NEUTROPHIL EXTRACELLULAR TRAPS FORMATION / INHIBITION IN VITRO IS DEPENDENT ON “WASH-EFFECT” OF INHIBITOR VS. STIMULATOR

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Discovery of neutrophil extracellular traps (NETs) opened a new field of immunology. However the knowledge about NET is still limited. Recently, a lot of attention has been focused on reactive oxygen species (ROS) in the course of NET generation. NETosis can be induced by 12-myristate 13-acetate (PMA). PMA mediated NET formation depends on the production of ROS by NADPH oxidase. Inhibition of NADPH oxidase with diphenyleneiodonium (DPI) blocked ROS formation and NETosis.

The goal of the present study was to find out how pretreatment or continuous exposure of neutrophils with DPI influenced the NET release after stimulation. After isolation granulocytes were preincubated with DPI. Subsequently DPI was either washed away or the media was unchanged. Finally PMA was added. NET formation was estimated qualitatively as well as quantitatively. Unstimulated cells were considered as a negative control. Stimulated cells in DPI-free media were used as a positive control.

Results have been represented as a mean ± standard deviation (SD) of 6 independent experiments. Significant differences were noticed between samples treated with various DPI concentrations (5 µM, 10 µM, 20 µM). Moreover, differences between DNA released from cells preincubated with DPI 10 µM, which was washed away and DNA released from cells with DPI remained within the sample, also were significant. Thus NET creation depends not only on inhibitor concentration, but also on washing step before stimulation. Our results show greater inhibition of NET formation, when DPI is removed from samples, than when cells are incubated with DPI and stimulator together.

key words: free radicals, NETosis, neutrophil function, NADPH oxidase inhibiton, neutrophil stimulation