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Circulating Hematopoietic Progenitors in Patients with Primary Lung Cancer

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The levels of circulating hematopoietic progenitors were measured in 28 patients with primary lung cancer. The average numbers of progenitors per milliliter of blood were 33 (range 0–360) for colony-forming unit-granulocyte macrophage (CFU-GM), 23 (range 0-140) for burst-forming unit-erythrocyte (BFU-E), and 4 (range 0–50) for colony-forming unit-mixed lineages (CFU-mix). No significant influence of age, sex, histological type, or clinical stage of the tumor on the progenitor levels was detected. After cytoreductive chemotherapy of the patients by treatment with cisplatin plus etoposide, the cells showed 6- to 50-fold rebound overshoots, but no rebound was observed after treatment with cisplatin alone, cisplatin plus mitomycin C or cisplatin plus vindesine plus mitomycin C, or in 4 of 5 patients treated with cyclophosphamide plus adriamycin plus vincristine. Peripheral blood hematopoietic progenitors should be useful as an alternative source of stem cells for lung cancer patients treated with marrow ablative chemotherapy.

Key words: Lung cancer — Hematopoietic progenitor — Chemotherapy

The reported incidence of lung cancer in Japan has recently been increasing and may exceed that of stomach cancer within 10 years. Even with improved therapeutic methods, the prognosis for patients with lung cancer is poor, the 5-year survival rate being around 10%. One reason for this poor prognosis is the development of resistance to various types of chemotherapeutic drugs, indicating the necessity for other treatments including high-dose chemotherapy with hematopoietic progenitor rescue. Small-cell type cancer of the lung is particularly sensitive to high-dose chemotherapy.[1-3] However, on autologous bone marrow transplantation (BMT), cancer cells may be present in the bone marrow, resulting in the recurrence of cancer, and in fact, so far reported results of this treatment have been discouraging.[4-6] Hematopoietic progenitors are known to be present in the peripheral blood of humans as well as rodents, dogs, and baboons,[7-14] and so there has been much recent interest in the use of autologous peripheral blood hematopoietic progenitors (PBHPs) for transplantation.[15-24] One advantage of the use of autologous PBHPs rather than bone marrows is their ready availability without the associated risk of anesthesia or the discomfort to patients arising from multiple bone marrow aspirations. Futhermore, PBHPs induce a faster hematopoietic recovery than marrow progenitors.[25,26] A further possible, but as yet unproved, advantage of PBHPs over autologous marrow cells is that they may contain fewer contaminating cancer cells. Autografts of PBHPs have so far been used mainly in patients with leukemia, multiple myeloma, or lymphoma, and, little is known about the kinetics of PBHPs in patients with solid tumors.[27,28] Therefore, in this work, we measured the numbers of colony-forming unit-granulocyte macrophage (CFU-GM), burst-forming unit-erythrocyte (BFU-E), and colony-forming unit-mixed lineages (CFU-mix) in the peripheral blood and bone marrow of patients with advanced lung cancer before and after chemotherapy.

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MATERIALS AND METHODS

Patients Numbers of PBHPs were measured in patients with histologically proven carcinoma of the lung. Studies were made after the following staging procedures of patients: chest X-ray examination; fiberoptic bronchoscopy with washing, brushing, and biopsies; bone marrow aspiration and biopsy; a bone scan; a CT scan of the brain; and abdominal echography. None of the patients had received previous chemotherapy or radiotherapy and all gave their informed consent to participate in the experiment.

PBHPs measurement Before chemotherapy, peripheral blood was obtained by venipuncture with a needle attached to a plastic syringe containing heparin. The blood was diluted 1:1 with calcium- and magnesium-free phosphate-buffered saline (PBS), and mononuclear cells were separated by centrifugation on a 60% Percoll cushion. For CFU-GM assay, the mononuclear cells were plated in 35 mm Petri dishes in Dulbecco's minimum essential medium (DMEM) supplemented with 0.8% methylcellulose, 20% fetal bovine serum (FBS), 1% bovine serum albumin (BSA), and 100 U/ml of purified granulocyte macrophage-colony-stimulating factor (GM-CSF, Chugai Co., Tokyo) at 2×10^5 cells per plate

and cultured in a humidified atmosphere of 5% CO_2 in air at 37°C. Duplicate cultures were set up and colonies (>40 cells) were scored under an inverted microscope after incubation for 14 days. For assays of BFU-E and CFU-mix, 1 U/ml of erythropoietin (Epo, Step III, Connaught, Toronto) and 10% phytohemagglutinin (PHA)-stimulated lymphocyte supernatant were added to the cultures instead of GM-CSF. BFU-E and CFU-mix were measured simultaneously in the same plate. Numbers of progenitors were calculated as total numbers of colonies per ml of blood sample.

