Brief report

Stem cell transplantation with reduced-intensity conditioning for hemophagocytic lymphohistiocytosis

Nichola Cooper, Kanchan Rao, Kimberly Gilmour, Lema Hadad, Stuart Adams, Cathy Cale, Graham Davies, David Webb, Paul Veys, and Persis Amrolia

Allogeneic stem cell transplantation (SCT) is curative for hemophagocytic lymphohistiocytosis (HLH). However, patients frequently have significant morbidity before transplantation and there is high transplant-related mortality (TRM). Because first-degree HLH is caused by immune dysregulation, a reduced-intensity conditioned (RIC) regimen might be sufficient for cure while decreasing the TRM. Twelve patients with HLH underwent RIC SCT from a matched family/unrelated or haploidentical donor. Eleven were conditioned with fludarabine/melphalan with additional busulphan for haploidentical grafts. One received fludarabine and 2-Gy total body irradiation (TBI). All patients showed engraftment at a median of 14 days. Nine of 12 (75%) are alive and in complete remission (CR) a median of 30 months (range, 9-73 months) after SCT. Two patients died from pneumonitis and one from hepatic rupture. Four patients developed acute graft-versus-host disease (GVHD) and 3 have chronic GVHD. Three of 9 survivors have mixed chimerism but remain free of disease. In summary, RIC compares favorably to conventional SCT with long-term disease control in surviving patients despite a significant incidence of mixed chimerism. (Blood. 2006;107:1233-1236)

Introduction

Primary hemophagocytic lymphohistiocytosis (HLH) is a disease of childhood that presents with fever, hepatosplenomegaly, and pancytopenia.[1,2] It is characterized by a selective deficiency of cellular cytotoxicity with hyperactivation of T cells and macrophages.[3-5] A number of genetic abnormalities, including perforin and *hMUNC13-4* defects in 20% to 40% of patients and mutations in *SH2D1A*/*SAP* have been described,[6-11] suggesting that a deficiency of triggering apoptosis may be responsible for the pathologic features.

Patients may achieve remission with immunomodulation consisting of epipodophyllin and steroids with or without cyclosporin A.[10,12] However, the only curative option is allogeneic stem cell transplantation (SCT).[13,14] Patients often have significant pretransplant morbidity and require intensive cardiorespiratory support before SCT. This toxicity results in a high transplant-related mortality (TRM) with conventional intensity conditioning, mostly from noninfectious pulmonary toxicity and veno-occlusive disease (VOD). In the HLH-94 study the TRM was 30%,[15] with a 3-year overall survival rate of 71% following matched related donor transplantation, 70% for matched unrelated, 54% for mismatched unrelated donor, and 50% for those with a haploidentical donor.

We hypothesized that SCT with reduced-intensity conditioning (RIC) might be sufficient to restore normal immune regulation and hence cure HLH, while decreasing TRM. In support of this, the HLH-94 registry reports that 8 of 43 evaluable patients receiving transplants have mixed chimerism, and all remain disease-free at a median of 3 years after bone marrow transplantation (BMT), demonstrating that mixed chimerism is sufficient for long-term cure. Our experience in children with primary immunodeficiencies suggests that RIC may decrease the TRM from SCT.[16] We therefore investigated if this approach could be used in children with HLH.

Study design

Patients

Twelve consecutive patients with primary HLH at Great Ormond Street Hospital underwent SCT from an unrelated (fully matched unrelated donor [MUD], n = 5, one antigen–mismatched unrelated donor [MMUD], n = 3) or phenotypically matched family (n = 1) or haploidentical (n = 3) donor using RIC between June 1999 and November 2004. Three patients had underlying primary immunodeficiencies and one child had Down syndrome. Although patients were prospectively enrolled, there were no formal entry criteria. Patients were chosen based on the clinician's judgment that TRM was likely to be unacceptably high with conventional conditioning because of significant coexisting morbidity. Patient characteristics, including pre-SCT organ dysfunction are described in Table 1. Three patients presented with multiorgan failure requiring ventilation, 3 had central nervous system (CNS) involvement, 2 had hypertrophic cardiomyopathy, 4 had liver disease, and one had renal infiltration with HLH. Four of 9 patients assessed had a detectable perforin mutation. One further patient had no detectable mutation, but absent perforin expression and an additional patient had absent *SAP* expression, with no detectable mutation. Details of perforin mutations are included in Table 1.

All patients had previously received the HLH-94 protocol. Eight of 12 were in complete remission (CR) and 4 in partial remission (PR) before

From the Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom.

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Reprints: Persis Amrolia, Great Ormond Street Hospital for Children NHS Trust, Great Ormond St, London WC1N 3JH, United Kingdom; e-mail: amrolp1@gosh.nhs.uk.

