Asian Pacific Journal of Allergy and Immunology (1996) 14 : 57-63

Successful Bone Marrow Transplantation in a Chinese Boy with Wiskott-Aldrich Syndrome

Miao-Tsu Huang, Chen-Cheng Chou, Shiann-Tarng Jou, Kai-Hsin Lin and Kue-Hsiung Hsieh

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/m^2/6 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 preconditioning 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.

Wiskott-Aldrich syndrome (WAS) is characterized by the clinical triad of eczema, thrombocytopenia and variable immunodeficiency. WAS was first described by Wiskott in 1937 and later by Aldrich in 1954 as a sex-linked recessive disorder with draining ears, eczema and bloody diarrhea.[1] Affected males usually died within the first two decades of life as a result of infections, hemorrhagic or neoplastic complications. Management is directed toward ameliorating the acute episodes of infection and hemorrhage. Supportive treatments in the past included splenectomy and transfusion therapy, but were unsatisfactory.[2]

Bone marrow transplantation (BMT) for treatment of Wiskott-Aldrich syndrome (WAS) was first attempted in 1968, using a conditioning regimen of cyclophosphamide and cytarabine. It resulted in a T-lymphocyte graft but no hematopoietic engraftment.[3] The first BMT which achieved complete lymphohematopoietic engraftment for WAS patients was reported in 1978, after preparing the patients with anti-thymocyte serum and total body irradiation with/without procarbazine.[4] BMT is considered the only therapy capable of producing hematological and immunological reconstitution. It has been extended to include matched unrelated donors as well as HLA-haploidentical donors. Successful transplantations for WAS patients with such donors has been described recently.[5,6]

In this paper, we report a successful outcome in a 17-month-old Chinese boy with WAS who received an HLA-matched sibling donor marrow transplantation after being conditioned with cyclophosphamide and busulfan. Clearance of eczema, complete hematological engraftment and gradual immunological reconstitution were documented. Good growth and development were also achieved after transplanation.

CASE REPORT

A 17-month-old boy was diagnosed as having WAS at the age

From the Department of Pediatrics, College of Medicine, National Taiwan University Hospital, 7 Chung-Shan S. Road, Taipei, Taiwan.

Correspondence : Kai-Hsin Lin

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

	Before BMT	50 days P BMT	198 days P BMT	238 days P BMT	Donor
Lymphocyte subset*					
ALC	2,136 ; 3,400	1,100	1,675	2,528	2,270
$CD3^+$ T cells (%)	65 ; 57 (62-69)†	81 (62-69)	81 (62-69)	66 (62-69)	69 (66-76)
$CD4^+$ T cells (%)	48 ; 23 (30-40)	16 (30-40)	34 (30-40)	31 (30-40)	31 (33-41)
$CD8^+$ T cells (%)	13 : 35 (25-32)	65 (25-32)	29 (25-32)	30 (25-32)	31 (27-35)
$CD19^+$ B cells (%)	4 : 3 (21-28)	3 (21-28)	8 (21-28)	16 (21-28)	14 (12-22)
$CD3^-CD16/56$ NK (%)	36 : 38 (8.0-15)	7 (8.0-15)	14 (8.0-15)	16 (8.0-15)	15 (9.0-16)
Serum immunoglobulin					
IgG (mg/dl)	2,510 (896 ±201)‡	1,610 (964 ±152)	1,160 (1083 ±163)	1,440 (1,083 ±163)	1,630 (1,396 ±328)
IgA (mg/dl)	1,510 (46 ±25)	189 (69 ±30)	110 (48 ±38)	146 (48 ±38)	163 (187 ±55)
IgM (mg/dl)	572 (138 ±36)	109 (149 ±53)	106 (159 ±50)	109 (159 ±50)	154 (183 ±48)
IgE (IU/ml)	ND	18 (WNL)	ND	ND	46 (WNL)
Serum isohemagglutinin					
Anti-A	1:4 (+)	1:4 (+)	1:2 (+)	1:2 (+)	1:32 (+)
Anti-B	1:4 (+)	1:4 (+)	1:2 (−)	1:2 (−)	1:64 (+)
Mitogen response					
Con-A, PHA, PWM	WNL	ND	ND	WNL	WNL
DTH					
Candida	Negative	ND	ND	Positive	Positive
PPD	Negative	ND	ND	Negative	Positive

* 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.
PBMT = Post bone marrow transplantation.
ALC = absolute lymphocyte count.
con A = concanavalin-A.
PHA = phytohemagglutinin.
PWM = pokeweed mitogen.
DTH = delayed-type hypersensitivity.
WNL = within normal limit.
ND = not determined.

