Recurrence of Aplastic Anemia Following Cyclophosphamide and Syngeneic Bone Marrow Transplantation: Evidence for Two Mechanisms of Graft Failure

By Frederick R. Appelbaum, Martin A. Cheever, Alexander Fefer, Rainer Storb, and E. Donnall Thomas

Two patients with aplastic anemia were treated with high-dose cyclophosphamide and marrow transplantation from their normal, genetically identical twin. Both patients rapidly recovered normal marrow function, but marrow failure recurred 13 and 18 months later. Because donor and host pairs were identical twins, these cases of graft failure could not have resulted from the usual cause of graft failure, i.e., immunological reactivity of host cells against unshared minor histocompatibility antigens of the donor. These results imply that there are at least two mechanisms responsible for graft failure after marrow transplantation for severe aplastic anemia.

© 1985 by Grune & Stratton, Inc.

RESULTS

Case 1

A 23-year-old man presented with petechiae in December 1980. His hematocrit was 38%, white cell count 2,100/μL with 20% granulocytes, platelets 15,000/μL reticulocytes 0.3%; bone marrow biopsy was markedly hypocellular. The diagnosis of idiopathic aplastic anemia was made, and the patient was transferred to the University of Washington. On Jan 22, 1981, he was transfused with 3.4 x 10^6 nucleated bone marrow cells/kg from his identical twin brother, without pretransplant immunosuppression. Evidence for monozygosity included identical physical appearance, HLA identity, and identical RBC grouping and enzymes performed on samples obtained prior to transfusion. Over the next two months, his granulocyte count increased slightly to 1500/μL; however, he remained severely thrombocytopenic and his bone marrow remained essentially empty. On April 6, 1981 (74 days from the first marrow infusion), the patient was started on cyclophosphamide 50 mg/kg for four days and on April 10, 1981, he received a second

From the Fred Hutchinson Cancer Research Center and the University of Washington School of Medicine, Seattle.

Supported by grants No. CA 30924, CA 18029, CA 15704, and CA 09319 from the National Cancer Institute, Department of Health and Human Services and Research Career Award No. AI 02425 from the National Institute of Allergy and Infectious Diseases (E.D.T.).

Submitted June 11, 1984; accepted Aug 29, 1984.

Address reprint requests to Dr Frederick R. Appelbaum, Room 318, Fred Hutchinson Cancer Research Center, 1124 Columbia St, Seattle, WA 98104.

© 1985 by Grune & Stratton, Inc.

0006-4971/85/6503-0007$03.00/0
infusion of twin marrow containing 1.9 x 10^6 nucleated marrow cells/kg. Within eight days, his peripheral granulocyte and platelet counts began to rise, and 26 days after the second transplant, his granulocyte count had risen to >1,500/μL and his platelet count had risen to >100,000/μL. After the second transplant, the patient did very well, with normal peripheral blood counts for 13 months; the petechiae then recurred. Reevaluation in July 1982 revealed that his hematocrit had fallen to 27%, WBC was 3,200/μL with 42% granulocytes, platelets were 20,000/μL, and bone marrow biopsy demonstrated an extremely hypocellular marrow. The patient was followed for six weeks and demonstrated no change in his peripheral counts or marrow cellularity. In September 1982, he was readmitted to the hospital and treated with ten days of horse anti-human thymocyte globulin (ATG), 15 mg/kg/d intravenously (IV). Approximately ten weeks after ATG therapy, the patient's reticulocyte count increased to 2.8%, his hematocrit increased to 32%, and his platelet count to 32,000/μL. His peripheral counts have slowly continued to improve and by September 1983, his hematocrit, white cell count, and granulocyte count were normal and his platelet count was 106,000/μL.

