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Successful Engraftment and Stable Full Donor Chimerism After Myeloablation With Thiotepa, Fludarabine, and Melphalan and CD34-Selected Peripheral Allogeneic Stem Cell Transplantation in Hemophagocytic Lymphohistiocytosis

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Allogeneic hematopoietic stem cell transplantation (HSCT) represents the only curative option for primary hemophagocytic lymphohistiocytosis (HLH), a rare disease of infants and young children, characterized by recurrent fever, hepatosplenomegaly, and cytopenia. We report a case of successful engraftment and stable full-donor chimerism in a patient with HLH who underwent peripheral allogeneic CD34-selected HSCT. The donor was his 1-antigen-HLA-mismatched grandmother. After a conditioning regimen based on the combination of thiotepa, fludarabine, melphalan, and rabbit antilymphocyte serum, the patient received a megadose of 26.3 × 10^6/kg of $CD34^+$ peripheral blood cells. Neutrophil (>0.5 × 10^9/L) and platelet (>50 × 10^9/L) engraftment was observed on days +16 and +12, respectively, and the patient was discharged home on day +24. No acute or chronic GVHD was observed. Infectious complications were the main causes of re-hospitalization in the first year after transplantation, but no significant morbidity was observed thereafter. Thirty-two months after HSCT, the patient is alive and well, still in complete clinical remission of his underlying disease with a durable engraftment, normal NK activity and full donor chimerism. This case suggests that a fludarabine-based conditioning regimen and CD34-selected peripheral allogeneic HSCT may be a feasible option in case of unavailability of a fully HLA-matched related or unrelated donor. Am. J. Hematol. 72:143–146, 2003. © 2003 Wiley-Liss, Inc.

Key words: thiotepa; fludarabine; melphalan; peripheral allogeneic stem cell transplantation; hemophagocytic lymphohistiocytosis

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is characterized by impaired immune regulation with the massive release of cytokines and histiocytic infiltration in multiple organs. Primary (or familial) HLH is inherited as an autosomal recessive disorder whose genetic defect has been located on chromosomes 9 and 10 by linkage studies [1,2], and recently it has been related to mutations of the perforin gene [3]. Primary HLH is almost inevitably fatal without treatment, but the estimated probability of survival at 5 years with chemotherapy is only 10%, as opposed to 66% for patients who undergo allogeneic bone marrow transplantation [4].

Etoposide is considered a key drug in the treatment of

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TABLE I. Main Clinical Data of the Patient*

	Diagnosis	Peripheral HSCT	Last follow-up
Age	3 months	8 months	40 months
Signs and symptoms			
Fever	39.5°C (max. value)	No	No
Hepatomegaly (cm below the right costal margin)	4.5 cm	2 cm	0
Splenomegaly (cm below the left costal margin)	6.5 cm	1 cm	0
WBC ($\times 10^9$/L)	2	3.8	7.1
PMN ($\times 10^9$/L)	0.4	0.6	3.5
HB (g/L)	6.4[a]	9.7	13.2
PLT	10[a]	236	229
Triglycerides (n.r., <2.26 mmol/L)	5.35	3.58	0.76[b]
Prothrombin time (n.r., 70–110%)	24	80	90[c]
Thromboplastin time (n.r., 23–35 sec)	59	29	32[c]
Fibrinogen (n.r., 1.5–4 g/L)	0.5	5.1	3.9[c]
Ferritin (n.r., 31–294 μg/L)	2243	216	35[d]
Natural killer activity	Severe deficit	n.d.	Normal[e]
Hemophagocytosis			
Bone marrow	Yes, diffuse	Absent	Absent[d]
Spleen (biopsy)	Yes, diffuse	n.d.	n.d.

*n.r., normal range; n.d., not done; HSCT, hematopoietic stem cell transplantation.
[a]Worst value in the week of diagnosis before the first transfusion and/or any chemotherapy.
[b]+13 months post-HSCT.
[c]+8 months post-HSCT.
[d]+12 months post-HSCT.
[e]+28 months post-HSCT.

HLH and is included both in front-line chemotherapy [5] and, at higher doses, in many conditioning regimens for bone marrow transplantation [6].

We report a case of successful engraftment and stable full-donor chimerism in a peripheral-CD34-selected hematopoietic stem cell transplantation using a fludarabine-based myeloablative conditioning regimen.

CASE REPORT

HLH was diagnosed in a 3-month-old baby that had previously been hospitalized for remittent fever, hepatosplenomegaly, anemia, and thrombocytopenia. Diagnostic work-up was consistent with a diagnosis of HLH (see Table I): progressive pancytopenia requiring platelet and blood transfusions, hypertriglyceridemia, coagulation disorders with low fibrinogen levels and prothrombin time, hemophagocytosis in bone marrow and spleen, pleocytosis and high protein levels in the cerebrospinal fluid, marked reduction of natural killer activity, hyperferritinemia, and hypercytokinemia: TNF, 263 ηg/L (normal range < 16); IL-6, 68 ηg/L (normal range 0.1–10); R-IL2, 25,130 U/mL (normal range 100–913); γ-IFN, 22 U/L (normal range < 1). The familial form of HLH was confirmed by the demonstration of mutation of perforin gene, as described by others [3].

