ImmunoTools *special* Award 2014

Florencia Cayrol, PhD student

Supervisor: Dr. Graciela Cremaschi

Instituto de Investigaciones Biomédicas, BIOMED-UCA-CONICET, Buenos Aires, Argentina

Thyroid hormones regulated antitumor immune response and contribute to the malignant phenotype in T cell lymphomas through nuclear and membrane-initiated transcriptional programs

The bidirectional interaction between the immune and neuroendocrine systems is involved in the immune homeostasis and physiology. In this context, our laboratory is committed to the study of the neuroendocrine regulation of immunity; and in the study of the contribution of thyroid hormones (TH) to the malignant phenotype of T cell lymphomas.

Thyroid hormones are important regulators of differentiation, growth and metabolism in different tissues. Both, thyroxine (T4) and triiodothyronine (T3) influence proliferation and differentiation in several cell types. There is further evidence suggesting that these hormones are also involved in cellular transformation, tumorigenesis and metastasis development, thus assuming a particular importance in the induction of tumor angiogenesis. Studies from our laboratory (*Barreiro Arcos 2011*) showed that TH can stimulate the proliferation of murine T lymphomas through different signaling pathways involving both genomic effects as nongenomic actions. However, the genes involved in THs genetic programs, yet were not characterized in normal and tumor T cells.

Particularly, in this project we hypothesized that TH-regulated membrane- and nuclear-initiated transcriptional programs could play a role in T-NHL survival and proliferation; and that by modulating these pathways, T-NHLs could be therapeutically targeted. Also, the anti-tumor immune response against T lymphomas could be modulated by thyroid hormones, so the pharmacological manipulation of thyroid status would also contribute to T lymphoma management.

Our results showed that THs could stimulate the proliferation of murine T-cell lymphoma through the THs nuclear receptor (TR) and a membrane receptor (mTR), represented by the integrin dimer $\alpha V\beta 3$ (CD51/CD61). In order to determine whether a similar effect was relevant for human T- cell lymphomas we first analyzed the expression of TH receptors in a panel of T-cell non-Hodgkin lymphomas (T-NHLs) cell lines representing the whole spectrum of the disease. This panel, that was characterized, both genetically (RNA-sequencing) and molecularly (through their surface markers: CD3, CD4, CD8, CD19, CD56, CD51, CD61), includes immature T cell lymphoma/leukemia and mature T-NHLs cell lines. We found that THs were able to increase cell proliferation in human T-NLHs. We also found an increase in the mRNA and protein levels of TR and mTR receptors compared to normal T cells from

human tonsils and peripheral blood. This data suggested that the blockade of its activity could constitute one of the first examples of targeted therapy for T-NHLs.

To determine whether integrin αvβ3 inhibition induce anti-lymphoma activity in T-NHL, we will conduct knockdown experiments using siRNA for integrins αv and β3 and perform proliferation and apoptosis (Annexin V) assays. We will determine how the inhibition of this receptor could improved the effect of the chemotherapeutic agents commonly used for this pathology.

We also work with animal models of hypo-and hyperthyroidism and studied if thyroid status is able to alter the lymphocyte activity by the analysis of cytokine secretion (IL-1, IL-2, IL-4, IL-6, IL-10, IFN-γ, TNFα, TGF-β) and distribution of lymphocyte subpopulations (T, B, Th1, Th2, Treg) . Also, we evaluated the action of thyroid status on T-cell lymphoma development. For this purpose, we inoculated eu-, hyper- and hypothyroidism mice subcutaneously with syngeneic lymphoma cell lines. To analyze the anti-tumor immune response we will obtained lymphoid populations from spleens and lymph nodes from tumor- bearing mice, as well as cell suspensions from the solid tumor to study tumor-infiltrating lymphocytes (TILs) that will be analyzed by flow cytometry in order to determine the distribution of lymphocyte subsets. We will determine the percentages of NK cells ($NK1.1^+$ $CD3^-$), B cells ($CD19^+$) and T cytotoxic ($CD3^+CD8^+$) or T helper ($CD3^+CD4^+$) lymphocytes. The distribution of immunosuppressive cells will also be determined in these organs. Percentages of myeloid derived suppressor cells ($Gr1^+$ $CD11b^+$) and T regulatory cells ($CD4^+$ $CD25^+$ $FoxP3^+$) will be determined by flow cytometry.

We plan to characterize in human samples of both tissue T cell lymphomas and blood the different subpopulation of immune cells, and their correlation to TH levels. Also, in blood samples we will analyze if THs are able to affect cell proliferation, apoptosis and the genetic programs triggered by THs.

Since T-NHL patients have poor prognosis, is really important focus efforts on the study of T cell lymphomas genetic nature and develop more effective therapies. Our studies will help and contribute to the improvement of therapeutic strategies for T-NHL patients. The ImmunoTools award would be a great help to work on this goal.

ImmunoTools *special* AWARD for **Florencia Cayrol** includes 25 reagents

FITC - conjugated anti-human CD3, Control-IgG2a, Annexin V,

PE - conjugated anti-human CD8, Control-IgG2a,

PerCP - conjugated anti-human CD4, CD20,

APC - conjugated anti-human CD61, CD62L, Control-IgG1, Annexin V,

human IL-4 ELISA-set for 96 wells (3 reagents),

recombinant human cytokines: rh IL-2,

FITC - conjugated anti-mouse CD4, NK-cells,

PE - conjugated anti-mouse CD8a, CD11b, CD19, isotype control IgG2b,

APC - conjugated anti- mouse CD3e, CD25, Gr-1,

recombinant mouse cytokines: rm IL-2

DETAILS more AWARDS