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ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN THE TREATMENT OF LEUKEMIA

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We reported 14 leukemia patients transplanted with allogeneic peripheral blood stem cells (Allo-PBSCT) of HLA identical sibling, AML 5, ALL 4, CML 5. There were 12 patients in their early stage of leukemia, 2 in late stage. They were 32 (25-42) years old, 10 male and 4 female. After conditioned with CTX and fTBI, G/GM-CSF mobilized donor's peripheral blood mononuclear cells (MNC) 4.80 (2.46-8.43) × 10^8/kg and CD34+ cells 3.96 (1.01-10.08) × 10^6 /kg were infused respectively. CSA and MTX used as GVHD prophylaxis. After Allo-PBSCT, WBC rose to 1.0 × 10^9 /L on day + 17 (+12 - +26) and platelet rose to 50 × 10^9 /L on day +27 (+19 - +35) respectively. The blood products support, the antibiotics used, and total environment protection duration decreased. Acute GVHD presented in 7 patients (50.0%). Four patients died in acute GVHD and infection on days of +73, +41 +57 and +49. The disease free survival days after Allo-PBSCT in the 10 patients was +137 (+91 - +240) days. It is suggested that Allo-PBSCT probably has some advantage than that of Allo-BMT.

Key words: Leukemia, PBSCT, Allogeneic

Transplantation of autologous peripheral blood stem cell (Auto-PBSCT) is a well-established method to reconstitute hematopoiesis in patients receiving myeloablative regimen in recent 10 years. But PBSCT is rarely used in allogeneic transplantation, because of questions regarding of durability of engraftment and graft-versus-host disease (GVHD).[1] G-CSF and/or GM-CSF mobilized peripheral blood stem cell from normal donor can be safely collected for allogeneic PBSCT.[2]

From 1995, we began the research of Allo-PBSCT in the treatment of leukemia.

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MATERIALS AND METHODS

Patients

Fourteen patients with leukemia, AML 5, ALL 4, CML 5, female: male was 4: 10, aged 25 — 42 (Median 32) years. 12 patients in their early stage of leukemia (CR1 in AL, CP in CML), two patients in their late stage (Rel in AL, BP in CML), as shown in Table 1.

Donors

All of them were HLA identical siblings of the patients . After injecting G-CSF and/or GM-CSF 5 μg/kg i h for 5 days (G-CSF in 10 patients, half dosage of G and GM-CSF each in 4 cases), leukapheresis by Baxter CS 3000 on day 5 and 6 to get the mononuclear cells for transplantation without cryopreservation, showed as figure 1. The median age of the donors was 32 (20 — 52) years old. The median number of leukapheresis was 1 (1-3 times, 10-30 L of blood processed).

Methods of Allo-PBSCT

The conditional regimen included TBI and CTX (Cyclophosphamide). The dosages of total body irradiation (TBI) were 4 Gy × 3 (in 9 cases) and 5 Gy × 2 (in 5 cases). The dosage of CTX was 60 mg/kg for 2 days. Cyclosporine A and short course of methotrexate were used for GVHD prophylaxis. All patients were given the total environment protection (Air lamina flow room; aseptic food; oral nonabsorptive antibiotics and skin decontamination) and parenteral nutrition support. Twelve patients were given G-CSF or GM-CSF after infused the mobilized MNC of the donor. The Allo-PBSCT preparation of the donor and the recipient must be done coordinately, as shown in Figure 1.

