

Session II: Restructuring of Immune Mechanism

1. Organism

Induction of autocytotoxic T cells with cyclosporine and interferon- γ for patients with non-Hodgkin's lymphoma after transplantation of peripheral blood stem cells

Shinji Nakao, MD,^a Yuji Miura, MD,^a Weihua Zeng,^a Akiyoshi Takami, MD,^a Naomi Sugimori, MD,^a Hirohito Yamazaki, MD,^a Akihiro Yachie, MD,^b Shintaro Shiobara, MD,^c and Tamotsu Matsuda, MD^a *Ishikawa, Japan*

Background: Autologous graft-versus-host disease is inducible after autologous bone marrow transplantation by means of administration of cyclosporine.

Objective: This study was performed to investigate the inducibility of autologous graft-versus-host disease after transplantation of peripheral blood stem cells (PBSCs).

Methods: Two patients with non-Hodgkin's lymphoma in remission underwent PBSC transplantation followed by administration of cyclosporine and low-dose interferon- γ .

Results: Although autologous graft-versus-host disease did not develop in either patient, T lymphocytes with cytotoxic activity against autologous lymphocytes appeared transiently in the early posttransplant period. Such autocytotoxic lymphocytes were not detectable in another patient who underwent PBSC transplantation who did not receive cyclosporine and interferon- γ . When CD4⁺ and CD8⁺ cells were sorted from the peripheral blood mononuclear cells of one of the two patients and tested for cytotoxicity against autologous lymphocytes, only CD8⁺ cells exhibited cytotoxic activity.

Conclusions: The results indicate that administration of cyclosporine and interferon- γ after PBSC transplantation can induce autocytotoxic CD8⁺ T cells, even though it may not produce autologous graft-versus-host disease. It is unclear whether induction of such autocytotoxic T cells among patients with non-Hodgkin's lymphoma who undergo PBSC transplantation has any antilymphoma effect. (*J Allergy Clin Immunol* 1997;100:S65-9.)

Key words: Non-Hodgkin's lymphoma, autologous graft-versus-host disease, cyclosporine, interferon- γ

High-dose chemoradiotherapy combined with autologous stem cell transplantation has been used successfully in the treatment of patients with malignant lymphoma.¹ A number of clinical studies have demonstrated that

Abbreviations used

HLA:	Human leukocyte antigen
LCL:	Lymphoblastoid cell line
PBMC:	Peripheral blood mononuclear cell
PBSC:	Peripheral blood stem cell
PHA:	Phytohemagglutinin
GVHD:	Graft-versus-host disease
IFN- γ :	Interferon- γ

among the therapeutic options available for relapsing lymphoma, autologous bone marrow transplantation is most effective in achieving long-term survival.² The use of peripheral blood stem cells (PBSCs) rather than bone marrow has markedly reduced the incidence of early death related to high-dose chemoradiotherapy.^{3,4} Autologous stem cell transplantation is being used increasingly as primary therapy for patients with malignant lymphoma who show only a partial response to conventional chemotherapy or as consolidation therapy to treat patients in remission with poor prognostic factors.⁵⁻⁷ Clinical trials have not demonstrated any advantage of autologous stem cell transplantation over conventional chemotherapy in the treatment of patients with non-Hodgkin's lymphoma who show a slow response to chemotherapy or patients with disease in remission but with poor prognostic factors.^{8,9} These findings suggest that raising the dose intensity of chemotherapy by use of autologous stem cell transplantation may not necessarily improve the outcome. For patients who do not achieve sustained remission after conventional chemotherapy, novel approaches such as immunotherapy seem to be needed.

One such approach is induction of autologous graft-versus-host disease after autologous stem cell transplantation. Autologous GVHD is inducible for 70% to 80% of recipients of autologous bone marrow by means of administration of cyclosporine in the early posttrans-

From the ^aThird Department of Medicine; ^bDepartment of Pediatrics; and ^cDivision of Transfusion, Kanazawa University School of Medicine.

Reprint requests: Shinji Nakao, MD, Third Department of Medicine, Kanazawa University School of Medicine, 13-1 Takaramachi, Kanazawa, Ishikawa 920 Japan.

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TABLE I. Clinical characteristics of the patients

	Patient 1	Patient 2	Patient 3
Age/sex	47/male	43/male	48/male
Clinical stage	IIIB	IVA	IIIA
Histologic composition	Angioimmunoblastic T-cell lymphoma	Peripheral T-cell lymphoma	Follicular cell lymphoma
International index	2	3	2
Chemotherapy before PBSC transplantation	CHOP 5 courses	CHOP 6 courses	CHOP 6 courses
Disease state at the time of PBSC transplantation	PR	CR	CR
CD34 ⁺ cell dose ($\times 10^6/\text{kg}$)	26.1	22.0	4.5

CHOP, Cyclophosphamide, doxorubicin, vincristine, and prednisolone; PR, partial remission; CR, complete remission.

