

II. Restructuring of immune mechanism

Induction of autologous graft-versus-host disease after autologous peripheral blood stem cell transplantation

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Background: Autologous graft-versus-host disease has been reported after the administration of cyclosporine in patients who have received autologous bone marrow transplantation. **Objective:** The purpose of this study was to determine whether autologous graft-versus-host disease could be induced in recipients of autologous peripheral blood stem cell transplantation and whether tacrolimus induced the disease instead of cyclosporine.

Methods: Twelve patients with acute leukemia and 5 patients with malignant lymphoma received either cyclosporine (1 mg/kg/day) or tacrolimus (0.05 mg/kg/day) orally after autologous bone marrow or peripheral blood stem cell transplantation.

Results: Autologous graft-versus-host disease of the skin, confirmed by histopathologic criteria, occurred in 40% of the patients at 8 to 25 days after transplantation and lasted 3 to 15 days. The frequency of autologous graft-versus-host disease was approximately the same (40%) irrespective of the source of the graft (bone marrow cells or peripheral blood stem cells) and the drug used for induction (cyclosporine or tacrolimus). **Conclusions:** This pilot study suggests that autologous graft-versus-host disease can be induced in recipients of autologous peripheral blood stem cell transplantation by cyclosporine or tacrolimus. (*J Allergy Clin Immunol* 1999;103:S457-61.)

Key words: Autologous graft-versus-host disease, autologous peripheral blood stem cell transplantation, tacrolimus

Allogeneic bone marrow transplantation (BMT) is currently the optimum treatment for patients with hematologic malignancies. Despite the morbidity and mortality rates associated with graft-versus-host disease (GVHD), there is a significantly lower probability of relapse after allogeneic BMT than after autologous BMT.^{1,2} The mechanism is possibly an immunologically mediated graft-versus-tumor effect occurring in association with GVHD.

However, a considerable number of patients have no HLA-matched donor, so they are treated with chemother-

Abbreviations used

BMT: Bone marrow transplantation
GVHD: Graft-versus-host disease
PBSCT: Peripheral blood stem cell transplantation
WBC: White blood cell count

apy alone or receive autologous BMT or autologous peripheral blood stem cell transplantation (PBSCT). Interest has recently focused on methods of stimulating immunologically mediated antitumor activity in such patients.³

Induction of an acute GVHD-like reaction (autologous GVHD) by a short course of cyclosporine after autologous BMT was initially reported in 5 patients with refractory lymphoma⁴ and more recently in 33 patients with acute myeloid leukemia.^{5,6} However, it is not known whether autologous GVHD can be induced after autologous PBSCT and also whether tacrolimus can induce autologous GVHD after autologous BMT or PBSCT. The present study was performed to address these issues.

METHODS

We investigated 17 consecutive patients who were treated at our institution between 1991 and 1997. They included 12 patients with acute leukemia and 5 patients with malignant lymphoma. Their median age was 32 years (range, 20-47 years). Clinical details are shown in Tables I and II. Informed consent was obtained from all patients, and the study was approved by the hospital Ethics Committee.

Before June 1994, 9 patients underwent autologous BMT. After receiving the conditioning regimens shown in Tables I and II, cryopreserved autologous bone marrow nucleated cells ($>3 \times 10^7/\text{kg}$ body weight) were infused on day 0.

After July 1994, 8 patients underwent autologous PBSCT. Before transplantation, hematopoietic progenitor cells were mobilized by high-dose cytosine arabinoside with or without recombinant human G-CSF and were collected by leukapheresis. The leukapheresis product was cryopreserved at -140°C until used. After patients received the conditioning regimens shown in Tables I and II, the leukapheresis product was thawed and CFU-GM units ($>1 \times 10^5/\text{kg}$ body weight) were infused on day 0. For induction of autologous GVHD, patients received either oral cyclosporine (1 mg/kg/day) or tacrolimus (0.05 mg/kg/day) from days 1 to 28.

