CRITICAL REVIEW

Current Status of Bone Marrow Transplantation for Aplastic Anemia and Acute Leukemia

By E. Donnell Thomas, Alexander Fefer, C. Dean Buckner, and Rainer Storb

Clinical results of marrow transplantation in the treatment of aplastic anemia and acute leukemia are reviewed. The principal problem areas in this field at this time are discussed.

Almost 30 yr have elapsed since studies in rodents began to lay the groundwork for marrow transplantation, and 20 yr have elapsed since the first efforts to carry out marrow transplantation in man. Although early efforts at marrow transplantation were not successful, work in the past 5 yr has led to the survival of a number of long-term marrow graft recipients. The principal clinical application of marrow transplantation to date has involved patients with severe combined immunologic deficiency disease, aplastic anemia, or acute leukemia. The basic principles of marrow transplantation biology and its clinical applications have been extensively summarized in a recent review.1 Marrow transplantation for immunologic deficiency disease has been reviewed elsewhere.2

PRINCIPLES OF MARROW TRANSPLANTATION

Marrow transplantation in man represents the clinical application of principles derived from studies in animals.1 These studies have involved the genetics of histocompatibility antigens, immunosuppressive regimens for prevention of graft rejection by the host, and approaches to the prevention or management of graft-versus-host disease (GVHD).

The major impediment to the transplant is the donor-host histoincompati-
bility, which is genetically determined. This barrier is not a problem when donor and recipient are genetically identical twins (a syngeneic graft)—then the infused marrow is readily accepted. This identity is the reason why simple infusion of twin marrow, without any immunosuppressive conditioning of the patient, is effective treatment for aplastic anemia. However, when donor and recipient are not genetically identical twins (an allogeneic graft), some degree of histoincompatibility must exist and constitutes a bidirectional immunologic barrier. First, the host may reject the graft. Therefore, the host immunologic reactivity must first be suppressed. Total body irradiation (TBI) and various chemotherapeutic agents have been used for this purpose. Unfortunately, marrow transplantation is also further complicated by the fact that immunologically competent cells contained in or derived from the donor marrow can react against the “foreign” host and cause the serious or fatal syndrome known as GVHD. Since, by analogy with animal data, it is assumed that the incidence and severity of GVHD in man might be less when donor and recipient are matched at the major histocompatibility complex (MHC), we have restricted marrow transplants to HLA-identical siblings only. Nevertheless, GVHD, usually managed with immunosuppressive agents, is difficult to prevent and even more difficult to treat.

APLASTIC ANEMIA

Marrow transplantation for the patient with severe aplastic anemia can now be considered the preferred form of treatment, provided there is a suitable marrow donor available. Two of the first four transplant recipients in the Seattle series are now well, more than 5 yr after grafting. A number of medical centers have reported successful marrow transplants for aplastic anemia. A prospective cooperative study has shown a significantly better survival for patients treated by transplantation as compared to those who are not transplanted because of the lack of a suitable donor. That there are many major problems remaining is indicated by the fact that only approximately one-half of the patients treated by marrow transplantation become long-term healthy survivors.

Definition of Severe Aplastic Anemia

In the Seattle series, patients have only been considered for transplantation for severe aplastic anemia, defined as a hypoplastic marrow and at least two of the following three characteristics: (1) platelet count less than 20,000, (2) granulocyte count less than 500, and (3) corrected reticulocyte count less than 1%, in the presence of anemia. This definition seems to identify a group of patients with a very poor prognosis on conventional treatment. However, occasional patients with severe aplastic anemia will recover partially or completely, while others with a mild aplastic anemia will progress to the severe form of the disease. Although some information is available concerning the prognostic parameters in aplastic anemia, more study is needed in order to identify with certainty those patients destined for a fatal outcome on conventional treatment.

Overall Clinical Results

In the Seattle series, 73 consecutive patients with severe aplastic anemia were conditioned with either cyclophosphamide (CY, 200 mg/kg) or TBI (1000 rads)
and given marrow grafts from normal HLA-identical siblings. Sixty-eight lived long enough to demonstrate engraftment. Twenty-one patients rejected the graft and 19 of these died. Forty-seven had sustained engraftment and 18 of these died. Sixteen deaths were associated with GVHD. Overall, 31 patients are alive with hematologic restoration 8 mo to 5 yr after grafting. A report from the Marrow Transplant Registry on 38 additional transplants indicates comparable results from a number of marrow transplant teams. A summary of the Seattle series is given in Table 1.

