ImmunoTools *special* Award 2014

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"Impact of Regulatory cytokines in Hematopoiesis"

Hematopoiesis controls the differentiation of hematopoietic stem cells (HSCs) into lymphoid and myeloid lineages, maintaining homeostasis in the organism. The ability of HSCs to perpetuate and generate new blood cells is regulated by several mechanisms, including signals derived from the stromal microenvironment. Such signals regulate proliferation and differentiation of HSCs by activating genetic programs that determine the specification of the precursors along the different lineages. Understanding the molecular mechanisms that regulate hematopoiesis is of key importance, because deregulation of this process leads to the development of diseases such as immunodeficiencies, leukemia or myeloproliferative disorders (MPD).

Several studies implicate Interleukins (IL) and other colony stimulating factors in the modulation of hematopoiesis. However, the role of anti-inflammatory cytokines, such as IL-10, has never been carefully addressed. IL-10 expression is tightly regulated and it exerts its anti-inflammatory effects in many cell types, having a prominent role in the regulation of immune responses. A role for IL-10 in hematolymphopoiesis is predictable, as several studies show the involvement of IL-10 in the pathogenesis and prognosis of hematopoietic disorders, such as B cell lymphomas.

Using genetically modified mice expressing IL-10 under the control of an inducible metallothionein (MT) promoter, pMT-10 mice, we found that high levels of IL-10 strongly impact hematopoiesis. Our initial studies showed that after 30 days of IL-10 overexpression, severe alterations in a number of hematological parameters were observed, namely a strong inhibition of bone marrow (BM) B-cell differentiation and increased myeloid cell production. pMT-10 mice developed splenomegaly with increased spleen weight and cellularity, accompanied by a structural disorganization of the tissue, with the appearance of cells phenotypically resembling megakaryocytes. The spleen and blood of induced mice also presented a substantial increase in myeloid cells. Overall, mice overexpressing IL-10 develop a phenotype reminiscent of that observed in animal models and patients with MPD. Therefore, further characterization of the pMT-10 model is very attractive, as it may represent an interesting mouse model for MPD induced by the anti-inflammatory cytokine IL-10.

We now want to build upon our findings and clarify how does IL-10 shift hematopoiesis towards the myeloid lineage. In this sense, a detailed phenotypical analysis is needed to define the populations affected by high IL-10 levels.

We plan to analyze the spleen, which is enlarged in pMT-10 mice, for the presence of multipotent and lineage specific hematopoietic progenitors, investigating in detail the occurrence of extramedullary hematopoiesis. We will examine, by q-RT-PCR, the transcriptional signature of these cell subsets. Moreover, we will study in further detail how IL-10 overexpression affects the BM compartment. For this we will need antibodies for flow cytometry to analyze the different hematopoietic progenitors that are represented in very small percentages in the BM. To test the effect of IL-10 on murine HSC *in vitro* during their differentiation, we will need to purify by flow cytometry that rare population.

Taken together, ImmunoTools reagents will be essential to allow us to enrich the HSC population, after depletion of the more mature forms by virtue of the expression of lineage specific markers, both in the BM and spleen. Culture of progenitor cells (*in vitro*) in the presence of defined cytokines will allow these cells to differentiate into various lineages, thereby establishing their multipotent potential.

Detailed characterization of the pMT-10 model is of great importance, as it may offer a good mouse model for MPD. Determining how IL-10 overexpression impacts hematopoiesis will provide new insights in the role that this cytokine exerts on hematopoiesis.