Expression and localization of adrenomedullin and its receptor RAMP2 in human thymus

G. Paliuri[1], S. De Martin[1], S. Bova[1]

[1] Dept. of Pharmaceutical and Pharmacological Sciences, University of Padua, Italy

Thymus cell differentiation and generation of the T cell repertoire are influenced and controlled by hormones and neuropeptides acting in autocrine/paracrine manner. Adrenomedullin (AM) is a multifunctional peptide endowed with a wide range of biological actions through the interaction with the plasma membrane receptor activity-modifying proteins 2 or 3 (RAMP2 and RAMP3) coupled to the calcitonin-like receptor (CLR). Animal studies suggest that AM may play a role in thymus growth and thymocytes differentiation *in vitro* by controlling proliferative and apoptotic events through RAMP2 and activation of the cAMP pathway. In this study we investigated the expression and localization of AM and its receptor CLR/RAMP2 in primary cultures of thymic epithelial cells (TECs) and thymocytes obtained from newborns undergoing open heart surgery.

In cultured TECS, double immunofluorescence coupled with confocal microscopy reveal that, AM is stored in cytoplasmatic vesicles, whereas RAMP2 can be detected in both cytoplasm and nucleus, but not on the cell membrane. CLR shows the same distribution of RAMP2, but can also be found on the cell membrane. RAMP2 and CLR localizations were confirmed by western-blot analysis and by the lack of cAMP production in response to AM exposure. AM, RAMP2 and CLR are present in some cultured thymocytes, in which they apparently show a cytosolic localization. As observed in TECs, no increase in cAMP production could be demonstrated following AM stimulation of thymocytes.

In conclusion, our results show that AM system is widely expressed in human thymus, in which AM could play a role in the modulation of TECs function by interacting with its intracellular receptor RAMP2 through a cAMP-independent mechanism.