of 8 monhts. Persistent thrombocytopenia with recurrent blood-streaked diarrhea and tarry stools were noted since the neonatal period. The platelet count was generally less than 60,000/mm^3 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 CD11/18 expression on leucocytes, adhesion, chemotaxis and NBT test were normal (data not shown). Immunological studies, hemogram, and measurements of

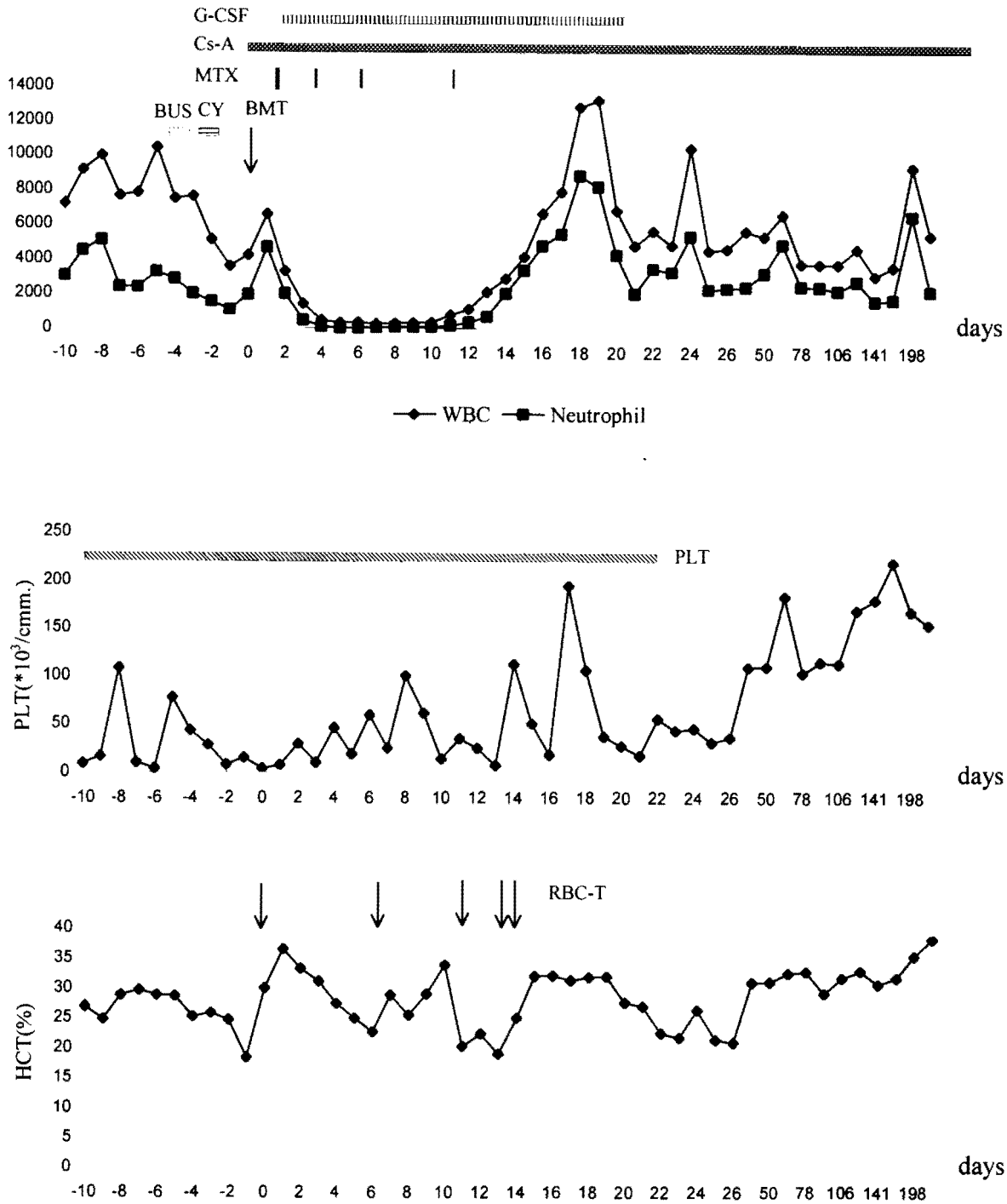

Fig. 1 Post transplant course. After being conditioned with busulfan (40 mg/m^2/6hours on day-5 and -4) and cyclophosphamide (60 mg/kg/day on day-3 and -2), the patient received 5.64 x10^8 nucleated marrow cells/kg from a HLA-identical sister. Transfusion of blood components and anti-GVHD medication 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/m^2 orally every 6 hours on days -5 and -4; cyclophosphamide 60 mg/kg day on days -3 and -2 (BU2CY2). A dose of 5.64×10^8 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/m^2/day on day 1 then 10 mg/m^2/day on days 3, 6, and 11; cyclosporin A 3 mg/kg/day iv since day -1. Acyclovir 500 mg/m^2/8 hours and IVIG 400 mg/kg/day were administered weekly from day -5 for prophylaxis of infection. Bactrim was prescribed to prevent *Pneumocystis carinii* pneumonia when the absolute neutrophil count reached 1,000/mm^3. G-CSF was given from day 0 until the WBC count exceeded 10,000/mm^3. 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 transplanation revealed a female origin in 16 of 17 mitoses. The platelet count remained above 100,000/mm^3 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.[7] The genetic defect for WAS has been mapped to the Xp11.2 region, and carrier detection with the hypervarible M27-β probe is new feasible.[8,9] 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.[10]

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 sialysated O-linked disaccharide units and appears to play a role in lymphocyte activation and proliferation.[11] 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.[12] 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 thrombocytopoiesis (disparity 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.[13-15] Evaluation of platelet function may be compromised by the number and volume of platelets. Ultrastructurally, platelets have abnormal

glycoprotein Ib, 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.[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.[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.[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.[26] Cyclophosphamide suppresses 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.[27-30] Mullen *et al.*[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.[31] Brochstein *et al.*[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.[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.[33] The youngest of three patients reported by Rumelhart *et al.*[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

