CLINICAL RESEARCH

Myeloablative Transplantation using either Cord Blood or Bone Marrow leads to Immune Recovery, High Long-Term Donor Chimerism and Excellent Survival in Chronic Granulomatous Disease

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The curative potential of hematopoietic stem cell transplantation in patients with chronic granulomatous disease depends on availability of a suitable donor, successful donor engraftment, and maintenance of long-term donor chimerism. Twelve consecutive children (median age, 59.5 months; range, 8-140 months) with severe chronic granulomatous disease (serious bacterial/fungal infections pretransplantation; median, 3; range, 2-9) received myeloablative hematopoietic stem cell transplantation using sibling bone marrow ([SibBM]; n = 5), unrelated cord blood (UCB; n = 6), and sibling cord blood (n = 1) at our center between 1997 and 2010. SibBM and sibling cord blood were HLA matched at 6/6, whereas UCB were 5/6 (n = 5) or 6/6 (n = 1). Recipients of SibBM were conditioned with busulfan and cyclophosphamide ± anti-thymocyte globulin (ATG), whereas 6 of 7 cord blood recipients received fludarabine/busulfan/cyclophosphamide/ATG. Seven patients received granulocyte-colony stimulating factor-mobilized granulocyte transfusions from directed donors. The first 2 UCB recipients had primary graft failure but successfully underwent retransplantation with UCB. Highest acute graft-versus-host disease was grade III (n = 1). Extensive chronic graft-vs-host disease developed in 3 patients. All patients are alive with median follow-up of 70.5 months (range, 12-167 months) with high donor chimerism (>98%, n = 10; 94%, n = 1; and 92%, n = 1). Myeloablative hematopoietic stem cell transplantation led to correction of neutrophil dysfunction, durable donor chimerism, excellent survival, good quality of life, and low incidence of graft-vs-host disease regardless of graft source.

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INTRODUCTION

Chronic granulomatous disease (CGD), an inherited disorder of phagocytic function secondary to a loss or deficiency of phagocyte membrane proteins gp91phox, p22phox, p47phox, or p67phox, occurs in about 1 in 200,000 individuals and often presents with recurrent life-threatening bacterial and fungal infections and/or granulomatous manifestations. Additionally, many patients develop aberrant immune-mediated inflammatory responses that can result in colitis, urinary tract obstruction, chorioretinitis, gastric outlet obstruction, and chronic dysphagia [1]. Almost three-fourths of patients present before age 5 [2], with a wide spectrum of clinical phenotypes [3,4]. The use of prophylactic and aggressive antimicrobial therapy and interferon-gamma has led to significant improvements in survival and reduction in morbidity. However, patients with severe phenotypes will have major life-threatening infections during childhood leading to significant mortality and morbidity particularly from fungal infections like aspergillosis, which may account for one-third of all deaths. Furthermore, patients with CGD with severe functional phenotype (lowest oxidant production) who survive childhood face high mortality after 20 years of age, likely a result

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of cumulative organ injury from recurrent infection and CGD-related inflammatory disease [5].

Hematopoietic stem cell transplantation (HSCT) is currently the only curative therapy for patients with CGD whereby engrafted donor cells correct the neutrophil killing defect and immune deficiency by replacing abnormal nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-producing phagocytic cells. The long-term benefit of HSCT depends on sustained high levels of donor chimerism and continuous production of donor-derived cells for the rest of the recipient's life. Barriers to successful outcomes of HSCT in CGD include increased resistance to engraftment, existing comorbidities related to prior infections and granulomas, and increased risk of transplantation-related mortality. Additionally, access to transplantation and the availability of a suitable donor are equally important factors. Most of the published data on HSCT for CGD pertains to the use of bone marrow grafts from HLA-matched siblings [6,7]. However, matched sibling donors are only available for about 25% of patients. For the remainder, alternative graft sources must be found. A recent article describes good outcome in 7 patients undergoing unrelated bone marrow transplantation (BMT) after myeloablative conditioning [7]. However, availability of unrelated bone marrow donors is limited for many racial and ethnic minority patients. Unrelated umbilical cord blood donors offer the advantages of faster availability, higher probability of finding a suitable match, lower incidence of graft-versus-host disease (GVHD), and no risk to the donor [8-10]. This report underscores the success of myeloablative HSCT using cord blood and bone marrow grafts from related and unrelated donors to treat pediatric patients with severe CGD and highlights the importance of early transplantation, good infection control, and aggressive supportive care.

MATERIALS AND METHODS

Patients

Twelve consecutive pediatric patients with CGD, with histories of multiple infections and thus considered to have clinically severe disease, underwent HSCT after myeloablative conditioning at Duke University Medical Center between August 1997 and June 2010. All patients with CGD who underwent HSCT during this period were included in the study. All patients were enrolled in a Duke University Medical Center Institutional Review Board approved protocol or treatment plan for transplantation. Written informed consent was obtained for all patients according to the Declaration of Helsinki. Preliminary data on patients no. 5 and no. 7 were previously published [11].