Chemotherapy The intravenous treatments used were (1) cisplatin (CDDP), (2) CDDP plus mitomycin C (MMC) or (3) CDDP plus MMC plus vindesine (VDS) for patients with non-small cell lung cancer (NSCLC), and (4) cyclophosphamide (CPM) plus adriamycin (ADM) plus vincristine (VCR) or (5) CDDP plus etoposide (VP-16) for treatment of small cell lung cancer (SCLC). These drugs were administered as follows: (1) CDDP at 80 mg/m^2 on day 1; (2) CDDP at 80 mg/m^2 on day 1, MMC at 8 mg/m^2 on days 1 and 8; (3) CDDP at 80 mg/m^2 on day 1, MMC 8 mg/m^2 on day 1, and VDS at 3 mg/m^2 on days 1 and 8; (4) CPM at 700 mg/m^2 on day 1, ADM at 50 mg/m^2 on day 1, and VCR at 1.4 mg/m^2 on day 1; and (5) CDDP at 80 mg/m^2 on day 1 and VP-16 at 75 mg/m^2/day on days 1 through 5. CDDP was administered with 2000 ml of Ringer's solution and 1000 ml of saline containing mannitol, metoclopramide, dexamethasone and furosemide for 13 h. Drug therapy was repeated every 3 to 5 weeks, and modified if the drugs appeared toxic. Blood samples were obtained by venipuncture on days 1, 8, 15, 22, 29 and 36 after initiation of chemotherapy and PBHPs levels were assessed. Bone marrow samples were obtained from some

Table I. Hematopoietic Progenitors of Peripheral Blood from Patients with Lung Cancer[a]

Case No.	Age	Sex	Histology	Stage	No. of progenitors/ml[b]		
					CFU-GM	BFU-E	CFU-mix
1	55	F	Adeno	IIIa	55	55	0
2	56	M	Adeno	IV	6	0	0
3	55	M	Adeno	IV	12	140	0
4	71	M	Adeno	IV	6	6	0
5	63	M	Adeno	IV	39	39	0
6	60	M	Squamous	I	360	0	0
7	53	F	Squamous	II	12	20	0
8	67	M	Squamous	IIIa	63	63	0
9	62	M	Squamous	IIIa	11	2	2
10	68	M	Squamous	IV	50	40	10
11	67	M	Squamous	IV	4	4	4
12	63	M	Squamous	IV	0	1	0
13	75	M	Squamous	IIIa	12	20	0
14	50	M	Squamous	IV	13	26	0
15	64	M	Squamous	II	0	0	0
16	77	M	Large	IIIb	14	6	2
17	61	F	Large	IV	33	21	27
18	68	M	Large	IV	56	46	10
19	67	M	Small	II	10	0	4
20	61	M	Small	IV	0	48	0
21	58	M	Small	IV	0	0	0
22	38	M	Small	IV	46	10	50
23	68	M	Small	IV	0	8	0
24	66	M	Small	IV	16	27	0
25	55	F	Small	IV	18	36	0
26	57	M	Small	IIIb	27	0	0
27	72	M	Small	IIIa	12	24	5
28	75	M	Small	IV	59	14	0
Mean±SD					33±66	23±29	4±10

a) All patients were untreated.
b) No. of progenitors was measured in 1 ml of peripheral blood.

patients by puncture of the anterior iliac crest with a needle fitted to a heparinized plastic syringe, and hematopoietic progenitors were assessed in the same manner except for seeded cell number (1×10^5 cells per plate).

Rebound overshoot phenomenon The rebound overshoot phenomenon was defined as a minimum of six-fold increase in the progenitor level over that before chemotherapy. When the number of progenitors was zero before chemotherapy, it was defined as a minimum peak of 288 progenitors/ml of blood after chemotherapy.

