INVESTIGATION OF GLUCOCORTICOID INDUCED MITOCHONDRIAL APOPTOTIC PATHWAY IN MOUSE THYMOCYTES

Lilla Prenek, Ferenc Boldizsar, Tímea Berki
University of Pécs, Medical Center, Department of Immunology and Biotechnology

Introduction: Glucocorticoids are essential in the development of T-cell in the thymus. Glucocorticoids play important roles in the selection and apoptosis of double-positive (DP) thymocytes. Classically, the ligand-bound glucocorticoid receptor (GR) regulating the gene expression after translocating into the nucleus. But next to these genomic effects, rapid non-genomic effects have emerged, for example the interaction of GR with cytoplasmatic proteins and the translocation of GR to the mitochondrium, which was described especially in apoptosis-sensitive cells. Mitochondrial translocation of activated GR in DP thymocytes has been demonstrated in our department. Based on these previous results we have researched the activation of the mitochondrial apoptotic pathway caused by the translocation of GR to the mitochondrium, and the association of GR with Bcl-2 family pro-apoptotic members.

Methods: Thymocytes were isolated from thymi of four-week-old mice, which were treated in vitro with 10^{-6}M dexamethason (DX) for an hour. DX is a synthetic steroid compound which has only glucocorticoid-like effects. After treatment the cells were lysed, cytoplasmic and mitochondrial fractions were separated. The apoptotic proteins: cytochrome c, activated caspase 3,8,9,12 and Bax were analysed by Western-blot. The association of GR with Bim, and Bcl-x were investigated by coprecipitation experiments.

Results: Elevated cytochrome c and activated caspase 3,8,9 were detected in thymocytes after DX treatment. The level of activated caspase 12 did not change after treatment. Accumulation of Bax in the mitochondrium was found and association of GR with Bim and Bcl-x were also observed.

Conclusion: Our results have supported our hypothesis that in the glucocorticoid-induced apoptosis of thymocytes the mitochondrial pathway plays an important role, which was proved by activation of caspase 9. Probably the interaction between GR and Bim and the acculmulation of Bax in the mitochondrium play also part in it. Interestingly the activation of caspase 8 was also detected, probably caused by the different glucocorticoid-induced apoptotic mechanism of non-DP cells, which are presented in lower portion in the thymus.