Graft Sources

Sibling donors were genotypic matches at 6/6 or 10/10 HLA loci. Bone marrow was harvested from the iliac crest using standard methods on the day of transplantation. Bone marrow from ABO-mismatched donors were depleted of red blood cells and/or plasma as needed before infusion to the recipient. Marrows were not manipulated in any other way. Cord blood donors were typed at low resolution for HLA class I (A and B loci) and high resolution for HLA-DRB1. A minimum match of 4/6 (using low to intermediate resolution matching at HLA class I and high-resolution matching at HLA class II) was required for unrelated cord blood donor/recipient pairs. Cord blood donors were procured through the National Marrow Donor Program "Be the Match" registry. On the day of transplantation, cord blood units were thawed and washed [12] before infusion.

Conditioning Regimens

All patients were cytoreduced using fully myeloablative regimens. As summarized in Table 1, pretransplantation conditioning regimens were as follows: sibling bone marrow (SibBM) patients (n = 5) received intravenous (IV) busulfan (1 mg/kg/dose or 40 m^2/dose every 6 hours × 16 doses over 4 days), cyclophosphamide (50 mg/kg/dose) daily for 4 days +/− antithymocyte globulin (ATG), sibling cord blood (SibCB) (n = 1), and 5 of 6 patients with UCB received fludarabine (25 mg/m^2/dose, daily for 3 doses), IV busulfan (1 mg/kg/dose or 40 mg/m^2/dose every 6 hours for 4 days), cyclophosphamide (50 mg/kg/day for 4 days), and equine ATG (30 mg/kg/dose, daily × 3 days). Busulfan pharmacokinetics was studied after the first dose, and subsequent doses were adjusted to maintain a steady state of 600 to 900 ng/mL. Three patients underwent 2 transplantations each. One (patient no. 3) had graft failure after a matched, related donor, T cell-depleted graft after reduced-intensity conditioning (RIC) with fludarabine and cyclophosphamide at another institution. He received fludarabine, busulfan, cyclophosphamide, and ATG for his subsequent transplantation. The other 2 patients had received myeloablative conditioning and cord blood graft at our institution. Patient no. 5 had initially received busulfan, cyclophosphamide, and ATG as preparation for his first transplantation. For the second transplantation, he received a single dose of total body irradiation (200 cGy), cyclophosphamide (50 mg/kg/dose) for 1 dose and fludarabine (40 mg/m^2/dose, daily for 5 days). Patient no. 7 had received fludarabine, busulfan, cyclophosphamide, and equine ATG as conditioning for his first transplantation. His second transplantation conditioning regimen consisted of campath (anti-CD52 antibody; 0.9 mg/kg/day for 5 days), fludarabine (25 mg/m^2/dose, daily for

Table 1. Graft and Transplant Characteristics

Patient Number	Donor Source	HLA Match (of 6)	Total Nucleated Cell Dose ($\times 10^7$/kg)	GVHD Prophylaxis	Granulocyte Infusions	Conditioning Regimen
1	SibBM	6	79.4	CSA/MTX	No	Busulfan/cytoxan
2	SibBM	6	16.6	CSA/MTX	No	Busulfan/cytoxan
3a[a]	SibBM	6	NA	CSA/TCD	No	Fludarabine/cytoxan + eATG
3b	SibBM	6	40	CSA/MTX	No	Fludarabine/busulfan/cytoxan + rATG
4	SibBM	6	34.8	CSA/MTX	No	Busulfan/cytoxan
5a[a]	UCB	5	4.15	CSA/steroid	Yes	Busulfan/cytoxan + eATG
5b	UCB	5	4.14	CSA/steroid	Yes	TBI (200 cGy)/fludarabine/cytoxan
6	SibBM	6	55	CSA/MTX	No	Busulfan/cytoxan + eATG
7a[a]	UCB	5	8.5	CSA/MMF	Yes	Fludarabine/busulfan/cytoxan + eATG
7b	UCB	4	6.87	CSA/MMF	Yes	Campath/fludarabine/cytoxan
8	UCB	5	3.47	CSA/MMF	Yes	Fludarabine/busulfan/cytoxan + eATG
9	UCB	5	4.8	CSA/MMF	Yes	Fludarabine/busulfan/cytoxan + eATG
10	SibCB	6	3.2	CSA/steroid	Yes	Fludarabine/busulfan/cytoxan + eATG
11	UCB	5	12.6	CSA/MMF	Yes	Fludarabine/busulfan/cytoxan + eATG
12	UCB	6	11.2	CSA/MMF	Yes	Fludarabine/busulfan/cytoxan + eATG

GVHD indicates graft-versus-host disease; SibBM, sibling bone marrow; CSA, cyclosporine A; MTX, methotrexate; NA, not available; TCD, T cell depleted; eATG, equine ATG; rATG, rabbit ATG; UCB, unrelated cord blood; TBI, total body irradiation; MMF, mycophenolate mofetil; SibCB, sibling cord blood.

[a]Patients who underwent 2 transplantations.

5 days), and cyclophosphamide (30 mg/kg/dose, daily for 2 days).