RESULTS

Hematopoietic progenitors in patients with primary lung cancer before chemotherapy There was a considerable variation in the progenitor levels, before chemotherapy: the average numbers of progenitors per milliliter of blood in the 28 patients with primary lung cancer were 33 (range 0–360) for CFU-GM, 23 (range 0–140) for BFU-E, and 4 (range 0–50) for CFU-mix (Table I). No significant influence of age, sex, histological type or clinical stage of cancer on the progenitor levels was apparant. Moreover, no significant correlation was found between the leukocyte count and the progenitor level (data not shown).

The numbers of progenitors per milliliter of blood and bone marrow were compared in five patients with primary lung cancer before chemotherapy (Table II). The average numbers of progenitors per milliliter of blood were 21 for CFU-GM, 23 for BFU-E, and 9 for CFU-mix. On the other hand, the average numbers of progenitors per milliliter of bone marrow were 14305 for CFU-GM, 1720 for BFU-E, and 1076 for CFU-mix. Thus the progenitor levels were higher in the bone marrow than in the peripheral blood by a factor of 681 for CFU-GM, 75 for BFU-E, and 120 for CFU-mix.

Effect of chemotherapy on hematopoietic progenitors The levels of PBHPs were markedly decreased 1 week after the completion of chemotherapy, and then recovered to the levels before treatment in 2 weeks. In some, but not all patients, there was a rebound overshoot phenomenon 3–5 weeks after the completion of chemotherapy. Figure 1 shows the kinetics of recovery of committed progenitors (CFU-GM) after chemotherapy with CDDP plus VP-16. The kinetics of recovery varied considerably in patients receiving different chemotherapeutic regimens. The rebound overshoots after five different types of chemotherapeutic regimens were evaluated (Table III). Twenty-one courses of chemotherapy were administered in 21 of 28 patients with primary lung cancer. Rebound overshoot was not observed in any patient treated with CDDP alone, CDDP plus MMC, or

Table II. Comparison between Hematopoietic Progenitors of Peripheral Blood (PB) and Bone Marrow (BM) from Untreated Patient with Primary Lung Cancer

Case No.		No. of progenitors/ml[a)]		
		CFU-GM	BFU-E	CFU-mix
10	PB	50	40	10
	BM	17860	1410	0
16	PB	14	6	2
	BM	10296	1188	2871
17	PB	33	21	27
	BM	750	500	1250
19	PB	10	0	4
	BM	1280	200	200
20	PB	0	48	0
	BM	41340	5300	1060
Mean±SD	PB	21±18	23±19	9±10
	BM	14305±14920	1720±1844	1076±1018

a) No. of progenitors was measured in 1 ml of peripheral blood or bone marrow.

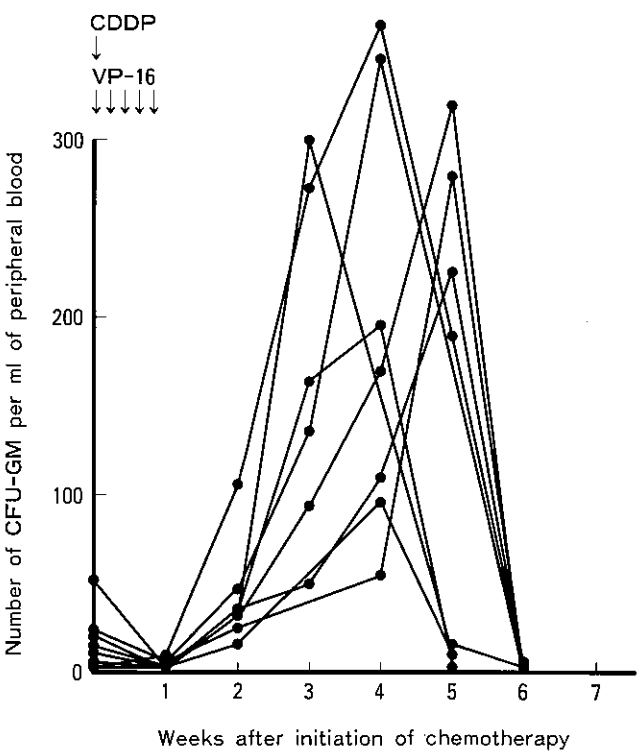

Fig. 1. Rebound overshoot phenomenon of colony-forming unit-granulocyte macrophage (CFU-GM) count following administration of cisplatin (CDDP; 80 mg/m^2 on day 1) and etoposide (VP-16; 75 mg/m^2/day on days 1 through 5) in patients with small cell lung cancer (SCLC).

