ImmunoTools *special* Award 2014

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Chronic Lymphocytic Leukemia: the role of sphingosine-1 phosphate and the microenvironment in the progression of the disease.

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia of adults which is characterized by a progressive accumulation of mature monoclonal B lymphocytes in peripheral blood and lymphoid tissues. The leukemic clone proliferates within permissive niches in lymphoid organs, where CLL cells were found in close contact with stromal cells, monocyte-derived nurse like cells (NLC) and activated T cells. Several *in vivo* and *in vitro* findings suggest that T cells, mainly $CD4^+CD40L^+$, provide a short-term support that enhances malignant B cell proliferation through cytokine secretion (i.e., IL-4 or IFNγ) and CD40/CD40L interactions. In addition, stromal cells and NLC provide a long-term support that favors the extended survival and accumulation of leukemic cells through cell–cell contact and/or the production of different factors, including BAFF, CXCL13, CCL19/21 and CXCL12. While numerous reports have studied the molecules involved in CLL entry to lymphoid tissues, less is known about the mechanism involved in the leukemic clone exit from this permissive niche to circulation. This is not a trivial issue because although in most cases conventional therapy effectively eliminates circulating CLL cells, disease usually persists in the lymph nodes and bone marrow. Therefore, numerous efforts have been made to mobilize tissue-resident CLL cells into the blood in order to sensitize them to cytotoxic drugs.

One of our projects is focused on **the response of CLL cells to sphingosine 1 phosphate (S1P),** a bioactive sphingolipid which mediates the exit of B and T

lymphocytes from lymphoid tissues to circulation. The normal exit of these cells depends on the different concentration of S1P, which is low in tissues and high in blood and lymph, and the regulated expression of one of its receptors, S1PR1. We hypothesize that the supportive microenvironment of lymphoid tissues will reduce the expression of S1PR1 on CLL cells and will impair the migratory response towards S1P, favoring their settle on the survival niche. To test this hypothesis we will culture CLL cells with different stimulus, such as CXCL12, CXCL13, CCL19, CCL21, soluble CD40L and the culture with autologous NLC or autologous T cells activated with anti-CD3 and anti-CD28, in order to mimic the supportive microenvironment. After 24 and 48hs the expression of S1PR1 in CLL cells will be evaluated by quantitative real time PCR (QPCR) and *in vitro* chemotaxis assay towards S1P will be performed by using the transwell system.

On the other hand, our group is studying **the expression of the molecules that regulate the levels of S1P**. S1P is generated from the conversion of ceramide to sphingosine by ceramidase and the subsequent phosporylation of sphingosine to S1P, in a reaction catalysed by two sphingosine kinases (SK), SK1 and SK2. S1P production by SK promotes cell growth, proliferation, migration, and survival in various cell types. By the contrary, ceramide and sphingosine are anti-mitogenic molecules generally associated with the induction of apoptosis. Furthermore, elevated expression of SK1 is observed in non-Hodking lymphomas, different leukemic cells lines and in multiple types of solid cancer, such as gastric, lung, brain, colon, kidney and breast cancers and associated with tumor grading as well as reduced patient survival. Moreover, the inhibition of SK1 and SK2 induces the death of different leukemic cells and solid tumor cells. On the other hand, S1P can also be irreversibly degraded by intracellular S1PL which can shift the balance towards cell death by diminishing S1P levels. As expected, low levels of S1PL were preferentially observed in different types of cancer cells, such as breast, ovarian and colon cancer cells.

While several studies have implicated the SK/S1P/S1PL pathway as an essential regulator of cell proliferation and survival in different cancer cells, there is no information in CLL. Thus, we will compare the expression level of SK1, SK2 and S1PL by QPCR and by western blot in purified B cells from CLL patients and from age matched healthy donors. Moreover, we will compare the expression of these molecules in control and activated CLL cells (the activation will be performed by

culturing CLL cells for 24 and 48hs as described above). Our hypothesis assumes that S1P and the microenvironment play a key role in CLL and participate in the progression of the disease. We hypothesize that CLL cells will express higher levels of SKs and lower levels of S1PL compared to healthy B lymphocytes. On the other hand, CLL growth and survival induced by the supportive microenvironment might involved an enhanced expression and activation of SKs and an impaired expression of S1PL, a scenario that would favor an increment in S1P intracellular levels.

This study will provide insights into the biology of the leukemic clone and will help to understand the impact of the protective microenvironment on the mechanism that regulates the exit of CLL cells from lymphoid tissues to peripheral blood. A better understanding of the role of S1P will provide insights into the progression of the disease and might help to find potential targets for novel therapeutic treatments. Obtaining the ImmunoTools Award would be of great benefit to our research.

Anti-CD45, CD19, CD20, CD5, CD3, CD56 and CD14 will be used to determine the percentage of leukemic cells ($CD19^{+}CD5^{+}$) and other accessory cells within peripheral blood mononuclear cells samples. The in vitro activation of CLL cells will be performed with BAFF, CCL19, CCL21, CXCL13 and sCD40L and will be checked by the upregulation of CD38, CD69, CD80 and CD86. The apoptosis of control and activated cells will be checked by Annexin V.

ImmunoTools *special* AWARD for **Romina Gamberale** includes 19 reagents

FITC - conjugated anti-human CD5, CD38, CD56, CD69, CD80, CD86, Control-IgG1, Annexin V,

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

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

recombinant human cytokines: rh BAFF/sCD257, rh MIP-3 / CCL19, rh CCL21, rh CXCL13, rh sCD40L / CD154 DETAILS more AWARDS