ImmunoTools *special* Award 2014

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Chronic Lymphocytic Leukemia: a study of AID mediated genetic alterations in tumor cells and of LPL protein as putative prognostic marker

Our work concentrates in the study of Chronic Lymphocytic Leukemia (CLL), the most common leukemia of the eldery in Western countries. This haematopoietic B-cell disease follows an extremely variable course and despite the fact that treatments often induce remissions, most patients relapse and CLL remains incurable. The dissection of the molecular basis of the interactions between cancer cells and their microenvironment is leading to the development of new treatment modalities which are aimed at manipulating the communication of tumor cells with their milieu. In this regard, CLL is an instructive example of how these relationships influence the natural history of a disease. Our work is framed by the haematology and tumoral immunology. It lies on the interface between biochemistry and the molecular and cell biology fields, which in combination with protein expression approaches constitute the core of our experimental designs.

In one of our projects we aim to develop new prognostic methods based on the differential expression of LPL protein in CLL B cells. In this regard, we recently demonstrated the expression of LPL protein in CLL patients and we obtained funds to carry out the project entitled *"Red-iberoamericana de Leucemia Linfoide Crónica: hacia el desarrollo de nuevos marcadores pronósticos"* at the International call CYTED (Centro Iberoamericano de Tecnología y Desarrollo). In addition, new methodologies such as Ribosome display and Affitins production by high-throughput screening are being incorporated to our unit in order to attain the best technology to carry out this research line.

On the other hand, CLL proliferative events occur in the tissues where leukemic cells are able to exploit microenvironment interactions to avoid apoptosis, acquire tumoral growing conditions and develop new genetic lesions, which allow the clone to become resistant to further treatments. Understanding the crosstalk between malignant B-cells and their milieu could give us new insights on the cellular and molecular biology of CLL that can finally lead to novel therapeutic strategies. We recently described that overexpression of AID enzyme in CLL is almost restricted to a subpopulation with a high proliferative potential. This small clonal subset also displays expression of different molecules that suggest a recent contact with the leukemic microenvironment. More importantly, the presence of this subpopulation is associated to a poor clinical outcome. The aggressive CLL cases, exhibiting high AID levels as reported in our study, represent an interesting model to evaluate progression characteristics in CLL or to discern how the AID enzyme, a clear biologically and pathologically relevant hallmark of this microenvironment stimulation, could be responsible for the leukemic progression in these patients. To attempt this, our group is advocated to better define the role of this proliferative subpopulation in CLL progression and treatment refractoriness and to also understand the consequences of AID over-expression in CLL tumoral development. To achieve this, we focus in the study of the genomic expression profile (mRNA and micro-RNAs), in the analysis of methylation profile as well as of genetic alterations events that could underpin disease progression in leukemic cells overexpressing AID. In addition, to this and to reinforce this research line, we recently acquired two transgenic mice lines: The first (Eμ/Tcl1) which mimic a progressive unmutated CLL human disease and the second, a transgenic line that overexpresses AID. In this context, genomic expression profile, deep sequencing techniques and confocal microscopy will be performed in order to investigate the origins of the proliferative potential of CLL B-cells overexpressing AID. Despite the fact that extrapolation results in cancer animal models may be controversial, we believe that this model could become relevant after confirmation in human cells. Since our group has this possibility, we believe that this is a good tool to demonstrate the role of AID overexpression in CLL as well as to further study of the role of microenvironment interactions in this leukemia.

We selected these human molecules from ImmunoTools because CLL cells will be activated to mimic tumor microenvironment conditions using CD40L, IL-4 and/or GM-CSF. Activation will be measured by CD86 and CD25 expression at the cell surface. CD3, CD14, CD38, CD20, CD5 and CD19 antibodies will be used to

discriminate CLL cells from T cells, NK cells or monocytes. Apoptosis of control and activated CLL cells will be evaluated by Annexin V stain.

We selected these mouse molecules from ImmunoTools because leukemic cells purified from TCL1 mice speen will be activated to mimic tumor microenvironment conditions using rm IL-4. Activation will be measured by CD25 expression. CD3e and CD19 antibodies will be used to discriminate leukemic cells.

ImmunoTools *special* AWARD for **Pablo Morande** includes 23 reagents

FITC - conjugated anti-human CD3, CD5, CD86, Control-IgG1, Control-IgG2a,

PE - conjugated anti-human CD14, CD19, CD38, Control-IgG1, Control-IgG2a,

PerCP - conjugated anti-human CD3, CD20, CD45,

APC - conjugated anti-human CD25, Control-IgG1, Annexin V,

recombinant human cytokines: rh GM-CSF, rh IL-4, rh sCD40L,

PE - conjugated anti-mouse CD19

APC - conjugated anti-mouse CD3e, CD25,

recombinant mouse cytokines: rm IL-4

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