Patients were examined daily for the presence and extent of skin rashes and other potential clinical indicators of GVHD. The complete blood count was obtained daily, and liver function tests were

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TABLE I. Induction of autologous GVHD in patients with acute leukemia

Patient	Age (y)/ sex	CR status at transplantation	FAB class	Conditioning regimen	Graft	Inducer	Autologous GVHD	Days to increase of WBC*/ ANC > 500†	Outcome
1	34/F	CR1	M1	BU/CY/CA/ETP	BM	C	+(d8-d18)	6/24	Died, 2 mo (liver failure)
2	35/M	CR1	M2	BU/CA/ETP	BM	T	+(d25-d39)	19/23	Remission, 69+ mo
3	22/M	CR1	L2	CA/CY/ETP	BM	C	—	7/10	Died, 29 mo (relapse)
4	46/F	CR2	L2	CA/CY/ETP	BM	C	—	5/11	Died, 14 mo (relapse)
5	23/M	Relapse	L1	BU/CA/ETP	BM	C	+(d14-d25)	6/24	Died, 9 mo (relapse)
6	22/F	Relapse	L1	CA/TBI	BM	C	—	13/NR	Died, 5 mo
7	30/M	PR	M2	CY/TBI	BM	C	+(d15-d18)	7/16	Died, 5 mo (relapse)
8	32/F	CR1	L2	CA/CY/ETP	PB	C	+(d21-d23)	11/17	Remission, 43+ mo
9	38/F	CR1	M2	BU/CA	PB	C	—	7/11	Remission, 40+ mo
10	20/F	CR1	M1	BU/CA/ETP	PB	T	—	6/14	Remission, 25+ mo
11	47/M	CR2	L2	BU/CY/ETP	PB	C	+(d13-d26)	6/10	Relapse, 2 mo
12	39/M	CR1	M2	BU/CA/ETP	PB	C	—	NR/NR	Died, 2 mo (sepsis)

CR, complete remission; FAB, French-American-British; ANC, absolute neutrophil count; BU, busulfan; CY, cyclophosphamide; CA, cytosine arabinoside; ETP, etoposide; BM, bone marrow cells; C, cyclosporine; d, days after transplantation; T, tacrolimus; TBI, total body irradiation; NR, not reached; PB, peripheral blood stem cells.

*Days after transplantation to increase in WBC from nadir.

†Days after transplantation to absolute neutrophil count more than 500 per microliter.

TABLE II. Induction of autologous GVHD in patients with malignant lymphoma

Patient	Age (y)/ sex	CR status at transplantation	Disease	Conditioning regimen	Graft	Inducer	Autologous GVHD	Days to increase WBC/ANC > 500	Outcome
1	21/F	CR1	HD	CA/CY/ETP	BM	C	—	7/10	Remission, 61+ mo
2	38/F	CR1	HD	CA/CY/ETP	BM	C	—	7/13	Remission, 58+ mo
3	22/F	CR1	NHL	MCNU/CBDCA/ETP/CY	PB	T	+(d15-d21)	5/10	Remission, 31+ mo
4	44/M	CR1	NHL	CY/ETP/MCNU	PB	T	—	7/11	Remission, 31+ mo
5	31/F	Relapse	HD	CY/ETP/DTIC/LPAM	PB	T	—	7/15	Remission, 27+ mo

CR, complete remission; ANC, absolute neutrophil count; HD, Hodgkin's disease; CA, cytosine arabinoside; CY, cyclophosphamide; ETP, etoposide; BM, bone marrow cells; C, cyclosporine; NHL, non-Hodgkin's lymphoma; MCNU, ranimustine; CBDCA, carboplatin; T, tacrolimus; d, days after transplantation; DTIC, dacarbazine; LPAM, melphalan.

