SUMMARY

We describe the successful use of HLA-compatible sibling bone marrow transplantation (BMT) in a 17-month-old Chinese boy in whom Wiskott-Aldrich syndrome (WAS) was diagnosed on the basis of eczema, thrombocytopenia, recurrent otitis media and abnormal immunological tests. The conditioning chemotherapy included 2 days' oral busulfan, 40 mg/m²/6 hours, and 2 days' intravenous cyclophosphamide, 60 mg/kg/day (BU2CY2). Complete hematological chimerism was achieved 3 weeks after transplantation. Eight months after his BMT the eczema has resolved, platelet count is normal, and he no longer has frequent infections. BU2CY2 as a pre-conditioning regimen gave complete lymphohematopoietic engraftment in this WAS patient with no evidence of graft-versus-host disease. The excellent clinical response of this patient and the inevitable fatal outcome of WAS support the opinion that where a histocompatible donor is available, BMT at the earliest opportunity is the best option. We believe this is the first case of successful BMT in a Chinese patient with WAS.
of 8 months. Persistent thrombocytopenia with recurrent bloodstreaked diarrhea and tarry stools were noted since the neonatal period. The platelet count was generally less than 60,000/mm³ without cycling, and platelet volume was reduced. The platelet-associated immunoglobulin G (PAIgG) level was normal. Bone marrow examination at the age of 8 months revealed no abnormality. Corticosteroids, intravenous immunoglobulin and danazol were unable to correct the platelet count. At least 3 episodes of otitis media occurred but serious infectious illnesses did not. Eczema predominating on the face, neck and back was also noted since early infancy. The family history of both parents, who were unrelated, was negative for bleeding disorders, eczema, or increased susceptibility to infection.

The immunologic findings in this patient are detailed in Table 1. Serum levels of IgG, IgA and IgM were elevated but isohemagglutinin was essentially absent. Intradermal skin tests for delayed-type hypersensitivity using Candida and PPD antigens were negative. Lymphocyte phenotyping at presentation was normal but reduced percentage of CD4⁺ cells with reversed CD4⁺/CD8⁺ ratio was subsequently noted. Lymphocyte responsiveness to each of three mitogens (concanavalin-A, phytohemagglutinin, and pokeweed mitogen) was within the normal range. Neutrophil function tests, including CD11b expression on leucocytes, adhesion, chemotaxis and NBT test were normal (data not shown). Immunological studies, hemogram, and measurements of

Table 1. Immune function studies before and after bone marrow transplantation.