TABLE II. Hematologic recovery and toxicity after PBSC transplantation

	Days after PBSC transplantation		
	Patient 1	Patient 2	Patient 3
Granulocytes $>0.5 \times 10^9/\text{L}$	9	10	9
White blood cell count $>2.0 \times 10^9/\text{L}$	10	10	10
Platelets $>50.0 \times 10^9/\text{L}$	11	11	14
Last platelet transfusion	9	10	13
Reticulocytes $>20.0 \times 10^9/\text{L}$	13	11	14
Maximum bilirubin (mg/dl)	0.6	0.8	0.8
Maximum creatinine (mg/dl)	1.0	0.9	1.3

plantation period.¹⁰ Results of clinical trials involving patients with acute myeloid leukemia or non-Hodgkin's lymphoma have suggested that induction of autologous GVHD can reduce the relapse rate.^{11, 12} This beneficial effect is likely to be caused by cyclosporine-induced autoreactive T cells directed against human leukocyte (HLA) class II antigens expressed on tumor cells.¹³ Recent clinical regimens for the management of breast cancer have included interferon (IFN)- γ , which has been shown to increase the severity of autologous GVHD when given with cyclosporine.¹⁴ We previously attempted to induce autologous GVHD for three patients with non-Hodgkin's lymphoma by means of administration of a low dose of cyclosporine (1 mg/kg a day) alone after PBSC transplantation using a method described by Jones et al.¹⁵ However, none of the patients contracted autologous GVHD (unpublished observation). After this failure, we initiated a new clinical study to induce autologous GVHD by administering a higher dose of cyclosporine in combination with a low dose of IFN- γ .^{14, 16} Although two patients treated with this regimen did not contract GVHD after PBSC transplantation, autocytotoxic T cells appeared in the blood in the early posttransplantation period.

METHODS

Patients

Three patients with non-Hodgkin's lymphoma were treated with high-dose chemotherapy consisting of ranimustine (200 mg/m² intravenously on days 8 and 3 before transplantation),

carboplatin (300 mg/m² intravenously on days 7, 6, 5, and 4 before transplantation), etoposide (500 mg/m² intravenously on days 6, 5, and 4 before transplantation), and cyclophosphamide (50 mg/kg intravenously on days 3 and 2 before transplantation) followed by PBSC transplantation. Table I summarizes the clinical characteristics. Cyclosporine (2.5 mg/kg a day) was administered intravenously to patients 1 and 2 from day 1 to day 28 after PBSC transplantation.¹⁴ Recombinant IFN- γ (100 μg ; Shionogi, Osaka, Japan) was administered subcutaneously to both patients on days 7, 14, 21, and 28. Patient 3 did not receive cyclosporine or IFN- γ . The dose of IFN- γ was set on the basis of a report that indicated the efficacy of IFN- γ at this dose in up regulating HLA expression of molecules on tumor cells.¹⁷ Granulocyte colony-stimulating factor (5 $\mu\text{g}/\text{kg}$ a day) was given to all three patients from day 1 after transplantation until the leukocyte count exceeded $5.0 \times 10^9/\text{L}$. This treatment protocol was reviewed and approved by the institutional review board, and informed consent was obtained from all patients.

Preparation of phytohemagglutinin-stimulated lymphocytes and lymphoblastoid cell line

Peripheral blood treated with heparin was collected from the patients before conditioning chemotherapy. Peripheral blood mononuclear cells (PBMCs) were separated by means of density-gradient centrifugation, suspended in RPMI 1640 medium (Gibco, Grand Island, N.Y.), and cryopreserved at -80°C after division into aliquots. To test the autocytotoxic activity of posttransplant lymphocytes, thawed PBMCs were cultured for 5 to 7 days in the presence of 1 $\mu\text{g}/\text{ml}$ phytohemagglutinin (PHA; Wellcome, Dartford, U.K.). Some PBMC aliquots from patient 1 were depleted of T cells by means of the rosette-forming method. Then 2 to 3×10^6 non-T cells were incubated in 10%

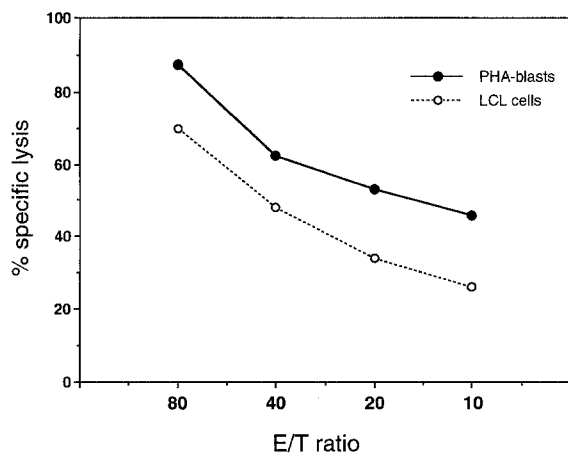


FIG. 1. Cytotoxicity for autologous lymphocytes of peripheral blood mononuclear cells obtained after peripheral blood stem cell transplantation. Phytohemagglutinin-stimulated lymphocytes (10^4 ; PHA-blasts) or B-lymphoblastoid cell line (LCL) cells were incubated with varying numbers of mononuclear cells obtained from patient 1 on day 13 after transplantation. *E/T ratio*, effector/target ratio.

