FLOW CYTOMETRY ANALYSIS OF CA^{2+} DEPENDENT OXIDATIVE BURST IN NEUTROPHILS

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Human neutrophils are differentiated cells in peripheral blood and the first cells recruited from the bloodstream to sites of infection. The increases in cytosolic Ca^{2+} precede the activation of neutrophils. This Ca^{2+} is kept in storage vesicles and obtained from extracellular influx. Following activation by bacterial products or other stimuli - LPS, fMLF, PMA, PAF - neutrophils execute several specialized functions that include chemotaxis, phagocytosis, and the generation of reactive oxygen species (ROS). The aim of this study was to compare the cell viability, stimulation and activation of the neutrophil in the presence and absence of extracellular Ca^{2+} using a flow cytometric assay, a semi-quantitative measure of the respiratory burst in neutrophils. The percentage of cells presenting fluorescence as a measure of respiratory burst was higher than control cells. In this regard, our results have shown that activation in the presence of Ca^{2+} resulted in an increase of 60% in the number of cells producing ROS for both PAF and fMLF, 30% for PAF plus fMLF and did not change for PMA. The average intensity of ROS production per cell also increased 4.6 fold for PMA, 3.1 fold for PAF, 31 fold for fMLF and 35 fold for PAF+fMLF. Furthermore, cell viability with PAF was of 98% and PMA was 94%.