<table>
<thead>
<tr>
<th>Lymphocyte subset *</th>
<th>Before BMT</th>
<th>50 days P BMT</th>
<th>198 days P BMT</th>
<th>238 days P BMT</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALC</td>
<td>2,136; 3,400</td>
<td>1,100</td>
<td>1,675</td>
<td>2,528</td>
<td>2,270</td>
</tr>
<tr>
<td>CD3⁺ T cells (%)</td>
<td>65; 57 (62-69)†</td>
<td>81 (62-69)</td>
<td>81 (62-69)</td>
<td>66 (62-69)</td>
<td>69 (66-76)</td>
</tr>
<tr>
<td>CD4⁺ T cells (%)</td>
<td>48; 23 (30-40)</td>
<td>16 (30-40)</td>
<td>34 (30-40)</td>
<td>31 (30-40)</td>
<td>31 (33-41)</td>
</tr>
<tr>
<td>CD8⁺ T cells (%)</td>
<td>13; 35 (25-32)</td>
<td>65 (25-32)</td>
<td>29 (25-32)</td>
<td>30 (25-32)</td>
<td>31 (27-35)</td>
</tr>
<tr>
<td>CD19⁺ B cells (%)</td>
<td>4; 3 (21-28)</td>
<td>3 (21-28)</td>
<td>8 (21-28)</td>
<td>16 (21-28)</td>
<td>14 (12-22)</td>
</tr>
<tr>
<td>CD3⁻CD16/56 NK (%)</td>
<td>36; 38 (8.0-15)</td>
<td>7 (8.0-15)</td>
<td>14 (8.0-15)</td>
<td>16 (8.0-15)</td>
<td>15 (9.0-16)</td>
</tr>
<tr>
<td>Serum immunoglobulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>2,510 (896±201)‡</td>
<td>1,610 (964±152)</td>
<td>1,160 (1083±163)</td>
<td>1,440 (1083±163)</td>
<td>1,630 (1,396±328)</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>1,510 (46±25)</td>
<td>189 (69±30)</td>
<td>110 (48±38)</td>
<td>146 (48±38)</td>
<td>163 (187±55)</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>572 (138±36)</td>
<td>109 (149±53)</td>
<td>106 (159±50)</td>
<td>109 (159±50)</td>
<td>154 (183±48)</td>
</tr>
<tr>
<td>IgE (IU/ml)</td>
<td>ND</td>
<td>18 (WNL)</td>
<td>ND</td>
<td>ND</td>
<td>46 (WNL)</td>
</tr>
<tr>
<td>Serum isohemagglutinin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-A</td>
<td>1.4 (+)</td>
<td>1.4 (+)</td>
<td>1.2 (+)</td>
<td>1.2 (+)</td>
<td>1.32 (+)</td>
</tr>
<tr>
<td>Anti-B</td>
<td>1.4 (+)</td>
<td>1.4 (+)</td>
<td>1.2 (+)</td>
<td>1.2 (+)</td>
<td>1.64 (+)</td>
</tr>
<tr>
<td>Mitogen response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con- A, PHA, PWM</td>
<td>WNL</td>
<td>ND</td>
<td>ND</td>
<td>WNL</td>
<td>WNL</td>
</tr>
<tr>
<td>DTH</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Candida</td>
<td>Negative</td>
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<td>ND</td>
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</tr>
<tr>
<td>PPD</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* Lymphocyte phenotyping before BMT was performed at 13 and 17 months old, respectively.
† Normal range for lymphocyte subsets.
‡ Reference level of serum immunoglobulins to patient's age in our laboratory, presented as mean±SD.
PBM T = Post bone marrow transplantation.
ALC = absolute lymphocyte count.
PHA = phytohemagglutinin.
PWM = pokeweed mitogen.
DTH = delayed-type hypersensitivity.
WNL = within normal limit.
ND = not determined.
Fig. 1  Post transplant course. After being conditioned with busulfan (40 mg/m²/6 hours on day -5 and -4) and cyclophosphamide (60 mg/kg/day on day -3 and -2), the patient received $5.64 \times 10^8$ nucleated marrow cells/kg from a HLA-identical sister. Transfusion of blood components and anti-GVHD medication are given as indicated.

Abbreviation: Bus, busulfan; CY, cyclophosphamide; BMT, bone marrow transplantation; G-CSF, granulocyte-colony stimulating factor; Cs-A, cyclosporin-A; MTX, methotrexate; PLT, platelet; RBC-T, red blood cells transfusion; WBC, white blood cells; Hct, hematocrit.
platelet size of the mother, elder sister and maternal uncle were essentially normal.

Tissue typing showed that a 9-year-old female sibling was HLA identical and bone marrow transplantation was planned. The patient was placed in protective isolation and given a diet low in bacterial content. Oral nonabsorbable antibiotics and antimicrobial skin care were administered daily. All blood products transfused were first irradiated. Conditioning before BMT was accomplished with busulfan 40 mg/m² orally every 6 hours on days -5 and -4; cyclophosphamide 60 mg/kg day on days -3 and -2 (BU2CY2). A dose of 5.64×10⁹ nucleated cells/kg of bone marrow from his histocompatible elder sister was infused on day 0. The donor and recipient are both of blood type O. Post-transplantation graft-versus-host disease (GVHD) prophylaxis consisted of methotrexate 15 mg/m²/day on day 1 then 10 mg/m²/day on days 3, 6, and 11; cyclosporin A 3 mg/kg/day IV since day -1. A single 600 mg/m²/8 hours and IVIG 400 mg/kg/day were administered weekly from day -5 for prophylaxis of infection. Bacitracin was prescribed to prevent Pneumocystis carinii pneumonia when the absolute neutrophil count reached 1,000/mm³. G-CSF was given from day 0 until the WBC count exceeded 10,000/mm³. Neutropenic fever developed on day 5 and empiric broad-spectrum antibiotics were given. A septic workup including blood, urine, and stool culture yielded no pathogen. Chest radiography was unremarkable. The febrile episode resolved promptly without complication. The posttransplantation course was otherwise uneventful.