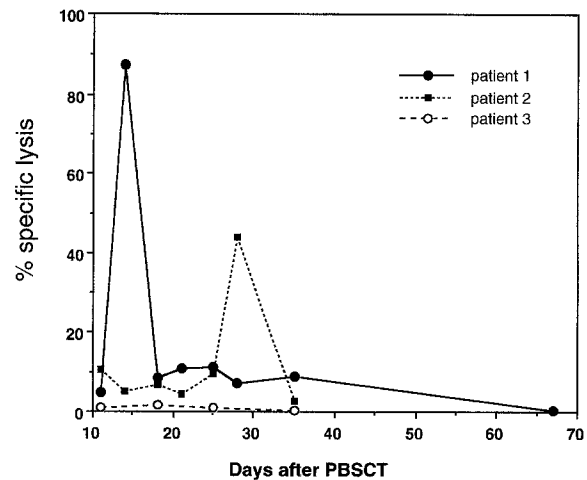


FIG. 2. Changes of PBMC cytotoxicity for autologous PHA-stimulated lymphocytes after PBSC transplantation. Cytotoxicity for autologous PHA-stimulated lymphocytes obtained at different time points after PBSC transplantation was determined at various times with an effector/target ratio of 80.

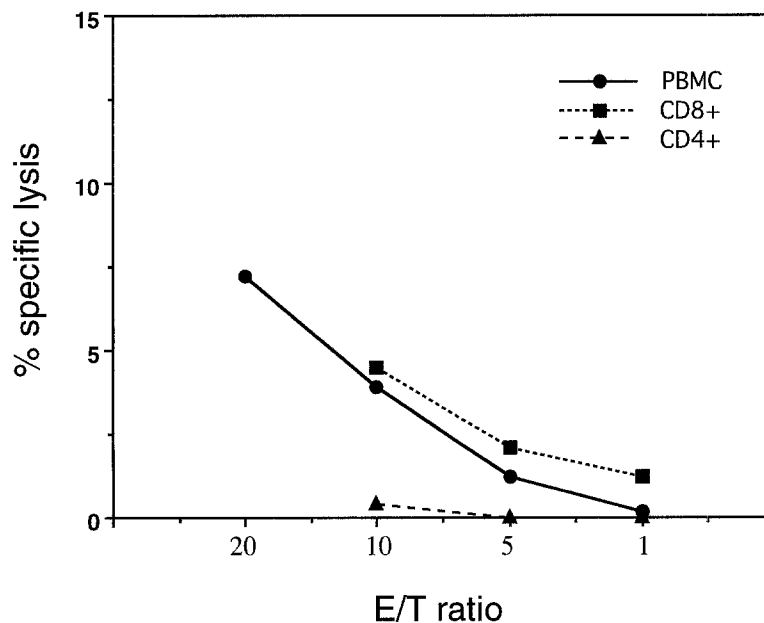


FIG. 3. Only CD8⁺ T cells exhibited cytotoxic activity comparable with that of unsorted PBMCs.

fetal calf serum (Gibco)-RPMI 1640 containing 10% conditioned medium of an Epstein-Barr virus-producing cell line (B95-8) at 37°C for 2 hours. The Epstein-Barr virus-infected cells were cultured for 3 weeks until transformed lymphoblastoid cell line (LCL) cells grew, and these cells were maintained by means of changing of the medium every 4 to 5 days. After washing with RPMI 1640, PHA-stimulated lymphocytes (PHA-blasts) and LCL cells were used for the ^{51}Cr release assay.

Cell sorting

PBMCs obtained from patient 1 on day 36 after PBSC transplantation were incubated with anti-CD4 or anti-CD8 monoclonal antibodies (Becton Dickinson, Mountain View,

Calif.), and the stained cells were sorted with an Epics C cell sorter (Coulter, Hialeah, Fla.).

