Split chimerism between nucleated and red blood cells after bone marrow transplantation for haemoglobinopathies

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Previous studies have shown that a stable presence of both donor and recipient haematopoietic derived cells after allogeneic haematopoietic stem cell transplantation (HSCT) occurs in approximately ten percent of the patients affected by β -Thalassemia. Once achieved this condition, defined as persistent mixed chimerism (PMC), the patients do not require additional red blood cells (RBCs) support and, regardless of the presence in some cases of an extremely low percentage of donor-derived nucleated cells, they are clinically cured by an incomplete, but functional graft. Most of the published papers have, however, investigated the impact of donor engraftment in the nucleated cells rather than in the mature erythrocytes. We have recently published a paper showing that in four long-term transplanted patients affected by hemoglobinopathies, characterized by the presence of few donor engrafted nucleated cells—both in the peripheral blood and in the bone marrow—the majority of the erythrocytes were of donor origin. Moreover we showed that the proportion of donor-derived erythroid precursors, determined by analyzing singularly picked-up burst-forming unit erythroid colonies, was equivalent to that observed in the mature nucleated cells rather than in the red blood cells. These results suggest that in patients characterized by the presence of PMC after HSCT a selective advantage of the donor erythroid precursors maturation might successfully contrast the problems bound to the recipient ineffective erythropoiesis. When genetically modified HSCT will be a possible

option for treating Thalassemia Major, the co-existence of the repaired cells with those still expressing the genetic defect will be an expected scenario, not in an allogeneic, but in an autologous environment.

Up to now, allogeneic haematopoietic stem cell transplantation (HSCT) is the only radical cure for β -Thalassemia major (β -Thal), the most common monogenic disorder due to mutations in the β -globin.¹⁻³ Data reported in literature by Lucarelli indicated a probability of overall survival and thalassemia free survival of 97% and 89% for β -Thal patients with no advanced disease and of 87% and 80% for patients with advanced disease.⁴ Engraftment of donor-derived cells represents a crucial clinical event following BMT even if the simultaneous presence of residual host cells (RHCs) and donor derived cells, referred to as mixed chimerism (MC), have been frequently observed after marrow transplantation for β -Thal. It has been previously shown that the presence of early chimerism determined within three months after HSC transplant represents a risk factor for thalassemia recurrence after bone marrow-derived HSCs.⁵ Moreover, different from what was observed by the Locatelli group in HLA identical sibling cord blood settings, we have shown that in β -Thal patients, after bone marrow transplantation, in order to obtain a durable complete or mixed chimerism an early engraftment of donor-derived cells larger than 90% is necessary.⁶⁻⁸ Therefore graft rejection probabilities were higher in patients with MC detected within the first months after the transplant and proportional to the amount

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Figure 1. Engraftment evolution over time of a patient with persistent mixed chimerism. PB, peripheral blood; BM, bone marrow; RBCs, red blood cells, BFU-E (BM). Burst forming unit erythroid colonies obtained from bone marrow.

of donor type hematopoiesis present in the recipient.⁹ It is likely that most of the patients with large amounts of RHCs early after HSCT face a prompt immunological reaction against the donor cells, although it has been shown that occurrence of transient MC does not necessarily lead to graft rejection. In fact, almost 10% of the transplanted patients achieved a stable state of persistent MC (PMC) with a number of donor marrow cells sufficient to produce enough β -globin chain synthesis and normal levels of hemoglobin in the peripheral blood so that transfusions to correct the anaemia were no longer required. In these patients PMC was observed also in the various peripheral lymphoid subsets, equally distributed in the different cell lineages.⁵ The reasons why in some patients MC represents a transient condition while in others the coexistence of donor and recipient cells persist through the entire post-transplant follow-up are still unknown. It is likely that in settings where the amount of RHCs present in the recipient is low, the donor and recipient cells have a chance to induce a status of reciprocal immunological tolerance allowing their co-existence and subsequently develop a status of PMC.¹⁰ Recent studies have shown the presence of high levels of IL-10 produced by peripheral blood mononuclear cells (PBMCs) of patients with PMC in comparison to normal donors and patients with complete chimerism.¹¹ Type 1 regulatory cell clones, of both donor and host origin, were able to inhibit the function of effector T cells of either donor or host origin in vitro.¹² These results suggest that interleukin-10 and Type

1 regulatory cells are associated with PMC and may play an important role in sustaining long-term tolerance in vivo.¹¹ Up to now, it is not clear if the mechanisms inducing an immunological tolerance in patients with PMC might also influence a different maturation of the donor erythroid precursors after HSCT for hemoglobinopathies. However, the presence of a split chimerism between mature erythrocytes and their progenitors has been recently described in persistent mixed chimera patients^{13,14} with a predominant erythroid engraftment in the peripheral blood, while the proportion of erythroid precursors (BFU-E—burst forming unit erythroid) colonies and nucleated cells of donor origin in the bone marrow were equivalent (Fig. 1). This observation confirming the presence of split chimerism between red blood cells and their erythroid progenitors supports the concept that a limited HSC engraftment—up to 20%—bearing effective erythropoiesis, is able to provide a sufficient amount of normal hemoglobin and mature erythrocytes.^{15–17} Moreover, this limited engraftment with normal erythropoiesis is able to inhibit, through unknown mechanisms, the expansion of the ineffective thalassemic erythropoiesis, as well as the well-known associated clinical consequences. These observations represent a strategic issue, particularly in the view of the future use of gene therapy for β -Thalassemia, since it is now evident that in patients affected by hemoglobinopathies, few donor normal engrafted cells are able to correct the genetic defect after HSCT.^{19,20}

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