Reconstitution of Normal Megakaryocytopoiesis and Immunologic Functions in Wiskott-Aldrich Syndrome by Marrow Transplantation Following Myeloablation and Immunosuppression With Busulfan and Cyclophosphamide

By Neena Kapoor, Dahlia Kirkpatrick, R. Michael Blaese, James Oleske, Margaret H. Hilgartner, R.S.K. Chaganti, Robert A. Good, and Richard J. O'Reilly

Three patients with Wiskott-Aldrich syndrome received transplants of marrow from their HLA-A, B, C identical siblings after myeloablation with busulfan, 2 mg/kg/day x 4 days, followed by immunosuppression with cyclophosphamide, 50 mg/kg/day x 4. Sustained engraftment of lymphoid and hematopoietic elements was documented in each case. Platelet counts in excess of 100,000/cu mm were restored 20–50 days posttransplant and remain in the normal range 6–12 mo later. Platelets exhibit normal size and in vitro aggregation. The patients produce isoagglutinins and antibodies to other polysaccharides. The use of busulfan in moderate dosages as a myeloablative agent, coupled with cyclophosphamide, may offer an improved alternative to the use of lethal total body irradiation as a preparative regimen for complete correction of Wiskott-Aldrich syndrome by marrow transplantation.

WISKOTT-ALDRICH syndrome (WAS) is an inherited X-linked disorder characterized by eczema, thrombocytopenia,1,3 immune deficiency of variable severity that involves both the T- and B-cell systems.4,5 Patients usually die of infection, hemorrhage, or lymphoreticular malignancy in early childhood. They rarely survive into adolescence.6,7 Patients with Wiskott-Aldrich syndrome who have received marrow transplants following immunosuppression with cyclophosphamide alone or in combination with antimetabolites have uniformly achieved engraftment of donor lymphoid elements and reconstruction of immune functions, but have not experienced engraftment of other donor hematopoietic elements or correction of thrombocytopenia.8,11 Failure to engraft myeloid, erythroid, or megakaryocyte elements may not be based on an immune response of the host against donor hematopoietic elements, since sustained functional engraftment of donor T lymphocytes has occurred in two of three patients prepared in this way.11 It would thus appear that nonimmune cellular interactions within the marrow itself may limit the engraftment or differentiation of nonlymphoid hematopoietic precursors.

Recently, sustained and complete engraftment of donor hematopoietic and lymphoid elements with correction of thrombocytopenia and immune abnormalities has been achieved in marrow transplant recipients following preparation with a combination of antithymocyte globulin (ATG), procarbazine, and total body irradiation (TBI).11 It has been postulated that only total body irradiation provides the degree of ablation of host hematopoiesis necessary to insure engraftment and function of nonlymphoid components of donor marrow.10,12 Experience with TBI in transplants for leukemia and aplastic anemia also suggests, however, that while high single dose TBI may not, of itself, constitute a major cause of interstitial pneumonia (as reflected in marrow transplants for leukemia between twins),1 its use may augment the severity of GVHD and interstitial pneumonia in the posttransplant period.13,14 Other late complications, e.g., cata- racs, are also incurred. Evaluation of less extreme preparative modalities thus seemed warranted.

In this article, we report complete engraftment of donor lymphoid and hematopoietic elements in three consecutive patients with WAS, who received marrow transplants after immunosuppression and myeloablation with cyclophosphamide and moderate doses of busulfan.

MATERIALS AND METHODS

HLA-A, B, C typing was performed using standard NIH two-stage microcytotoxicity techniques.15 Mixed lymphocyte cultures were performed according to the method of Dubois et al.16 In vitro lymphocyte transformation responses to mitogens and antigens were performed using methods previously described.11 Immunoglobulins
Table 1. Immunoglobulin Levels in Wiskott-Aldrich Syndrome Following Marrow Transplantation