Eczema resolved gradually over the subsequent 2 weeks, before the hemogram had fully recovered. Hematological data are summarized in Fig. 1. The peripheral blood neutrophil count improved gradually from day 11 and no platelet transfusion was needed after day 22. Karyotype analysis of bone marrow lymphocytes with PHA twenty days after transplantation revealed a female origin in 16 of 17 mitoses. The platelet count remained above 100,000/mm³ from day 36 after transplantation. Karyotype analysis of peripheral blood lymphocytes at this time revealed only female cells. The mean platelet volume normalized; it had been too broadly distributed to be measured by our laboratory before transplant (10.1 fl). Blood counts of all three series were normal within two months after the transplant.

Immunological studies of lymphocytes before and after transplantation are summarized in Table 1. Fifty days after transplantation, serum IgG and IgA levels were still elevated while IgM level was normal. IgG and IgA levels returned to nearly normal range about six months later. Isohemagglutinin titers appeared but remained depressed after eight months. Lymphocyte phenotyping 50 days after transplantation showed a reversed CD4⁺/CD8⁺ ratio, thought to be a transient manifestation of bone marrow recovery. The reversed ratio resolved six months later. A delayed-type hypersensitivity skin test 8 months after transplantation showed a positive response to Candida antigen.

**DISCUSSION**

The annual incidence of WAS in the United States was estimated to be 4.0 cases per million live male births. However, the incidence may be underestimated because of the common failure to diagnose WAS in patients with milder, variant forms of the syndrome. The genetic defect for WAS has been mapped to the Xp11.2 region, and carrier detection with the hypervariable M27-β probe is new feasible. Life expectancy in patients born before 1935 was 8 months and increased to 6.5 years in patients born after 1964. With the progress made during the last decade, the average age of living patients in Sullivan's series had increased to 11 years.

The genetic origin of WAS in our patient is unclear, since it is an X-linked disorder and the family history was negative. Female carriers of WAS are phenotypically normal because of selective inactivation of the X chromosome bearing the defective gene; it is therefore probable that the mother is a carrier of the WAS gene. A gene probe helps to identify female carriers; but was not performed in our case. Sporadic spontaneous mutations can not be excluded.

Lymphocytes from affected patients are deficient in a 115,000-dalton glycoprotein gp L115 (CD43), named sialophorin, which is characterized by its high content of sialylated O-linked disaccharide units and appears to play a role in lymphocyte activation and proliferation. Sialophorin deficiency is not the primary defect since the gene encoding CD43 was mapped to chromosome 16 and T cell lines from WAS patients do not express defects in CD43. The gene defective in the WAS may encode a protein that normally functions to maintain or regulate the blood cells' cytoskeletal structure because both peripheral lymphocytes and T cell lines exhibit severe morphological abnormalities.

Thrombocytopenia has been attributed to either rapid elimination of defective platelets or ineffective thrombopoiesis (dispersity between marrow substrate and circulating product). Splenic function is contributory to the production of the small WAS platelets because thrombocytopenia and mean platelet volume frequently improve within a few days of splenectomy. Evaluation of platelet function may be compromised by the number and volume of platelets. Ultrastructurally, platelets have abnormal
glycophosphatidylinositol (GPI) anchor, lack granules and mitochondria, and contain less than 60% of the calpain (Ca^2+–dependent neutral protease) content of normal platelets. The cytoarchitectural defect in WAS platelets may permit abnormal or accelerated microvesiculation and explain the reduction in platelet size.\(^\text{16,17}\) In our patient, thrombocytopenia presented with recurrent bloody diarrhea beginning during the neonatal period. Platelet size was small at that time. Frequent platelet transfusions before BMT resulted in a broad range of platelet sizes spanning the normal range. PAIgG levels during neonatal period and at 8 months old were normal and became markedly elevated before transplant. This, along with the failure of IVIG and corticosteroids to elevate the platelet count, exclude PAIgG as a major cause of thrombocytopenia. Repeated platelet transfusions were thought responsible for this change. Abnormal platelet size, PAIgG level, and platelet count all normalized after transplantation.

