Tyrosine Phosphorylation Regulates Activation and Inhibition of Apoptosis in Human Eosinophils and Neutrophils

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Early signalling events which control the process of programmed cell death are largely unknown. Tyrosine phosphorylation plays a major role in transmembrane signal transduction via most cell surface receptors. Granulocyte/ macrophage colony-stimulating factor (GM-CSF), a cyto-kine released by activated T cells, has been shown to increase tyrosine phosphorylation in several cells and to inhibit granulocyte cell death in vitro. In this study, we demonstrate that the effect of GM-CSF on granulocyte cell death can be blocked by the tyrosine kinase inhibitor genistein (fig. 1) suggesting that increases in tyrosine phosphorylation are essential to inhibit cell death. To analyze the role of tyrosine phosphorylation for the regulation of granulocyte cell death more precisely, we increased levels of tyrosine phosphorylation using the protein-tyrosine phosphatase inhibitor phenylarsine oxide (PAO). Similarly to GM-CSF, treatment of the cells with PAO was followed by large increases in tyrosine phosphorylation and inhibition of programmed cell death in human eosinophils and neutrophils (fig. 2). Strikingly, at low concentrations of the inhibitor and

low induction of tyrosine phosphorylation, acceleration of apoptosis was observed (fig. 2). Genistein and herbimycin A reversed the effects of PAO on tyrosine phosphorylation and granulocyte apoptosis (fig. 2). These results suggest that programmed eosinophil and neutrophil death is regulated by early events of signal transduction pathways such as tyrosine phosphorylation.

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-□- Medium alone
 O GM-CSF
 ·~0~· GM-CSF and genistein
 -Δ- Genistein
 20 40 60
 Hours in culture
 -□- Neutrophils
 O Eosinophils
 -O- Neutrophils with genistein
 -A- Eosinophils with genistein
 0.01 0.1 1
 PAO (μM)

Fig. 1. Effects of GM-CSF on eosinophil death and its reversibility by genistein. Eosinophil viability without and in the presence of 10 ng/ml GM-CSF was assessed by uptake of ethidium bromide and flow cytometry. In addition, experiments were performed in the presence of 50 μg/ml genistein. GM-CSF increased the in vitro survival of human eosinophils. Pretreatment with genistein almost completely abolished the effect of GM-CSF. Genistein alone had no effect. Fig. 2. Effects of PAO on granulocyte death and its reversibility by genistein. Relatively small changes in the PAO concentration led to opposite effects on 18-hour granulocyte survival. PAO-induced cell death and prolonged survival were abolished by genistein.