### Factors Associated with Graft Rejection

The 68 patients in the Seattle series who lived long enough to demonstrate engraftment were analyzed by a proportional hazards regression model in order to identify factors that correlated with marrow graft rejection. Only 2 of the 24 factors were strongly associated with graft rejection: (1) detection of recipient lymphocyte reactivity against donor cells, either by a positive relative response index in mixed leukocyte culture or by a 51Cr release test, or (2) a low number of marrow cells used for transplantation (less than 3 x 10^6 cells/kg).

Studies in the dog have indicated that a prospective marrow graft recipient could be immunized by blood transfusions so that a marrow graft from a DLA-matched littermate would be rejected. Presumably the human patients also have been sensitized to minor transplantation antigens contained in the transfused blood and, by chance, present in the donor but not in the recipient. Of the 68 patients, only 3 had not been transfused prior to transplantation. All 3 grafts were successful and were not rejected. Obviously, more untransfused patients need to be studied in order to determine whether or not the in vitro evidence of host-versus-donor reactivity is a reflection of sensitization or a part of the disease process or both.

### Factors Associated with GVHD and Survival

The 47 patients in the Seattle series with sustained engraftment were also analyzed by a proportional hazards regression model directed at identifying factors that predicted the presence or absence of GVHD and survival. Twenty-four factors were entered into the analysis and only two strongly correlated

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**Table 1. Seattle Marrow Transplant Summary as of February 1977**

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Includes only transplants done before November 1975. ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia.
with survival: (1) sex match of donor and recipient (p < 0.01), and (2) absence of refractoriness to random donor platelets at the time of transplantation (p < 0.05). These data suggested that X and Y chromosome-associated transplantation antigen systems were important determinants of the outcome of marrow grafts between HLA-identical siblings. We do not know why refractoriness to random donor platelets influenced survival.

Comments

The patients described above have had a variety of possible etiologic factors for aplastic anemia. The results of transplantation, however, seem to be the same regardless of etiology. Consistent engraftment in the vast majority of cases indicates that most cases of aplastic anemia are due to an acquired disorder of the stem cell that can be corrected by transplantation of normal marrow cells.

At the present time it appears that marrow transplantation for aplastic anemia could be curative for virtually all patients with an HLA-identical sibling donor if the transplant were carried out before clinical deterioration, if the recipient did not show in vitro evidence of sensitization to the donor, if a relatively large number of marrow cells were used for transplantation, and if donor and recipient were of the same sex. However, each restriction in selection means that marrow transplantation will be of benefit to a smaller and smaller group of patients.

We should therefore consider what measures might be taken to improve the overall clinical results of marrow transplantation when an HLA-matched sibling is available. First of all, and perhaps most important, the physician should be alert to the possibility of transplantation when a patient with aplastic anemia is first seen. HLA typing of the patient and the family should be carried out immediately. If a matched sibling is not available, marrow transplantation is not now a reasonable option and conventional treatment can be instituted. If, on the other hand, a matched sibling is available, early transplantation should receive prime consideration. Transfusions of blood products should not be given unless indicated by urgent medical necessity. All transfusions from family members should be avoided. Immediate efforts should be made to find a bed for the patient on a marrow transplant unit. A period of observation of 10–14 days may be necessary in order to be certain that the process is not transient and that it is severe. It often takes at least that length of time to arrange a place on a transplant unit for the patient.