GVHD Prophylaxis

GVHD prophylaxis (Table 1) consisted of standard cyclosporine A (CSA) with methotrexate on days 1, 3, 6, and 11 for all SibBM transplantations [13]; cord blood transplantation recipients received CSA with either 45 mg/kg/day mycophenolate mofetil (n = 5) or 1 mg/kg/day of methylprednisolone (n = 2). Methylprednisolone was preferred in patients who had been on prior steroids due to a history of colitis or other immune or inflammatory problems. In cord blood recipients, CSA was administered for 9 months and then tapered over 2 to 3 months, if there was no active GVHD. In SibBM, cyclosporine was tapered after 6 months. Methylprednisolone or mycophenolate mofetil was continued for 2 to 3 months in patients without ongoing GVHD. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were scored according to standard criteria [14,15].

Supportive Care Measures

Patients were admitted to reverse isolation rooms on a dedicated pediatric transplantation unit with positive pressure ventilation and high-efficiency particulate air filtration. Standard prophylaxis against viral pathogens and *Pneumocystis carinii* was used. Fungal prophylaxis was administered with low-dose amphotericin-B until 1999 and with voriconazole thereafter. Empiric broad-spectrum antibiotic therapy was started with the first fever. IV immunoglobulin (500 mg/kg per dose) was given weekly until day 100 and then "as needed" to maintain immunoglobulin levels greater than 500 mg/dL until discontinuation of GVHD therapy and documentation of antibody production. Veno-occlusive disease prophylaxis was continuous infusion heparin (100 units/kg/day) from day -10 to day +28. Patients received total parenteral nutrition and transfusions of leukocyte-depleted and irradiated packed red cells and platelets as needed. Granulocyte colony-stimulating factor (G-CSF) (10 mcg/kg/day) was given IV daily starting on day 0 and continued until a white blood cell (WBC) count of 5×10^9/L or higher was achieved.

Granulocyte Transfusions

G-CSF-mobilized, irradiated granulocyte transfusions were used during neutropenia to prevent serious infections in patients undergoing cord blood transplantations. Granulocyte donors were either parents or other family members who underwent medical and blood product donor clearance followed by the placement of a central venous line. G-CSF (10 mcg/kg) was given subcutaneously the night before pheresis, which was performed twice weekly, 3 to 4 days apart. Donors were supported with oral iron, vitamin K, and calcium supplements. Each pheresis product was irradiated to prevent engraftment and subsequently divided into 3 aliquots with a maximum dose of 5×10^9 cells/kg of recipient body weight. Granulocytes were plasma and/or RBC depleted if the blood types of the recipient, granulocyte donor, and stem cell donor were not compatible. Twice-weekly pheresis allowed daily granulocyte transfusions 6 days per week. Patients received granulocyte infusions until sustained engraftment with a WBC count over 5,000 and no active infections. Engraftment was evaluated based on rising WBC counts beginning the day after the patient did not receive granulocytes.

Follow-up

Patients underwent neutrophil functional assays, blood chimerism testing, immune function studies,

Table 2. Patient Characteristics

Patient Number	Age at HCT (months)	Gender	Mutation	Inheritance	Pretransplantation Infections[a]
1	8	F	Unknown	AR	Colitis, bronchiolitis, thrush
2	25	M	Unknown	AR	Diffuse Candida peritonitis, thrush
3	56	M	gp91^{phox}	X-linked	Multiple pneumonias, apthous ulcers
4	17	M	Unknown	AR	Burkholderia pneumonia
5	122	M	gp91^{phox}	X-linked	Pneumonias, lung aspergilloma, multiple osteomyelitis, salmonella gastroenteritis
6	109	M	p22^{phox}	AR	Serratia osteomyelitis, mycobacterium skin nodules, resistant lung acid-fast bacilli, bladder granulomas
7	18	M	gp91^{phox}	X-linked	Staphylococcal skin infection, lymphadenitis, pulmonary nodules
8	100	M	gp91^{phox}	X-linked	Kidney granulomas, pneumonia, colitis
9	140	M	gp91^{phox}	X-linked	Nocardia pneumonia, gut mucormycosis, sinusitis, colitis
10	73	M	gp91^{phox}	X-linked	Klebsiella abscess, pulmonary nodules, multiple pneumonias, gingivitis, colitis
11	14	M	gp91^{phox}	X-linked	MRSA liver abscess, *Staphylococcus aureus* neck abscess, mediastinal cyst
12	46	M	gp91^{phox}	X-linked	Serratia abscess, apthous ulcers

HCT indicates hematopoietic cell transplantation; AR, Autosomal Recessive; X-linked, X Chromosome linked; MRSA, methicillin-resistant *Staphylococcus aureus*.

[a]Patients had a median of 3 serious infections (range, 1-6) by the time they underwent stem cell transplantation.

and other routine posttransplantation multisystem evaluations at day 100, 6 months, 9 months, 12 months, and then yearly posttransplantation or whenever clinically indicated.

