TITLE: SIGMA FACTOR RPOE IS CRUCIAL FOR CELLULAR ADHESION AND PHAGOCYTOSIS RESISTANCE IN KLEBSIELLA PNEUMONIAE

AUTHORS: SANTOS, T.E.P.; PEREIRA, F.M.; SIQUEIRA, N.M.G.; FERRAZ, L.F.C.

INSTITUTION: LABORATÓRIO DE BIOLOGIA MOLECULAR DE MICRORGANISMOS, UNIVERSIDADE SÃO FRANCISCO, BRAGANÇA PAULISTA, SP (AVENIDA SÃO JOSÉ, 218, CEP 12916-900, BRAGANÇA PAULISTA – SP, BRAZIL)

ABSTRACT:
Klebsiella pneumoniae is a Gram-negative bacterium responsible for hospital-acquired infections, especially in immunosuppressed patients. This is mainly due to the virulence factors and the capacity of this pathogen to survive in the intracellular environment of cell hosts. The Sigma factor RpoE regulon acts to maintain the integrity of the cellular envelope in bacteria submitted to stress conditions. In addition, in many bacteria the Sigma factor RpoE plays an important role in mechanisms of resistance to phagocytosis. However, little is known about the role of Sigma factor RpoE in *K. pneumoniae* pathogenicity. In this sense, this study aimed to investigate an RpoE mutant strain of *K. pneumoniae* in the processes of adhesion, phagocytosis and intracellular survival in macrophages. Macrophages RAW 264.7 cultured in 6 wells plates were infected with either the wild-type or the mutant *K. pneumoniae* strains at a multiplicity of infection of 200: 1 (bacteria: RAW 264.7). Adhesion, phagocytosis and intracellular survival steps were assessed after incubation periods of 30 min, 1 and 3 hours, respectively. At each point, the infected RAW 264.7 cells were lysed for counting bacterial colony forming units and were submitted to nitric oxide quantification. The modulation of the inflammatory response in the infected RAW 264.7 cells were assessed by Real-Time PCR. The absence of Sigma factor RpoE rendered a mutant strain with less capacity to survive phagocytosis by the macrophages when compared to the wild-type *K. pneumoniae* strain. The RpoE mutant strain of *K. pneumoniae* induced a three times greater production of Nitric Oxide in comparison to the wild-type strain during cell adhesion and triggered an upregulation of *iNOS*, *Il1b* and *Tnf* genes in the RAW 264.7 cells. The results indicate that the mutant strain induces a much more intense inflammatory response in macrophages than the wild-type, which may explain the deficiency of the mutant bacteria in surviving the in vitro phagocytosis assay. These results suggest that in *Klebsiella pneumoniae* the Sigma RpoE factor also play a crucial role in defense mechanisms against phagocytosis.

Keywords: *Klebsiella pneumoniae*, Host-Pathogen Interactions; Sigma Factor, Phagocytosis, Gene Silencing.

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