Other measures that may be undertaken in order to improve the overall results are currently being studied on an experimental basis. The Seattle group is attempting to obtain more marrow cells from the donor and also evaluating the effect of giving additional donor buffy-coat cells. For the patient with evidence of reactivity against the donor in vitro, efforts are being made to use more vigorous immunosuppressive regimens prior to transplantation. Treatment of GVHD with antithymocyte globulin (ATG) is being compared with high-dose steroids and other agents. Our experience indicates that about one patient in ten with a matched sibling will have a second matched sibling. In the past the sibling of opposite sex has been chosen because of the convenience of the cytogenetic marker in following engraftment. It is obvious now that a donor of the same sex should be chosen whenever possible.
ACUTE LEUKEMIA

Marrow transplantation for acute leukemia involves the same general transplantation biology problems that are encountered in aplastic anemia, except that graft rejection is rare. The eradication of the population of malignant cells, however, is an additional major problem. Despite these problems, an ever increasing number of long-term survivors is being documented. Until recently, marrow transplantation for treatment of these patients has been undertaken only after failure of conventional and experimental combination chemotherapy. As a consequence, most of these patients have been in far advanced relapse with a heavy body burden of leukemic cells. Despite these obstacles the longest disease-free survivor is now $6\frac{1}{2}$ yr post-transplantation, and in the United States there are 15 leukemia-free patients who are now more than 2 yr posttransplantation. It should be emphasized that these patients have not been on any form of maintenance chemotherapy following transplantation.

Analysis of Survival

In the Seattle series, 10 patients were prepared for transplantation by the administration of 1000 rads of TBI and 100 patients were prepared with CY (60 mg/kg on each of two days) followed by TBI. An actuarial analysis of the survival curve of these patients by the method of Kaplan and Meier indicated three periods of interest. The first period was the first 120 days following transplantation. There was a rapid loss of patients during this time, apparently due to the problems of advanced illness, GVHD and associated infections, and, to a lesser extent, recurrent leukemia. The second period extended from approximately 120 days to 2 yr after grafting. There was a much slower rate of loss of patients during this time and the loss was due primarily to recurrent leukemia. The third period, which now extends from 2 to $6\frac{1}{2}$ yr, was almost flat, with a negligible loss of patients and no recurrent leukemia. This flat portion of the curve, which comprised about 15% of the patients, constituted an operational definition of cure for these patients. It is therefore reasonable to conclude that marrow transplantation can be considered to be curative for some patients even in the endstages of acute leukemia.

Efforts to Eradicate the Leukemic Cell Population

The first ten patients in Seattle were prepared only with TBI because of our experience with irradiation and marrow transplantation in the dog, because irradiation was known to be effective in destroying leukemic cells, and because irradiation could penetrate the "privileged sites" where residual leukemic cells might be lurking. When a significant incidence of recurrent leukemia was observed, we added the two large doses of CY prior to TBI. In addition, a number of patients received added chemotherapy, principally rubidomycin and cytosine arabinoside, in an effort to achieve maximal "cytoreduction" of the leukemic cell population. Although the number of patients was too small for critical analysis, added chemotherapy increased the time of maximal pancytopenia and drug toxicity, and instances of recurrent leukemia continued to be observed.

Other investigators have utilized vigorous chemotherapy instead of TBI before transplantation. Santos et al. employed CY, 50 mg/kg on each of 4 days,
and Graw et al.26 utilized the same regimen with 45 mg/kg of CY. Although these CY regimens were well tolerated, all patients who survived developed recurrent leukemia. Accordingly, Graw et al.27 developed a bischloroethyl-nitrosourea, cytosine arabinoside, CY, 6-thioguanine (BACTA) regimen. The BACT regimen proved to be very toxic with many early deaths. However, one patient with endstage acute myelogenous leukemia (AML) is now alive and well 5 yr after transplantation.28

Gale et al.29 have developed a 6-thioguanine, CY, cytosine arabinoside, rubidomycin, TBI regimen (SCARI) which employs high-dose chemotherapy followed by TBI. Although the SCARI regimen has proved to be very toxic for patients over the age of 18, preliminary results are encouraging in that only two patients have suffered a relapse of leukemia. The number of patients treated by the SCARI regimen and the duration of their survival is as yet inadequate for a valid comparison with the Seattle regimen.

