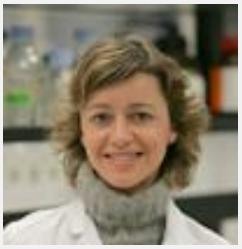
ImmunoTools *special* Award 2014



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Development of new strategies to inhibit T regulatory cells for improving immunotherapy in cancer and viral disease.

The efficacy of current immunotherapeutic protocols against cancer and chronic infectious diseases is unfortunately very poor. Immunosuppressive mechanisms induced by tumours or infectious agents might be responsible for this low efficacy. T regulatory cells (Treg) are considered as one of the main players generating this immunosuppressive environment able to impair the activation of an efficient immune response. Thus, the development of molecules able to block the activity of Treg is crucial for the design of more efficient therapies. In a previous project we developed peptide inhibitors for TGF beta as well as for the transcription factor FOXP3, which are crucial for Treg activity. *In vitro* and *in vivo* assays demonstrated that these peptides inhibited Treg activity and enhanced antitumor and antiviral immunotherapies in mice. One of the aims of this project is to develop a viral vector for the *in vivo* delivery of these peptides to improve their anti-tumor/antiviral efficacy in relevant *in vivo* models. The second objective is the identification of new peptide inhibitors for the surface molecules LAG-3 or BTLA/CD160, which may play an important immunosuppressive role during T cell activation. The third objective of this project is to identify new targets to inhibit Treg activity. Preliminary experiments in our lab showed that FOXP3 is activated on effector T cells after suboptimal antigen stimulation. We propose to study TCR signalling pathways implicated in FOXP3 activation and thus identify new potential targets for Treg inhibition. We believe that the results of this project will be a valuable tool to optimize the current immunotherapeutic protocols against cancer or infectious diseases.

For this project we will use specific antibodies for T regulatory cells both in human and mouse (CD3, CD4, CD25, FOXP3, LAG-3, GITR, CTLA4, CD160) and well as classic

activation markers on effector T cells (surface and intracellular markers like CD45, CD69, IFN gamma, IL-2, TNF alpha, IL-6, PD1).

The culture and expansion of Treg requires recombinant IL2, both in human and mice, and in some experiments TGF beta also, to carry out conversion experiments from T effector cells to Treg.

Regarding the TCR signalling pathways, antibodies against phospo-proteins could be useful like LAT, LCK, ZAP70, ERK, SLP76 …

To study the general immune response induced, we will measure IFN gamma produced by CD8 and CD4 lymphocytes (by ELISA or Elispot) as well as maturation surface markers on dendritic cells (CD11c, CD80, CD86, class II, CD54).

Due to the importance of the edge Treg/Th17 lymphocytes, we are also interested in measure Th17 production by ELISA or flow cytometry and also the specific Th17 markers like RORgammaT.

Flow cytometry and cell expansion with ImmunoTools will be crucial in this project, and it could develop a methodology easily transferable to human studies in which Treg play an important role such cancers and chronic infections.

Earlier evidence suggested that Treg accumulate in tumors and the peripheral blood of patients with cancer and through suppression of anti-tumor immune responses promote tumor growth. However, more recent data indicate that in certain cancers, such as colorectal carcinoma, Treg suppress bacteria-driven inflammation which promotes carcinogenesis and thus benefit the host. Treg appear to play a dual role in cancer. This might explain why the frequency and functions of Treg are associated with a poor prognosis in some cancers but with favorable outcome in others. With our work we hope to shed any light on this controversy.

ImmunoTools *special* AWARD for **Noelia Casares** includes 22 reagents

FITC - conjugated anti-human CD80, CD54, Annexin V,

PE - conjugated anti-human IL-6, IFN-gamma,

CD4 FITC / CD3 PE / CD8 PerCP
CD3 FITC / CD4 PE / CD45 PerCP

CD3 FITC / CD8 PE / CD45 PerCP

CD3 FITC / CD4 PE / CD19 APC

human IL-6 ELISA-set for 96 wells, (3 reagents),

FITC - conjugated anti-mouse CD8a, CD11b, CD25, NK-cells,

PE - conjugated anti-mouse CD19, CD62L,

APC - conjugated anti-mouse CD62L, NK-cells,

recombinant mouse cytokines: rm EGF, rm IL-2 DETAILS more AWARDS