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Table 1. Patient characteristics before transplantation

Patient no.	Family history	Other diseases	Perforin or SAP defect	Status at SCT	Organ toxicity before SCT
1	No	XLP	Absent SAP expression, no SAP or perforin mutation detected	CR	Hepatic HLH
2	No	Granulomatous disease	Absent perforin, perforin mutation (*dupc.667-692/Y296H*)	CR	Granulomatous skin disease, jaundice, cholangitis, multiple CNS lesions
3	No	Chediak-Higashi	—	CR	Pulmonary infiltrates requiring ventilation, probable HLH; multiple infections, renal HLH
4	No	None known	Perforin expressed, no perforin mutation, *munc 13-14* expressed	CR	Hepatic HLH, recurrent chest infection, pulmonary infiltrates possible HLH
5	No	Down syndrome	Perforin expressed, *munc 13-14* expressed	PR	Mild left ventricular hypertrophy, purpura fulminans, pulmonary infiltrates requiring ventilation, possible HLH
6	No	None known	SAP expressed, *munc 13-14* expressed	CR	Microcephaly, intracranial hemorrhage, developmental delay, multiorgan failure requiring ventilation, CNS HLH
7	No	Phenylketonuria	Absent perforin expression, no mutation found	CR	None known
8	No	None known	No perforin mutation	PR	Developmental delay, pancreatic insufficiency, partial villous atrophy, previous SVC and IVC thrombosis
9	No	None known	Absent perforin, perforin mutation (*L17X/C279G*)	CR	None known
10	Yes	None known	—	PR	CNS HLH
11	Yes	None known	Absent perforin, perforin mutation (*C393X/C393X*)	PR	Hypertrophic cardiomyopathy
12	No	None known	Absent perforin, perforin mutation (*L17X/L17X*)	CR	Hepatic HLH, pleural effusions, impaired renal function

SVC indicates superior vena cava; IVC, inferior vena cava.

SCT. The median age at transplantation was 14 months (range, 4-151 months). SCT was performed at a median of 6.5 months from diagnosis (range, 3-14 months).

Approval was obtained from the Great Ormond Street Hospital Ethics Committee for the use of the RIC regimen. Informed consent was provided according to the Declaration of Helsinki from all parents or next of kin.

Preparative regimen, transplantation, and supportive care

Eleven patients were conditioned with fludarabine 30 mg/m^2 for 5 days (days −7 to −3) and melphalan 140 mg/m^2 (day −2) in the patients receiving matched family/unrelated donor (MFD/MUD) transplants or 125 mg/m^2 (day −1) in the patients receiving haploidentical grafts. Patients receiving MFD/MUD transplants received serotherapy with alemtuzumab 0.2 mg/kg for 5 days (day −8 to −4) and those receiving haploidentical transplants received antithymocyte globulin (ATG) 5 mg/kg for 5 days (day −5 to −1) together with busulphan 4 mg/kg for 2 days (days −9 to −8) as additional myelosuppression. One patient, who was ventilated at the time of transplantation, received fludarabine 30 mg/m^2 for 3 days (day −4 to −2) and 2 Gy total body irradiation (TBI) in a single fraction. Grafts were T-replete marrow (for MFD/MUD) or peripheral blood stem cells (PBSCs for MMUD) and granulocyte colony-stimulating factor (G-CSF)–mobilized CD34 selected using the CliniMACs system (Miltenyi Biotec, Bisley, United Kingdom) for the haploidentical donors.

The median number of bone marrow mononuclear cells infused was 4.1×10^8/kg (range, $1.7\text{-}7.5 \times 10^8$/kg) in unrelated donors and the median of CD34$^+$ selected cells infused was 15.5×10^6/kg (range, $13.8\text{-}32.0 \times 10^6$/kg) in the haploidentical transplants. Graft-versus-host disease (GVHD) and antimicrobial prophylaxis were as described.[16]

Analysis of chimerism

Engraftment was assayed on the peripheral blood using variable numbers of tandem repeats (VNTRs) analysis with multiple informative alleles. Lineage-specific chimerism analysis was performed after selection of CD3/CD15/CD56/CD19$^+$ cells using the magnetically activated cell sorting (MACS) system (Miltenyi Biotech, Surrey, United Kingdom).

Results and discussion

Engraftment and chimerism

At a median of 14 days (range, 9-26 days), all patients had neutrophil engraftment and at a median of 16 days they had an unsupported platelet count more than 20×10^9/L (range, $4\text{-}34 \times 10^9$/L). All patients had 100% donor cells at engraftment. Three of the 9 survivors subsequently developed mixed chimerism. Lineage-specific chimerism was analyzed in mixed chimeras and is shown in Table 1. Chimerism was stable after 6 months following SCT. No patient has rejected the graft or relapsed.

Toxicity and survival

Three patients had severe mucositis; none developed VOD. Two patients developed pneumonitis. Six patients had cytomegalovirus (CMV) reactivation and 3 reactivated Epstein-Barr virus (EBV). Nine of 12 (75%) children are alive and in CR a median of 26 months from transplantation (range, 5-69 months). One patient each had TRM from CMV pneumonitis, multifactorial pneumonitis following T-cell sequestration and CMV disease on the background of previous pulmonary HLH, and hepatic rupture after transjugular liver biopsy.