Table III. Rebound Overshoot Phenomenon of Peripheral Blood Progenitors Depending on Chemotherapeutic Regimens

Regimen	No. of evaluable courses	No. of courses with rebound overshoot (%)
CDDP	2	0 (0)
CDDP+MMC	4	0 (0)
CDDP+VDS+MMC	2	0 (0)
CPM+ADM+VCR	5	1 (20)
CDDP+VP-16	8	8 (100)
Total	21	9 (43)

Table IV. Correlation between Leukocytopenia Induced by Chemotherapy and Rebound Overshoot Phenomenon of Peripheral Blood Hematopoietic Progenitors (CFU-GM)

Rebound overshoot	No. of evaluable chemotherapy courses	Nadir of leukocytopenia	
		Leukocyte count/μl	Days
Yes	9	1889 ± 638[a, b]	17.3 ± 2.3[c]
No	12	2992 ± 1096	17.4 ± 5.2

a) Mean±SD.
b) Significant difference from values for chemotherapy courses without rebound overshoot phenomenon ($P<0.05$).
c) Days after initiation of chemotherapy.

CDDP plus VDS plus MMC or in 4 of 5 patients treated with CPM plus ADM plus VCR. But rebound overshoot was observed in all patients treated with CDDP plus VP-16.

The correlation between post-chemotherapy leukocytopenia and the rebound overshoot phenomenon was evaluated. Results showed that the extent of cytopenia after chemotherapy was greater in patients who showed a rebound than in those who did not (Table IV). The increase in the number of progenitors preceded the increases of the neutrophil and monocyte counts and was followed by a gradual decrease over a period of 4–5 weeks, after which the next course of therapy was started. Typical kinetics of the recovery of blood cells and progenitors in a patient are illustrated in Fig. 2.

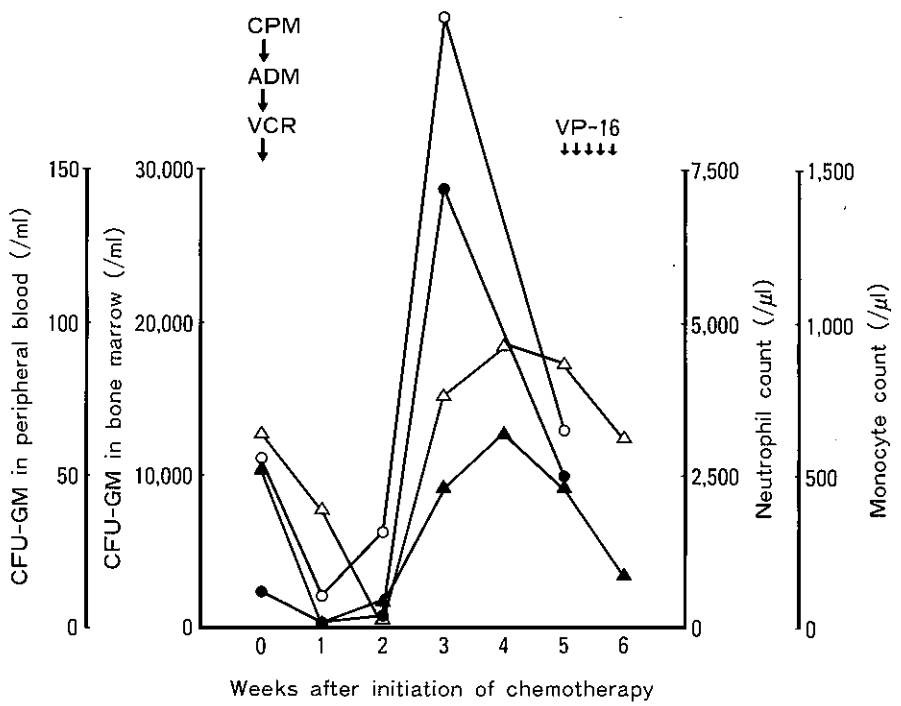

Fig. 2. Effect of chemotherapy on neutrophil count (△), monocyte count (▲), and colony-forming unit-granulocyte macrophage (CFU-GM) count of peripheral blood (●) and bone marrow (○). Cyclophosphamide (CPM) at 700 mg/m^2 on day 1, adriamycin (ADM) at 50 mg/m^2 on day 1, vincristine (VCR) at 1.4 mg/m^2, and etoposide (VP-16) at 75 mg/m^2/day on days 35 through 39 were administered.