Table 1. The clinical manifestations of the 14 leukemia patients

Case	Sex	Age (Yr.)	Diag	Stage	Diag-Tx month	Relation donor	MNC $\times 10^8$/kg	Acute GVHD	Chronic GVHD	Survival (days)
1	M	42	CML	CP	6	Bro	7.70	2	-	+73 Died
2	F	35	ALL	CR1	6	Bro	3.44	1	+	+240 DFS
3	M	29	AML	CR1	5	Sis	2.46	2	-	+229 DFS
4	F	20	ALL	CR1	4	Sis	6.56	-	+	+215 DFS
5	M	29	AML	CR1	9	Sis	5.40	-	+	+165 DFS
6	F	25	AML	REL	48	Bro	3.88	3	-	+41 Died
7	M	32	CML	CP	4	Sis	6.52	-	-	+143 DFS
8	M	20	ALL	CR1	5	Sis	8.43	-	-	+131 DFS
9	M	36	CML	BP	24	Bro	4.60	2	-	+57 Died
10	M	40	CML	CP	9	Bro	7.00	2	-	+117 DFS
11	M	32	CML	CP	9	Bro	4.57	-	-	+103 DFS
12	F	32	AML	CR1	8	Sis	4.90	4	-	+49 Died
13	M	37	AML	CR1	7	Bro	4.80	-	-	+93 DFS
14	M	26	ALL	CR1	6	Bro	2.80	-	-	+91 DFS

DFS=Disease Free Survival; CP=chronic phase; BP=Blastic phase; CR=Complet remission; TX=Transplantation

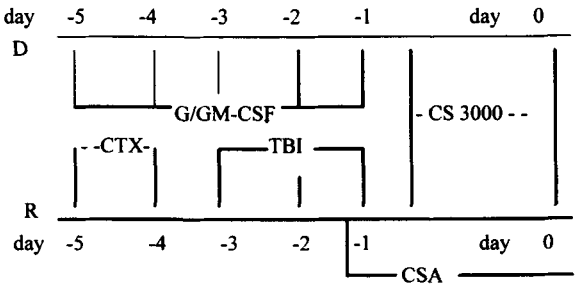

Fig 1. The conditional regimen of the recipient and the preparation of the donor: must be done coordinately

RESULTS

Mobilized Mononuclear Cells and Leukapheresis

Leukapheresis of the donor: After mobilizing by G/GM-CSF, the donors were leukapheresised by cell separator: of Baxter CS 3000 for 1-3 times (Median 1) (10 — 30L blood, median 10L was treated). The mononuclear cell (MNC) were 2.46 — 8.43 × 10^8/Kg (Median 4.80). The MNC were further analysed by flow cytometric analysis. The infused cells were as follows: CD34+, CD34+ /CD38+, CD34+/CD38 - . The cells were 3.96 × 10^6/Kg (1.01 - 10.08), 3.75 × 10^6/Kg (0.99 - 8.55) and 0.23 × 10^6/Kg (0.02 - 0.91) respectively in the 10 patients. The CD3 cells

infused in two assayed patients were 56.9 × 10^6/Kg and 61.0 ×10^6/Kg respectively.

Haematopoiesis Reconstitution

The haematopoiesis reconstituted in all patients after Allo-PBSCT. The leukocytes rose to 1.0 × 10^9/L. is on day 17 (12 — 26) after infusing the MNC. Platelet counts rose to 50 × 10^9/L on day 27 (19 — 35) after Allo-PBSCT. The engraftment of the donor's stem cells were demonstrated by sex chromosome (5 patients), RBC type (3 patients), GVHD present (4 patients) and DNA analysis (2 patients).

Graft-Versus-Host Disease

Seven patients developed acute GVHD with grade 1 (1 patient), grade 2 (3 patients), grade 3 (2 patients) and grade 4 (1 patient) after Allo-PBSCT (50.0%). The GVHD severe than grade 2 were in 42.8% patients.

Survival State of the 14 Luekemia Patients after Allo-PBSCT

Four patients died of acute GVHD and infection on days 73, 41, 57 and 49. Ten patients have disease free survival for 137 days (91 — 240 days).

