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Hematopoietic stem cell transplantation: 40 years of continuous progress and evolution

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The importance of an innovative medical event can be measured by the insights and the clinical consequences that the event itself produces for the benefit of human beings. In this regard, there is no doubt that hematopoietic stem cell transplantation (HSCT) has represented one of the most innovative treatments of the last decades, as well as one of the most significant medical feats of human bio-solidarity. This year is the right time to celebrate a significant anniversary of HSCT, as the first two successful transplants were performed just 40 years ago, in 1968.[1,2] The first patient had a form of sex-linked lymphopenic immunological deficiency, and, at five months of age, was transplanted with bone marrow (BM) cells of his sister, aged eight years.[1] The second was a child with Wiskott-Aldrich syndrome, who, at the age of two years, received the allograft from a sister, herself an X-trisomic (47, XXX) child.[2] The demonstration of histocompatibility between donor and recipient was obtained through the tests of mixed lymphocyte culture, and of lymphocytotoxic assay in the first case,[1] while in the second pair, the reciprocal non-stimulation between the patient's lymphocytes and those of his sister was verified repeatedly, also with extensive controls.[2]

Behind this accurate attention paid to histocompatibility between donor and recipient, which was emphasized in both scientific reports, we find the following observation of Bach and colleagues:[2]

"*In all reported cases of bone-marrow transplantation in man, the histocompatibility relationship between donor and recipient has not been well defined. Proven chimerism following bone-marrow transplantation in man has been rare.*"

The following remark of Gatti and colleagues about a lack of histocompatibility, in the case of previous transplants performed on patients with lymphopenic immunological deficiency, provides a rather similar concept:

"*Unfortunately, the introduction of allogeneic immunologically competent cells has consistently produced fatal graft-versus-host reactions because patients with this disease, being unable to reject the grafted immunologically competent cells, cannot prevent an immunological assault by the donor lymphocytes on the host's cells and tissues*".[1]

If, certainly, HLA identity in the donor/recipient pair was deemed an essential condition, which could not be eluded before a transplant could be programmed, there was another prerequisite to be satisfied for an allograft to be successful, namely the need to administer to the transplanted subject, despite the demonstrated HLA identity with the donor, an immune suppressive drug able to prevent the attack of donor immune cells on recipient tissues, what is widely known as graft-versus-host disease (GvHD) prophylaxis. This prophylaxis of HSCT-related immune complications, together with careful monitoring of the clinical signs heralding GvHD, in turn permitting a prompt start of an immune suppressive treatment whenever needed, was immediately recognized as a key element for successfully transplanting humans.[1]

Today, after 40 years of unceasing progress, full HLA identity between donor and recipient remains mandatory in cases of unmanipulated BM or peripheral blood stem cell (PBSC) transplantation, but we have also learned how to cross the HLA barrier, through the use of megadoses of $CD34^+$ cells, coupled with profound T-cell depletion of the graft,[3] or through the use of unrelated donor cord blood transplantation (CBT).[4] This year is, by the way, also the twentieth anniversary of CBT, which was first successfully performed by Eliane Gluckman and colleagues in Paris on an American child with Fanconi anemia, using the cryopreserved cells of

his HLA-identical sister collected at time of birth.[5]

GvHD has also been revisited, as, over time, we have learnt its pathophysiology,[6] and it has become evident that, in patients with hematologic malignancies, this complication may be associated with a beneficial effect able to prevent disease recurrence, namely the so-called graft-versus-leukemia (GvL) effect, so that tailoring of strategies of prophylaxis may be considered according to the different risks of leukemia relapse in patients transplanted in different phases of disease.[7-9]

All this is already history: the history of a progressive improvement that has resulted in the *cure* of an increasing proportion of patients given HSCT who, following the natural history of their disease, would not have survived or, for some patients with non-malignant disorders, who would have survived with a significantly worse quality of life.