Factors Affecting Survival

Graft rejection has not been a problem in patients with acute leukemia. Of the 100 patients prepared with CY plus TBI only 1 patient rejected the graft.23 Presumably, the patient with acute leukemia may not be sensitized by blood transfusions because of the nature of the disease, because blood transfusions are usually given during periods of intensive chemotherapy with immunosuppressive drugs, or because of greater immunosuppression by the CY-TBI regimen. Although the patients with acute lymphoblastic leukemia (ALL) have had a longer disease history before coming to transplantation than the patients with AML, the survival and the relapse rate after transplantation are not different between the two groups. The most significant factor affecting survival is the clinical condition of the patient at the time of transplantation. A prospective study involving overall clinical evaluation at the time of transplantation has shown that patients in good condition have a much better chance of surviving than patients who are in poor condition.23

GVHD and Interstitial Pneumonia

In patients receiving marrow grafts for leukemia or for aplastic anemia, GVHD is clinically recognizable in approximately two-thirds of the patients and in one-fifth of the patients GVHD is life threatening. GVHD involves the skin, gut, and liver as target organs and, in addition, is usually associated with severe immunologic deficiency. Consequently, the usual terminal event in patients with GVHD is an infection by bacterial, fungal, or viral opportunistic organisms. Treatment of GVHD with ATG is only partially successful.30

Interstitial pneumonia, most often seen in association with GVHD, is a major clinical problem in the recipients of allogeneic marrow transplants.31,32 About 40% of the cases of interstitial pneumonia are associated with cytomegalovirus, about 10% are associated with Pneumocystis carinii, and, except for a few cases of varied opportunistic infections, the remainder are idiopathic. Although the search for an etiologic agent in these idiopathic cases continues, intensive chemotherapy, irradiation, or GVHD may play a role in the pathophysiology of idiopathic interstitial pneumonia. Current protocols involving
prophylactic adenine arabinoside, prophylactic ATG, and a randomized trial of therapy with ATG versus high-dose steroids may help resolve these problems. Three of the 14 long-term survivors grafted for leukemia and five of 31 grafted for aplastic anemia have developed chronic GVHD, involving skin and occasionally liver, but not the gut. Therapy of chronic GVHD with ATG or high-dose steroids has not been successful. Efforts are being made to shed light on the pathophysiology of chronic GVHD and to develop rational approaches to its prevention or treatment.

Comments

Two main approaches are available for reducing the incidence of recurrent leukemia and increasing the number of long-term survivors. The first involves efforts to improve the antileukemic potency of the preparative regimen before marrow grafting. The SCAR! regimen described above is one example of this approach. More vigorous chemotherapy with non-cycle-dependent drugs seems warranted, as is now being done in Baltimore with myleran and in Seattle with dimethyl myleran. It should be pointed out that these efforts may in fact be misdirected, since in an exponential killing process it is difficult to kill the last leukemic cell. In fact, there is as yet no statistically valid difference between the patients treated with TBI, the patients treated with CY plus TBI, or the patients treated with the SCAR! regimen. The apparent cures may be due to eradication of the last leukemic cell by an immunologic mechanism involving minor transplantation antigens and/or leukemia-associated antigens.

The second approach involves a resort to marrow transplantation before the patient reaches the endstages of the disease. The obvious advantages of this approach include (1) treatment before the leukemic cell population becomes resistant to therapeutic modalities, (2) treatment at a time when the body burden of leukemic cells is minimal, and (3) treatment when the patient is in excellent clinical condition and, therefore, better able to tolerate the transplantation regimen.

In January 1976 a Seattle protocol for early transplantation was approved by the Human Subjects Review Committee of the University of Washington. This protocol involved CY plus TBI and marrow transplantation for patients with AML in the first remission and for patients with ALL in the second or subsequent remission. In the first half of 1976 nine patients underwent marrow transplantation in remission. In all patients the early posttransplantation course was relatively benign. Three patients subsequently developed interstitial pneumonia and died. One patient with ALL transplanted in the third remission relapsed at 6 mo. The other five patients are well now 5–11 mo posttransplantation. Although these early results are encouraging, we must await the long-term results in this series of patients.

SYNGENEIC MARROW TRANSPLANTATION

The patient with an identical twin is particularly fortunate because marrow transplantation from the syngeneic donor cannot result in either graft rejection or GVHD. In Seattle, three patients with aplastic anemia have been cured by an intravenous infusion of marrow from a syngeneic donor without immuno-
suppression of the recipient. The longest survivor is now 16 yr posttransplantation. Several similar successful experiences have been reported. In addition, one irradiation accident victim has been successfully treated by a syngeneic marrow transplant.