The patient was started on the HLH-94 protocol [4] and, according to Baker et al. [5], achieved a clinical remission defined by normalization of blood counts, triglyceride and fibrinogen levels, liver enzymes, and the reduction of hepatosplenomegaly. The fact that no HLA-identical related or unrelated donor was identified within 6 months of the diagnosis led to the decision to perform a peripheral blood CD34-selected HSCT using the best-related donor available: the child's 39-year-old grandmother who was found to have 1 antigen mismatched on class II. HLA typing of the patient and the donor were as follows: A* 24, B* 40 (60), DRB1* 12; A* 26, B* 13, DRB1* 16; A* 24, B* 40 (60), DRB1* 12; A* 26, B* 13, DRB1* 04, respectively. The patient was conditioned with the myeloablative regimen used at our center for haplo-identical transplantation in non-malignant diseases, i.e., thiotepa 10 mg/kg (day −9), fludarabine 40 mg/m^2/day × 4 days (from day −8 to day −4), melphalan 140 mg/m^2 (day −1) , and rabbit antilymphocyte serum, 3 mg/kg/day for 5 days (from day −6 to day −2). The donor was stimulated with G-CSF, 300 μg × 2/day for 4 days (from day −4 to day −1) and underwent leukapheresis on day 0. As prophylaxis against graft-versus-host disease, T-depletion was performed with a CD34-positive magnetic microbead selection (CliniMacs), but no immunosuppressive drugs were administered thereaf-

ter. The patient received a megadose of 26.3×10^6/kg of $CD34^+$ peripheral blood cells, the total dose of $CD3^+$ cells being 0.8×10^4/kg.

Neutrophil ($>0.5 \times 10^9$/L) and platelet ($>50 \times 10^9$/L) engraftment was observed on days +16 and +12, respectively, and the patient was discharged home on day +24. No acute or chronic GVHD was observed, and stable full-donor chimerism was achieved from day +55.

In the first 12 months after HSCT, the patient was hospitalized for 62 days in all because of the following infectious complications: CMV reactivation (day +36), 2 episodes of FUO (days +52 and +262), EBV post-transplant lymphoproliferative disease [EBV-PTLD] (day +88), syncytial respiratory virus pneumonia (day +134), periorbital cellulitis (day +156), and CVC-related sepsis due to *Staphylococcus epidermidis* (day +189). CMV reactivation was treated first with foscarnet for 7 weeks (120 mg/kg/day for 2 weeks as attack and 90 mg/kg/day for 5 weeks as maintenance) and then, due to an allergic reaction to foscarnet, with ganciclovir (5–10 mg/kg/day) for another 4 weeks. Syncytial respiratory virus pneumonia was managed with supportive measures only. The EBV-PTLD was treated with 4 doses of monoclonal anti-CD20 (Rituximab, 375 mg/m^2), while the other conditions responded promptly to broad-spectrum antibiotics. No significant morbidity was reported in the second year after HSCT.

At latest follow-up, 30 months since HSCT, the patient is alive and well, still in remission of his underlying disease with a complete immunological recovery ($CD3^+$ 3.2, $CD4^+$ 1.2, $CD8^+$ 1.7, $CD19^+$ 0.3, $CD16^+$ 0.8, all $\times 10^9$/L, respectively; ratio $CD4^+/CD8^+$ 0.7, normal response to phytohemagglutinin stimulation test), normal NK activity, and full-donor chimerism (determined by variable number of tandem repeats method).

DISCUSSION

Primary HLH is invariably a progressive disease without treatment and only allogeneic HSCT offers a real chance of long-term cure [4]. Current chemotherapy for HLH, based on dexamethasone, etoposide, cyclosporine, and intrathecal methotrexate, aims to reduce disease activity and prolong survival with a view to implementing HSCT as soon as the best available donor is identified. A recent review of HLH treated so far with allogeneic bone marrow transplantation showed that the preparatory regimen included busulfan, etoposide, and cyclophosphamide in 86% of cases [7]. There is nothing to show that this combination is superior to other myeloablative regimens in terms of disease eradication or long-term engraftment. On the other hand, busulfan-based conditioning carries a high risk of severe transplant-related hepatic morbidity and mortality due to veno-occlusive disease [8–10]. In addition, the typical liver involvement at diagnosis of HLH and at HSCT, in cases transplanted with active disease despite prior chemotherapy, may exacerbate the risk of liver toxicity [11,12].

Durken et al. reported 12 HLH patients alive and free of disease 8–70 months after HSCT with a conditioning regimen based on busulfan, etoposide, and cyclophosphamide [12]. In this series, 6 patients developed severe, non-lethal toxicity: 5 patients had VOD and 1 patient had mucositis with airway obstruction that required mechanical ventilation. According to the Bearman criteria [13], lethal grade III VOD was observed in 2 of our previous patients undergoing allogeneic bone marrow transplantation (1 from a sibling donor and 1 from an unrelated donor) using the combination of busulfan, etoposide, and cyclophosphamide as the conditioning regimen (personal data).

Overall, experience of HSCT in HLH is very limited, but our case suggests that successful myeloablation and stable engraftment are achievable in HLH with less toxic conditioning regimens. Indeed, purine analogues such as fludarabine are being used more and more for myeloablative or sub-myeloablative purposes due to their low extra-hematological toxicity [14–16], and the engraftment is not affected. Moreover, it may not be essential to use high doses of etoposide, with its severe toxic effects on the oral and gastrointestinal mucosa, to obtain long-term cure of the disease. GVHD has been reported as the cause of death in 13% of the cases of HSCT-HLH [6], and its prevention is essential especially in the HLA-mismatched setting. In our case, the $CD34^+$-positive selection was successful in avoiding any risk of acute or chronic GVHD, but the high risk of PTLD or other infectious complications makes it essential to monitor patients closely throughout the first year after transplantation or until an adequate immunological recovery is achieved.

In conclusion, our case suggests that a fludarabine-based conditioning regimen and CD34-selected peripheral allogeneic HSCT may be a feasible option in case of unavailability of a fully HLA-matched related or unrelated donor. Further studies are warranted to confirm any advantage of this choice over the use of unmanipulated related or unrelated HSCT.

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