Case 2

The initial part of this patient's history has been reported previously. In brief, this 44-year-old man presented in August 1976 with severe anemia. The diagnosis of pure red cell aplasia was made. Treatment with androgens and prednisone produced no response; over the next 14 months, he required frequent RBC transfusions. He was referred to the University of Washington in December 1977, at which time his physical exam revealed only pallor, his hematocrit was 17% with no reticulocytes, his WBC was 4,100/μL with 50% granulocytes, and his platelet count was 80,000/μL. A bone marrow aspirate and biopsy revealed 25% cellularity with less than 10% erythroid precursors and increased plasma cells and lymphocytes. The diagnosis of aplastic anemia primarily affecting red cells and platelets was made. On Dec 16, 1977, the patient received 0.75 x 10^6 nucleated bone marrow cells/kg from his identical twin brother. Monozosity was determined by the family history of a monochorionic twin placenta, HLA identity, and the presence of congenital hip dysplasia in both twins. RBC phenotyping could not be performed because the patient had been recently transfused. During the next three months, there was no change in his clinical course; he continued to require RBC transfusions to maintain a hematocrit over 20%, his reticulocyte count remained below 0.3%, his platelets remained below 100,000/μL, and his marrow remained hypocellular. On March 20, 1978, he was started on cyclophosphamide 50 mg/kg/d for four days, and on March 24 he received 1.8 x 10^6 nucleated bone marrow cells/kg from his twin brother. Within four weeks of the second transplant, his hematocrit had recovered to 34% and his reticulocyte count to 4.8%; his platelets were 120,000/μL. He did well, with normal peripheral counts from May 1978 until September 1979, when he again developed anemia with a hematocrit of 23%, a white blood count of 3,200/μL with 70% granulocytes, and platelets of 80,000/μL. He was treated with RBC transfusions, but his peripheral counts continued to worsen; by March 1980, his hematocrit was 30% (post RBC transfusion), his WBC was down to 1,100/μL, his platelets were 59,000/μL, and a bone marrow biopsy revealed less than 5% cellularity, confirming the diagnosis of severe aplastic anemia. A trial of low-dose oral cyclophosphamide was unsuccessful. On April 21, 1980, the patient received a third marrow infusion from his identical twin after preparation with cyclophosphamide 60 mg/kg for two days and 1,000 rad total body irradiation. Within four weeks, the patient's
hematocrit was 36%, his white blood count was 3,200/μL with 50% granulocytes, his platelet count was 52,000/μL, and a bone marrow aspirate revealed a recovering bone marrow. The patient subsequently recovered normal peripheral blood counts and did well until May 1983, when he once again developed anemia with a hematocrit of 24%, a white count of 4100/μL with 56% granulocytes, and platelets of 140,000/μL. He has since continued to require red cell transfusions.

**DISCUSSION**

Graft failure after marrow transplantation for severe aplastic anemia in previously transfused patients has usually been viewed as the result of sensitization of the host to unshared non-HLA antigens on donor cells. This view is supported by studies in mice, dogs, and humans, all of which demonstrate that exposure to blood products prior to marrow transplantation increases the risk of marrow graft rejection.6,12 The reason for graft failure in untransfused patients is less clear but could reflect a similar mechanism, except that the sensitization to non-HLA antigens takes place in vivo after the transplant.

The two patients presented here received grafts from their identical twins. In this setting, there are no genetic markers to prove initial engraftment. However, the pattern of recovery of hematopoiesis after transplantation was similar to that which occurs after allogeneic transplantation; therefore, we have assumed that engraftment occurred. Given this qualification, the clinical courses of the two patients presented here demonstrate that host reactivity against unshared non-HLA antigens on donor cells is not the only mechanism accounting for graft failure. Because the patients and their donors were identical twins, unshared non-HLA antigens could not have existed. Therefore, some other reason for graft failure must exist.