A number of patients with hematologic malignancies have been treated by syngeneic marrow transplantation following high-dose chemotherapy and TBI with or without an attempt at immunotherapy. Of the 16 patients reported in 1974, 6 are living and well in complete remission on no maintenance chemotherapy now 4-6 yr posttransplantation.

Obviously, only a relatively small number of patients will prove to have an identical twin. Nevertheless, the physician must be particularly alert for these cases because of the unique opportunity of benefit for the patient.

THEORETICAL CONSIDERATIONS DERIVED FROM MARROW TRANSPLANTATION

Although marrow transplantation is usually thought of as a therapeutic procedure designed to aid the patient with a fatal illness, important insights into the pathophysiology of disease have been gained. For example, cure of aplastic anemia by marrow transplantation provides strong evidence that the disease is the result of an acquired stem cell defect. Although some cases of aplastic anemia might be due to a disorder of the microenvironment, successful engraftment in the vast majority of marrow transplant recipients indicates that aplastic anemia is not usually a disorder of the microenvironment of the marrow nor is it due to a persisting toxin or lack of an essential nutrient. Although an immune mechanism may be involved in the etiology of aplastic anemia in some patients, prompt recovery following the intravenous infusion of syngeneic marrow, without immunosuppression of the host, argues against the presence of an ongoing “autoimmune” disorder.

Paroxysmal nocturnal hemoglobinuria (PNH) is a disorder that has long fascinated hematologists. Cure of PNH by marrow transplantation confirms the hypothesis that this disorder is also an acquired stem cell defect. The first patient with PNH transplanted in Seattle is now living and well more than 5 yr postgrafting without evidence of the disease. Further insight into the pathophysiology of PNH has been provided by a unique opportunity to study a patient who had a normal identical twin. Simple intravenous infusion of marrow from the normal twin resulted in cure of the disease (follow-up now more than 3 yr), indicating that the PNH stem cell does not have a competitive advantage with regard to normal stem cells.

In leukemia, the most startling observation concerned recurrence of leukemia in donor cells observed in two cases. Both patients were females with ALL who had a marrow graft from an HLA-matched brother. The recurrent leukemia was clearly shown by cytogenetic studies to be in male cells. Although many hypotheses have been considered to explain the recurrence in donor cells, it is apparent that reinduction of a malignant cell population can occur in some patients with leukemia. Skipper et al. indicated many years ago that kinetic considerations of the killing of leukemic cells by chemotherapeutic agents was valid only if reinduction was not taking place. Reinduction may indeed be a factor in the relapse of leukemia after chemotherapy of the disease.
CONCLUSIONS

Marrow transplantation, once undertaken as a desperate therapeutic maneuver in terminally ill patients, has now become established as a therapeutic option of definite benefit to some patients with aplastic anemia or acute leukemia. Some progress is being made toward resolution of the problems of transplantation biology that occur even though the sibling donor-recipient pairs are matched at the major histocompatibility complex. Efforts to reduce the incidence of recurrent leukemia by more intensive antileukemic therapy or by transplantation in remission are underway.

It is now feasible to extend marrow transplantation to the therapy of patients with other hematologic malignancies and to genetic disorders of hematopoiesis. Through advances in tissue typing, marrow transplantation can be expected to become a therapeutic option for the patient without a matched sibling. One such successful case has already been reported.44

Marrow transplantation is a complex undertaking that requires a skilled team of physicians of many disciplines, nurses, and technologists. Because of the critical illness of the patients and the complexity of the undertaking, marrow transplantation is expensive. By analogy to experience in the past decade with hemodialysis and open heart surgery, the cost of marrow transplantation can be expected to decrease appreciably in the coming years. The relatively short period of hospitalization required for the patients with acute leukemia recently transplanted in remission is one example of a reduction in costs. Pending development of methods for prevention and for specific treatment of the hematologic disorders amenable to therapy by marrow transplantation, marrow transplantation is likely to be undertaken for an ever increasing number of patients in the coming decade.

REFERENCES

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