

THE MECHANISM OF INTERLEUKIN-1 CYTOTOXICITY IN HeLa CELLS

MAŁGORZATA DOSZCZAK¹, ARKADIUSZ PIERZCHALSKI¹, HELMUT HOLTSMANN³ and JACEK BIGDA^{1,2}

¹Department of Cell Biology, Intercollegiate Faculty of Biotechnology UG-MUG, ²Department of Histology and Immunology, Medical University of Gdańsk, ul. Dębinki 1, 80-211 Gdańsk, ³Institute of Molecular Pharmacology, Medical University of Hannover, Germany

Interleukin-1 (IL-1) is a pleiotropic cytokine playing a major role in the regulation of inflammatory and immune responses. It also participates in the regulation of growth, differentiation and many metabolic processes. IL-1 may exert cytotoxic activity in a limited number of cell types, usually in the presence of metabolic inhibitors. The mechanism of IL-1-induced cell death remains unknown. The aim of this study was to analyze the cytotoxic effect mediated by IL-1 in the presence of the translation inhibitor cycloheximide (CHX) in HeLa cells. We found that the cytotoxic action of interleukin-1 reveals significant similarities to apoptosis induced under the same conditions by tumor necrosis factor (TNF). Both effects involve caspase-dependence and show a similar DNA degradation pattern. Following the application of IL-1/CHX, we found TNF-like activity in supernatants and a substantial increase of cell-associated TNF in HeLa cells. This was confirmed via antibody-blocking experiments, specific ELISA and FACS. Thus, the cytotoxic effect of IL-1 in HeLa cells may be dependent on the release of TNF. The transmembrane form of TNF may also be involved in the mediation of the IL-1/CHX effect, as demonstrated via experiments with the use of TAPI-1, the inhibitor of TNF sheddase (TACE). The mechanism of TNF appearance in HeLa cells exposed to IL-1/CHX is unknown. We demonstrated that interleukin 1 and CHX activate the transcription factor NF κ B, involved in the regulation of TNF gene expression, as well as in the increase in the level of specific transcript. However, the cytotoxic effect and the presence of TNF in the cell extract were also observed following the treatment of HeLa cells with IL-1 in the presence of the transcription inhibitor actinomycin D. Additionally, the measurement of ³⁵S-labeled methionine levels revealed the inhibition of protein biosynthesis to app. 3-7% of the physiological level in less than 20 minutes. These results indirectly indicate that *de novo* TNF synthesis in these conditions is unlikely. It seems that stimulation of HeLa cells with IL-1 in the presence of CHX leads to a release of preformed TNF. TNF appearance is blocked by SB203580, TPCK and TLCK, which indicates the involvement of p38 MAPK and serine proteases in the regulation of TNF release.