DISCUSSION

We know that there are haematopoietic stem cells in peripheral blood for a long time.[3] In chronic myeloid leukemia transplantable haematopoietic stem cells are also present in peripheral blood. The haematopoietic stem cells are present in peripheral blood of other leukemia and lymphoma especially after chemotherapy.[4] Peripheral blood stem cells can be mobilized by G-CSF or GM-CSF. Sheridan, et al. reported that after giving G-CSF 12 μg/kg/day, GM-CFU the peripheral blood can be increased 58 times of the baseline.[5] The PBSC can be transplanted in autologous in the treatment of solid tumors and leukemia. PBSC rarely used in allogeneic transplantation until recent years. 180 cases of Allo-PBSCT had been reported in 1994 in EBMT.[6] There were three consecutive reports of Allo-PBSCT in the treatment of leukemia and Editorial in "Blood" in 1995.[7] In 26th — 28th Oct. 1995, the 1st International Symposium of Allogeneic Peripheral Blood Progenitor Cell Transplantation held in Geneve, Switzerland. In that Meeting more than 200 cases of Allo-PBSCT were reported.

In Allo-PBSCT the recipient and the donor preparation must be in the same time, showed as figure 1. The condition regimen of the 14 patients were mainly CTX and fTBI lasting for 5 days. The mobilization preparation of the blood stem cell of the donors were lasting 5 days also. The minimum numbers of G/GM-CSF mobilized normal donor MNC and CD34+ cells necessary to achieve consistent sustained engraftment using HLA-matched siblings are unknown. The lowest numbers of MNC infused in our 14 patients was 2.46 × 10^8/Kg. The lowest number of CD34+ cell infused 1.01× 10^6/Kg (10 patients). All patients engrafted the donor's stem cells immediately. Azevedo, et al. reported that the lowest of MNC of their 17 patients was 1.8 × 10^8/Kg and they believed that the number of MNC was a rely on criteria of engraftment.[8] We think that more than 2 × 10^8/Kg of MNC or more than 2 × 10^8/Kg of MNC or more than 2 × 10^6/Kg of CD34+ cells are enough for the hematopoietic reconstitution of HLA identical sibling recipient. CD34+/CD38- cells are a small sub-population of CD34+ cells, they represent the long term culture-initiating cells (LTC-IC) and are the early haematopoietic stem cells component.[9] In our last 10 patients the number of CD34+/CD38-cells infused was 0.23 × 10^6/Kg (0.02 - 0.91). After Allo-PBSCT, the blood stem cells engrafted in all the 14 leukemia patients. WBC rose to 1 × 10^9/L on day 17 (day 12 — 26), platelet count rose to 50 × 10^9 /L on day 27 (day 19 - 35, 11 patients). In the same period for allogeneic bone marrow transplantation (BMT) the WBC rose to 1.0 × 10^9/l on day 29 (day 19 — 42). These results are similar to the other's reports.[8] Because of the shorter period of the restore of haematopoiesis, the blood product support, the antibiotics used, and the TEP duration are decreased. The median number of platelet transfusion for the 14 patients was 2 (1 — 5) bags of apheresised platelet. The median days of TEP after Allo-PBSCT was 31 (27 — 41) days. They were far less than that of BMT patients. The incidence of acute GVHD was 7/14 (50.0%), 5/14 (42.8%) >grade 2. They were not greater than that of BMT patients. After G/GM-CSF mobilization, the T lymphocytes content in peripheral blood is ten times more than that of bone marrow.[10] Much more HLA matched T lymphocytes infusion did

not cause acute GVHD,[11] although T cells depletion BMT can decrease the incidence and the severity of acute GVHD. The incidence and severity of acute GVHD in our 14 patients was similar to that of Allo-BMT patients and the Allo-PBSCT reported of the others.[8] The incidence of chronic GVHD probably much higher than that Allo-BMT.[12] Auquier, et al. made a comparison between anxiety, pain and discomfort in leukacytapheresis and bone marrow harvest, and conclude that the former was better than the latter.[13] The long term results of Allo-PBSCT was probably better than that of Allo-BMT, because of the large amount of T lymphocytes and NK cells infused.[14]

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