GVHD

Four patients developed acute GVHD; 3 had grade 2 (skin and gut) and one had grade 4 (skin). Two patients have developed limited skin chronic GVHD and one has low-risk extensive chronic GVHD of the skin and gut.

Immune reconstitution

Eight patients are alive with more than 6 months of immunology follow-up. All have achieved normal T-cell function, as assessed by

Table 2. Outcome after transplantation

Patient no.	Type of transplant	Chimerism at 1 mo, %	Chimerism at last follow-up, % donor	Lineage chimerism, %				Chronic GVHD	Lansky score, %		Clinical status	Time from transplantation, mo
				CD3	CD56	CD15	CD19		Before SCT	After SCT		
1	MUD	100	100	—	—	—	—	No	60	100	CR	33
2	MUD	100	76	81	NT	64	NT	No	70	90	CR	30
3	MMUD	100	100	—	—	—	—	—	70	—	Died of pneumonitis	—
4	MMUD	100	100	—	—	—	—	Yes, skin	70	100	CR	25
5	MUD	100	100	—	—	—	—	Yes, skin	20	100	CR	32
6	MUD	100	100	—	—	—	—	—	40	—	Died from a hemorrhage	—
7	MUD	100	100	—	—	—	—	Yes, skin, gut	90	90	CR	18
8	Haplo	100	84	100	NT	79	56	No	40	90	CR	65
9	Haplo	100	100	—	—	—	—	—	100	—	Died of pneumonitis	—
10	Haplo	100	2	100	0	0	0	No	80	100	CR	73
11	MFD	100	100	—	—	—	—	No	50	100	CR	18
12	MMUD	100	100	—	—	—	—	No	90	100	CR	9

NT indicates not tested; —, not applicable (linear chimerism is not required for patients who are 100% donor, and CGVD and Lansky scores are not provided for patients who have died).

the phytohemagglutinin (PHA) stimulation index, a median of 7.5 months (range, 4-13 months) from SCT. Seven have normal age-adjusted CD3 counts a median of 8 months (range, 5-16 months) from SCT, and 8 have normal CD4 counts (median, 8 months; range, 6-16 months) from SCT. Two patients have normal B cells 8 and 13 months from SCT. Six have normal IgM levels. Patient 1 with X-linked lymphoproliferative (XLP) disease had decreased natural killer (NK) T cells ($CD3^+CD56^+$) before transplantation, which increased to normal levels after transplantation (2.9×10^5/L to 2.08×10^6/L), and remains in remission.

Quality of life

Nine of 12 patients are alive and in CR with Lansky scores of 90% to 100% a median of 30 months from SCT (Table 2).

Discussion

Our results with RIC SCT in patients with HLH and significant pre-SCT morbidity are encouraging. Although too small for statistical analysis, the overall survival data compare favorably with historical data, particularly for patients receiving mismatched transplants. In our cohort, 4 of 5 patients receiving cells from a MUD and 5 of 6 from an HLA-mismatched donor survive in CR, compared with corresponding figures of 70% and 52% in the HLH-94 study.[15] Viral reactivations were frequent, as has previously been reported with the immunosuppressive RIC,[16] but only one patient died of directly infectious causes.

Three surviving patients remain in long-term remission despite having mixed chimerism. All had high-level donor T-cell engraftment. Patient no. 10 is especially intriguing, in that he remains disease-free 6 years after SCT despite having only donor T-cell and not NK cell engraftment. These data suggest the presence of donor T cells is sufficient to restore normal immunoregulation and prevent hypercytokinemia and macrophage activation and that myeloablation is not critical for cure of HLH. We have not performed cellular cytotoxicity assays after SCT because the majority of our patients were 100% donor engrafted. In future studies, it would be of interest to assess T-cell and NK cell cytotoxicity in patients with mixed chimerism.

Although it is predicted that the incidence of end-organ damage, infertility, and growth retardation may be lower than with conventional intensity conditioning, demonstrating this will require longer-term follow-up. One particular concern is the potential for etoposide-associated therapy-related acute myeloid leukemia (t-AML) in patients with residual recipient myelopoiesis. However, data from patients not receiving transplants with EBV-associated HLH[17] have shown that this risk is very low and we believe it is likely to be even lower in patients undergoing SCT, who receive a much lower cumulative dose of etoposide.

Given these results, we would recommend RIC SCT in patients with severe organ toxicity before transplantation. Larger studies are indicated for standard-risk patients undergoing unrelated donor SCT. We have previously noted a higher incidence of rejection in patients with immunodeficiency receiving transplants from a matched sibling donor using the alemtuzumab/fludarabine/melphalan protocol, reflecting the reduced donor-versus-host alloreactivity. Given the risk of rejection (which may predispose to relapse[18]) in such patients and in those given a transplant from a haploidentical donor, we would recommend conventional intensity conditioning in these patients at present, unless precluded by significant pre-SCT morbidity.

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