TITLE: Phagocytosis, persistence and cytokines production upon stimulation of human macrophages with *Staphylococcus aureus* of different lineages.

AUTHORS: PEREIRA, M.F.; BARCELOS, E.C.S.; ARDISSON, J.S.; GOMES, D.C.O.; KITAGAWA, R.R.; SCHUENCK, R.P.

INSTITUTION: Universidade Federal do Espírito Santo – Laboratório de Biologia Molecular e Virulência Bacteriana – Departamento de Patologia – Av. Marechal Campos 1468, Vitória - ES, Brasil.

ABSTRACT: *Staphylococcus aureus* is an important human pathogen that can cause a wide variety of diseases, ranging from superficial skin infection to severe systemic complications. While generally considered an extracellular pathogen, this bacterium has the ability to invade and survive within different cell types, including phagocytic cells. *S. aureus* strains present great genetic variability and exclusive combinations of virulence genes. Protection from primary staphylococcal infections is mainly dependent on innate rather than adaptive immune responses. Therefore, understanding the innate immune mechanisms of the host against this bacterium may aid in the development of therapies for the treatment of *S. aureus* infections. In the present work, we compared the phagocytosis, persistence, cytokines and nitric oxide (NO) production upon stimulation of THP-1 macrophages with 11 *Staphylococcus aureus* of different lineages. THP-1 macrophages were infected with *S. aureus* strains (1:25), incubated at 37 °C in a 5% CO_2 atmosphere for 15, 60 and 120 min for evaluation of phagocytosis and 24, 48 and 72 h for analysis of persistence. The supernatants were collected to detect NO concentration by Griess assay, and cytokines TNF-α, IL-6, IL-1β and IL-10 production using ELISA. The results were compared and analyzed using ANOVA. *P* values lower than 0.01 were considered as statistically significant. All *S. aureus* strains exhibited similarities in their interactions with THP-1 macrophages as to phagocytosis and persistence. The phagocytic activity increased significantly at 60 and 120 min post infection compared to 15 min. The intracellularly viable bacterial count (persistence) was elevated at 24, 48 and 72 h after phagocytosis. The 11 *S. aureus* strains induced the production of TNF-α, IL-1β and IL-10 in the 4 and 24 h post infection. However, two genetically distinct strains present significantly lower TNF-α and IL-1β production as compared to the others. All strains similarly stimulated IL-10 production in the 4 h post infection, however, during the 24 h, one strain of the group presented high levels of induction, unlike the others. A significant difference in NO induction was observed in the 4 h post infection. These findings suggesting difference in immunomodulation among *S. aureus* presenting different genetic background and can benefit the pathogenesis of some lineages.

KEYWORDS: cytokines, intracellular survive, nitric oxide, phagocytosis, *Staphylococcus aureus*.

DEVELOPMENT AGENCY: Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)