ImmunoTools *special* Award 2014

Pinelopi Samara
PostDoc

Department of Animal and Human Physiology, Faculty of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, 15784, Athens, Greece

Prothymosin alpha and related peptides as immunotherapeutic and diagnostic tools for cancer and inflammation

Prothymosin α (proTα) is a polypeptide located in the nucleus of all mammalian cells. Although its precise role is not yet elucidated, existing literature suggests a dual role for the polypeptide: an intracellular, associated with cell proliferation and apoptosis, and an extracellular, concerning cell-mediated immunity phenomena. During cell apoptosis, proTα is cleaved by caspases in D99, releasing the decapeptide proTα(100-109). We developed a specific ELISA which determines the concentration of proTα(100-109) in biological fluids (*Samara et al., J. Immunol Meth 2013*). In parallel, we showed that the detected levels of proTα(100-109) in blood serum of mice infected with bacteria are associated with the induction and the extent of cell death. We have also shown that proTα(100-109) is the immunoreactive fragment of the proTα, since it is as effective as the intact molecule, while both proTα and the decapeptide, in the presence of antigen, act as adjuvants, stimulating antigen-reactive immune cell functions (*Ioannou et al., CII 2012*). Specifically, both molecules enhance the basic functions of neutrophils derived from healthy donors and breast cancer patients (*Samara et al., Int Immunoph 2013*), while they signal through Toll like receptor 4, maturing functionally immunocompetent dendritic cells (DCs) (*Ioannou et al., BMC Immunol 2013*). We intend to elucidate the exact role of proTα and proTα(100-109) and, thus, link the intracellular to the extracellular action of the polypeptide. In parallel, we will investigate the potential clinical application of our results for the improvement of cancer immunotherapeutic protocols and for diagnosing conditions associated with extensive cell death (eg. sepsis).

<u>*In vivo* study of proTα and proTα(100-109) as adjuvants</u>

C57BL/6 and BALB/c mice will be inoculated with melanoma and colon cancer cells, respectively. With the appearance of palpable tumors, animals will be administered with rmGM-CSF (positive control), proTα or proTα(100-109) in combination with surface antigens extracted from the melanoma and colon cancer cells. We will assess: (a) the progress of tumor growth and the overall survival of mice; (b) the expansion of tumor-reactive T cells in the spleen and in the peripheral blood of the animals (cytotoxicity and proliferation assays); (c) the infiltration of the tumor

microenvironment by immune cells (immunohistochemistry), (d) the secreted cytokines and the type of the induced immune response (Th1 [IL-2/IFN-γ], Th2 [IL-4/IL-10], Th17); and (e) the percentage of suppressor cells like MDSCs ($CD11b^+Gr\text{-}1/Ly\text{-}6G^+$) and regulatory T cells ($CD4^+CD25^+FoxP3^+$).

In vivo model of lethal septicemia with *Klebsiella pneumoniae* strains

CD-1 mice will be intraperitoneally infected with *Klebsiella pneumoniae* strains. We will determine the levels of proTα(100-109) in the serum of the animals by our specific ELISA at specific time points over the course of infection and the levels of IL-17 and IFN-γ. Spleens will be aseptically removed from selected animals, splenocytes will be isolated and tested for their phenotype, activation of caspases and the type of the induced cell death after staining with annexin V/PI.

In vitro study on the mode of infection of immune cells by *Klebsiella pneumoniae* strains

Monocytes of healthy donors will be *in vitro* differentiated to dendritic cells (using IL-4 and GM-CSF) and macrophages (M-CSF), and will be further incubated with labeled *Klebsiella pneumoniae* bacteria for various time points. We will study the ability of the different types of immune cells to phagocytose or not, the bacterial strains, as well as the induced type of cell death (staining with annexin V/PI and flow cytometry analysis).

ImmunoTools *special* AWARD for **Pinelopi Samara** includes 22 reagents

FITC - conjugated anti-human CD1a, CD33, CD86, Annexin V,

PE - conjugated anti-human CD11c, CD80, Annexin V,

CD4 FITC / CD8 PE / CD45PerCP

APC - conjugated anti-human CD40,

human ELISA-set for 96 wells: human IL-6, human IL-12p40 differential (each 3 reagents),

recombinant human cytokines: rh IL-4, rh M-CSF,

FITC - conjugated anti-mouse CD3e,

PE - conjugated anti-mouse CD11b, CD25,

APC - conjugated anti-mouse Gr-1,

recombinant mouse cytokines: rm GM-CSF DETAILS more AWARDS