (gp43), which is secreted during the infective phase and contains epitopes that induce delayed type hypersensitivity (DTH) in patients with PCM. It may also be involved in the virulence of this pathogen, since it has been shown that a decrease in the expression of gp43, using genetically modified *P. brasiliensis*, resulted in lower pathogenicity in murine experimental model. Recently, it was described that *P. brasiliensis* is able to induce Neutrophil Extracellular Traps (NETs), a structure released by activated neutrophils (PMNs) and composed by fibers containing decondensed chromatin, histones and several other granular proteins. NETs were also identified in tegumentary lesions of patients with PCM, and it could be involved in defense as well as in pathogenesis. In this context, the objective of this study was to evaluate the participation of gp43 in NETs formation *in vitro*. For this, human PMNs were stimulated with gp43 in different concentrations (50 µg/mL, 1 µg/mL, 100 ng/mL and 10 ng/mL) or PMA (positive control) for 2 hours and analyzed by Confocal Microscopy. NETs components were identified by anti-elastase and anti-histone monoclonal antibodies, and DNA was stained with DAPI. Our results showed that gp43 is able to induce NETs by human PMNs, and analyzing the images it seems that this release was probably dose dependent. In cultures stimulated with gp43 10 ng/mL, NETs seemed to be thinner and more delicate than those observed in cultures stimulated with higher doses of gp43 (50 µg/mL and 1 µg/mL). The assays to quantify these structures and demonstrate that this release is dose dependent are being conducted in our lab. The implication of gp43 in NETs release by PMNs brings relevant new information that could be related with the pathogenesis of this disease.

Keywords: *Paracoccidioides brasiliensis*, gp43, neutrophils, NETs