

P007 Apoptosis-induced proteinase 3 membrane expression is not dependent from degranulation : implication in ANCA-vasculitis
Stéphanie Durant, Magali Pederzoli, Patrick Nusbaum,
Philippe Lesavre and Véronique Witko-Sarsat
INSERM U507, Hôpital Necker, 161 rue de Sévres, 75015 Paris, France

Proteinase 3 (PR3) and human neutrophil elastase (HNE) are serine proteinases stored in the azurophilic granules of neutrophils. PR3 is the target of anti-neutrophil cytoplasm antibodies (ANCA) in Wegener's granulomatosis. The mechanisms leading to the membrane expression of PR3 appear to be critical to understand the pathophysiological role of ANCA. RBL cells were stably transfected with either HNE or PR3 or an inactive mutant of PR3 (PR3S203A). Using the calcium ionophore A23187 as secretagogue, PR3 was expressed at the plasma membrane in RBL/PR3 and RBL/PR3S203A, the inactive mutant of PR3. In contrast, no membrane HNE could be detected in RBL/HNE. Apoptosis was then induced by etoposide and was evaluated by i) DNA fragmentation, ii) by the presence of cytoplasmic histone-associated-DNA-fragments and iii) by annexin V labeling. No membrane HNE was detected in RBL/HNE. In contrast, in RBL/PR3 and in RBL/PR3S203A, the membrane expression of PR3 increased with etoposide concentrations and appeared closely related to annexin V labeling. Our data strongly suggest that membrane PR3 originates from two distinct pools, either the granular pool or a plasma membrane pool mobilized upon apoptosis.