Donor Leukocyte Infusions for the Treatment of Leukemia Relapse After Allogeneic Hematopoietic Cell Transplantation with Myeloablative Conditioning

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ABSTRACT

Leukemic relapse after allogeneic transplantation is a difficult problem. Conventional treatment modalities or second transplants have not provided the sustained complete remissions on the whole. Reinfusion and activation of cytotoxic T lymphocytes of the donor seem to be effective and it has been understood that the success of the transplantation depends mainly on graft versus leukemia effect. Thirteen patients with leukemia (8 CML, 5 AML) who had relapsed after allogeneic hematopoietic cell transplantation (HCT) with myeloablative conditioning, have received donor leukocyte infusions (DLI). The median time between transplantation and relapse was 18 months (4-57 months). For CML patients who had cytogenetic or hematologic relapse, IFN alpha 2b was started at a dose of 5 million units/m^2/d for every consecutive days. Starting from the fifth week of this treatment, unprimed donor peripheral blood mononuclear cells were infused to the patients once a week for four weeks. IFN treatment was not cessated during these infusions, and was given for 12 weeks totally. For relapsed acute leukemia patients, standart chemotherapy regimens as for AML were used. After the treatment, donor lymphocytes which were obtained from the original HCT donor who was primed with G-CSF were given. After recovery, IFN alfa2b was started 5 million U/m^2/d each consecutive day until the GVHD findings were observed. GVHD prophylaxis was not made after DLIs. Acute GVHD was seen in 11 of 13 patients. Four patients developed chronic GVHD. Among 13 patients, four patients are alive and they have been in complete remission for 23 to 76 months. The other patients were not alive due to mostly disease progression. Two patients died because of advanced GVHD. In our practice, the patients with progressive disease were not the well responded ones. These observations suggest that there is a limit for the immune effect regarding the number of the tumor cells and their proliferative capacity. Chemotherapy, which does not suppress immunity, may give time and chance to allogeneic lymphocytes to affect.

Key Words: Donor leukocyte infusion, Leukemia relapse, Allogeneic hematopoietic cell transplantation.

INTRODUCTION

To overcome leukemia, allogeneic hematopoietic cell transplantation (HCT) has been a hopeful treatment for many years. Improving survival after alloHCT depends mainly on developing supporting measures. But leukemic relapse after allogeneic transplantation is still a difficult problem. Selection of the patient, timing of transplantation, type of conditioning regimen are the major determinants of the probability of relapse. While this probability is between 10% and 40% in the patients with standart transplantation indication, it increases as high as 70% with the advanced disease, T cell depletion, and the absence of graft versus host disease (GVHD). The clonogenic malign cells of the recipient, which escape from the effects of high dose chemoradiotherapy and graft versus leukemia (GVL) effect, are responsible for leukemic relapse. Leukemia which arises from donor cells, especially after conditioning with radiotherapy and in the late posttransplant period, has not been seen commonly. Leukomogenesis which is precibitated by radiation; de novo leukemic transformation due to the recurrent leukemogenic stimulus; and transfer of oncogenic genetic material to donor cells from recipient cells are some examples of the mechanisms which have been accused[1]. Conventional treatment modalities or second transplants are far from the sustained complete remissions[2-4]. Stopping immunosuppressive agents which are used in transplant setting; using immunomodulator agents like interleukin-2 (IL-2) and interferon (IFN) alpha; and other techniques which have been used incidentally have not been with good results either. Cessation of cyclosporin (CsA) when early relapse is determined has been reported to provide hematologic and/or cytogenetic remission in patients with leukemia[5]. GVHD was observed in almost all of those patients. Granulocyte colony stimulating factor (G-CSF) administration at the time of the relapse could make hematologic or cytogenetic stabilization or remission[6,7]. It was showed that donor originated cells in the recipient's marrow were stimulated predominantly in these patients. But if there was circulating blasts or extramedullary relapse, response has not been seen usually, furthermore G-CSF administration might have increased disease progression[8]. IFN-alpha inducts immune mediated antileukemic activity directly or via cytokine activation[9]. It also increases T-cell and NK cell activity and minor histocompatibility antigen expression on cell surfaces. Another effect of IFN-alpha is on adhesion of chronic myeloid leukemia (CML) progenitor cells to bone marrow stromal cells[10]. IL-2 is another immune modulator cytokine, which has been used especially in the postremission therapy of acute leukemias, has also been tried incidetally for posttransplant leukemia relapses[11].

In eighties, donor buffy-coat infusions were applied soon after bone marrow transplantation (BMT), which was performed to hematologic malignities, for strengthening adoptive immunotherapy[1]. But it caused increased risk of transplant related mortality. In the following years, antileukemic activity of peripheral blood leukocytes of donor in relapsed disease has come into view. Reinfusion and activation of cytotoxic T lymphocytes of the donor seem to be effective and it has been understood that the success of the transplantation depends mainly on GVL effect[12]. Minor histocompatibility antigens (mHA) (tissue or lineage restricted or non-restricted) and antigens that are accepted as leukemia specific, have been the candidates of target antigens in GVL effect[13]. The ratio of difference of mHA expression between donor and patient is one of the major determinants that effects graft versus host activity. The most important complications of donor leukocyte infusion (DLI) are GVHD (especially chronic) and marrow aplasia. If DLI is given after hematologic relapse, immunologically reactive cells of donor may eliminate not only leukemic cells but also non-leukemic cells of the recipient and it may cause aplasia[14,15]. If relapse can be detected in the very early phase, like CML cytogenetic relapse, administration of DLI at this time may decrease the probability of this complication[16]. Indeed, being able to apply DLI in the very early phase of relapse may be one of the reasons about why DLI is the most successful in posttransplant CML relapse. In advanced phase leukemic relapses DLI may not be efficient at first stage because progression of the disease is more rapid than donor cells' immune activity. Conventional dose or non-myeloablative chemotherapies

may be useful in this condition for gaining time for this immune effect, and they have been showed not to suppress graft versus host reactions[17,18].

As being a major transplantation center which has performed transplantations for a long time, we have faced leukemic relapse in increasing frequency. Donor lymphocyte infusions, mostly with IFN, have been given to these patients since the first leukemic relapse occurred. We are presenting the results of these applications.

MATERIAL and METHODS

PATIENTS

Thirteen patients with leukemia who had relapsed after allogeneic HCT with myeloablative conditioning, have received DLIs since 1994. Type of HCT was BMT for nine patients and peripheral blood (PB) HCT for four patients. Eight patients had CML chronic phase and five had acute myeloid leukemia (AML). All the patients have had allogeneic HCT from their HLA identical siblings. One of them, a woman with AML, received BM from her monozygotic twin. Table 1 shows the patients' characteristics. GVHD prophylaxis was made by CsA and MTX. T cell depletion was not performed. Infection prophylaxis was made by ciprofloxacin, TMP-SMX (before transplantation and after the engraftment), acyclovir and fluconasole. Conditioning regimens consisted of busulfan 4 mg/kg/d, p.o. for four days plus cyclophosphamide 60 mg/kg/d, i.v. for two days. Their relapse was diagnosed either after deterioriation of their health status or in routine control examination. Relapses were seen as hematologic or cytogenetic. The median time between transplantation and relapse was 18 months (4-57 months).

METHOD

For CML Patients Who Had Cytogenetic or Hematologic Relapse

Interferon alfa 2b was started at a dose of 5 million units/m^2/d for every consecutive days. Dose escalation or the cessation of the application was made according to the cell counts and drug toxicity. Starting from the fifth week of this treatment, unprimed donor peripheral blood mononuclear cells were infused to the patients once a week for four weeks. IFN treatment was not cessated during these infusions, and was given for 12 weeks totally. Except toxicity, aggravated or newly developed GVHD was the only indication for stopping IFN. If there was a high leukocyte count, hydroxyurea was used in the beginning, for the patients with hematologic relapse.

For Relapsed Acute Leukemia Patients

Standart chemotherapy regimens as for AML induction were used for these patients. They contained standart or intermediate dose Ara-C plus one of the anthracyclines or mitoxantrone. Since the aim was to achieve maximal leukemic cell reduction with minimal tissue damage and to avoid disturbing mixed chimeric status, the decision of further chemoradiotherapy (preferably high dose Ara-C; one patient received melphalan 140 mg/m^2) before DLI was made on individual base. After the treatment, donor lymphocytes, which were obtained by leukapheresis from the original HCT donor (except one patient) who was primed with G-CSF at a dose of 2.5 mcg/kg/d or 5 mcg/kg/d for five or more days, were given. For the patient transplanted from her monozygotic twin, her HLA identical other sibling was used as lymphocyte source. After recovery, IFN alfa2b was started 5 million U/m^2/d each consecutive day. Dose escalation was made according to the degree of cytopenia and drug toxicity. IFN treatment continued until the GVHD findings were observed.

Follow-up

Standart remission criteria were evaluated frequently for each patient. Further techniques like cytogenetic or molecular detection of specific abnormalities, and follow-up of the chimeric status were carried out in a conventional manner. Diagnosis and grading of acute GVHD were based on standart clinic and histopathologic definitions. When complete remission was not achieved or when further relapses occurred, DLI with or without chemotherapy was reapplied if the patient had any donor chimerism or the status of GVHD allowed.

RESULTS

Table 1. Patients' characteristics

No	Patients initials	Sex (R/D)	Age (R/D)	Diagnosis	Stem cell source	Diagnosis-tx time (mo)	Relapse type	Tx-relapse Time (mo)	Post tx. GVHD
1	S D	F/M	22/30	CML-1st CP	BM	8	Cytogenetic	21	-
2	M G	M/M	27/21	CML-1st CP	BM	8	Cytogenetic	20	-
3	M ‹	M/M	28/30	CML-1st CP	BM	10	Hematologic (AP)	22	Acute Grade I
4	A E	F/F	37/25	CML-1st CP	BM	24	Cytogenetic	24	-
5	H B	M/M	21/24	CML-1st CP	BM	4	Hematologic	57	Chronic cutaneous
6	S E	M/M	23/30	CML-1st CP	PBHC	12	Hematologic	4	-
7	R D	M/M	14/16	CML-1st CP	BM	6	Hematologic	6	-
8	G Y	F/F	35/44	CML-1st CP	PBHC	24	Hematologic	9	Acute Grade II
9	E E	M/M	24/40	AML-1st CR	BM	20	Hematologic	20	-
10	F A	F/F	39/32	AML-1st CR	PBHC	12	Hematologic	18	-
11	M K	F/F	28/28	AML-1st CR	BM-SYN	10	Hematologic	16	-
12	Z K	F/F	16/10	AML-1st CR	PBHC	7	Hematologic	4	-
13	G B	F/F	34/24	AML-1st CR	BM	8	Hematologic	12	Acute Gr 1, cut.

Abbr: M: Male, F: Female, R: Recipient, D: Donor, CML: Chronic myeloid leukemia, CP: Chronic phase, AP: Accelerated phase, CR: Complete remission, AML: Acute myeloid leukemia, BM: Bone marrow, PBHC: Peripheral blood hematopoietic cells, SYN: Syngeneic

Overall Outcome

Among 13 patients, four patients are alive and they have been in complete remission (CR) for 23 to 76 months (Table 2). The other patients were not alive due to mostly disease progression. Three of the seven complete responders were CML patients, and two of them were with cytogenetic relapse only. In two patients with AML and CML, relapse occurred in central nervous system (CNS). Two patients died because of advanced GVHD.

Graft versus Host Disease

GVHD prophylaxis was not made after DLIs. Acute GVHD was seen in 11 of 13 patients. One patient died due to grade IV acute GVHD. Grade II to III acute GVHD was seen in nine patients. Four patients developed chronic GVHD. Three of them were extensive. One of them died because of pulmonary complication. Corticosteroid, with or without CsA was used at first step when GVHD developed.

Postrelapse Therapies Applied With DLIS

Severe and lasting toxic effects were not observed after IFN-alpha administration. A 39 year-old woman with AML relapsed 18 months after allogeneic PBHCT. Her PB and BM samples showed mixed donor chimerism. The immunophenotyping features of blastic cells included high CD38 and PCA1 positivity. Standart dose Ara-C and idarubicine did not provide remission. Melphalan 140 mg/m^2 was given before DLI. Donor priming was made with ten days administration of G-CSF at a dose of 2.5 mcg/kg/d. After recovery, CR and complete donor chimerism were established. She developed grade III acute GVHD and it was controlled with steroid and CsA administration. The patient was diagnosed as having isolated CNS relapse five months after DLI and treated with CNS radiotherapy and intrathecal chemotherapy. After healing of CNS relapse, hematologic relapse occurred. The second session of DLI was performed without conditioning and donor priming, and then she had CR again with complete donor chimerism. Several months after this, she was diagnosed as having isolated CNS relapse again without hematologic relapse and died.

A 24 year-old male patient with AML relapsed 20 months after allogeneic BMT. Mixed donor chimerism was confirmed by PCR analysis. He had second CR after receiving standart dose Ara-C and idarubicine. While his donor was primed with G-CSF 2.5 mcg/kg/d for 10 days, he was given Ara-C 3 g/m^2 twice daily for four days. After this treatment, DLI was given. No GVHD prophylaxis was used. After recovery, IFN alfa was started and stopped on day + 53 in which acute GVHD was observed. GVHD resolved after steroid therapy. He faced with second relapse after BMT 36 months after the first DLI. He had third CR after treating with Ara-C+mitoxantrone and second DLI from primed donor. The third relapse was observed six months after the second DLI. No response was obtained after Ara-C and etoposide administration. He died due to the disease progression[17].

DISCUSSION

After a long time which includes very big and significant studies about treating leukemias; the question of which treatment is favorable or can obtain cure, can not be answered clearly. The treatment schedules based on conventional chemotheurapetic agents whether they are used in standart or high doses, with or without hematopoietic growth factors and/or autologous hematopoietic cell support, have limited effect on both obtaining and maintaining remission. Immunotherapy which can be added to these regimens is mainly based on enhancing immunocompetent cells' antileukemic effects, or transplanting them from healthy donor or both. Allogeneic HCT was firstly accepted as providing chance for applying high dose chemotherapy and then for changing all of the disordered bone marrow. But it is understood that the major and dominant effect of allogeneic transplantation is graft versus leukemia effect, and this effect is accepted to be responsible for all better results of DFS in leukemias with BMT. But this distinguished but not well discriminated effect has been questionable after having relapses following allotransplants.

The relapse of leukemia after allotransplant has been a very hard condition to be solved. Rat-

Table 2. Results

No	Patient initials	MNC (x 10^8/kg)	T cell (x 10^8/kg)	GVHD Acute/chronic	Chimerism status	Outcome
1	SD	3.4		Acute Grade I	CC	+ 76 mo, CR
2	MG	4.1		Acute Grade III chronic extensive	CC	+ 75 mo, CR
3	M‹	2.5		Acute Grade II	ND	+ 2 mo, ex, disease progression
4	AE	4.2		Acute Grade II chronic extensive	MC	+ 7 mo, ex, GVHD, pulmonary failure
5	HB	6.0	3.2	Acute Grade II chronic extensive	CC	+ 35 mo, CR
6	SE	9.3	3.9	No	ND	+ 2 mo, ex, disease progression
7	RD	15.8	7.6	No	ND	+ 15 mo, ex, disease progression
8	GY	10.9	8.9	Acute Grade III	ND	+ 2 mo, ex, CNS relapse
9	EE	16.0	5.1	Acute Grade II chronic limited	CC	+ 43 mo, ex, disease progression
10	FA	6.1	3.4	Acute Grade III	CC	+ 24 mo, ex, CNS relapse
11	MK	4.8		Acute Grade II	ND	+ 40 day, ex, disease progression
12	ZK	3.7	2.2	Acute Grade III	CC	+ 23 mo, CR
13	GB	2.5	1.1	Acute Grade IV	CC	+ 3 mo, ex, GVHD

Abbr: MNC: Mononuclear cells, MC: Mixed chimerism, CC: Complete chimerism, Mo: Month, ND: Not done

her than conventional or high dose chemotherapies or second transplants, enhancing the immunologic effect against leukemic cells has been the main choice for fighting relapse recently. For this purpose; stopping immunosuppressive agents which are used in transplant setting; using immunomodulator agents like IL-2 and IFN-alpha; and other techniques have been tried. In the recipient who is in complete or mixed chimerism at the time of the relapse (which means there are partly donor originated immunopoiesis) it is logical to provide reinforced and activated donor originated immunopoiesis rather than struggle hopelessly to use chemotheurapetics. After the realisation of this feature, beyond the applications described above, infusions of donor leukocytes have been performed. Recent developments in immunology, application of hematopoietic growth factors, and automated blood cell seperators have made transfusion of immunologically reactive cells possible. It has been very successful especially in the patients with minimal disease; i.e. patients with cytogenetic relapse of CML[16]. Although the expectancy that DLI is more effective in the chronic phase of the CML than in more advanced disease; regarding the hopelessness of the situation,

it should be tried for the patients who have advanced disease also because this is the only treatment option that promises cure for them[5,12,16,19-23]. Different studies demonstrated over 70% response rate for CML in cytogenetic and hematological relapse and in most of those patients responses were durable. In advanced diseases and acute leukemias response rates have found to be very low (approximately below 20%) and not sustainable. Especially in patients with CML, IFN alpha has been given with DLI to augment GVL effect in many of the studies. IL-2 was also used for activation of donor peripheral blood lymphocytes both in vitro and in vivo in some studies[24]. To overcome the poor prognosis of relapsed acute leukemia and the risk of cytopenia due to DLI, donors were primed with G-CSF and DLIs were given after standart dose antileukemic chemotherapy[17,23]. It was also showed that DLI provided reduction in marrow fibrosis in relapsed chronic idiopathic myelofibrosis following alloPBHCT[25]. Acute GVHD were seen in 30 to 80% of patients in different series. Developing GVHD have not always associated with complete response.

In a retrospective analysis which determined the effects of unrelated DLI in 58 patients who relapsed after unrelated BMT, toxicity including GVHD seemed acceptable[26]. They reported that response rates were higher in CML patients, and in patients with acute leukemia response rates were not as poor as with matched sibling DLI, however the numbers of the patients were small. They stated that only a longer interval from BMT to relapse and BMT to DLI was associated with improved survival and disease free survival, respectively. It was shown in a former study that infusion of cells from HLA matched volunteer donors did not increase the risk of GVHD compared with infusion of cells from HLA identical siblings in patients with CML who relapsed after alloBMT[27].

In a study which was performed on relapsed CML patients after alloBMT, predominance of donor lymphopoiesis at the time of the relapse was found, and it was explained by a state of tolerance to recipient's cells[28]. It means that they do not mediate an efficient immune effect. It was accepted that this is caused by mixed T cell chimerism which may allow donor/host tolerance. DLI after relapse may break this tolerance. If mixed chimerism with normal recipient hematopoietic cells persists after transplantation, it was shown that this will be with increased risk of relapse of hematologic malignancies[29]. It was shown that small numbers of donor cells (< 10^6/kg recipient body weight-RBW) can eradicate EBV lymphoproliferative disease but may not mediate GVL effect after T-cell depleted BMT in patients with CML[30]. In escalating dose DLI regimen, which was started with lower cell doses (for sibling donors from 1 x 10^7/kg RBW; for unrelated donors from 1 x 10^6/kg RBW), the incidence and severity of acute and chronic GVHD were shown to be lower than bulk dose regimen in patients who relapsed after allogeneic transplantation for CML[31]. It was shown that starting T cell dose has to be 1 log higher for multiple myeloma patients in one study[32]. Relapses with more tumor load can respond this treatment when chemotherapy for tumor reduction is applied first. Standart dose chemotherapy, which was given after DLI for post-allotransplant relapse in two multiple myeloma patients, was shown not to effect graft-versus-host reactions[18]. This finding is important, because the disease which relapsed after allotransplant may progress rapidly. Chemotherapy, which does not suppress immunity, may give time and chance to allogeneic antimalignancy lymphocytes to affect. High number of tumor cells may cause aplasia after DLI if donor is not stimulated with growth factors. Growth factor treatment of the donor before lymphocyte harvesting results in increase of CD34(+) cells. The use of G-CSF for cell mobilization in healthy donors caused marked increase of HLA-DR and CD34 expression and decrease of CD10, CD15, CD16 expression on neutrophil granulocytes[33]. These were accepted as the features of immaturity. There was an increase in CD71 and CD14 and they indicated the proliferation and increased functional activity. All these changes were reversible and returned to levels that were prior to G-CSF administration in a month. It was demonstrated that G-CSF mobilized cells reduce severity of acute GVHD by diminished inflammatory cytokine response which was involved in the development

of acute GVHD, and they preserve CTL activity for GVL effect[34]. Although it was shown that repeated cell mobilization from healthy donors reduce stem cell yield within a short time, adequate number of stem cells can be obtained repeatedly within two months[35]. Ex vivo T cell depletion of the donor marrow graft followed by DLI for the treatment of disease relapse to reduce transplant related complications was presented as a successful approach[36]. In this kind of approach, CD34+ cell dose was found to be predictive for relapse and survival[37].. High stem cell dose was supposed to have potential benefit in lowering transplant related mortality and relapse rate after alloHCT.

In our practice, the patients with progressive disease, like accelerated phase relapse of CML patients and relapsed AML patients, were not the well responded ones. These observations suggest that there is a limit for the immune effect regarding the number of the tumor cells and their proliferative capacity. Tissue damage as a result of disease progression and the toxicity of chemotherapy may play a role in the immune complications seen after DLI. Remaining GVH tolerance in the mixed chimeric status and the absence of a toxic conditioning regimen are the possible reasons for expecting mild GVHD. We have not come across DLI related aplasia. But signs of chronic graft versus host disease caused depraved quality of life in some of our patients. Although there is controversy about the therapeutic role of HCT for CNS leukemia, it was showed that donor lymphocytes cross blood-brain barrier more easily after administration of total body irradiation (TBI) containing conditioning regimens than under normal conditions[38]. One of the reasons of the higher CNS relapse rate in our patients may be the low migration rate of infused cells to the cerebrospinal fluid due to our conditioning regimens which did not contain TBI. Effects of recipient age, sex, diagnosis, prior therapies, infection status, diagnosis-transplantation time, transplant type, donor characteristics, donor priming, conditioning regimen, features of graft components, existence of posttransplant GVHD, transplant-relapse time, dose of infused T cells on response rate and durability, and postinfusion complications should be evaluated by multivariate analysis when we reach sufficient number of patients.

In fact, this is not a rescue operation, but one of the steps in overcoming malignancy. There will always be some minimal residual disease which can be detected or not. It is seen that, some patients have had remission with complete chimerism or some people have been refractory and died; but another group of patients shows mixed or triple chimerism with persistent or slowly progressing disease. For those patients or for those who have been relapsed; repeating DLI's are being tried[39]. As number of the patients who receive DLI for posttransplant disease relapse has increased, to provide sensitive statistical estimate of the effects of DLI on patients' outcome, different models were developed like multistage model which describes nine health states that a patient may be in after transplant;

0. Alive in first remission,
1. Dead before relapse,
2. Relapsed after transplant waiting for DLI,
3. Dead after relapse before DLI,
4. Relapsed with DLI,
5. Dead after DLI before second remission,
6. In second remission after DLI,
7. Dead while in remission after DLI,
8. Relapsed after second remission[40].

Unless the mechanisms which cause leukemia fully come into sight and the solutions which are straight forward to these mechanisms are found, fight with existing malign cells can only be made with immune system. In theory, this fight longs for a life time. Separating the immune components that act only against tumor cells, characterisation of the molecular targets of GVL effect, idendification of responsible cells, and proper stimulation of them both in quantity and selective targeting are the forthcoming aims. Until those aims are realized, unwanted immunologic reactions should be prevented without suppressing GVL effect.

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