ImmunoTools *special* Award 2014

Norberto W. Zwirner, PhD

Institute of Biology & Experimental Medicine,
Vuelta de Obligado 2490, C1428ADN-Buenos Aires
ARGENTINA

Endogenous and exogenous factors that regulate NK cell effector functions and that shape tumor immunity

Natural killer (NK) cells are lymphoid cells with a critical role during immunity against viruses and tumors, through cytotoxic responses and secretion of chemokines and proinflammatory cytokines, such as IFN-γ and TNF-γ. NK cells can discriminate healthy from damaged or infected cells through the engagement and signaling from an array of inhibitory and activating receptors. Additionally, activation, maturation and survival of NK cells are also controlled by different cytokines, microbial products and stress signals.

Besides their direct effector functions NK cells are essential as a bridge between innate and adaptive immune responses, because of their ability to set up a cross-talk with dendritic cells (DCs) and macrophages, shifting the adaptive immune response towards a Th1/pro-inflammatory profile.

The general aim of our laboratory is focused on the study of different factors that regulate the activity of NK cells in different physiopathological situations. We have several research projects carried on by different PhD students:

One of our projects is focused on the study of NK cell-DC cross-talk in humans and mice. We will generate immature DC (iDC) from healthy human monocytes (cultured in the presence of IL-4 and GM-CSF) and from mouse bone marrow progenitors (cultured in the presence of Flt3L or GM-CSF), and differentiate them towards mature (mDC) or regulatory (rDC) phenotype. After co-culture with resting or activated purified NK cells we will analyze different characteristics of both cell populations: expression of costimulatory molecules and secretion of cytokines (IL12 p70, IL10) in DCs and IFN-γ production, cytotoxic responses against target cells and expression of activating receptors in NK cells. To gain insight in the molecules involved in the cross-talk we will use different blocking antibodies during the co-cultures.

Another project of our group is to study the role of NK cells during an anti-tumor immune response. NK cells are essential players of the immune surveillance not only because they can directly recognize and eliminate tumor cells through a cytotoxic

response but also because they can secrete a large number of cytokines upon recognition of tumor cells, that acting on DCs can impact on the nature and quality of an adaptive immune response. In this context, one of our goals is to determine the role of NK cells in the priming phase of a spontaneous anti-tumor adaptive immune response in vivo. We will analyze early tumor-specific T cell priming (IFN-γ production after ex vivo re-stimulation with tumor antigens) in mice depleted of NK cells compared to undepleted controls. In those animals we will also analyze the activation status of the different DC subpopulations in tumor draining lymph nodes and in tumor infiltrating cells. As we are also interested in the dynamics of immune cell recruitment to the tumors, we will evaluate the impact of the administration of different chemokines on T cell priming.

To complement the results obtained in mice we have set up a collaboration to get human samples of renal cancer and blood from patients. We will disaggregate the tumor samples to obtain single cell suspensions and we will purify peripheral blood mononuclear cells (PBMCs) from the blood samples and we will quantify and analyze phenotypic characteristics of NK cells, DC and T cells in the different samples. As gating strategy we will use the CD45 marker (immune cells) and the CD10 marker (tumor cells).

Our studies have translational implications because the knowledge that we intend to generate could contribute with the development and optimization of therapeutic strategies for patients with cancer and pathologies with immunological background trough the manipulation of the biological activity of NK cells. Obtaining the the ImmunoTools Award would be of great benefit to our research.

ImmunoTools *special* AWARD for **Norberto Walter Zwirner** includes 22 reagents

FITC - conjugated anti-human Annexin-V

PE - conjugated anti-human CD56, Granzyme B,

PerCP - conjugated anti-human CD45,

APC -conjugated anti-human CD10, Annexin-V

recombinant human cytokines rh IL-4, rh GM-CSF

FITC - conjugated anti-mouse CD44,

PE - conjugated anti-mouse CD19, TCR gamma-delta

APC -conjugated anti-mouse CD25, CD62L,

recombinant mouse cytokines rmCCL3, rmCCL4, rmCCL5, rmFlt3L, rm GM-CSF, rm G-CSF, rm CXCL10, rm IL-4, rm IFN gamma

DETAILS