TITLE: Involvement of ROS and PI3K/Akt pathways during infection of human endothelial cells by *Streptococcus agalactiae*

AUTHORS: Oliveira, J.S.S.; Santos, G.S.; Ferreira, B.J.; Moraes, J.A.; Saliba, A.M.; Barja-Fidalgo, T.C.; Mattos-Guaraldi, A.L.; Nagao, P.E.

INSTITUTION: Instituto de Biologia Roberto Alcantara Gomes, Rio de Janeiro, RJ (Avenida 28 de setembro, 87 Fds, Térreo, CEP 20551-030, Rio de Janeiro – RJ, BRAZIL).

ABSTRACT:

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is an important human pathogen responsible for invasive diseases such as pneumonia and meningitis in newborns, immunocompromised adults and elderly individuals. *S. agalactiae* serotype III are prevalent in newborn infections and invasive diseases. Previous works of our group demonstrate an increase in glutathione S-Transferase, a protein involved in cell protection against reactive oxygen species (ROS), after the interaction between *S. agalactiae* and human umbilical endothelial cell (HUVEC). These reactive oxygen species contribute to endothelial and neuronal injury and NADPH oxidase is an important source of these molecules. ROS production is correlated with activation of Akt in myeloid cell, but the mechanism by which this process occurs in endothelial cells remains unknown. In this study we explored the role of ROS production in HUVEC infected by invasive *S. agalactiae* 90356-III strain. The HUVEC treatment with DPI and apocynin (NADPH oxidase inhibitors) were used to evaluate ROS production and NADPH oxidase activation. The expression of NADPH oxidase subunits (p22*phox*, p47 *phox* and p67 *phox*) was assessed by real time PCR and the phosphorylation profiles of p47*phox* and Akt proteins were analyzed by *Western blotting*. Data showed that *S. agalactiae* induced ROS production in HUVEC with increased expression of p47*phox* subunit. It was also observed the translocation of p47 *phox* subunit from the perinuclear region to the plasmatic membrane. Experiments using HUVEC treated with DPI or apocynin showed a reduction of *S. agalactiae* invasion and the remodeling of actin cytoskeleton in HUVEC treated with LY294002 (PI3k inhibitor). We also observed increased of Akt phosphorylation. In this work, we showed for the first time the ROS production in endothelial cells infected by *S. agalactiae*, and the involvement of PI3k/Akt signaling pathway, suggesting that it plays a key role in *S. agalactiae* pathogenicity.

Keywords: *Streptococcus agalactiae*, HUVEC, p47*phox*, reactive oxygen species, PI3K/Akt pathway