BONE MARROW TRANSPLANTATION: A HISTORICAL REVIEW*

TRANSPLANTE DE MEDULA OSSEA: UMA REVISÃO HISTÓRICA

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ABSTRACT: Bone marrow transplantation has evolved over a period of 50 years. Laboratory observations and animal studies defined the essentials of transplantation biology. The first attempts to transfer these studies to patients met with little success. The definition of the complexities of the human leukocyte antigen (HLA) system made it possible to select compatible sibling donors and more recently unrelated donors. Transplantation of stem cells from marrow, blood, or cord blood is now the treatment of choice for a variety of hematological and genetic diseases. Transplantation using less toxic preparative regimens to induce mixed chimerism makes possible an application to autoimmune diseases. Laboratory and clinical research directed toward induction of tolerance and elimination of malignant cells point the way to a wider application of hematopoietic cell transplantation in the next decade.

UNITERMS: Bone Marrow Transplantation; history.

Dr. Thomas received the 1990 Nobel Prize in Medicine from King Carl XVI Gustaf of Sweden for his pioneering work in bone marrow transplantation.

The Hutchinson Center performs more life-saving bone marrow transplants than any institution in the world.

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INTRODUCTION

The story of marrow transplantation began in 1949 with the studies of Jacobson et al.[36] who found that shielding the spleen of a mouse during otherwise lethal irradiation permitted survival. Lorenz et al.[45] reported that irradiated mice could also be protected by an infusion of spleen or marrow cells. Initially, it was thought that the "irradiation protection" phenomenon was due to humoral factors. However, in 1954 Barnes and Loutit reviewed their own and other experiments and stated "the chemical hypothesis has not been proved by the complete exclusion of the cellular hypothesis" [3]. Strong support for the cellular hypothesis came in 1955 when Main and Prehn[47] reported that irradiated mice protected by an infusion of allogeneic marrow subsequently displayed tolerance of a donor skin graft. Shortly thereafter, Ford et. al.[26] showed that lethally irradiated mice protected by a subsequent marrow infusion had marrow cytogenetic characteristics of the donor.

EARLY STUDIES IN ANIMALS

In the 1950s, fundamental observations were made in murine systems as detailed in the book by van Bekkum and DeVries[95]. It was found that allogeneic marrow cells successfully engrafted could mount an immune attack against the host, resulting in a wasting syndrome known as "secondary disease". The disease was the result of an immune reaction of the engrafted lymphoid cells against the tissues of the host, now known as graft-versus-host disease (GVHD)[6].

Uphoff reported that in allogeneic transplants the severity of the immune reaction of donor cells against the host was controlled by genetic factors[93]. Also, Methotrexate (MTX), as an immunosuppressive agent, could prevent or ameliorate the graft-versus-host (GVH) reaction [43,94].

In the 1960s, studies in canines provided important information about bone marrow transplantation (BMT) in outbred species applicable to humans. It was found that dogs could survive 2 to 4 times the lethal exposure to total body irradiation (TBI) if given an intravenous infusion of marrow cells set aside or cryopreserved before the TBI[49]. Dogs given supralethal irradiation and allogeneic marrow demonstrated the problems of failure of engraftment, graft rejection, engraftment with GVHD and, in some dogs, stable engraftment without GVHD, i.e., tolerance[86]. Dogs could be successfully engrafted without TBI using chemotherapy with cyclophosphamide (CY) or dimethyl busulfan[71]. Marrow grafts between littermate pairs matched for dog leukocyte antigens (DLA) were often successful with the recipients becoming healthy chimeras[22,23]. Hematopoietic cells for engraftment could be obtained from the blood as well as the bone marrow [15]. Blood transfusions from the marrow donor or unrelated dogs could sensitize the intended recipient to transplantation antigens resulting in graft failure[72].

Proof of the importance of leukocyte groups in hematopoietic cell transplantation (HCT) came from studies of the DLA system[23,75]. Dogs given supralethal irradiation and marrow from a DLA mismatched littermate died of graft rejection or GVHD. Most recipients of DLA matched marrow, especially those given some post-grafting MTX to suppress the GVH reaction, became long-term healthy survivors.