1. Aldrich RA, Steinberg AG, Campbell DC. Pedigree demonstrating a sex-linked recessive condition characterized by draining ears, eczematoid dermatitis and bloody diarrhea. Pediatrics 1954; 13 : 133-8.
2. Standen GR. Wiskott-Aldrich syndrome : a multidisciplinary disease. J Clin Pathol 1991; 44 : 979-82.
3. Bach FH, Albertini RJ, Anderson JL, Joo P, Bortin MM. Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. Lancet 1968; 2 : 1364-6.
4. Parkman R,Rappeport J, Geha R, *et al.* Complete correction of the Wiskott-Aldrich syndrome by allogeneic marrow transplantation. N Engl J Med 1978; 298 : 921-7.
5. Lenarsky C, Weinberg K, Kohn DB, Parkman R. Unrelated donor BMT for Wiskott-Aldrich syndrome. Bone Marrow Transplantation 1993; 12 : 145-7.
6. Rumelhart SL, Trigg ME, Horowitz SD, Hong R. Monoclonal antibody T-cell-depleted HLA-haploidentical bone marrow transplantation for Wiskott-Aldrich syndrome. Blood 1990; 75 : 1031-5.
7. Perry GS, Spector BD, Schuman LM, *et al.* The Wiskott-Aldrich syndrome in the United States and Canada (1892-1979). J Pediatr 1980; 97 : 72-8.
8. Peacocke M, Siminovitch KA. Linkage of the Wiskott-Aldrich syndrome with polymorphic DNA sequences from the human X chromosome. Proc Natl Acad Sci USA 1987; 84 : 3430-3.
9. Goodship J, Carter J, Espanol T, *et al.* Carrier detection in Wiskott-Aldrich syndrome : combined use of M27β for X-inactivation studies and as a linked probe. Blood 1991; 77 : 2677-81.
10. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott-Aldrich syndrome. J Pediatr 1994; 125 : 876-85.
11. Shelley CS, Remold-O'Donnell E, Davis AE, *et al.* Molecular characterization of sialophorin (CD43), the lymphocyte surface sialoglycoprotein defective in Wiskott-Aldrich syndrome. Proc Natl Acad Sci USA 1989; 86 : 2819-23.
12. Molina IJ, Kenney DM, Rosen FS, Remold-O'Donnell E. T cell lines characterize events in the pathogenesis of the Wiskott-Aldrich syndrome. J Exp Med 1992; 176 : 867-74.
13. Lum LG, Tubergen DG, Corash L, Blaese RM. Splenectomy in the management of the thrombocytopenia of the Wiskott-Aldrich syndrome. N Engl J Med 1980; 302 : 892-6.
14. Corash L, Shafer B, Blaese RM. Platelet-associated immunoglobulin, platelet size, and the effect of splenectomy in the Wiskott-Aldrich syndrome. Blood 1985; 65 : 1439-43.
15. Weiden PL, Blaese RM. Hereditary thrombocytopenia : relation to Wiskott-Aldrich syndrome with special reference to splenectomy. J Pediatr 1972; 80 : 226-34.
16. Grottum KA, Hovig T, Holmsen H, Abrahamsen AF, Jeremic M, Seip M. Wiskott-Aldrich syndrome : qualitative platelet defects and short platelet survival. Br J Haematol 1969; 17 : 373-88.
17. Kenney DM, Reid R, Parent DW, Rosen FS, Remold-O'Donnel E. Evidence implicating calpain (Ca^{2+}-dependent neutral protease) in the destructive thrombocytopenia of the Wiskott-Aldrich syndrome. Br J Haematol 1994; 87 : 773-81.