<table>
<thead>
<tr>
<th>J.R.</th>
<th>M.A.</th>
<th>L.H.</th>
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<tbody>
<tr>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>IgG</td>
<td>1320</td>
<td>147</td>
</tr>
<tr>
<td>IgA</td>
<td>530</td>
<td>166</td>
</tr>
<tr>
<td>IgM</td>
<td>12</td>
<td>315</td>
</tr>
<tr>
<td>IgE</td>
<td>107</td>
<td>33</td>
</tr>
<tr>
<td>Red cell type</td>
<td>A</td>
<td>O</td>
</tr>
<tr>
<td>Isohemagglutinins</td>
<td>Anti-A</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>Anti-B</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>MLC</td>
<td>30%</td>
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G, A, and M were quantitated by radial immunodiffusion and IgE by radioimmunoassay. Isohemagglutinins were titers by standard serologic techniques. Antibodies to pneumococcal polysaccharides were quantitated by a radioimmunoassay by G. Schiffman, Ph.D. Platelet size was measured by the method of Murphy et al. Epinephrine, adenosine diphosphate (ADP), and collagen induced platelet aggregation was assessed in vitro using standard methodologies. Red cell phenotypes were established by standard serologic techniques before transplant and at least 4 mo after the last transfusion posttransplant. Donor and host chromosome markers were established from a study of sex chromosomes or quinacrine-stained metaphase cells derived from PHA-stimulated peripheral blood lymphocytes and spontaneous dividing marrow cells. The chromosome preparations, quinacrine staining, and fluorescence microscopy were performed following conventional methods. Patients were transplanted in laminar air flow isolation after chromosomal preparations, quinacrine staining, and fluorescence microscopy were performed following conventional methods. Patients were transplanted in laminar air flow isolation after 4 mo after the last transfusion posttransplant.

CASE REPORTS

Case 1

J.R., a 5-yr-old male with Wiskott-Aldrich syndrome, was admitted for a marrow transplant on 4/23/79. Severe thrombocytopenia and eczema were detected in early infancy. Thereafter, the patient had recurrent otitis media and required multiple hospitalizations for treatment of pneumonia and for severe epistaxes that necessitated multiple platelet and red cell transfusions. Assays of immunologic and hematologic functions, summarized in Tables 1 and 2, revealed severe thrombocytopenia, small platelets, abnormal platelet aggregation, a deficiency of IgM, increased levels of IgA, reduced in vitro transformation responses to allogeneic cells, antigens and pokeweed mitogen, and absent isohemagglutinins.

Prior to transplantation, the patient was prepared with oral busulfan (2 mg/kg/day) for 4 days followed by cyclophosphamide (50 mg/kg/day) intravenously for an additional 4 days. Two days thereafter the patient received an infusion of marrow (2.98 x 10^8 nucleated marrow cells/kg body weight) from his HLA-A, B, C, D identical brother. Methotrexate prophylaxis was not administered.

The posttransplant course is summarized in Fig. 1. First evidence of hematopoietic engraftment was detected at day 14. By 3 wk, the neutrophil count had increased to 2000/cu mm, and platelets were self-sustaining at >30,000/cu mm. At this time the patient developed severe graft versus host disease, manifested by desquamating erythrodema, severe diarrhea, and hepatitis. The patient was treated with prednisone (2 mg/kg/day). GVHD manifestations resolved completely by day 40. Thereafter, platelet counts rose rapidly to >200,000/cu mm. His course was otherwise uncomplicated until 6 mo posttransplant when he developed an asthma-like syndrome with chronic wheezing and significant exercise intolerance. Chest roentgenograms revealed hyperaeration with increased perihilar markings. A lung biopsy revealed focal alveolitis with mononuclear cell infiltrates. Neither proliferative endarteritis nor interstitial fibrosis was detected. Cultures failed to define a bacterial, viral, or fungal pathogen. P. carinii was not detected. The process was unaltered by empirical therapy with antibiotics and responded minimally to bronchodilating agents. The patient was, therefore, treated with prednisone (2 mg/kg/day), which produced significant and continuing improvement in pulmonary function. The patient is now 12 mo posttransplant. Prednisone is being tapered; he is at home living normally without exercise intolerance.

Case 2

M.A., a 7-yr-old male, developed petechiae and enteritis during the neonatal period. Thereafter, he had multiple episodes of otitis media, impetigo, and pneumonia. The diagnosis of Wiskott-Aldrich syndrome was made at the age of 10 mo, on the basis of his recurrent infections, severe thrombocytopenia, and eczema. From that time until his admission for transplantation, he had been maintained on prophylactic antibiotics.