EARLY ATTEMPTS TO TREAT LEUKEMIA INVOLVING BMT

In 1956 Barnes et al. reported the treatment of leukemic mice by supralethal irradiation followed by infusion of normal mouse marrow[2]. At almost the same time attempts to treat human patients with TBI or chemotherapy and a marrow infusion were reported[89]. Large quantities of marrow, anticoagulated and screened to break up particles, could be infused intravenously without ill effect. The only successful transplants, however, utilized an identical twin donor[88]. In the 1950s and 1960s almost all attempts to achieve allogeneic grafts in humans were unsuccessful[9]. Mathé et al.[52] achieved the first persistent allogeneic marrow graft in a patient with leukemia, but the patient died with many problems that probably were due to the complications of chronic GVHD.

HISTOCOMPATIBILITY

Techniques for defining tissue antigens in humans were crucial to the development of BMT. In 1954 Miescher[55] recognized antibodies induced by transfusions or pregnancy that reacted with antigens on white blood cells. Dausset [18] and van Rood and colleagues[96] used such antibodies to describe human leukocyte antigen (HLA) groups. In the subsequent years continuing progress has been made in the characterization of the immunogenetics of these antigens[8].

It is now known that these antigens provoke immune reactions when tissues are grafted from one individual to another and that genetic control of these antigens resides on chromosome 6 in a "super gene" region known as the major histocompatibility complex (MHC). In this region class I antigens involve three loci important in transplantation, HLA-A, -B and -C, while class II antigens are governed by HLA-DR, -DP and -DQ. These regions on chromosome 6 are tightly linked and constitute a "haplotype". Multiple alleles at each of these loci account for the very large number of possible haplotypes in the human population. Other antigens important in transplantation, as yet poorly defined, are governed by genes outside the MHC[50].

In the 1970s and 1980s most BMTs involved sibling donor-recipient pairs. Each parent has two HLA haplotypes, and each child inherits one haplotype from each parent. Thus, there is one chance in four that a sibling will be matched with a patient. These genotypically matched pairs could be recognized even by poorly defined serological techniques.

In the 1990s serological typing techniques have been replaced by molecular techniques enabling a precise characterization of genes of the MHC. These DNA techniques have disclosed an even greater heterogeneity of the MHC but have made possible matching of unrelated individuals[54]. The establishment of large panels of volunteer donors has made it feasible to find matched donors despite the heterogeneity of the MHC[35]. Success of BMTs between matched unrelated individuals now approximates that between matched siblings[34].

THE BEGINNING OF THE MODERN ERA OF BMT

By the end of the 1960s, platelet transfusion support, improved antibiotics and more effective anticancer agents had been developed. Increasing knowledge of human histocompatibility antigen systems led to renewed attempts at allogeneic marrow grafting in human patients. Gatti et al.[27] reported a successful allogeneic marrow graft in a patient with severe combined immunological deficiency using a sibling donor presumed to be HLA identical with the patient. Subsequent typing, however, showed that the patient and donor differed by one HLA antigen. Two similar successes were reported at almost the same time[1,21].The patients did not require immunosuppressive therapy since they were already immunoincompetent because of their disease. All three were alive and well 25 years later[10].

In 1969 the Seattle marrow transplant team began a series of marrow transplantations using HLA matched sibling donors for patients in the end stages of leukemia[14] or aplastic anemia[84]. In 1975, a review article summarized the state of knowledge of BMT at that time[91]. The article described the results in 37 patients with aplastic anemia and 73 with leukemia, all transplanted after failure of conventional therapy. Engraftment was successful in some patients with aplastic anemia and survival with grafts in remission was observed in a few patients with leukemia.

In the 1970s evaluation of the role of hematopoietic cell transplantation (HCT) in the treatment of leukemia was difficult because almost all patients had been transplanted for advanced disease after failure of conventional therapy. In 1977 the Seattle team reported 100 patients with advanced acute leukemia who were prepared with CY and TBI and given marrow from an HLA matched sibling[81]. At the time of the report, 17 of the 100 were alive 1 to 3 years later. Eight of these 17 are alive and well now more than 23 years after transplantation. The early demonstration of a plateau in a Kaplan-Meier plot of disease-free survival had indicated that some patients with advanced leukemia might be cured[87].

BMT AS ACCEPTED TREATMENT

Success with some patients with advanced disease made it possible to consider BMT before the terminal stage of the disease. In the late 1970s, transplants for leukemia in first remission or at the first sign of relapse quickly demonstrated a greatly improved overall survival[5,82]. Of the first 19 patients with acute myeloid leukemia (AML) transplanted in first remission in Seattle, 8 are alive and well now 19 to 21 years later. In the 1980s and 1990s many similar observations rapidly led to the application of marrow grafting to patients with a variety of malignant diseases having in common a high probability of failure of other forms of therapy.

