Subcellular Mechanisms of Eosinophil Degranulation: The Role of RANTES, Interleukin-5 and Tumor Necrosis Factor- α

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Toxic proteins, contained in the matrix and the core of secondary granules of eosinophils, were recently shown to be actively involved in the propagation of the inflammatory process in atopic diseases, particularly atopic dermatitis [1,2]. Only selected soluble mediators, such as platelet-activating factor or C5a, are capable of inducing the progressive release of these proteins [3]. However, little is known about the complex process of 'piecemeal degranulation' in vivo. Particularly, cytokines which are known to stimulate eosinophil metabolic activity, such as interleukin (IL)-5, tumor necrosis factor (TNF) α , and RANTES are ineffective [3-5]. Therefore, in the present study, the subcellular mechanisms of this process were investigated by immune electron microscopy (IEM) and histochemistry [5]. For this purpose, highly purified eosinophils [4] were stimulated with C5a (10⁻⁷ M), IL-5 (102U/ml), RANTES [100ng/ml] or TNF- α [102U/ml] for 60 min at 37°C in the presence or absence of cytochalasin B. Thereafter, activated eosinophil cationic protein (ECP) and eosinophil perox-*id*ase (EPO) were detected using specific monoclonal antibodies. Upon stimulation with IL-5 or RANTES, significant staining of activated ECP was detected in the matrix of eosinophil secondary granules, whereas EPO was found homogenously distributed in the cytoplasm of the cells as detected by IEM and histochemistry (DAB technique). Preincubation of eosinophils with cytochalasin B before addition of IL-5 and RANTES or incubation with cytokines for ≥ 2 h resulted in an intracellular shift of activated ECP to the small dense granules, a characteristic structure of activated eosinophils. In contrast, EPO was only found in intracellular vesicles near the plasma membrane. A similar distribution of activated ECP and EPO was observed with C5a as stimulus. However, in contrast to IL-5 or RANTES, these findings were evident within 30 min incubation, even in the absence of cytochalasin B. Only small amounts of ECP and EPO were detected in the matrix of secondary granules of cells stimulated with TNF- α or un-stimulated cells. These results clearly show that eosinophil-activating cytokines, such as IL-5 or RANTES, stimulate the intracellular mobilization and activation of eosinophil granule proteins. These cytokines, however, are unable to trigger the exocytosis of toxic proteins even in the presence of

cytochalasin B. Therefore, a second stimulus, probably provided by the inflamed tissue, may be required to induce the last step of degranulation in activated eosinophils.

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