Statistics

Descriptive analyses were conducted using patient data obtained through July 2011. Patient listings are provided as are time-to-event analyses. The Kaplan-Meier estimator was used to describe overall survival (OS) and event-free survival (EFS) [16]. OS considered a patient death as the event and censored on the last date of contact, whereas EFS considered either graft failure or death as the event. The cumulative incidence estimator [17] was used to describe the probability of an event occurrence such as neutrophil engraftment, platelet engraftment, or GVHD. Engraftment was defined as the first day of an absolute neutrophil count of $>0.5 \times 10^9$/L for 3 consecutive days not secondary to granulocyte infusions, and platelet engraftment was defined as platelet counts $>20 \times 10^9$/L or $>50 \times 10^9$/L for 7 consecutive days without platelet transfusions. For patients receiving granulocytes (days 1-6 of the week), engraftment was determined on the basis of absolute neutrophil count on the day after the "off" day for granulocyte transfusion. Intra- and posttransplantation complications, respiratory burst assays, and performance status were evaluated using descriptive measures.

RESULTS

Patient Demographics

All patients were diagnosed by respiratory burst assays. Mutation analysis data was available for 9 of 12 patients. Patient characteristics are presented in Table 2. Eight patients had the X-linked Gp91phox mutation of the *CYBB* gene; the remaining 4 had autosomal recessive inheritance. Eleven patients were men, and 8 were white. Three patients were cytomegalovirus (CMV) seropositive pretransplantation. There were 2 sets of affected siblings within the cohort. The patients underwent transplantation at a median age of 59.5 months (range, 8-140 months) and weighed a median of 16.9 kg (range, 9-60 kg). Their weight-for-age percentile ranged from <5th to the 75th. However, 5 patients were below the 5th percentile at the time of transplantation. At the time of transplantation, all patients had a performance status score of 80 to 100 (Lansky) with cardiac, lung, and renal function in the normal range. One patient had a history of urethral polyps, and 4 of 12 patients had a history of extensive colitis requiring treatment with systemic corticosteroids before transplantation.

Pretransplantation Infections

All patients had suffered multiple episodes of serious bacterial and/or fungal infections before transplantation (Table 2). These infections included Candida peritonitis, *Klebsiella* abscess, hepatic abscess, gut mucormycosis, peritonitis, osteomyelitis, and multiple pneumonias. One patient presented with a large invasive lung and chest wall aspergilloma. This patient required treatment with interferon-gamma, voriconazole and caspofungin, and granulocyte transfusions for almost a year to successfully control his aspergilloma before proceeding to HSCT. One pair of siblings (patients no. 8 and no. 9) had histories of fungal pneumonias partially treated with fluconazole, itraconazole, and posaconazole at the time they presented for transplantation. Patient no. 10 presented with a long history of pulmonary nodules, which had been previously biopsied. At presentation to our institution, he had multiple areas of scarring and ground-glass appearance before transplantation. Infections were

Table 3. Transplantation Outcomes

Patient Number	Neutrophil Engraftment Day	Platelet Engraftment Day	Follow-up Duration (years)	aGVHD Grade	aGVHD Location	cGVHD	Transplant Complications	Donor Chimerism
1	18	40	13.9	0		None	None	98%
2	20	59	13.5	0		None	Coagulase negative Staphylococcal bacteremia	98%
3	16	33	8	2	Gut	Limited skin	None	94%[a]
4	11	39	7	0		None	*Streptococcus viridans* bacteremia, parainfluenza 3	98%
5	32	74	6.33	3	Skin	Extensive gut & skin	Autoengrafted initial transplantation, urine polyoma virus, ITP	>98%[a]
6	16	40	6.5	1	Skin	None	Pancreatitis	>98%
7	16	-	5.4	1	Skin	None	Autoengrafted initial transplantation	>98%[a]
8	29	170	3.6	2	Skin	Extensive gut & skin	Gram-positive cocci bacteremia, stool *C. difficile* infection, bladder granuloma, pericardial effusion	>98%
9	43	36	3.6	2	Skin	Extensive skin	CMV viremia, urine polyoma virus, ITP	>98%
10	29	72	2	1	Skin	None	CMV viremia, urine polyoma virus infection, idiopathic pneumonia syndrome	92%
11	18	70	1.4	2	Skin	None	ITP	>98%
12	19	43	1	2	Skin	None	Parainfluenza 3	98%

aGVHD indicates acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; ITP, Immune Thrombocytopenic Purpura; CMV, cytomegalovirus.

[a]Chimerism after second transplantation, patient no. 7 did not have platelet aplasia during his second transplantation.

adequately treated before transplantation, and no patient had a clinically active infection at the time of starting cytoreduction. All patients had failed prophylaxis with antibiotics and 8 of 12 had also failed prior treatment with interferon.

Graft Characteristics

Graft sources (Table 1) were matched SibBM in 5, unrelated donor cord blood (UCB) in 6, and SibCB in 1 patient. SibBM and SibCB donors were genotypic HLA matches at 6/6 or 10/10. UCB units for the first transplantation matched at 5/6 (n = 5) or 6/6 (n = 1) loci using low-resolution typing for HLA-A and HLA-B and high-resolution typing for HLA-DRB1. The HLA matching of UCB units used for second transplantation were 4/6 (n = 1) and 5/6 (n = 1). The cord blood units contained a median pre-cryopreservation total nucleated cell dose of 4.87×10^7 cells/kg (range, 3.2-12.6 $\times 10^7$ cells/kg) and a median CD34 dose of 2.0×10^5 cells/kg (range, 1.1-5.0 $\times 10^5$ cells/kg). SibBM contained a median total nucleated cell dose of 4.03×10^8 cells/kg (range, 1.66-7.94 $\times 10^8$ cells/kg) and a median CD34 dose of 6.02×10^6/kg (range, 3.86-22.71 $\times 10^6$ cells/kg). ABO mismatched bone marrows underwent red cell and/or plasma depletion and were washed before transplantation.

