

**TITLE:** ETHANOL TREATMENT IMPAIRS THE RESPONSE OF HUMAN MONOCYTE-DERIVED MACROPHAGES AGAINST *P. BRASILIENSIS*.

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**ABSTRACT:**

Macrophages present a preponderant role in the initial immune response in Paracoccidioidomycosis (PCM). Some studies have shown that ethanol consumption, could interfere with the effector's functions of these cells and, consequently, increase the susceptibility to infections. In PCM, alcoholism is considered a risk factor, mainly in the chronic form (CF), however, there are no studies that have demonstrated the ethanol effects on the human immune response. Therefore, we aimed to investigate the effects of ethanol treatment on macrophages functions in response to the fungus *Paracoccidioides brasiliensis* (Pb). Macrophages were differentiated from monocytes purified from peripheral blood obtained from healthy individuals by treatment with GM-CSF for 5 days. After the differentiation, cells were pretreated with ethanol (25mM, 75mM or 150mM) for 4 hours and stimulated with Pb yeast cells or LPS for 24 hours. First, we evaluated the expression of molecules related to pathogen recognition and antigen presentation. We found that the treatment with ethanol diminished the expression of MHC-I, MHC-II, CD80 and CD86, but does not interfere with the expression of dectin-1 and TLRs (TLR2 and TLR4). However, ethanol treatment diminishes the phosphorylation of Syk protein (necessary for dectin-1 signalling). We also evaluated the effects of ethanol treatment on the phagocytic activity and in the production of reactive oxygen species (ROS) and cytokines by macrophages stimulated with Pb. Our data showed an increment in the phagocytosis, but a diminishment in ROS production after the treatment. In relation to the production of cytokines, we observed that the treatment of cells with ethanol diminished the production of IL-1beta in a dose dependent manner, and increased the production of IL-6 and IL-10. In conclusion, our data showed that ethanol exposure could interfere and modulate the expression of molecules related to antigen presentation, the phosphorylation of protein related to fungus recognition, the ROS production and the production of cytokines by macrophages. The reduced production of ROS and inflammatory cytokines (IL-1beta), essential for fungal contention, associated with the increased production of suppressive cytokines (IL-10), and the diminished expression of molecules associated with antigen presentation, can result in the impairment of the immune response and contribute to the susceptibility to infection observed in patients with the CF of PCM.

**Keywords:** Ethanol, Macrophages, *Paracoccidioides brasiliensis*.

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