As already briefly mentioned, while for many years an HLA-matched sibling was the only type of donor routinely employed, more recently, matched unrelated volunteers, unrelated umbilical cord blood units and HLA-haplotype mismatched family members are largely utilized to transplant patients lacking an HLA-identical relative.[10] Therefore, nowadays, an alternative donor can be found for virtually all patients and the decision whether to use either an unrelated volunteer, an HLA-mismatched cord blood unit, or a full-haplotype disparate relative depends on many patient-, disease, donor- and center-related factors.[11] Each of these options has advantages and limitations, but rather than being considered competing alternatives, they should be regarded as complementary options, to be chosen after a careful evaluation of the relative risks and benefits in the patient's best interest. None of the first two of these alternative types of transplantation would have been possible without, on the one hand, the diffusion of a culture of bio-solidarity leading to disinterested, anonymous donation of hematopoietic progenitors, and, on the other hand, a tremendous organizational effort which has resulted in the creation and development of registries of volunteers for the donation of BM cells (the first one, the Anthony Nolan Bone Marrow Trust, having been established in England in 1974), as well as of banks for the storage of cryopreserved cord blood units. The number of donors enrolled in the BM registries is already over 12 million and it is continuously growing, while it can be estimated that, today, more than 300,000 cord blood units are available in more than 40 banks worldwide.[11]

The model of primary immune deficiencies

Primary immune deficiencies (PIDs), although rare diseases, have always been pivotal for several fields of medicine, that of HSCT being no exception. Indeed, the scientific work carried out in the '50s to first diagnose and cure children affected by PIDs provided precious information on the way the immune system works, as well as, later, on the genetics of hereditary disease.[12] Through the observation of these children with PIDs, who are real *experimenta naturae*, we learned what it means for a human being to be obliged to live without antibodies or without the cells, namely T-lymphocytes and natural killer (NK) cells, which protect an individual against the attacks coming from the environment. We now know how to treat PIDs, through HSCT or more conventional therapies, but also, from what we have learned, we now have a much deeper knowledge of the role played by cells of the immune system in curing or preventing the occurrence of other diseases with a significantly higher social impact (i.e. leukemia and solid tumors).[13] While studying PIDs, moreover, the role of molecular basis of disease became evident[14,15] and opened the way to novel therapeutic approaches, such as pre-natal cell therapy (i.e. *in utero* HSCT) and gene therapy.[16-20]

Revisiting in an historical perspective the close link between PID and HSCT, besides the obvious, already commented upon remark that the first two HSCTs were performed in children with inherited defects of the immune system,[1,2] it must also be noted that in the '80s, the first demonstration that the HLA-compatibility barrier could be by-passed by a profound T-cell depletion of the graft was provided by Reisner and O'Reilly in a patient with severe combined immune deficiency (SCID).[21] This seminal case, proving the possibility of successfully using HLA-partially matched family donors, raised the hope that the transplant option could be offered to virtually any patient in need of an allograft. However, many years had to elapse before haploidentical HSCT became a routine, although still highly sophisticated, transplant option, successfully employed in patients with malignancies. In this regard, besides the already mentioned use of mega-doses of $CD34^+$ cells needed for promoting engraftment, gaining an understanding of the NK alloreactivity in preventing leukemia recurrence, GvHD and graft failure,[22] as well as the development of adoptive cell therapy for restoring immune competence,[23,24] has been fundamental.

PIDs, and in particular SCID, have also been the first diseases successfully treated with *in utero* transplantation of T-cell depleted, parental $CD34^+$ cells, a result obtained without using any cytotoxic drug in preparation to the allograft.[16] *In utero* transplantation of hematopoietic progenitors has been employed to treat other pre-natally diagnosed genetic disease of blood, such as thalassemia or metabolic diseases.[17,25] The clinical results have been much less encouraging in these disorders, as no or only transient engraftment of donor cells was obtained.[17,25] This finding was not completely unexpected, as even in children with SCID given *in utero* transplantation, engraftment was largely limited to donor T cells,[16] and we have limited knowledge on the period of intrauterine life during which a foetus develops immune mechanisms of rejection of foreign cells/tissues, thus losing the possibility of being tolerant to donor hematopoietic progenitors. This latter consideration justifies the experimental work in progress on animal models of *in utero* HSCT.[26] Another disease which could be a selective target of *in utero* transplantation is *osteogenesis imperfecta*, a genetic defect of osteoblasts, which can benefit from engraftment of another type of somatic stem cells, namely mesenchymal stem cells (MSCs), which are increasingly attract-

ing the attention of many researchers involved in the optimization of different approaches for reparative/regenerative cell therapy, as well as in the perspective of modulating the immune response against alloantigens (with a dramatic effect on acute GvHD) or even to autoantigens.[27-29] *In utero* transplantation of *ex vivo* expanded MSCs in a fetus with *osteogenesis imperfecta* changed the severity of the disease, significantly decreasing the number of pathological fractures.[30] Data obtained in experimental animal models are no less exciting.[31]