Cytotoxicity assay

PHA-stimulated cells or LCL cells were suspended in 100 μl of ^{51}Cr (400 to 1200 Ci/g chromium; Du Pont NEN Products, Boston, Mass.) for one hour. After washing with phosphate-buffered saline solution, 10^4 cells were cultured with varying numbers of autologous PBMC or sorted T cells obtained after PBSC transplantation. After incubation for 4 hours, release of ^{51}Cr into the medium was measured with a gamma counter. Specific lysis (mean $\pm$ SE) was determined as a percentage in triplicate cultures as follows: $100 \times (\text{experimental release in}$

cells per minute – spontaneous release in cells per minute) ÷ (maximum release in cells per minute – spontaneous release in cells per minute).

RESULTS

Hematologic recovery and treatment-related toxicity after PBSC transplantation

Table II summarizes the hematologic recovery and treatment-related toxicity after PBSC transplantation among the three patients. Both cyclosporine-treated patients showed rapid recovery of hematopoiesis, which was comparable with that for patient 3, who received no cyclosporine or IFN- γ . There was no increased toxicity related to administration of IFN- γ , except for transient fever after its injection. However, GVHD was not induced in either of these patients.

Autocytotoxic activity of lymphocytes after PBSC transplantation

Cytotoxicity of PBMCs for autologous lymphocytes was detectable for both patients treated with cyclosporine and IFN- γ . The results of the ^{51}Cr release assay of PBMCs from patient 1 on day 13 are shown in Fig. 1. The PBMCs exhibited cytotoxicity against both PHA-stimulated lymphocytes and LCL cells in a concentration-dependent manner. Fig. 2 illustrates the changes in the autocytotoxic activity of lymphocytes over time after PBSC transplantation. Peak cytotoxicity was observed on day 16 for patient 1 and on day 28 for patient 2. Lymphocytes with autocytotoxic activity were no longer detected after day 36 for both patients and were never detectable for patient 3.

Phenotype of autocytotoxic T cells

CD4 $^{+}$ and CD8 $^{+}$ T cells were sorted from the PBMC obtained from patient 1 on day 36 and tested for cytotoxicity against autologous PHA-stimulated lymphocytes. As shown in Fig. 3, only CD8 $^{+}$ T cells exhibited cytotoxic activity comparable with that of unsorted PBMCs.

DISCUSSION

In this preliminary study, we tested the inducibility of autologous GVHD using cyclosporine and low-dose IFN- γ to treat patients with non-Hodgkin's lymphoma after PBSC transplantation. This regimen is reported to be capable of inducing autologous GVHD among approximately 80% of recipients of autologous bone marrow transplants. However, our two patients did not manifest any signs of GVHD. The failure to induce GVHD in our patients may have been due to the use of PBSCs rather than bone marrow. Pati et al. reported that the incidence of autologous GVHD among patients treated with cyclosporine after PBSC transplantation was only 30%.¹⁸ Because the leukapheresis product used as a source of PBSCs contained a high number of T cells, it is possible that PBSC grafts may possess more T cells that down regulate the development of autologous GVHD compared with bone marrow.¹⁰ Recipients of

PBSC transplants may be less sensitive to the induction of autologous GVHD with cyclosporine compared with recipients of bone marrow transplants. However, this possibility has to be investigated among a larger number of patients undergoing PBSC transplantation.

Although GVHD did not develop in our patients, T cells with autocytotoxic activity were detectable in both patients treated with cyclosporine plus IFN- γ . Such autocytotoxic T cells were present for a limited time after PBSC transplantation and were no longer detectable from day 36 after PBSC transplantation. The transient appearance of lymphocytes with autocytotoxic activity in the early posttransplant period is consistent with the findings of Hess et al., who reported that cytotoxicity against autologous PHA-stimulated lymphocytes was detectable in PBMCs obtained from days 7 through 15 after transplantation.¹⁹ Our finding that the cytotoxicity of PBMCs from patient 1 was mediated by CD8 $^{+}$ T cells also agrees with the report of Hess et al.¹⁰ Thus administration of cyclosporine plus IFN- γ to our patients appears to have induced autoreactive T cells similar to those that mediate autologous GVHD. It is unknown whether induction of autocytotoxicity without manifestations of GVHD is helpful in preventing the relapse of non-Hodgkin's lymphoma, as is the case for induction of autologous GVHD. Among recipients of allogeneic bone marrow with acute leukemia, the rate of relapse is known to be lower than that among recipients of syngeneic bone marrow, even when these patients do not contract GVHD.²⁰ This has been believed to be due to the graft-versus-leukemia effect mediated by T cells that recognize a minor histocompatibility antigen or leukemia-specific antigen on leukemic cells. Autocytotoxic CD8 $^{+}$ T cells induced by means of administration of cyclosporine and IFN- γ may exert an antitumor effect even if there is no overt autologous GVHD. Long-term follow-up study involving a large number of patients treated in the same way is needed to determine the efficacy of inducing autocytotoxic T cells in the management of non-Hodgkin's lymphoma.

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