Paracoccidioides brasiliensis INDUCES CYTOKINE SECRETION BY ACTIVATING PARs IN EPITHELIAL CELLS

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Introduction: Protease-activated receptors (PARs) are expressed in many cell types and comprise a family of G protein-coupled receptors (PAR1, PAR-2, and PAR-4), which are activated by proteases that cleave the extracellular amino terminus of PAR, and expose a new tethered ligand domain that binds to receptor and triggers multiple signaling cascades. Objectives: In this work, we investigate whether the pathogenic fungus Paracoccidioides brasiliensis (Pb) secretes proteases which activate PARs, promoting cytokine secretion by epithelial A549 cells. Materials and Methods: A549 cells were incubated with Pb in a transwell system to verify whether this fungus secretes components that induce cytokine secretion. IL-6 and IL-8 levels in culture supernatants were determined by ELISA. Serine proteases in Pb culture supernatants were purified by p-aminomethylbenzamidine-Sepharose (pABA), and 10 Fractions were obtained by this process. Fluorescence resonance energy transfer (FRET) peptides derived from sequences that span the activation sites of human PARs were synthesized, incubated with Fractions 1-10 and analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI-MS). Next, to block the activation of PARs, A549 cells were incubated with antagonist peptides of PAR-1, PAR-2 and PAR-4, and then, with Fraction 4. Next, IL-6 and IL-8 levels were determined by ELISA. Discussion and Results: We verified that Pb secretes proteases that induce the activation of PARs and increase IL-6 and IL-8 levels in culture supernatants of A549 cells. By LC/ESI-MS, we observed that Fraction 4 of pABA column (termed PbP, P. brasiliensis Protease) hydrolyzed PAR-1, PAR-2 and PAR-4 at amino acid residues corresponding to the activation site of these receptors. In addition, ELISA assay demonstrated that PAR-1 and PAR-2 antagonists reduced significantly IL-6 and IL-8 levels in culture supernatants of A549 cells. Conclusions: Taking together, these results suggest that Pb secretes proteases that activate PARs, promoting IL-6 and IL-8 secretion by A549 cells, and in this way this fungus may manipulate the host immune response.

Key Words: Protease-Activated Receptors, Paracoccidioides brasiliensis, Cytokines.
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