Finally, PIDs have been pivotal also for testing the efficacy (and the safety as well) of what can be considered a bridge between cellular and molecular therapy, namely gene therapy. In the early '90s, a new era began with the demonstration that, knowing the genetic defect underlying a form of SCID due to adenosine deaminase (ADA) deficiency, it was possible to transduce patient T cells with a retroviral vector carrying the correct gene.[32] Later, a much more sustained correction of the genetic defect over time was obtained through the transduction of hematopoietic stem cells of children with ADA-deficient SCID.[33] A number of clinical trials followed, aimed at extending the use of gene therapy to other inherited disorders and acquired hematopoietic malignancies, but, as often happens when experiments in the animal model are transferred to humans, the clinical results have so far been disappointing.[19] Moreover, the report of clonal lymphoproliferative disorders developing in children with SCID due to the defect of γ-chain given autologous cells corrected of the genetic defect through the use of a retroviral vector raised major concerns about the safety of gene therapy.[34,35] This neither means that gene therapy is a hazardous therapy for genetic disorders, nor that we should abandon this therapeutic approach: it only indicates that much more information on the site of preferential insertion of certain vectors, as well on the consequences that this insertion has on the regulation of genes crucial for cell proliferation and differentiation, has to be gathered, and that we still have a long way to go before this treatment can be considered a widely favored option.

The ageless principle: science must always be at the service of human beings

Discussing the unwanted side effects of gene therapy leads us to briefly consider the fundamental issue of the ethical approach to those highly sophisticated therapies in continuous evolution of which HSCT is a paradigm. There is little doubt that, like any other medical treatment, allogeneic HSCT must be applied with full adhesion to the founding principles of bioethics, satisfying its two most pragmatic criteria, namely that its use has always to be *in the best interest* of the patient and that the biological cost for the donor be largely compensated by the benefit to be derived from the procedure for the patient.[36] Allogeneic HSCT is of benefit to a subject (the recipient) different from the subject (the donor) who provides the transplant. However, unlike what happens when a solid organ (e.g. a kidney) is transplanted into a recipient from a donor who chooses to be deprived of it for life and agrees to undergo a surgical intervention for donation, HSCT does not imply such problems of permanent impairment to the donor. Given that, there are also no doubts that such a procedure, like any *donation*, is based on a form of bio-solidarity that provides gratification to the donor, who, during all his/her life will carry a positive recollection of his/her choice. It is to be considered highly ethical that this bio-solidarity be implemented and that, especially when the donor is a minor, a praiseworthy role is recognized for it in the context of filial/familial ethics.[37,38]

To the ethics of familial bio-solidarity is also closely linked the issue, felt particularly for genetic disorders, of *programming* the birth of a healthy, HLA-compatible child for the purpose of collecting hematopoietic stem cells (from BM,[39] or more recently, cord blood)[40] to be used for transplanting a sibling in need of an allograft. To this purpose, the technique introduced by medical science known as *in vitro fertilization and pre-implantation genetic diagnosis (PGD)* has proved to be highly functional. There is a substantial current of thought providing arguments and indicating the merits of this approach and substantiated indications, which include in particular *pre-implantation HLA matching* to program a cord blood cell donor for a sibling with a disease curable by transplantation of hematopoietic stem cells.[41,42] However, one could easily argue against this approach, as other embryos, which may be healthy but not HLA-compatible, would be discarded. In fact, PGD for HLA compatibility with the prospect of transplantation has led to many ethical reservations, especially among followers of the Catholic faith.[43] Leaving aside religious concerns, clearly it is a preliminary unavoidable condition that this child be loved not only as a *donor* (capable of saving the life or improving the quality of life of a sibling) but also for himself, and, as a child, with a personal dignity deserving love and respect.

A final consideration must be made in the light of what could be falsely considered as a possible, future evolution of collection, storage and use of cord blood hematopoietic progenitors; namely the *auto-dedication* of cord blood. This option had found strong resonance and support in the media, which launched campaigns favoring the elaboration by parents (or by other entities) of projects of cryopreserving and storing one's child's cord blood cells with the perspective of possible, future use in approaches of reparative/regenerative medicine. This policy contrasts with any approach of bio-solidarity and with any reasonable prevision of a successful result; in fact, just in the field of hematology, one's cord blood cells are useless if HSCT is used to treat genetic disease, while, for malignant disease, the use of autologous cells does not provide any GvL effect. Do we really want to undermine the culture of donation and bio-solidarity which has allowed so many lives to be saved of patients with leukemia or other life-threatening hematologic disorders, just to pursue the myth of curing non-hematologic diseases, such as myocardial infarction, Alzheimer disease, etc. with cord blood hematopoietic stem cells? For every scientist the answer is more than evident…..

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