The patient was admitted for marrow transplantation on 7/17/79 and placed in protective isolation. He was prepared for transplantation with the same busulfan and cyclophosphamide protocol as described for patient J.R. and 48 hr thereafter received an intravenous infusion of 9.45 x 10^8 nucleated bone marrow cells/kg body weight from his HLA-A, B, C, D identical ABO compatible brother. Methotrexate prophylaxis was administered for the first 3 wk posttransplant. The course of engraftment was unremarkable. By day 23, the leukocyte count had risen to 2000/cu mm with 31% neutrophils. Platelet counts were self-sustaining at >30,000/cu mm. The patient was discharged 45 days posttransplant. At this time, eczema was completely resolved, hematologic indices and marrow cellularity were normal, with platelet counts >150,000/cu mm. GVHD was not detected. In the 8 mo since transplantation, the patient has experienced two mild upper respiratory tract infections, which he handled normally, and has been otherwise well.

Case 3

L.H., a 3-yr-old male, was admitted for marrow transplantation on 11/21/79. In his case, the diagnosis of Wiskott-Aldrich syndrome was suspected at birth when he presented with petechial
epistaxis, recurrent otitis media, three documented episodes of pneumonia, and one episode of orbital cellulitis.

On admission, the patient was placed in protective isolation. He was prepared for transplantation with busulfan and cyclophosphamide as previously described. On 12/7/79, he received an infusion of $9.2 \times 10^8$ nucleated marrow cells/kg from his ABO compatible HLA-A, B, C, D identical sister. Methotrexate prophylaxis was administered for 3 wk posttransplant. Engraftment was documented by marrow karyotype 2 wk posttransplant. By day 15, the leukocyte count was 1400/cu mm with only 25% neutrophils but the platelet count was self-sustaining at >60,000/cu mm. He was discharged on day 34 posttransplant with a normocellular marrow, a leukocyte count of 3500/cu mm with 65% neutrophils, and a platelet count of 161,000/cu mm. In the 6 mo since transplants, eczema has completely resolved. He has experienced neither GVHD nor infections in the posttransplant course.

**RESULTS**

Evidence for engraftment of lymphoid and hematopoietic elements is summarized in Table 3. In each case, spontaneously dividing marrow elements and PHA-stimulated peripheral blood lymphocytes are exclusively of donor origin, as shown by cytogenetic analyses.

In patient J.R., donor cells could be easily distinguished from host cells by the characteristic quinacrine-fluorescent heteromorphisms present on their no. 13 chromosomes. Both no. 13 chromosomes of the patient had brightly fluorescent short arms, while one no. 13 chromosome only of the donor had a brightly fluorescent short arm (p 11) and satellites (p 13). In patient M.A., donor cells could be easily distinguished from host cells due to the presence of brightly fluorescent satellites on chromosomes no. 14 and 15 (p 13), which were absent on the host chromosomes. The donor for patient L.H. was his sister, allowing differentiation of donor and host cells by sex chromatin markers. Engraftment of donor erythroid elements was also documented by red cell phenotype in patients J.R. and M.A. Patient L.H. had received a blood transfusion shortly before referral for transplantation. His own red cell phenotype was therefore not evaluable.

As shown in Table 2, each patient was severely thrombocytopenic prior to transplantation. In addi-

<table>
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<th>Table 3. Evidence of Engraftment</th>
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<tbody>
<tr>
<td>J.R.</td>
</tr>
<tr>
<td>Red cell antigen</td>
</tr>
<tr>
<td>Cyto genetic</td>
</tr>
<tr>
<td>PHA-stimulated lymphocytes</td>
</tr>
<tr>
<td>Platelets</td>
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NE, not evaluable.
tion, platelets were abnormally small in size and volume in the two patients studied (patient platelets: 1.3–3.0 cu μ; control: 4.01–9.02 cu μ). Because of severe thrombocytopenia, accurate assessments of platelet aggregation could not be performed. Following transplantation, each patient has developed platelet counts that are consistently >150,000/cu mm. The platelets are now normal in size. In addition, the platelets from each patient when studied 6–12 mo posttransplantation, exhibited normal in vitro aggregation responses when stimulated with epinephrine, ADP, or collagen.

Assays of immunologic function, performed prior to and following transplantation, are summarized in Table 1. Prior to transplantation, each patient exhibited a marked deficiency of IgM. In patients J.R. (A+), and L.H. (O+), isoagglutinins were not detected. Each patient exhibited normal in vitro lymphocyte transformation responses to the mitogens PHA and Con-A. However, responses to allogeneic cells were only 10%–30% of normal. Following transplantation, IgM levels have increased to normal in each case. In vitro transformation responses to allogeneic cells are 60%–80% of normal in all the patients. Both L.H. and J.R. now produce isoagglutinins.