Marrow transplants for non-malignant diseases other than immunodeficiency disorders began with patients with aplastic anemia[76,84]. Survival was poor for the early patients who were transplanted after failure of multiple transfusions and other therapies. Results improved dramatically when transplants were

carried out earlier in the course of the disease[73]. marrow transplant technology led to the first cures of thalassemia major[46,83] and of sickle cell disease[37].

The results of marrow transplantation for all the various diseases are given in the clinical chapters of the book *Hematopoietic Cell Transplantation*[80].

ALTERNATIVE SOURCES OF HEMATOPOIETIC STEM CELLS (HSCs) FOR BMT

The use of HSCs from sources other than the marrow has occasioned the shift in terminology from bone marrow transplantation (BMT) to hematopoietic cell transplantation (HCT).

Transplantation with peripheral blood stem cells (PBSCs) rather than marrow began with the demonstration of these cells in the blood of mice[32], dogs[15], and non-human primates[74]. It was found that the number of PBSCs in circulation could be increased by chemotherapy[38] and by the administration of hematopoietic growth factors (GM-CSF or G-CSF)[28]. The donor need not to go to the operating room, as for a marrow transplant, since the cells can be collected by vein. Although the greatest use of PBSCs has been for autologous grafting, they can also be used for allogeneic grafting but with an increased risk of chronic GVHD[65,79].

The demonstration of the presence of HSCs in cord blood suggested the use of these cells for HCT[13]. The first successful HCT using cord blood stem cells (CBSCs) was reported by Gluckman et al. in 1989[30]. Banks of cryopreserved and HLA typed CBSCs have now been established in many institutions[31,61]. The utility of CBSCs is being evaluated, particularly the suggestion that these cells may be immunologically immature and less likely to cause GVHD.

THE PREPARATIVE REGIMEN

In the early human BMTs for leukemia, irradiation was a logical choice as a preparative regimen since leukemic cells were known to be highly sensitive to irradiation, and irradiation had been widely used in animal studies of marrow transplantation. Since high voltage machines were not available, opposing cobalt-60 sources were used in an attempt to get uniform TBI in large animals and human patients[24]. Because the patients were desperately ill with advanced leukemia, TBI was given in a single exposure in an effort to establish a graft quickly. The Seattle team used 1000 cGy TBI administered at 7 cGy per minute. The few patients successfully engrafted showed early recurrence of leukemia. The recurrences indicated that irradiation alone would not be sufficient to eradicate all leukemic cells as Barnes *et al*. had anticipated[2].

Santos and Owens reported that CY was a potent immunosuppressive agent in the rat model[62]. CY was also an antileukemic agent, and Santos et al. used CY as the only preparative regimen for human patients. Despite successful engraftment, they observed early recurrence of leukemia[63]. The Seattle team gave CY, 60 mg/kg, on each of 2 days before TBI. This regimen produced the first long-term disease-free allogeneic recipients [91]. As patients were transplanted in earlier stages of leukemia and supportive care improved, immediate transplantation was not necessary, fractionation of the TBI became feasible, and a randomized study showed the superiority of fractionation[20,85].

Santos et al. devised a preparative regimen consisting of high-dose busulfan (BU) and CY[64]. A randomized comparison of the BU/CY regimen with the CY/TBI regimen in patients with chronic myeloid leukemia (CML) showed no difference in disease-free survival and that both were effective[16]. A regimen of TBI with etoposide and CY has shown promise in high-risk leukemia[44] or in CML[68]. Many transplant teams are using irradiation and chemotherapeutic agents in various combinations for preparative regimen. Randomized studies of most of these preparative regimens have not been reported[4,66].

GRAFT-VERSUS-HOST DISEASE

Billingham and Brent defined the immunological basis of the graft-versus-host reaction in murine studies[6]. Extensive studies in murine systems have defined the role of T cells and of histocompatibility system)[41].

The magnitude of the problem in human patients was not appreciated until consistent engraftment of donor marrow was achieved in the early 1970s. Even with an HLA matched sibling donor, GVHD occurred in one-half of the patients. Prevention or treatment with MTX and/or glucocorticoids was only partially effective[90]. Studies in the canine model indicated that the addition of cyclosporine to a short course of MTX resulted in improved prevention of GVH[19]. The

effectiveness of the combined drug regimen was subsequently demonstrated in randomized studies in human patients[69,70].

Reisner et al., Filipovich et al. and Prentice et al. described the amelioration of GVHD in human patients by the removal of T cells from the marrow innoculum[25,58,59]. It is now recognized that removal of T cells from the graft can prevent GVHD but at the cost of graft failure, delayed immunological recovery and loss of the graft-versus-leukemia (GVL) reaction [51]. Advances in immunology are providing new approaches to prevention of GVHD without necessarily impairing immunological recovery or inhibiting the GVL reaction [Reviewed in[7]].

THE GVL REACTION

In the first article on treatment of murine leukemia by x-rays and homologous marrow transplantation, Barnes and colleagues pointed out that cure might depend on the capacity of the donor cells to destroy by a reaction of immunity the residual leukaemic cells[2]. Studies in a murine system indicated the existence of a GVL effect that could be separated from the GVH reaction[11]. The apparent existence of a GVL effect in human patients was reported by Weiden et al. who described a statistically significant reduced incidence of recurrent leukemia in 79 recipients of allogeneic marrow with GVHD as compared to 117 without GVHD[97].

DONOR LYMPHOCYTE INFUSIONS

In the late 1980s investigators began to explore cautiously the possible anti-leukemic effect of donor lymphocyte infusions for patients who relapsed after transplantation [reviewed in[67]]. In 1986 in Jerusalem, a patient with relapsing acute lymphoid leukemia after BMT was treated by graded infusions of donor lymphocytes which resulted in a remission of more than 8 years duration. Kolb et al. reported the achievement of long-term remissions and possible cure of relapsed CML by donor lymphocyte infusion together with interferon alpha[40]. Cullis et al. showed that donor lymphocyte infusion alone could induce long-lasting remission[17]. Numerous reports have confirmed the anti-leukemic effect of donor lymphocyte infusions but with risks to the host of marrow suppression and GVHD [reviewed by Kolb [39]]. Of great importance is the demonstration that immunoblastic lymphomas occurring after allogeneic BMT can be cured by donor lymphocyte infusion[56]

AUTOLOGUS BMTs

Numerous studies in mice, dogs and primates demonstrated the effectiveness of autologous BMT in protecting the animals against otherwise lethal irradiation (see above). Autologous marrow grafts for human patients were used in the 1950s[42,53]. These grafts protected against marrow toxicity, but the clinical benefit was unclear because of ineffective eradication of the disease in the patients. Autologous HCTs are now being used following high-dose chemo-irradiation therapy of both hematological malignancies and solid tumors[57]. Genetic marking techniques make it possible to identify recurrent disease originating in cells contaminating the marrow innoculum[12,33].

MIXED CHIMERISM

In the initial applications of BMT to the treatment of hematological malignancies, high-dose chemo-irradation was administered to kill the malignant cells with marrow replacement to prevent death from marrow aplasia. Despite the consequent regimen-related toxicity, many patients became complete chimeras with only donor cells in the marrow. However, some patients with non-malignant diseases were treated with less toxic regimens, and their marrow subsequently showed a mixture of donor and host cells. Recent studies in dogs[78] and in human patient[29] have demonstrated that it is possible to get marrow grafts with less cytotoxic regimens if combined with sufficient immunosuppression and a generous infusion of HSCs. For patients with malignant disease, mixed grafts make it possible to take advantage of the graft-versus-tumor effect, with or without donor lymphocyte infusions. However, this approach is particularly attractive for non-malignant diseases. Many transplant centers are now considering mixed chimerism as an approach to the treatment of genetic diseases, such as sickle cell disease[77]. In the 1950s, before the development of good immunosuppressive drugs, consideration was given to preceding a kidney graft with a marrow graft from the same donor as could be done in animals[48]. If marrow grafts that are mixed donor-host can now be done with safe non-toxic regimens, this approach may again be considered.

ADOPTIVE IMMUNOTHERAPY

Mathé et al. first proposed that the graft-versus-host reaction might be used to destroy a tumor with subsequent treatment of the GVHD to prevent death of the host. He later coined the term adoptive immunotherapy to describe the therapeutic effect of transplanted cells of the immune system[52]. Adoptive immunotherapy at that time was not possible because of the inability to secure grafts or the inability to control the graft-versus-host reaction once established. With the achievement of long-lasting BMTs it became possible to recognize a GVL effect associated with GVHD and to enhance this effect with additional donor lymphocyte infusions (see above). With the availability of cytokines such as IL-2, it became possible to clone antigen-specific lymphoid cells from the donor, to grow them in quantity, and to demonstrate their survival in the host after a BMT[60]. Clearly, adoptive immunotherapy is now feasible, and many BMT teams are exploring it for enhancing the defense against infections, for therapy against tumor-specific antigens or antigens that are up-regulated on tumors or for correction of an ailing immune system.