A number of such reasons can be hypothesized. Inadequate marrow cell dose resulting in stem cell exhaustion has been described in animal studies, but is an unlikely problem in these patients in that they received marrow cell doses similar to those given to patients with sustained engraftment. Neither is there any evidence that the transplanted marrow stem cells were qualitatively defective in that they grew normally in CFU-C and BFU-E cultures. Furthermore, both donors remain perfectly healthy with normal hematologic values. Drugs, radiation, and viral infections all have been associated with recurrent aplasia, but no such exposure history could be elicited from our patients. Of course, reexposure to an unrecognized causative agent is possible. Although either lack of an essential nutrient or a defect in the microenvironment has been mentioned as a possible cause of aplastic anemia, it is difficult to imagine why these problems should occur after 13 to 18 months of sustained engraftment.

The most likely mechanism leading to graft failure in these two patients is reemergence of a population of abnormal cells (perhaps T cells) that were responsible for creating the original aplasia. How these cells originally developed is an enigma, although several hypotheses have been developed,13,14 but there is strong evidence that such a population exists. Both patients failed to respond to initial treatment with simple marrow infusion from their twin, but a second transplant following cyclophosphamide preparation led to prompt hematologic recovery. The previous experience with twin transplants for aplastic anemia is that in half the cases, twin marrow infusion without preparation is successful, but in the other half, cyclophosphamide preparation appears to be required for successful engraftment.13,14,15 This observation suggests that in some cases cyclophosphamide is necessary to ablate a population of cells responsible for suppression of hematopoiesis. Several studies have demonstrated that the removal of T cells from the blood and marrow of patients with aplastic anemia can markedly increase in vitro colony formation.16-18 These studies have been interpreted as suggesting that some cases of aplastic anemia are initiated or maintained by T cell-mediated immune suppression of stem cells. This view is further supported by the observation that ATG therapy for severe aplastic anemia results in hematologic improvement in 25% to 60% of patients.19-22 In the first case presented here, the patient was treated with ATG after developing recurrent aplasia; he appeared to recover normal hematopoiesis. Case 2 was treated with cyclophosphamide and total body irradiation and also recovered temporarily. Given the presumptive evidence for a role for abnormal T cell function in aplastic anemia, a reasonable interpretation of the clinical courses of our two patients is that their aplastic anemia was initially caused by reactivity of a population of host T cells that was significantly but not completely depleted with the initial cyclophosphamide preparative regimen, but that regrew once again, resulting in the recurrence of the disease. However, without cytogenetic or other markers it is impossible to demonstrate that such an abnormal population of cells was of host rather than donor origin.

The pattern of graft failure seen in the two patients presented here was distinct from that usually seen after allogeneic transplantation in that they rejected their grafts at 13 and 18 months after transplantation. After allogeneic transplantation, rejection is usually seen within the first 100 days.1,23 The incidence of graft
failure more than one year after allogeneic transplantation is extremely small; in the first 175 allogeneic graft recipients for aplastic anemia treated in Seattle, late failure was seen only once. Whether the observation of two late failures among the eight twins transplanted for aplastic anemia in Seattle represents a chance occurrence or a real increase in frequency is unknown. It may be that one of the mechanisms responsible for the success of allogeneic marrow transplantation for aplastic anemia is an allogeneic reaction against T cell populations in the host responsible for the disease that would otherwise recover following high-dose cyclophosphamide. This assumption is in keeping with the proven utility of viable donor buffy coat in sustaining engraftment in previously transfused allogeneic graft recipients.

ACKNOWLEDGMENT

The authors thank the physicians, nurses, ward clerks, secretaries, and technologists of the University of Washington and the Fred Hutchinson Cancer Research Center, and the patients’ referring physicians for their cooperation in this study.

REFERENCES

Recurrence of aplastic anemia following cyclophosphamide and syngeneic bone marrow transplantation: evidence for two mechanisms of graft failure

FR Appelbaum, MA Cheever, A Fefer, R Storb and ED Thomas

Updated information and services can be found at:
http://www.bloodjournal.org/content/65/3/553.full.html

Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml