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Allogeneic Bone Marrow Transplantation in Aplastic Anemia, Acute Leukemia and Malignant Lymphoma

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Abstract Allogeneic bone marrow transplantation was performed in 3 patients of severe aplastic anemia, 3 patients of acute lymphocytic leukemia and 1 patient of malignant lymphoma. One patient of aplastic anemia, 2 patients of acute leukemia and 1 patient of malignant lymphoma are alive for from 200 days to 3 years after transplantation.

Two patients of aplastic anemia and 1 patient of acute leukemia caused graft rejection and failure of sustained engraftment, and ultimately died of fungal and cytomegalovirus infections. The clinical courses and the outcomes of these patients were presented referring to the recent progress of bone marrow transplantation.

Key Words : allogeneic bone marrow transplantation, aplastic anemia, acute leukemia, malignant lymphoma

Introduction

The treatment of allogeneic bone marrow transplantation (BMT) had now been established as the effective and potentially curative therapeutic modality of leukemia and severe aplastic anemia in the world and also in Japan. The long-term survival rates after BMT of acute leukemia now attained approximately to 60%[1], and 80% in the untransfused patients of aplastic anemia[2]. These excellent results were obtained by overcoming many problems such as graft rejection, acute and chronic graft versus host disease, relapse of leukemia and infection.

We started allogeneic BMT in the Yamaguchi University Hospital since 1983. The purpopse of this paper is to present our results of allogeneic BMT referring to the recent progress of BMT.

Materials and methods

1. Patients (Table I-1)

Three patients of severe aplastic anemia, 3 patients of acute lymphocytic leukemia (ALL), and 1 patient of non-Hodgkin malignant lymphoma with leukemic change, had been transplanted for allogeneic BMT from HLA-A, B, C

and DR compatible and MLC non-reactive sibling donors.

2. Methods

The patients were cleaned and entered in the laminar air flow room before preconditioning of BMT, and isolated until granulocyte recovered more than 500/mm³ after transplantation. The patients were served sterile diet and also performed intravenous hyperalimentation. The patients took unabsorbable antibiotics of tobracin, vancomycin and nystatin for intestinal sterilization and also inhalated those regimens. All items entering the laminar air flow room were gas or steam sterilized. Staffs entered downwind after donning sterile cap, mask, gown, gloves and shoes.

The bone marrow cells were aspirated from the iliac crests and infused by the method of Thomas and Storb[3]. The preconditioning regimens of BMT in aplastic anemia was cyclophosphamide (CY) (50mg/kg for 4 days) and total body irradiation (TBI) (300 rad by linear accelerator)[4]. Those of leukemia and lymphoma were originally by CY (60mg/kg for 2 days) and TBI (1,000 rad, fractioned for 3 days)[5]. In the 2 cases of acute leukemia, they were preconditioned additionally with high dose of cytosine arabinoside (HDAR) (3g/m²x2/day for 4 days)

The grade of graft versus host disease (GVHD) were classified from 0 to IV according to the severiety of the disease[5]. The clinical performance status of the patients after GVHD was evaluated by the Karnofsky score[6].

The prophylaxis for GVHD was initially performed according to the mathod of Thomas et al by methotrexate (MTX) (15mg/m² on day 1, 10 mg/m² on days 3, 6, 11 and then weekly until discharge). Recently, the prophylaxis was performed by cyclosporin A (CYA)[7]. The patients received CYA 3.0-5.0mg/kg/day intravenously in two divided doses beginning on the day before transplantation, and when the oral medication could be tolerated, 5.0-12.5mg/kg/day was orally administered in two divided doses. Beginning on day 50 after transplantation, the dose was gradually decrased by 5% per week and treatment was discontinued six monthos after transplantation[7]. The dose of CYA was adjusted by monitoring serum CYA levels, aiming trough level of more than 50-200ng/ml and maximum level of less than 500ng/ml, and also the dose of CYA was reduced in the patients with impaired renal function. CYA was used also for the treatment of the established GVHD, as well as prednisolone, 2mg/kg/day, until the disease was ameliorated[8].

Trimethoprim (80 mg)-sulfamethoxazol (400 mg) was given orally every 8 hours as prophylaxis against pneumocystis carinii infection on days -7 through 0 prior to transplantation and from +30 to +100 days after transplantation. Commercially available immunoglobulins which had high titer to cytomegalovirus (CMV) were administered for the prophylaxis of CMV infection, 10g/day/week until discharge. Blood products from seronegative donors for CMV were used as frequently as possible for the prophylaxis of CMV.

For the prophylaxis of the hemorrhagic cystitis, most of the patients were performed hydration and bladder irrigation with concomitant use of 2-mercaptoethane sulfonate (mesna) during CY administration.

Results

1. ABO-mismatch (Table I-1)

In 2 patients of major mismatch, they were successfully traeted for acute hemolysis after infusion of bone marrow cells (1 patient was treated by immunoadsorption for the removal of blood group antibody[9] (UPN 2), and in the other patient the bone marrow mononuclear cells were collected by IBM 2,991[10](UPN 6)), however, both patients died shortly after BMT of graft rejection or failure of sustained engraftment. In a patient of minor mismatch, the plasma was removed from the donor bone marrow aspirate and there was no problem (UPN 5).

2. Preconditioning and grafting (Table I-2)

Among the 3 patients of aplastic anemia who were preconditioned with CY and low dose TBI, 2 patients were grafted, however, only 1 patient could maintain the engraftment (UPN 1). In the 2 patients who were preconditioned with CY and TBI, 1 patient of ALL (UPN 4) and 1 patient of malignant lymphoma (UPN 7), both were grafted. In the 2 patients of ALL who were

preconditioned with CY, TBI and HDAR, both patients could be grafted, however, 1 patient failed to maintain the engraftment (UPN 6).

3. Hematological reconstitution (Table I -2, 3)

The infused bone marrow cells were rejected in 1 patient of aplastic anemia (UPN 2). Among the 6 patients whose donor bone marrow cells were grafted, granulocyte count recovered more than 500/mm³ in 17.7± 1.6 days and platelet count recovered more than $2x10^4$/mm³ in 20.2±6.6 days (mean±SD) after transplantation. However, 2 patients subsequently failed to maintain the engraftment, and anemia and thrombocytopenia in a patient (UPN 3) and pancytopenia (UPN 6) occurred. The other 4 patients could maintain the engraftment.

4. Infectious complication (Table I -4)

Herpes zoster infection occurred about 100 days after BMT (UPN 1 and 4). Herpes simplex stomatitis (by HSV type 1) occurred soon after (UPN 6 and 7) and about 100 days after BMT (UPN 1). Generalized CMV infection occurred during the course of acute GVHD (UPN 3), Fungal pneumonia of undetermined pathogen occurred before acute GVHD (UPN 2), and generalized candida albicans infection occurred shortly after acute GVHD (UPN 6). Hemorrhagic cystitis by adenovirus type 11 occurred about 30 days after BMT (UPN 5 and 6).

5. The immunosuppression by MTX or CYA (Table I -5)

MTX was effective for the prevention of GVHD and rejection (UPN 1 and 4). However, the adverse reaction of MTX appeared soon after BMT as oral mucositis especially in the patients who were preconditioned with 1,000 rad TBI (UPN 4 and 5). CYA was effective for those purposes (UPN 5 and 7), and it was also effective for the treatment of the established chronic GVHD (UPN 4). The one of the serious adverse reactions of CYA was nephrotoxicity, and trough and maximum serum CYA levels frequently exceeded over the therapeutic levels during intravenous administration and during renal damage appeared. However, nephrotoxicity was reversible and elevated BUN, blood creatinine or decreased creatinine clearance returned to normal soon after decreasing the dose.

6. GVHD (Table I -5)

Acute GVHD was extremely severe in a patient of aplastic anemia (grade IV in UPN 3), with generalized erythroderma with bullous formation and desquamation, liver damage, diarrhea and intestinal hemorrhage. Acute GVHD was not so severe in other patients. Chronic GVHD was severe and extensive in a patient, with skin involvement just like of systemic lupus erythematosus, wasting and occular symptom of dryness and oral symptoms of stinging with acidic foods and dryness (UPN 4). In the other patients, the symptoms of chronic GVHD were of minimum grade and limited, and included hyperkeratotic papules of the palms and foot soles, dry skin, hyperpigmentation, oral and occular dryness, stinging with acidic foods and nail deformity (UPN 1, 5 and 7). The grade of the clinical performance status of all of the survived patients was 100%.

7. The survival of the patients after BMT (Table I -5)

Among the 3 patients of aplastic anemia, 1 patient are alive for more than 3 years. The other 2 patients died shortly after BMT (UPN 2 and 3). Among the 3 patients of ALL, 1 patient is alive for more than 2 years (UPN 4) and the other patients are for more than a year (UPN 5). One patient died 72 days after transplantation (UPN 6). One patient of non-Hodgkin malignant lymphoma with leukemic change survived for more than 200 days (UPN 7). Relapse of leukemia or lymphoma was not recognized during these observation periods.

8. Immunological reconstitution (Figure I)

The decrease of $OKT4^+$ cells, increase of $OKT8^+$ cells reversal of $OKT4^+$ to $OKT8^+$ cells, ratio (Figure I, 1-3), and depressed responses to the mitogens of PHA, Con A and PWM (Figures I, 4-6), were observed through 2 to 3 years after BMT. The maximum suppression was observed about half a

Table I (1-5) : The lists of the patients profiles and the results of allogeneic bone marrow transplantation.

Table I -1

Case No	Age	Diagosis	Status	Sex		ABO
				Recipient	Donor	(Procedure)
1	20	AP A	Severe	F	F	Match
2	35	AP A	Severe	F	F	Major Mismatch (Immuno Adsorb)
3	14	AP A	Severe	F	M	Match
4	17	ALL	Ist CR	F	F	Match
5	6	ALL(T)	Ist CR	M	F	Minor Mismatch (Removal of Plasma)
6	28	ALL(T)	Ist CR	M	F	Major Mismatch (IBM 2,991)
7	17	Malignant Lymphoma	Ist CR	M	M	Match

AP A : aplastic anemia
ALL : acute lymphocytic leukemia
T : T cell phenotype

Table I -2

Case No	Prior Transfusion	Pre-Conditioning	Infused Cell No (10^8/kg)	Engraftment
1	R 9U	CY+Low Dose TBI	9.6	Take
2	R 30U	CY+Low Dose TBI	6.8	Rejection
3	R 6U, P 4U	CY+Low Dose TBI	7.0	Take--Failure (Anemia, Thrombocytopenia)
4		CY+TBI	6.4	Take
5		HDAR+CY+TBI	7.5	Take
6		HDAR+CY+TBI	0.3 (MNC)	Take--Failure (Pancytopenia)
7		CY+TBI	6.4	Take

R : red blood cell
P : platelet
U : unit
CY : cyclophosphamide
TBI : total body irradiation
HDAR : high dose of cytosine arabinoside (are-C)
MNC : mononuclear cell
Failure : failure of sustained engraftment

Table I-3

Case No	Hematological Recovery (Days)		Stay in LAF (Days)
	Granulo 500/mm³	Plt 2x10⁴/mm³	
1	16	10	39
2	NE	NE	18
3	17	25	58
4	16	16	23
5	19	25	30
6	20	27	29
7	18	18	20

Granulo : granulocyte
Plt : platelet
LAF : laminar air flow room
NE : not examined

Table I-4

Case No	Infectious complication	Date of Onset (Days)
1	Herpes Simplex, Oral Cavity	95
	Herpes Zoster, Extemity	100
2	Fungus, Lung	Before Acute GVHD
3	Cytomegalovirus, Generalized	During Acute GVHD
4	Herpes Zoster, Neck	142
5	Hemorrhagic Cystitis	32
6	Herpes Simplex, Oral Cavity	26
	Hemorrhagic Cystitis	30
	Candida Albicans, Generalized	After Acute GVHD
7	Herpes Simplex, Oral Cavity	15

GVHD : graft versus host disease

Table I-5

Case No	GVHD			Karnofsky Performance Rate	Survival
	Prophylaxis	Acute	Chronic		
1	MTX	I	Limited	100%	+3Y
2	MTX	NE	NE		18 Days
3	MTX	IV	NE		58 Days
4	MTX	I	Extensive	100%	+2Y
5	MTX···CYA	0	Limited	100%	+1Y
6	CYA	II	NE		72 Days
7	CYA	I	Limited	100%	+200 Days

MTX : methotrexate
CYA : cyclosporin A
--- : MTX was switched to CYA

Fig. I (1-9) : Immunologic recovery after allogeneic bone marrow transplantation

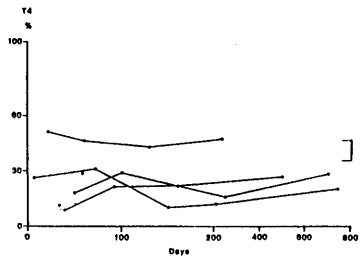

Fig. I-1

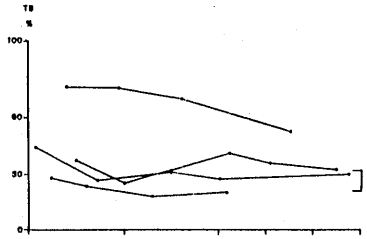

Fig. I-2

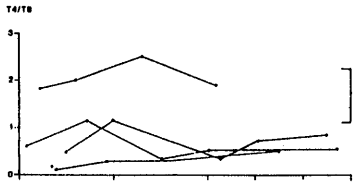

Fig. I-3

1-3 : The percentage of OKT4$^+$ cells, OKT8$^+$ cells, and their ratio (T4/T8)
] : normal range

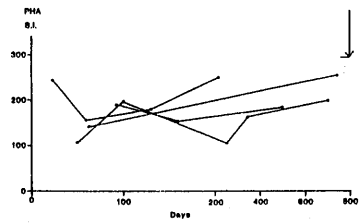

Fig. I-4

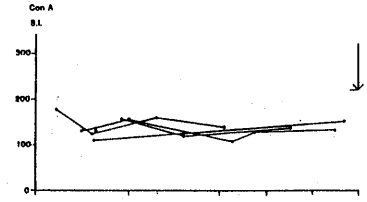

Fig. I-5

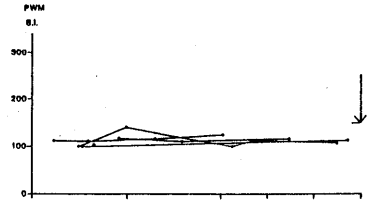

Fig. I-6

4-6 : Mitogen reaponse
S. I. : stimulation index
↓ : normal range, more than the level

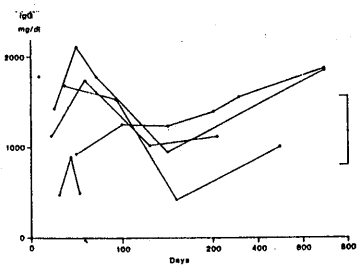

Fig. I-7

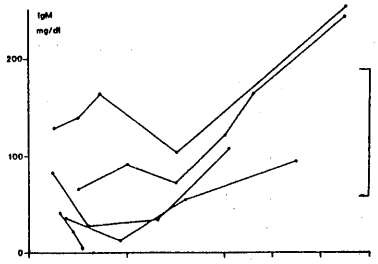

Fig. I-8

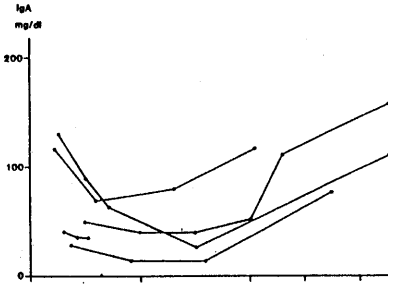

Fig. I-9

7-9 : Immunoglobulins
] : normal range

year after BMT, and the gradual recovery was observed thereafter although the values were subnormal. As for the immunoglobulin production, each immunoglobulin level was depressed severely after a year of BMT, and then the levels of IgG and IgM gradually increased to normal after a year, however, the level of IgA remained low long after BMT. On the contrary, increased levels of IgG and IgM were observed in the long-term survivors (Figure I, 7-9).

9. Psycological problems

All of the patients were treated in the laminar air flow room (for 31±14 days, mean±SD, Table I-3). In addition to the stress by confinement in the room, most of the patients had great anxieties to the outcome of BMT. When the patients recovered steadily after transplantation, the mental states of the patients were in good condition having hope for the future life. However, when the patients had the complications of acute GVHD, graft rejection or infection, the mental states of the patients were deteriorated and had great fear about the outcome of BMT.

Discussion

Transfusion induces sensitization is believed responsible for most of graft rejections with severe aplastic anemia who are prepared with cyclophosphamide alone[11]. Patients who received multiple blood transfusions before 1976 had long-term survival rates of 43 to 50%, a graft rejection rate of 38%, and rejection related mortality rate of 30%[12]. Attempts to prevent rejection in sensitized patients by additional immune suppression with antithymocyte globulin[8], or with total body irradiation[13] had limited success. Higher survival rates had been reported with the addition of total lymphoid irradiation[14], or with the use of buffy coat cell infusion after transplantation[12], and these procedures increased the long-term survival rate to 70% and decreased rejection related mortality rate to 10%, however, the latter procedure increased a risk factor of GVHD. In the recent report of the untransfused patients, the incidence of graft failure was only 10%, with related mortality rate of 4% and long-term survival rate of 82%[2]. Our patients were preconditioned with CY and low dose TBI because of the probability of sensitization by prior blood transfusion, however, only 1 patient could maintain the engraftment. The reason of the failure of the engraftment remains unknown, since HLA and non-HLA antibodies against donor leukocytes could not be detected in the sera of these recipients before and shortly after transplantation judged by leukocyte cytotoxicity test (data not shown).

The three year probability of disease free survival of acute non-lymphocytic leukemia was 55% for the first remission transplant[1,15], 54% for the second remission, 33% in the third remission, and 10.3% in the relapsed group[1]. Patients with acute lymphocytic leukemia in second or subsequent remission had significantly increased disease free interval and greater survival rate than the conventionally treated patients[16]. The improved survival seen in the early remission group was due to a significant decrease in the incidence of relapse posttransplant, as was also observed in our patients. In the allogeneic BMT in acute leukemia, the graft versus leukemia (GVL) effect plays the role of eradicating the residual leukemic cell proliferation in the recipients[17,18]. It was shown that those transplant recipient in whom significant GVHD developed had a decreased risk of leukemia relapse compared to the patients in whom GVHD did not develop[17,18], and natural killer cell might play an important role for antileukemia activity[19]. However, the survival of the patients was comparable since the lesser probability of recurrent leukemia in patients with severe GVHD was offset by a greater probability of other causes of death[17,18]. The preconditioning regimens for leukemia were also increased in the doses of irradiation and the additional chemotherapy to eradicate more neoplastic cells in the recipients, and the latter included HDAR,

VP-16, busulfan and melphalan[20,21]. The advantage for increasing the doses of preconditioning regimens is not certified yet[20,21], and these procedures might, in turn, cause pulmonary and cardiac toxicities, oversuppress and delay the hematological and immunological reconstitution after transplantation, and might increase the risk of complications such as infection and hemorrhage, as was observed in our patient (UPN 6) and in the other patient of our autologous BMT (data not shown).

CYA treated patients had a significantly earlier engraftment, shorter hospitalizations, earlier platelet transfusion independence and infrequent oral mucositis than MTX treated patients[7], however, the immunological reconstitution in CYA treated patients was not accelerated over that of MTX treated patients[22], with no or only marginal reductions in the incidence of acute GVHD and no improvement in survival[7]. Recent report of the combination of MTX and CYA was superior to CYA alone in the prevention of acute GVHD[23]. Our experience of the use of CYA is limited, however, it is evident that CYA was effective for the prevention of graft rejection and GVHD and was also effective for the treatment of the established GVHD. Oral mucositis was infrequent than the MTX treated patients especially in the patients who were preconditioned with 1,000 rad TBI. The nephrotoxicity as a adverse reaction of CYA was reversible and was not serious. GVHD is caused by the donor T cells, and the treatment of the donor bone marrow cells with monoclonal anti T cell antibody and complement had been performed to deplete T cells for the prevention of GVHD[24,25]. Patients having T-cell depleted bone marrow cells had lower incidence of acute GVHD and related mortality, however, the incidence of graft failure was increased than in the control group, and further evaluation is required for the recurrence of leukemia[24,25].

The severe and prolonged GVHD after BMT leads to the prolonged immunosuppression[26-30]. In addition to the T cell defects, such as depleted $OKT4^+$cells, normal or increased $OKT8^+$cells, reversal of $OKT4^+$: $OKT8^+$cells ratio and impaired responses to the mitogens[26-28], the defective B cell function and the lack of helper T cell and predominant suppressor T cell activities on B cell activation, lead to the impaired immunoglobulin production[29,30]. On the average, a diminished concentration of IgG persisted until 6 months, IgA through 12 months and IgM until 6 months after transplantation[30]. In contrast, hypergammaglobulinemia was found in the patients with chronic GVHD[31]. The same tendency of the immunological reconstitution was observed in our patients.

The activated T cells appeared after BMT regulate the proliferation of the hematopoietic stem cells[32,33]. Our patients (UPN 3 and 6) were grafted once but subsequently failed to maintain the engraftment, and those patients might have increased suppressor T cell activity on hematopoiesis.

The infectious complications after BMT are the bacterial infection during granulocytopenia, and viral, fungal or pneumocystis carinii infection during immunosuppression after transplantation. There were no problems of bacterial infections in our patients as long as the patients were admitted in the protective environment. For the prevention of interstitial pneumonia by pneumocystis carinii, the prophylactic use of trimethoprim-sulfamethoxazol was effective[34] as in our patients. The most serious infection after BMT is cytomegalovirus (CMV) infection. The incidence of CMV infection was siginificantly low in the patients who were seronegative for antibody to CMV infection before transplantation and received seronegative blood products[35]. In contrast, the use of seronegative blood products did not prevent CMV infection among the patients with seropositive recipients, since most of the CMV infection is caused by the reactivation of the CMV[35]. Furthermore, CMV infection was strongly associated with acute GVHD, and the severiety and duration of the ac-

companied immunoincompetence may be more critical than the route of exposure[36]. Augmentation of GVHD by CMV had also been demonstrated in the experiments of the mice[37]. One of our patients (UPN 3) had severe GVHD and generalized CMV infection, and thus, these complications could potentiate each other. Attempts to prevent CMV infection after transplantation had been performed by using CMV immune globulins[35,38-40], however, the the clinical usefulness of CMV immune globulins was not confirmed, and could not be recommended for the routine use without additional study[35].

The hemorrhagic cystitis occurred in our experience in only the patients in which the bladder irrigation was omitted despite the use of mesna in all of our patients for the prevention of the hemorrhagic cystitis, and the usfulness of mesna, waits for further evaluation.

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