COMPARATIVE ANALYSIS OF TLR4 EXPRESSION ON MONOCYTES IN INTERACTION WITH ISOLATES OF KLEBSIELLA PNEUMONIAE ISOLATED

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TLR4 is the main recognition receptor of *K. pneumoniae*, as it is able to recognize lipopolysaccharide present in the cytoplasmic membrane. Comparative analysis of TLR4 expression on immune cells when cultured with microorganisms will allow the relative pathogenic potential of the isolates to be assessed. This study presents the results of evaluation of the effects on monocytes of *K. pneumoniae* isolated from clinical material and environmental objects

Keywords: K. pneumoniae, monocytes, TLR4, flow cytofluorimetry.

Klebsiella pneumoniae is a species of Gram-negative facultative-aerobic bacilliform immobile bacteria capable of causing a wide range of infections. *K. pneumoniae* is responsible for 0.5-5% of all cases of pneumonia, usually resulting in a high incidence of complications and increased mortality [1].

TLR4 are expressed both in cells of hematopoietic origin and in stromal cells, including lung epithelium. When *K. pneumoniae* enters the lungs, bacteria-specific TLR4 on epithelial cells are activated, resulting in the release of cytokines and chemokines. This attracts and activates monocytes, which phagocytize the bacteria. Activation of TLR4 by lipopolysaccharide stimulates the production of proinflammatory cytokines and chemokines, which determine the intensity of the inflammatory response and, consequently, the pathogenicity of the microorganism [1,2].

The aim of the study was to compare the pathogenic potential of two isolates of *K. pneumoniae* isolated from clinical material and environmental objects by flow cytofluorimetry.

We evaluated differences in the ability of isolates to activate TLR4 to characterize the pathogenic potential of isolates.

The model immune cells were monocytes isolated on a density gradient from peripheral blood mononuclear cells obtained from a healthy donor. The monocytes were incubated with *K. pneumoniae* suspensions for 24 hours. The main experimental evaluation was performed by flow cytofluorometry using a panel of specific monoclonal antibodies (MAB). Antibodies to TLR4 were used as MABs for monocytes.

The baseline TLR4 expression in the negative control was 94.9%, while in the positive control it was 82.6%. When cultured with the clinical isolate of *K. pneumoniae*, the TLR4 expression levels were: 89.6% for the concentration (10^3), 91.1% (10^4) and 90.3% (10^5). For the isolate obtained from the environment, these values statistically decreased to 83.1% (10^3), 77.2% (10^4) and 80.4% (10^5).

Based on the statistical analysis of the obtained results, reliable differences in the levels of TLR4 expression on monocytes were established during interaction with the clinical isolate of *K. pneumoniae* and the isolate of *K. pneumoniae* isolated from the environment ($p=0.001894$). Thus, the obtained data indicate a more pronounced ability of the isolate of *K. pneumoniae* from clinical material to activate TLR4-mediated mechanisms of innate immunity, which indicates its greater pathogenicity compared to the isolate from the environment.

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