Inflammation and clinical immunology

HL-60 cells as a model for studies on the mechanism of neutrophil extracellular traps formation

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Neutrophils release Neutrophil Extracellular Traps (NETs) to fight microorganisms and this process is regulated by reactive oxygen species formation. However, the role of related family of compounds - reactive nitrogen species (RNS) has not been widely elucidated. We aimed: (1) to check, whether RNS formation is vital for NETs release and (2) to find an experimental model employing differentiated HL-60 cells for studies on the role of RNS in NETs release.

Neutrophils were isolated from buffy coats by density gradient centrifugation. HL-60 cells were incubated with all-trans retinoic acid (ATRA, 1μM), dimethylsulfoxide (DMSO, 1.25%) or N,N-dimethylformamide (DMF, 70 mM) for 5 days to differentiate toward granulocyte-like-cells. NETs release was stimulated with PMA (100 nM), calcium ionophore (4 μM) or S-nitroso-N-acetylpenicillamine (SNAP, 500μM; nitric oxide - NO donor). Alternatively, cells were preincubated for 1 hour with nitric oxide synthase (NOS) inhibitors (aminoguanidine or L-NAME). Three hours after stimulation DNA release was measured fluorometrically and NETs were visualized with fluorescent microscope (DNA and myeloperoxidase staining). NO synthesis was measured fluorometrically with DAF-FM DA probe.

Using peripheral blood neutrophils, we showed that NO is synthesized during NETs release, NOS inhibition diminishes formation of NETs and that NO is sufficient to stimulate NETs release. Although DMF-differentiated HL-60 cells were the most effective source of granulocyte-like-cells releasing NETs, none of the experiments on the role of RNS was successfully repeated with these cells.

Nitrosative stress is a factor modulating intensity of NETs release. Differentiated HL-60 cells cannot be a model for studies on the role of RNS.