Survival and Quality of Life

At the time of the analysis, all patients were alive and disease free with a median follow-up of 70.5 months (range, 12-167 months) as shown in Table 3. No new infection episodes were seen in any patient after 8 months posttransplantation. All but 1 patient with cGvHD (Lansky 60) have Lansky scores of 80 to 100. All school-age children returned back to full-time school within 18 months after transplantation.

Engraftment

All patients engrafting after their first transplantation achieved neutrophil engraftment by day 43 post-first transplantation with a cumulative incidence of 83.3% (95% confidence interval [CI], 55.2-100.0; Figure 1). Two patients failed to engraft with their first transplantation but engrafted after a second transplantation that was performed on days 62 and 63 post-first transplantation, respectively. In the patients engrafting after the first transplantation, neutrophil engraftment occurred in a median of 16 days (range, 11-20 days) in bone marrow and 29 days (range, 16-43 days) in cord blood recipients. Overall, the median time to neutrophil engraftment was 20 days with a range of 11 to 43 days. When considering graft failure as an event, the 6-month EFS probability was 83.3% (95% CI, 48.2% to 95.6%).

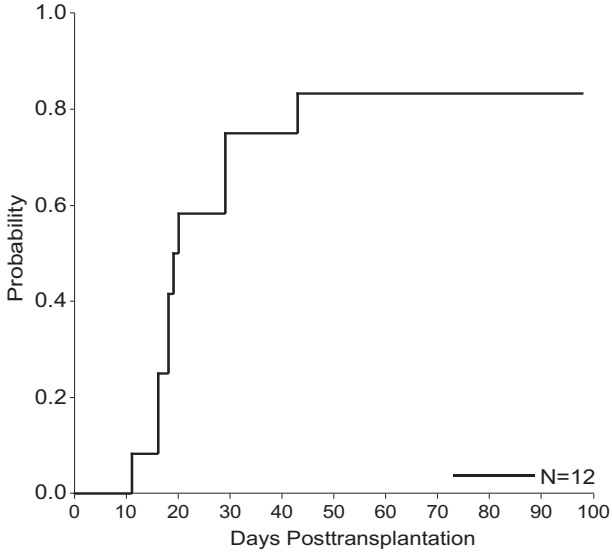

Figure 1. Cumulative incidence of neutrophil engraftment to 0.5 $\times$ 10^9/L after the first transplantation.

All patients engrafting after the first transplantation achieved platelet engraftment >20,000 $\times$ 10^9/L (20K) and >50,000 $\times$ 10^9/L (50K) within 180 days. Platelet engraftment >20K occurred in a median of 34 days (range, 26-160 days) in the whole group; 31.5 days (range, 29-54 days) in the bone marrow group, and 42.5 days (range, 26-160 days) in the cord blood group, respectively. Platelet engraftment >50K occurred in a median of 43 days (range, 33-170 days) in the whole group; 40 days (range, 33-59 days) in the bone marrow group, and 71 days (range, 36-170 days) in the cord blood group, respectively. Two UCB transplantation recipients had graft failure as described earlier, but both engrafted successfully after the second UCB transplantation [11].

Granulocyte Transfusions

Seven patients underwent granulocyte transfusion in the posttransplantation period. The median number of transfusions per patient was 24 (range, 12-36) with a median duration of 4 weeks (range, 2-6 weeks).

GVHD

AGVHD (Figure 2A) developed in 9 patients and was limited to the skin in 8 (grade 1 in 3 patients and grade 2 in 5 patients). The overall cumulative incidence estimate by day 100 of grade II to IV aGVHD was 33.3% (95% CI, 7.3-59.3). Grade III aGVHD of the gut developed in 1 patient who had undergone a second transplantation. Five patients developed cGVHD, 2 limited to the skin and 3 with extensive disease. One of these patients initially developed extensive skin and gut cGVHD, which progressed to a macrophage-activation-like syndrome with multifocal arthritis, fevers, and cytopenia. He responded to interleukin-1 receptor antagonist therapy. The cumulative incidence estimate of overall cGVHD at 2 years was 33.3% (95% CI, 7.3-59.3) and of extensive cGVHD was 26.7% (95% CI, 1.8-516; Figure 2B). Three patients developed immune thrombocytopenia in the posttransplantation period. They were successfully treated, 2 with steroids and rituximab and 1 with steroids alone.