DISCUSSION

In 1978, Parkman et al. reported successful engraftment of both lymphoid and hematopoietic elements with full correction of the platelet and lymphocyte abnormalities characterizing Wiskott-Aldrich syndrome in two patients prepared for transplantation with lethal body irradiation, procarbazine, and antithymocyte serum. They postulated that total body irradiation is necessary to achieve the degree of myeloablation required to allow engraftment of nonlymphoid hematopoietic elements. To date, five patients with Wiskott-Aldrich syndrome have been transplanted with a protocol incorporating lethal body irradiation. Three have achieved a full correction, one remains thrombocytopenic and one died with interstitial pneumonia and severe graft versus host disease. One reconstituted child has developed cataracts in the posttransplant period. The degree of correction achieved in four of five patients treated with this protocol is clearly superior to that achieved in patients prepared with cyclophosphamide alone, suggesting that myeloablation or more intense immunosuppression is indeed necessary to achieve full correction of this disorder.

We reasoned, however, that a myeloablative regimen might be developed that would insure full engraftment yet obviate the necessity for using lethal body irradiation. The immunosuppressive activity of cyclophosphamide appeared adequate, since two of three patients with Wiskott-Aldrich syndrome prepared for transplantation with this agent alone have achieved sustained engraftment of donor lymphocytes. The choice of busulfan, which is a potent myeloablative agent but has few other toxicities and minimal immunosuppressive activity, was suggested by the studies of Santos et al. and Tutschka et al. These investigators showed that whereas myelosuppression with busulfan or immunosuppression with antithymocyte globulin alone was not sufficient preparation to permit engraftment of allogeneic marrow in normal rats, use of a combination of these agents regularly led to full and sustained engraftment. The busulfan dose was selected from experience with this agent in patients transplanted for leukemia, as that which will regularly induce aplasia in man without producing other toxicities, particularly damage to the lung, which might contribute to significant morbidity or mortality in the posttransplant period.

As documented in the three patients presented in this report, the combination of moderate doses of busulfan with high-dose cyclophosphamide is, indeed, sufficient to insure full hematopoietic and lymphoid engraftment with correction of immunologic deficiencies and abnormalities of platelet number and function in patients with Wiskott-Aldrich syndrome. It remains to be seen whether this regimen is also adequate to abrogate the known susceptibility of these patients to lymphoreticular malignancies.

The combination of busulfan and cyclophosphamide as used in these cases is well tolerated. Immediate side effects were limited to nausea and vomiting on the days of drug administration. Stomatitis developed in only one of the three patients and was documented to be due to a Herpes simplex virus infection. This patient also developed GVHD and later an acute pulmonary disease, both of which completely responded to short-term steroid therapy. While chronic administration of busulfan has been shown to produce pulmonary disease, specifically interstitial fibrosis and microvascular disease, this complication has not been described in individuals receiving short courses of this agent at the low doses used. Indeed, the lung biopsy obtained from patient J.R. revealed no evidence of interstitial fibrosis or other changes associated with busulfan lung. Late complications of lethal total body irradiation, such as cataracts, have not as yet been observed with this protocol.

We, therefore, suggest that the use of this combination of busulfan and cyclophosphamide is an effective and probably less toxic alternative to lethal total body irradiation as a preparative regimen for marrow transplantation in patients with Wiskott-Aldrich syndrome. It remains to be determined whether this region would also serve as adequate preparation of patients with...
other lethal congenital disorders of the hematopoietic system that do not affect the overall cellularity of the marrow, particularly since such patients may not suffer from the gross immunodeficiency that characterizes Wiskott-Aldrich syndrome. To pursue this issue, we have recently transplanted a patient with congenital osteopetrosis after preparation with busulfan and cyclophosphamide. In the year posttransplant, the child has enjoyed engraftment and progressive reconstruction of bone and intramedullary sites of hematopoiesis, comparable to that achieved with cyclophosphamide and total body irradiation.

REFERENCES

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Reconstitution of normal megakaryocytepoiesis and immunologic functions in Wiskott-Aldrich syndrome by marrow transplantation following myeloablation and immunosuppression with busulfan and cyclophosphamide

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