performed once a week until day 28. Biopsy specimens were obtained from any skin rashes and were histologically assessed by one of the authors (S.I.) according to published criteria.⁷

Peripheral blood samples were taken from the patients before and serially after transplantation. Serum was separated and stored frozen in aliquots at -80°C until assay. Serum soluble IL-2 receptor, interferon- γ , and IL-12 levels were measured by sandwich ELISAs with the following kits: Soluble IL-2 receptor was measured with a kit from T cell Diagnostics, Cambridge, Mass (detection limit, 100 U/mL), interferon- γ with a kit from Otsuka Pharmaceuticals, Tokyo, Japan (detection limit, 10 pg/mL), and IL-12 p70 with a kit from Genzyme Diagnostics, Cambridge, Mass (detection limit, 10 pg/mL).

RESULTS

Tables I and II summarize the results of this study. One patient with acute leukemia died of sepsis associated with persistent aplasia; the other 16 patients showed engraftment after transplantation. Autologous GVHD of the skin developed in 7 patients at a median of 15 days after transplantation (range, 8-25 days) and lasted for a median of 10 days (range, 3-15 days).

An erythematous macropapular rash affected the palms and soles of all 7 patients and the extremities in 1

patient. Autologous GVHD of the skin was confirmed histologically by skin biopsy (Fig 1). Vacuolar degeneration of epidermal basal cells, dyskeratosis and eosinophilic necrosis of single cells in the epidermis, and slight lymphocytic infiltration were observed. The rash resolved spontaneously in all patients. No patient had diarrhea or a raised serum bilirubin level suggestive of extracutaneous GVHD.

Six of the 12 patients (50%) with acute leukemia and 1 of the 5 patients (20%) with malignant lymphoma experienced the development of autologous GVHD of the skin (Tables I and II). The frequency of such autologous GVHD was approximately the same (40%) irrespective of the source of the graft (bone marrow cells or peripheral blood stem cells) and the drug used for induction (cyclosporine or tacrolimus; Table III). Moreover, the frequency of autologous GVHD of the skin was the same (50%) in patients with acute leukemia receiving cyclosporine after autologous BMT and after autologous PBSCT (Table III).

In patients who experienced the development of a skin rash, the median time to the onset of the rash was 15 days (range, 8-25 days), whereas the median time to an increase in the white blood cell count from the nadir was

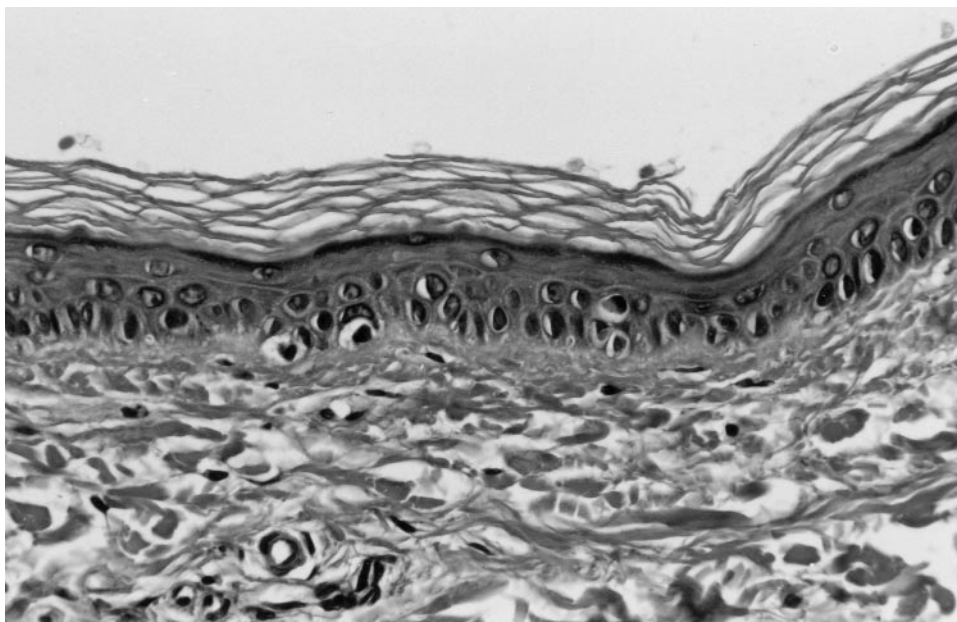


FIG 1. Skin biopsy specimen obtained 34 days after autologous BMT from patient 2 with acute leukemia who received tacrolimus (Hematoxylin-eosin stain; original magnification, $\times 100$).

6 days (range, 5-19 days). The median time to an absolute neutrophil count of more than 500 per μL was 17 days (range, 10-24 days) after transplantation (Table IV).

The outcome of the patients is summarized in Tables I and II. One patient died of transplantation-related complications. Five patients with acute leukemia had relapse (1 first complete remission, 2 second complete remission, 1 partial remission, and 1 relapse) at a median of 7 months (range, 1-20 months) after transplantation. Patient 6 with acute leukemia remained in relapse after transplantation. None of the patients with malignant lymphoma have experienced relapse. Four patients with acute leukemia in first complete remission are alive in remission at a median of 41.5 months (range, 25-69+ months) after transplantation. All 5 patients with malignant lymphoma are alive in remission at a median of 31 months (range, 27-61+ months) after transplantation.

Serum levels of soluble IL-2 receptor, interferon- γ , and IL-12 were measured serially after autologous BMT in 2 patients with and without cyclosporine administration. Both soluble IL-2 receptor and interferon- γ increased markedly in the patients (Fig 2, A and B). In contrast, there was a slight but significant increase of the serum IL-12 level concomitantly with the development of autologous GVHD (Fig 2, B).

DISCUSSION

The main cause of failure of autologous BMT or PBSCT is tumor recurrence. Current cytotoxic preparative regimens for autologous BMT or PBSCT are near to dose-limiting toxicity levels, so further dose escalation is not feasible. However, immunologic manipulation

remains an attractive approach to improve disease-free survival by decreasing the relapse rate. Because most acute leukemia cells and malignant lymphoma cells express Ia antigen⁸ and autologous GVHD induced by cyclosporine is associated with the appearance of anti-Ia cytotoxic T lymphocytes in rodents⁹ and in patients who have received autologous BMT,⁴ autologous GVHD could theoretically have a clinically significant antitumor effect.

Histologic examination of skin biopsy specimens revealed the features of acute GVHD with a severity between grades 1 and 2 (Fig 1). Mild cutaneous manifestations resembling acute GVHD have previously been reported in some recipients of autologous BMT or PBSCT without cyclosporine administration.^{10,11} Because such cutaneous manifestations appear within 1 to 2 days of the time when the white cell count shows an increase from its nadir of 100 to 200 per μL and because biopsy specimens show the histologic features of grade 1 or 2 acute GVHD, this is referred to as the engraftment syndrome¹⁰ or autoaggression syndrome.¹¹ In our study, however, the median time to onset of the skin rash (day 15) was 9 days later than the median time to an increase of the white blood cell count (WBC; day 6) after transplantation, and the median WBC at onset of the skin rash was relatively high (1,850 per μL ; Table IV). The frequency of such cutaneous manifestation is reported to be 7% (7 of 96 patients with hematologic malignancies).¹² However, we have not previously observed such cutaneous manifestation in 32 patients with acute leukemia who underwent autologous BMT or PBSCT without cyclosporine administration. Thus autologous GVHD induced by cyclosporine or tacrolimus appears later than the engraftment syndrome, and its frequency seems to be

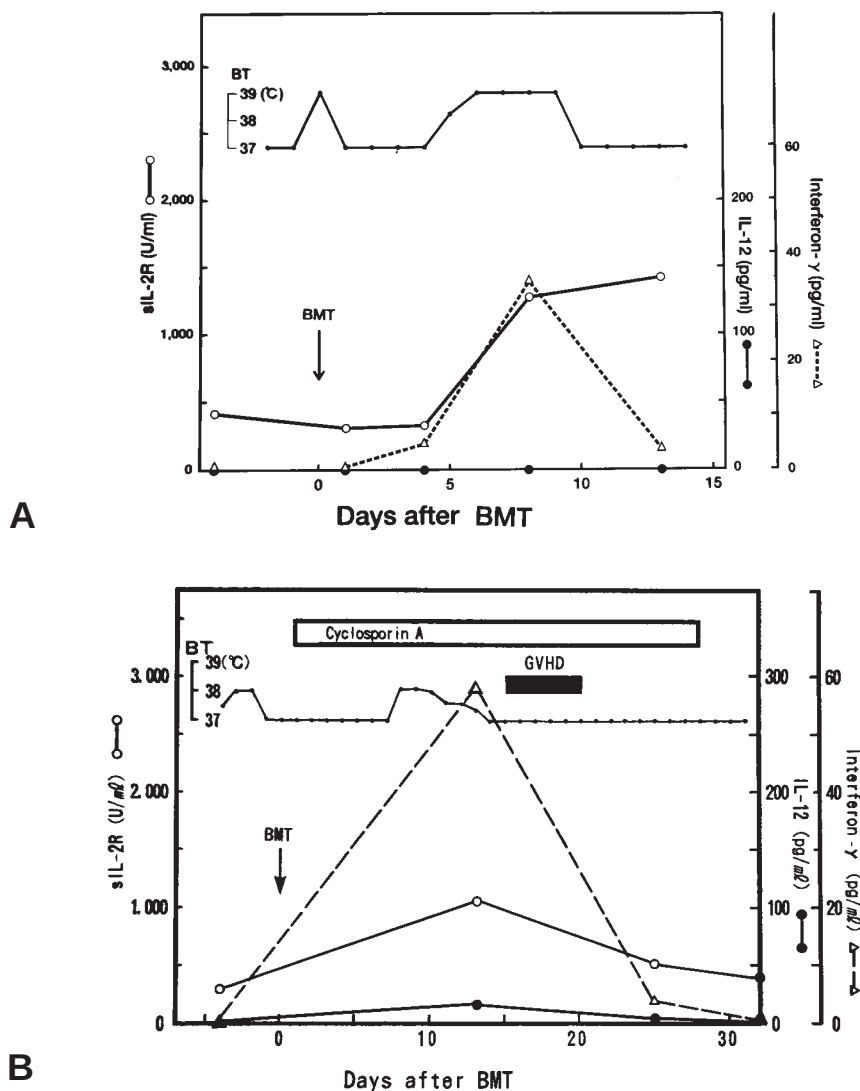


FIG 2. **A**, Profiles of serum soluble IL-2 receptor, interferon- γ , and IL-12 in a patient with acute myeloid leukemia (AML) who underwent autologous BMT. Cyclosporine was not administered. *BT*, Body temperature; *sIL-2R*, soluble IL-2 receptor. **B**, Profiles of serum soluble IL-2 receptor, interferon- γ , and IL-12 in a patient with AML who underwent autologous BMT. Cyclosporine was administered orally and induced autologous GVHD of the skin (black bar).

TABLE III. Frequency of autologous GVHD

Graft/inducer	No. of patients	Autologous GVHD+ (%)
Autologous BMT	9	4 (44)
Autologous PBSCT	8	3 (38)
Cyclosporine	12	5 (42)
Tacrolimus	5	2 (40)
Patients with acute leukemia receiving cyclosporine		
Autologous BMT	6	3 (50)
Autologous PBSCT	4	2 (50)

TABLE IV. Relationship of skin rash to engraftment in all 7 patients with autologous GVHD

Skin rash/engraftment	Median days after transplantation (range)
Time to skin rash	15 (8-25)
Time to increased WBC*	6 (5-19)
Time to ANC > 500 per μ L	17 (10-24)
WBC at onset of skin rash	1850 per μ L (32-8,630 per μ L)

ANC, Absolute neutrophil count.

*Days to increase in WBC from nadir.

higher than that of the engraftment syndrome. The relationship between autologous GVHD and the engraftment syndrome remains to be elucidated.

Because Talbot et al¹³ reported that oral cyclosporine (1 mg/kg/day) can induce autologous GVHD of the skin almost as efficiently as intravenous cyclosporine, cyclosporine (1 mg/kg/day) or tacrolimus (0.05 mg/kg/day) was administered orally in this study. Six of the 12 patients (50%) with acute leukemia and 1 of the 5 patients (20%) with malignant lymphoma experienced autologous GVHD of the skin at a median of 15 days (range, 8-25 days) after transplantation (Tables I, II, and IV). Talbot et al¹³ reported that autologous GVHD of the skin occurred in 2 of 5 patients (40%) with acute myeloid leukemia when oral cyclosporine (1 mg/kg/day) was administered for 28 days after autologous BMT. More recently, Yeager et al¹⁶ reported that 15 of 19 patients (79%) with acute myeloid leukemia experienced autologous GVHD of the skin when intravenous cyclosporine (1 mg/kg/day) was administered for 28 days after autologous BMT. The frequency, time to onset, and duration of GVHD were similar among the 3 cyclosporine doses (1, 2.5, and 3.75 mg/kg/day) administered intravenously.

Cooper et al¹⁴ showed that autologous GVHD can be induced by tacrolimus in rats, with at least 75% of animals developing autologous GVHD of the skin after intramuscular injection of tacrolimus (0.25 to 1 mg/kg/day) at 40 to 45 days after syngeneic BMT. In addition, the incidence of autologous GVHD increased with increasing doses of tacrolimus.

The present pilot study showed that oral tacrolimus (0.05 mg/kg/day) can induce autologous GVHD of the skin as efficiently as oral cyclosporine (1 mg/kg/day) and that the frequency of autologous GVHD induction, in patients who received autologous PBSCT, is approximately the same as in patients who received autologous BMT (Table III). Because autologous PBSCT has recently been used in place of autologous BMT and peripheral blood stem cells contain 10 times as many lymphocytes as bone marrow cells, this result is quite interesting.

No valid conclusions can be drawn concerning the potential of antitumor effect of autologous GVHD. Obviously, new strategies to increase the frequency of autologous GVHD and to enhance its antitumor effect are required.

Because autologous GVHD is associated with the appearance of Ia-specific cytotoxic T cells,^{4,9} cytokines such as IL-2, interferon- γ , and IL-12 may play an important role in this process.

The soluble IL-2 receptor level was measured instead of IL-2, which has a short half blood life. Serum levels of soluble IL-2 receptor, interferon- γ , and IL-12 were determined serially in 2 patients with and without cyclosporine administration in the early postautologous BMT period. Serum soluble IL-2 receptor and interferon- γ levels were markedly increased in both patients (Fig 2, A and B), suggesting that the occurrence of high fever may be related to this increase.

Siebert et al¹⁵ reported an increase of serum soluble IL-2 receptor levels in patients after allogeneic BMT with high-grade fever. We have also found a significant correlation between the increase of both soluble IL-2 receptor and interferon- γ levels and the occurrence of high fever after allogeneic BMT (unpublished observation). In contrast, a slight but significant increase of serum IL-12 level was observed in a patient with autologous GVHD (Fig 2, B). IL-12 is a cytokine with an immunoregulatory role in the generation of type I T-helper cells and cytotoxic T cells.¹⁶ Whether the serum IL-12 level can be used as a marker of autologous GVHD remains to be established.

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