DISCUSSION

In this work we analyzed the PBHPs in patients with lung cancer before and after various types of chemotherapy. The use of autologous stem cells has a great advantage over the use of allogeneic transplantation because it avoids the graft versus host reaction and the difficulty of finding a suitable donor. Thus, autologous BMT is preferable to allogeneic transplantation when the stem cells can be freed of metastatic cancer cells. An unresolved problem with autologous BMT is the possibility of contamination of the marrow in remission with lung cancer cells.[29, 30] PBHPs might be less likely than marrow cells to be contaminated with tumor cells,[31] although this has not yet been proved. Other advantages of the use of PBHPs rather than bone marrow cells are that they can be obtained without the use of anesthesia and without the discomfort involved in multiple bone marrow aspirations. The clinical application of PBHPs is not limited by the patient's age[32] and PBHPs are potentially available for all patients with malignant diseases.

In this study, peripheral blood samples from patients with lung cancer were found to contain significant levels of progenitors before chemotherapy, although much lower levels than in the bone marrow. After antineoplastic chemotherapy, some patients showed 6- to 50-fold increases (rebound overshoot phenomenon) in CFU-GM levels in the recovery phase after myelosuppression. This rebound overshoot was dependent on the chemotherapeutic regimen used and the degree of leukocytopenia after chemotherapy; namely, it was observed most frequently in patients who had received combination chemotherapy with CDDP plus VP-16, and in patients who subsequently developed severe leukocytopenia. The degree of leukocytopenia after chemotherapy is an important factor for the overshoot phenomenon because severe myelosuppression must be followed by a rapid supply of progenitors and consequent blood cells. The reason why this rebound overshoot was dependent on the chemotherapeutic regimen used is not clear. Possible explanations of this phenomenon are as follows. 1) The degree of leukopenia after chemotherapy is very different among the chemotherapeutic regimens used in this study. 2) The timing of administration of drugs may be very important for inducing an overshoot. When the second course of chemotherapy is given before the first course of chemotherapy induces the overshoot, no overshoot phenomenon due to the first course of chemotherapy may be seen. 3) The mechanisms of drug action on hematopoietic progenitors are different among the drugs used.

To *et al.*[33] reviewed the reconstitutive capacity of PBHPs and concluded that at least 3×10^5 CFU-GM/kg must be infused to attain sustained hematopoiesis. Watanabe *et al.*[34] reported that infusion of 1.5×10^4 CFU-GM/kg could reconstitute hematopoiesis in children with malignant diseases. The variations in the numbers of circulating CFU-GM necessary for autografts may result from selective expansion of the committed stem cells over pluripotent stem cells in the recovery phase after myelosuppression. Before chemotherapy, the patients with lung cancer had an average of 48 CFU-GM per milliliter of blood, whereas after combination chemotherapy with CDDP plus VP-16, their average level was 380 CFU-GM per milliliter of blood. These data suggest that at least 40–800 ml of autologous blood/kg must be processed to collect sufficient cells for sustained hematopoiesis in patients with lung cancer after combination chemotherapy with CDDP plus VP-16. This should be practicable by repeated leukapheresis.

Autologous BMT has been used as supportive therapy to reduce the duration of aplasia after very-high-dose chemotherapy including CPM and VP-16 in the treatment of SCLC.[35, 36] In particular, autologous BMT has been used with late intensive high-dose chemotherapy after some courses of conventional chemotherapy for treatment of patients with SCLC.[37–41] Late intensification of chemotherapy was found to increase the complete remission rate and the relapse-free survival time significantly, but it did not improve the overall survival significantly. Few reports are available on its effects in NSCLC, but so far results have been disappointing, showing no increase of the complete remission rate or prolongation of survival.[42] The final goal of our work is the use of PBHPs instead of bone marrow cells after late intensive therapy for complete remission of SCLC and after intensive chemotherapy of conventional-chemotherapy-resistant NSCLC.

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