BMT FOR AUTOIMMUNE DISEASES[92]

Autoimmune diseases are characterized by threat to life and particulary by chronic, painful and debilitating courses that warrant aggressive therapy. Selection of patients will be difficult because of the variable disease course and the necessity to choose patients who still have reversible disease. The safety of both autologous and allogeneic stem cell grafting has progressed to a point where, in many cases, the risks of the disease far outweigh those of transplantation. In my opinion we should proceed cautiously with both autologus and allogeneic stem cell grafts. Purified stem cells, which have the advantage of being free of lymphocytes should be used for the first series of autologous studies. The studies are atractive because of the low risk of transplant related complications but are less likely to be curative. Allogeneic grafts from perfectly matched donors have the advantage of providing a completely new immunological environement. For this reason, I believe that curative results are most probable after allogeneic stem cell engraftment. Emphasis should be on the identification of patients with HLA matched siblings. Initially, these studies will be carried out in patients with advanced disease, as was the case in the early days of transplantation for leukemia. In considering more aggressve treatment for autoimmune diseases it appears there are three possible approaches. First, and most conservative, is to store peripheral blood stem cells for possible future morrow rescue and then to give higher doses of immunosuppressive agents, specially in combination, to see whether there is an improvement over conventional doses. Second, for those who prefer a conservative approach to stem cell transplantation, is to give myeloablative and lymphoablative chemotherapy followed by purified (lymphocyte-free) hematopoietic stem cells. Third, and most likely to be curative, is myeloablative and lymphoablative therapy followed by stem cells from an HLA identical matched family member with subsequent short methotrexate and cyclosporine treatment to control GVHD. There are differences of opinion about whether the preparative regimen should include irradiation. It should be pointed out that total body irradiation is a most effective way to destroy lymphoid cells throughout the body. Careful monitoring of accumulating clinical results will pilot future investigation.

SUMMARY

Fifty years have gone by since the first experiments in mice that were to lead to the wide application of human hematopoietic cell transplantation. The solution of problems recognized in human patients came from animal research ranging from mice to dogs to non-human primates. In the early days these cellular transplantations were carried out only in terminally ill patients. Now in many diseases transplantation is carried out early in the course of the disease with greatly improved results. Research designed to continue improvement of the application to human patients includes the achievement of engraftment without lethal marrow ablative regimens, the use in autoimmune diseases, ex vivo culture of hematopoietic stem cells, gene transfer studies, and the development of techniques for inducing tolerance for solid organ grafting. The development of effective anti-viral and anti-fungal drugs and the shift to outpatient care has resulted in dramatic reduction of the cost of transplantation as well as improved long-term survival. Pending the development of highly specific curative agents, hematopoietic cell transplantation will continue to be used increasingly during the early years of the third millenium.

THOMAS ED. Transplante de medula óssea: uma revisão histórica. **Medicina, Ribeirão Preto, 33**: 209-218, ju./set. 2000.

RESUMO: O transplante de medula óssea evoluiu bastante nos últimos 50 anos. Observações laboratoriais e estudos experimentais definiram os elementos essenciais da biologia dos transplantes. As primeiras tentativas para replicar esses estudos em pacientes tiveram pouco sucesso. A definição de complexidade do sistema HLA humano tornou possível selecionar, como doadores, irmãos compativeis e, mais recentemente, doadores não relacionados. Transplantes de celulas tronco hematopoéticas da medula óssea, sangue periférico ou cordão umbilical são, agora, o tratamento de escolha para várias doenças hematológicas e genéticas. Transplantes usando regimes de condicionamento menos tóxicos para induzir quimerismo misto tornaram possível sua aplicação em doenças auto-imunes. Investigações clínicas e laboratoriais dirigidas à indução de tolerância imunologica e eliminação de células malignas abrem caminho para aplicações mais amplas do transplante de células tronco hematopoéticas na próxima década.

UNITERMOS: Transplante de Medula Óssea; história.

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