Phenotype and Function of Alkaline Phosphatase Cells in a Murine Model of Radio-Induced Marrow Aplasia

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Bone marrow aplasias are characterized by a peripheral blood cells deficit resulting from a deficiency in the production of one or several blood cells lineages in bone marrow. Some of these aplasias are able to regenerate, others don't respond to treatment. In most of the cases, spontaneously regenerating bone marrows are characterized by the presence of a great number of fibroblastic cells presenting a strong membranous alkaline phosphatase activity (ALP), this activity has completely disappeared in irreversible bone marrow aplasias. The aim of our work is to analyze phenotypic and functional characteristics of these ALP positive reticular cells. In this aim, we used a murine radio-induced aplasia model: a 4 Gy gamma rays total body irradiation induced an important but reversible bone marrow aplasia.

In a first step, we have demonstrated that the density of ALP network and the number of ALP positive cells increase strongly in the first three days following irradiation. This increase precede haematopoietic regeneration and isn't a result of cell proliferation. In vitro studies have also demonstrated that this increase in ALP activity is due to the expression of TNSALP (tissue non specific alkaline phosphatase). Next, we have established a relationship between these ALP positive reticular cells and mesenchymal stem cells. In vitro observations, as well as phenotypic analysis, indicate that TNSALP+ cells differentiated from mesenchymal stem cells. Although expressing an early osteoblastic marker, cbfa1, they keep self-renewal and differentiation capacities. To test their function in haematopoiesis, we have realized co-cultures of CFU-Fs and haematopoietic precursors (ckit+, sca-1+, lin-). We observed that, CFU-Fs from irradiated bone marrow, characterized by a high proportion of TNSALP positive cells, strongly stimulate the proliferation and the differentiation to the myelo-monocytic and granulopoietic lineage. In accordance with this observation, bone marrows of TNSALP deficient mice, at a late embryonic stage, are characterized by a deficit in granulopoiesis.

In order to understand the effects of TNSALP in granulopoiesis, we added recombinant TNSALP (Enobia, Montréal Canada) on TNSALP negative CFU-Fs from control mice and observed that it has an effect on the differentiation process without any effects on the proliferation.

All these data suggest that TNSALP expressed by reticular cells, directly derived

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from mesenchymal stem cells plays a role in normal haematopoiesis and in regeneration of bone marrow aplasia by inducing differentiation of myelo-monocytic and granulocytic precursors. Recombinant TNSALP (Enobia, Montréal Canada) will be tested in murine models of bone marrow aplasias.