Immunologic abnormality and clinical course vary from one patient to another. Abnormalities of lymphocyte, neutrophil, monocyte, and platelet function have all been described in WAS.\(^\text{18–24}\) Reduced numbers of T cells, poor lymphocyte proliferative responses to mitogens, and impaired delayed hypersensitivity often can be demonstrated. In our case, lymphocyte classification at the age of 14 months was normal but decreased CD4^+ and increased CD8^+ with a reversed CD4^+/CD8^+ ratio was found immediately before BMT. The reversed CD4^+/CD8^+ ratio persisted until 6 months post-transplant. T-cell function is thought to be intact early in the disease and then declines progressively. Older patients particularly after 1 year of age, will generally have the quantitative immunoglobulin abnormalities typically associated with WAS. These are elevated IgA and IgE and depressed IgM. Our patient had elevated rather than low serum IgM in addition to increased IgG and IgA levels. However, isohemagglutinin levels were nearly absent, implying that immunoglobulin function was deficient. Serum immunoglobulin abnormalities progress with time to contribute to a severe and often fatal immunodeficiency.

Our patient’s eczema resolved soon after transplant, accompanying the initial recovery of peripheral blood cells. Abnormal T-lymphocyte function is thought responsible for eczema because eczema resolves only in patients who undergo BMT and establish T-lymphocyte grafts. However, the mechanism by which T-lymphocytes mediate eczema remains unknown.\(^\text{25}\)

Splenectomy and BMT were reported to reduce the incidence of bleeding per patient-year by 81% and 99%, respectively. These two treatment strategies are not directly comparable because BMT provides additional benefit by treating the immunodeficiency of WAS.

Both myeloablative and immunosuppressive therapy are necessary to allow complete engraftment.\(^\text{26}\) Cyclophosphamide supresses the immune capacity of patients to reject HLA-identical marrow but does not adequately reduce the recipient’s hematopoietic stem-cell population. Either TBI or busulfan can be used as ablative treatment. Busulfan and cyclophosphamide were used to prepare patients receiving histocompatible marrow successfully in 1979 to avoid the toxic effects of TBI, and thus allow BMT in very young WAS patients. Our patient was prepared with busulfan and cyclophosphamide and had no adverse effects during treatment and no difficulty in marrow engraftment.

The results of HLA-identical sibling BMT for WAS has been encouraging.\(^\text{27–30}\) Mullen et al.\(^\text{31}\) retrospectively analyzed the medical records of 69 WAS patients observed in the Metabolism Branch of the NIH Clinical Center during the years 1966 to 1992 and reported disease-free survival in twelve of 12 patients receiving HLA-matched sibling marrow. Only two of seven who had received either haploidentical parental or matched unrelated marrow had survived more than 1 year after BMT at the time of writing.\(^\text{31}\) Brochstein et al.\(^\text{32}\) had similar findings from a review of 17 patients having undergone allogeneic BMT between 1979 and 1989 at Memorial Sloan-Kettering Cancer Center. Long-term disease-free survival was observed in 10 of 11 patients receiving HLA-identical sibling transplants and in one of five who had received HLA-disparate parental marrow.\(^\text{32}\) The treatment of patients lacking an HLA-identical familial donor has been extended through the application of T-cell depleted marrow from haploidentical parental donors or closely matched unrelated individuals after a more immunosuppressive preparative regimen.\(^\text{33}\) The youngest of three patients reported by Rumelhart et al.\(^\text{6}\) was only 13 months old, and the patients had survived 332-895 days at the time of the report. Transplantation before severe clinical illness developed is recommended.

Because of the inevitable poor outcome in untreated WAS patients and the lack of any other curative treatment modality. BMT should be performed whenever an HLA-identical donor is available. It is now 8 months since transplantation and our patient’s eczema has resolved. He has made a complete hematological recovery and has improved immunologic function. Clinically, he is free of frequent infections, no longer has any bleeding tendency and has good growth and development. Our experience with this
patient strongly supports early BMT for WAS patients.

REFERENCES