Infections and Other Posttransplantation Complications

Two patients (no. 9 and no. 10) had CMV reactivation requiring treatment, 3 patients (no. 2, no. 4, and no. 8) had uncomplicated gram-positive bacteremia, 3 patients (no. 5, no. 9, and no. 10) had urine polyoma virus infections, 1 patient (no. 6) had mild pancreatitis,

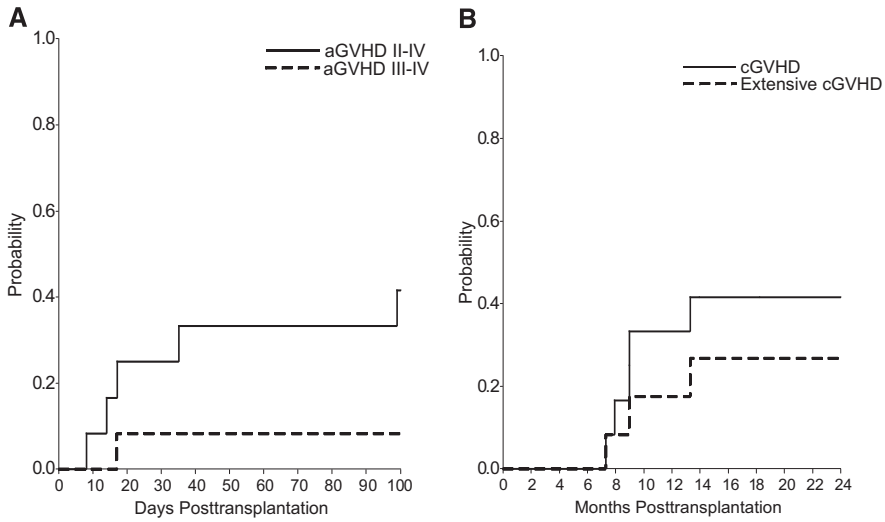

Figure 2. (A) Cumulative incidence of grades II to IV and III to IV acute graft-versus-host disease (GVHD). (B) Cumulative incidence of overall chronic GVHD (cGVHD) and of extensive chronic GVHD.

1 patient (no. 8) had pericardial effusion requiring pericardiocentesis, and 1 patient (no. 10) required treatment for idiopathic pneumonia syndrome. Patient no. 10 with idiopathic pneumonia syndrome, is currently 18 months posttransplantation, physically active with no oxygen requirement, and has normal lung function tests. Pericardial fluid in patient no. 8 was exudative in nature and negative by PCR for HHV-6, EBV, CMV, adenovirus, parvovirus B-19, and AFB smear, bacterial, viral, and fungal cultures. One patient developed macrophage-activation-like syndrome, as described in the previous section. No patient developed any new infections after 8 months posttransplantation.

Chimerism

By day 100, all patients had >90% donor chimerism, and at their most recent follow-up, donor chimerism in whole blood was >98% in 10 patients, 94% in 1 patient (no. 3), and 92% in another patient (no. 10). At the last follow-up, all patients had normal respiratory burst assays and/or dihydrorhodamine fluorescence cytometry and were free of infection.

DISCUSSION

Results from our cohort of patients with severe CGD undergoing HSCT showed that myeloablative HSCT is successful and results in long-term survival, low incidence of GVHD, freedom from infections, and improved quality of life if performed early in the course of the disease and with aggressive supportive care. Most of the published data describe the use of related or unrelated bone marrow in patients who underwent transplantation at multiple centers. Our study involves a consecutive series of patients including 7 with cord blood grafts who underwent transplantation at a single center utilizing uniform supportive care and cytoreduction.

Soncini et al. [18] reported the outcome in 20 patients who underwent transplantation between 1998 and 2007 using matched SibBM (n = 9), matched SibCB (n = 1), unrelated matched bone marrow (n = 6), unrelated matched peripheral blood stem cell (PBSC; n = 2), mismatched PBSC (n = 1), and mismatched UCB (n = 1). Four patients underwent the RIC regimen. Overall survival was 90%, and 2 patients required a subsequent bone marrow boost to maintain adequate neutrophil oxidative burst. The 2 patients that did not survive underwent RIC conditioning and died from disseminated fungal infections [18]. Schuetz et al. [19] reported the outcomes of 12 patients with CGD, 6 of whom underwent transplantation with matched unrelated bone marrow, 3 with matched PBSC, and 3 with matched SibBM, 9 received myeloablative conditioning, and 3 received RIC. OS was 75%, with 67% survival in the group undergoing myeloablative conditioning. In addition, 1 of the 3 patients undergoing transplantation with RIC had autologous recovery [19]. In a retrospective registry-based study of 27 patients, Seger et al. [20] reported the European experience from 1985 to 2000 from 14 centers. Twenty-three patients had received myeloablative, and 4 had received RIC. The OS at a median follow-up of 24 months was 92% and 75% in the myeloablative and reduced-intensity cohorts, respectively. It is crucial to note that only half of the patients receiving RIC had successfully engrafted. Martinez et al. [7] reported excellent outcomes of 11 children who underwent transplantation at a single center. With 7 receiving unrelated matched bone marrow and 4 receiving matched SibBM, all patients are alive at a median of 2.5 years, and 9 of the 11 patients achieved full-donor chimerism [7]. Güngör et al. [21] reported the outcomes of 11 patients receiving RIC using busulfan at a total dose of 8 mg/kg, fludarabine at a total dose of 180 mg/m^2 and 4 days of ATG, undergoing bone marrow (n = 10) or PBSC (n = 1) transplantations. There was 1 death in that cohort [21]. Outcomes of our cohort as measured by OS and sustained donor chimerism compare favorably with the published reports of HSCT using matched sibling, unrelated bone marrow, as well as unrelated PBSC in patients with CGD.