18. Wolff JA. Wiskott-Aldrich syndrome : clinical, immunologic, and pathologic observations. J Pediatr 1967; 70 : 221-31.
19. Blaese R, Strober W, Brown RS, Waldmann TA. The Wiskott-Aldrich syndrome : a disorder with a possible defect in antigen processing or recognition. Lancet 1968; 1 : 1056-60.
20. Cooper MD, Chase HP, Lowman JT, Krivit W, Good RA. Wiskott-Aldrich syndrome : an immunologic deficiency disease involving the afferent limb of immunity. Am J Med 1968; 44 : 499-513.
21. Ochs HD, Slichter SJ, Harker LA, Von Behrens WE, Clark RA, Wedgwood RJ. The Wiskott-Aldrich syndrome : studies of lymphocytes, granulocytes, and platelets. Blood 1980; 55 : 243-52.
22. Lau YL, Jones BM, Low LCK, Wong SN, Leung NK. Defective B-cell and regulatory T-cell function in Wiskott-Aldrich syndrome. Eur J Pediatr 1992; 151 : 680-3.
23. Blaese RM, Strober W, Levy AL, Waldmann TA. Hypercatabolism of IgG, IgA, IgM, and albumin in the Wiskott-Aldrich syndrome. J Clin Invest 1971; 50 : 2331-8.
24. Inoue R, Konda N, Kuwabara N, Orii T. Aberrant patterns of immunoglobulin levels in Wiskott-Aldrich syndrome. Scand J Immunol 1995; 41 : 188-93.
25. Kue-Hsiung Hseih. Atopic dermatitis. Clin. Immunother 1995; 3 : 88-93.
26. Parkman R, Rappeport JM, Hellman S, *et al.* Busulfan and total body irradiation as antihematopoietic stem cell agents in the preparation of patients with congenital bone marrow disorders for allogeneic bone marrow transplantation. Blood 1984; 64 : 852-7.
27. Ochs HD, Lum LG, Johnson FL, Schiffmans G, Wedgwood RJ, Storb R. Bone marrow transplantation in the Wiskott-Aldrich syndrome. Transplantation 1982; 34 : 284-8.
28. Meuwissen HJ, Bortin MM, Bach FH, *et al.* Long-term survival after bone marrow transplantation : a 15-year follow-up report of a patient with Wiskott-Aldrich syndrome. J Pediatr 1984; 105 : 365-9.
29. Rimm IJ, Rappeport JM, Bone marrow transplantation for the Wiskott-Aldrich syndrome : long term follow-up. Transplantation 1990; 50 : 617-20.
30. Beard LJ, Toogood IRG, Pearson CC, Ferrante A. Early bone marrow transplantation in an infant with Wiskott-Aldrich syndrome. Am J Pediatr Haematol/Oncol 1991; 13 : 310-4.
31. Mullen CA, Anderson KD, Blaese RM. Splenectomy and/or bone marrow transplantation in the management of the Wiskott-Aldrich Syndrome : long-term follow-up of 62 cases. Blood 1993; 82 : 2961-6.
32. Brochstein JA, Gillio AP, Ruggiero M, *et al.* Marrow transplantation from human leucocyte antigen-identical or haploidentical donors for correction of Wiskott-Aldrich syndrome. J Pediatr 1991; 119 : 907-12.
33. Fischer A, Friedrich W, Fasth A, *et al.*

Reduction of graft failure by a monoclonal antibody (anti-LFA-1 CD 11a) and HLA nonidentical bone marrow transplantation in children with immunodeficiencies, osteopetrosis, and Fanconi's anemia : a European group for immunodeficiency/European group for bone marrow transplantation report. Blood 1991; 77 : 249-56.