

THE PROTEIN HLYX OF *LEPTOSPIRA INTERROGANS* SEROVAR
COPENHAGENI DELAYS APOPTOSIS AND INCREASES THE EXPRESSION
OF CD18 (MAC-1) IN HUMAN POLYMORPHONUCLEAR CELLS

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Leptospirosis is a widespread human and animal disease caused by pathogenic leptospires. The HlyX protein identified from the *Leptospira* genomes is found only in pathogenic leptospires, which means that this protein is probably associated with infection. In this work, our aim is to study whether HlyX protein possesses activity on human polymorphonuclear cells (PMN). The PMNs were isolated from peripheral blood by Ficoll Hypaque ($\delta=1077$) gradient centrifugation and dextran sedimentation. PMNs were treated with 3, 6 and 12 $\mu\text{g}/\text{mL}$ of recombinant LPS-free HlyX (cloned and expressed in *Escherichia coli* and then purified by Ni⁺²-charged chelating Sepharose). Analyses performed by Fluorescence Microscopy (morphology nuclein) and Flow Cytometry (hypodiploid cells and CD18 expression) showed that HlyX increases the viability of PMN and the expression of CD18 in their surface, which would increase the cellular rolling in effect vascular endothelium. Moreover, these effects were diminished when HlyX-murine specific neutralizing serum was previously added to the reaction mixture. HlyX can be considered a proinflammatory protein that is involved in delaying the apoptosis and recruiting this defense cells during the leptospirosis disease.

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