Patients with CGD are prone to graft failure after HSCT, which may largely be explained by exaggerated immune responses [22]. The first reported transplantation for CGD developed graft rejection 2 months posttransplantation [23]. Prior infections and resultant organ dysfunctions may also increase transplantation-related complications, particularly if patients undergo transplantation later in the course of their disease. In recent years, there has been significant interest in RIC as an option to decrease chemotherapy-related toxicity and limit transplantation-related mortality and morbidity. However, there has been limited success with this modality. In a study published in 2001, 10 patients with CGD were treated with T cell-depleted, matched-sibling PBSC transplantations. With a median follow-up of 17 months (range, 8–26 months), 1 patient had graft failure, 1 had graft rejection, and there were 3 fatalities. Whereas 7 of 10 patients in this cohort were alive at the time of publication, the rate of high (>90%) donor chimerism in the myeloid, as well as lymphoid fractions was low [24]. Various investigators have considered the use of RIC to decrease the short- and long-term toxicity of transplantation. In the European cohort, 3 of 4 patients who underwent RIC transplantations developed graft failure [20] with 1 patient recovering donor chimerism after donor leukocyte infusion. With the success of HSCT in CGD critically dependent on achieving a high level of donor chimerism and maintaining it over the long term, limited experience from RIC-HSCT suggests that

lower immunosuppression in this context is associated with a higher incidence of graft failure.

All patients with severe CGD are at high-risk regimen-related toxicity because of previous infections, altered and hyperactive immune system, and organ dysfunctions. Despite the young age of our patient cohort, all had developed multiple serious infections before being referred for transplantation. These infections required hospitalizations, surgery, and prolonged antimicrobial therapy. We elected to support some of these patients with supplemental transfusions of irradiated, directed donor G-CSF mobilized granulocytes to help reduce or control infections during their aplasia in the first month posttransplantation. This approach may have contributed to improved outcomes, particularly in recipients of cord blood grafts.

The negative impact of pretransplantation infections on transplantation outcomes was highlighted by Segar et al. [20] in a registry-based European Group for Blood and Marrow Transplantation retrospective study of 27 BMT performed between 1985 and 2000 at 14 centers [20]. Sixteen patients were considered high risk due to either disseminated infection or significant pulmonary restrictions. Four of the 16 underwent RIC. The disease-free survival rate in the high-risk group was 69%, and the OS was 75%. No patient died in the low-risk group, whereas in the high-risk group, 4 patients died, and 3 had autologous recovery [20]. It may be possible to lower regimen-related toxicity by performing transplantations early in the course of the disease before repeated infections and cumulative effects of an overactive immune system have taken a toll on various organ systems and pushed the patients in the "high-risk" category. The outcomes could also be improved by providing excellent supportive care including optimization of pretransplantation infection control, aggressive management of intra transplantation infections, and the use of granulocyte transfusion during the neutropenic period for select patients. For example, patient no. 5, a 10-year-old boy who presented with a visible deformity of his chest wall secondary to a large active aspergilloma, was biopsied and then aggressively treated with caspofungin, voriconazole, and interferon-gamma, followed by granulocyte infusions during the transplantation period. He successfully underwent transplantation and is currently over 6 years posttransplantation and disease free. Additionally, the impact of pulmonary issues on the outcomes is particularly important. Lungs are frequently diseased in patients with CGD due to repeated bacterial and fungal infections, noninfectious granulomas, and sometimes from the impact of surgeries. Most of these complications increase with age, and it is likely that a younger patient with CGD will, in general, have healthier lungs. High rate of survival and low incidences of complications in our patients reflects transplantation at a younger age, high

Table 4. Summary of Current Series and Published Data on Bone Marrow Transplantations for CGD

Source	Center or Group	Donor Source (no. of patients)	Conditioning (no. of patients)	Median Follow-up (months)	OS %	Engraftment %	Patients Achieving High (>90%) Donor Chimerism
Tewari, 2012	Duke University Medical Center	R-M-BM (5)	MAC	64	100	100	100
Martinez, 2012	Texas Children's Hospital	R-M-BM (4), UM-BM (7)	MAC	2.5	100	100	81
Goździk, 2011	University Children's Hospital Cracow	R-M-BM (2), U-M-BM (3), U-MM-BM (1)	MAC (5), RIC (1)	Mean 20	100	100	100
Soncini, 2009	UK Northern Supra Regional HSCT Unit	R-M-BM (9), U-M-BM (6), U-M-PBSC (2), U-MM-PBSC (1)	MAC (14), RIC (4)	61	88 MAC (100), RIC (50)	MAC 100 RIC 75	NA
Schuetz, 2009	University Hospital Ulm	R-M-BM (3), U-M-BM (6), U-M-PBSC (3)	MAC (9), RIC (3)	53	75 MAC (67), RIC (100)	MAC 100 RIC 66	92
Güngör, 2005	University Children's Hospital of Zurich	M-U-BM (1), M-R-BM (2)	RIC	17	100	100	100
Seger, 2002	EBMT	R-M-BM (25), U-M-BM (2)	MAC (23), RIC (4)	24	85 MAC (92), RIC (75)	MAC 95 RIC 50	85
Horwitz, 2001	National Institutes of Health, Bethesda	R-M-BM (10)	RIC	17	70	80	60

OS indicates overall survival; R-M-BM, related matched bone marrow; MAC, myeloablative conditioning; U-M-BM, unrelated matched bone marrow; U-MM-BM, unrelated mismatched bone marrow; RIC, reduced-intensity conditioning; HSCT, hematopoietic stem cell transplantation; U-M-PBSC, unrelated matched peripheral blood stem cells; U-MM-PBSC, unrelated mismatched peripheral blood stem cells; NA, not available; EBMT, European Group for Blood and Marrow Transplantation.

Table 5. Summary of Current Series and Published Data on CBT for CGD

Source	Center or Group	Donor Source (no. of patients)	Conditioning	Median Follow-up (months)	OS (%)	Engraftment (%)	Patients Achieving High (>90%) Donor Chimerism
Tewari, 2012	Duke University Medical Center	U-M-CB (5), U-MM-CB (1), R-M-CB (1)	MAC	44	100	100	100
Jaing, 2010	Chang Gung Children's Hospital	U-MM-CB (1)	MAC	8	100	100	100
Goussetis, 2010	Aghia Sofia Children's Hospital	R-M-CB (2)	MAC	15.5	100	100	100
Soncini, 2009	UK Northern Supra Regional HSCT Unit	R-M-CB (1), U-MM-CB (1)	MAC	61	100	100	50
Mochizuki, 2009	Fukushima Medical University School of Medicine	U-MM-CB (1)	RIC	36	100	100	100
Suzuki, 2007	Sapporo Medical University School of Medicine	U-M-CB (1)	RIC	14	100	100	100

CBT indicates cord blood transplantation; CGD, chronic granulomatous disease; OS, overall survival; U-M-CB, unrelated matched cord blood; U-MM-CB, unrelated mismatched cord blood; R-M-CB, related matched cord blood; MAC, myeloablative conditioning; HSCT, hematopoietic stem cell transplantation; RIC, reduced-intensity conditioning.

pretransplantation Lansky scores, near-normal organ function in most patients, and implementation of aggressive supportive care measures. While it is understandable that physicians and parents may be reluctant to refer children who seem well for a complex therapy like HSCT, our data suggest that early transplantation when organs are still in a good functional state is optimal and can lead to excellent outcomes.

Most of the published data on HSCT in CGD involves the use of matched-SibBM (Table 4) [18-20,24-26]. However, the probability of finding a matched sibling donor in most populations is about 25%, and in fact, even lower within siblings of patients with genetic diseases. For the remainder of patients, an alternate donor source including unrelated bone marrow, haploidentical related donor, or UCB units should be found. The reported survival and long-term donor chimerism after unrelated BMT and PBSC is relatively lower [27], and there are no published data on haploidentical transplantation in CGD. Published data on the use of cord blood as a graft source in CGD is limited to a total of 7 patients in 5 case reports describing 3 unrelated and 4 related cord blood transplantions (CBT) [18,28-32] (Table 5). It is certainly possible that other cases of CBT with poor outcomes have not been reported in the literature. Various cytoreductive regimens and GVHD prophylaxis were used in the published reports. In contrast, our cohort consists of 6 unrelated and 1 related CBT patient who underwent transplantation using a uniform regimen and, to our knowledge, is the largest cohort of CBTs for CGD. It is important to note that all CBT recipients are alive and well with high donor chimerism. Despite small numbers, our study highlights the potential of using this valuable graft source to achieve long-term success. We believe that the use of unrelated UCB is a feasible option for patients lacking matched sibling donors.

CGD has a wide clinical spectrum ranging from patients with trivial, infrequent infections to those with repeated life-threatening infections early in life. This clinical variability must be taken into consideration when making therapeutic decisions regarding the use of prophylactic antibiotics, various supportive care measures, interferon, and HSCT. Despite extensive laboratory research, gene therapy is currently not available for clinical use leaving HSCT as the only curative option. Criteria for identifying appropriate transplantation candidates, in particular those that are early in the disease course, are yet to be developed. Our data suggest that patients with severe phenotype CGD who develop early immunologic or inflammatory problems or multiple serious infections should undergo transplantation early. In this report, we present evidence that HSCT using a variety of donor and graft sources including UCB units after myeloablative conditioning results in durable engraftment and excellent long-term survival, regardless of graft source. Correction of aberrant phagocytic function and immune-mediated inflammation, prevention of new infections, with an acceptable incidence of transplantation-related morbidity was seen. We conclude that HSCT, including those using UCB donors, should be considered early in pediatric patients with severe phenotype CGD early in the course of their disease.

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Joanne Kurtzberg was involved in conceptualizing the study, collection of clinical data, editing the manuscript, and direct contribution in the generation of graft data; Suhag H. Parikh, Timothy A. Driscoll, Kristin M. Page, and Paul L. Martin were involved with collection of clinical data and preparation of the manuscript; Harry L. Malech provided critical input in preparation and editing of the manuscript and is supported by the Intramural Program of the National Institute of Allergy and Infectious Diseases, NIH.

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