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Autografting with peripheral blood CD34-positive cells following high-dose chemotherapy against breast cancer

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We report autologous $CD34^+$ cell transplantation performed in 3 cases of recurrent breast cancer. The hematological recovery in these cases was assessed by comparing with that in the previous cases of autologous hematopoietic stem cell transplantation performed with the same high-dose chemotherapy regimen. Patient 1 was a 32-year-old woman with pulmonary and skeletal metastases; patient 2, a 55-year-old woman with pulmonary metastases; and patient 3, a 48-year-old woman with hepatic metastases. On day 1, cyclophosphamide 1000 mg/m^2 and epirubicin 130 mg/m^2 were administered concurrently with granulocyte colony-stimulating factor, and peripheral blood stem cells were harvested on days 14-16. These stem cells were processed using anti-CD34 monoclonal antibody and an immunomagnetic bead device, Isolex 300i™. The high-dose chemotherapy regimen consisted of cyclophosphamide 2000 mg/m^2/day, div, and thiotepa 200 mg/m^2/day, div on day -5, -4, and -3. The harvested $CD34^+$ cells numbered $3.9 \pm 2.8 \times 10^6$/kg (range: 0.73-7.8/10^6/kg), and the CFU-GM, $8.3 \pm 5.6 \times 10^5$/kg (range: 1.2-15.1/10^5/kg). After the separation, the percent of $CD34^+$ cells was $81.9 \pm 11.6\%$ (range: 65.8-96.4%), the $CD34^+$ cell yield, $71.8 \pm 30.2\%$ (range: 46.0-129.6%), and the CFU-GM yield, $48.9 \pm 9.1\%$ (range: 35.3-62.0%). At the time of transplantation, the number of nucleated cells was $0.55 \pm 0.31 \times 10^5$/kg, and that of CFU-GM, $31.2 \pm 17.8 \times 10^5$/kg. Comparison of the hematological recovery in these three cases with that in patients receiving an identical high-dose chemotherapy regimen revealed recovery rates significantly faster than in patients having bone marrow transplants, and approximately identical with that in peripheral blood stem cell transplantation cases.

Abbreviations: $CD34^+$, CD34-positive; AHSCT, autologous hematopoietic stem cell transplantation; G-CSF, granulocyte colony-stimulating factor; PBSC, peripheral blood stem cells; HDC, high-dose chemotherapy

Keywords : CD34-positive selection, Breast cancer, High-dose chemotherapy

INTRODUCTION

Researches on multidisciplinary treatment techniques for various types of malignant tumor have been conducted using high-dose chemotherapy (HDC), and the usefulness of HDC in combination with autologous hematopoietic stem cell transplantation (AHSCT) has been examined [1] in cases of advanced or recurrent breast cancer. Currently, the principal type of AHSCT is peripheral blood stem cell (PBSC) transplantation (PBSCT). Problems including bradycardia, hypertention, nausea, vomiting and arrhythemia, occasionally occur with PBSCT, depending on the total amount transfused [2]. In Japan, no studies have been conducted on positive selection of hematopoietic stem cells and on the combinated use of HDC and AHSCT for positive selection in cases of breast cancer. The device, Isolex300i™ is suitable for processing large-volume PBSC to isolate and transplant small-volume peripheral blood CD34-positive ($CD34^+$) cells. We have been carrying out HDC with AHSCT for breast cancer [3]. In

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the present work, we utilized Isolex300i™ in three recurrent breast cancer cases and compared the hematological recovery with that of AHSCT without selection in which the identical HDC regimen was followed. It has been pointed out that contamination with tumor cells may occur following AHSCT, and such contamination has also been reported with PBSC [4]. $CD34^+$ positive selection may reduce the number of contaminating tumor cells without damage to normal hematopoietic stem cells. Because the amount of positively selected cells was very small, we limited this investigation to assessing the hematopoietic recovery relative to that of AHSCT.

PATIENTS AND METHODS

Patients

Peripheral blood $CD34^+$ cell transplantation was performed on three patients between December 1995 and November 1996. The first was a 32-year-old woman with pulmonary and skeletal metastases noticed four years after surgery. She was treated with four cycles of CAF (cyclophosphamide (CY) 500 mg/m^2, iv, adriamycin (ADR) 40 mg/m^2, iv, 5FU 500 mg/ m^2, iv), and four cycles of FMV (5FU 800 mg/m^2 cont.div, vindesine 2.6 mg/m^2, iv, and mitoxantrone 6 mg/m^2, iv). The second was a 55-year-old woman diagnosed as having advanced breast cancer. She was administered two cycles of neoadjuvant chemotherapy as follows; 5FU 500 mg/body. div, and CY 100 mg/day, p.o. for 14 days, and then underwent radical mastectomy. As adjuvant postoperative therapy, five cycles of the same chemotherapy (5FU and CY) were given and, nine months later when the disease recurred in the lung, four cycles of FMV were administered. The final of the three patients was a 48-year-old woman in whom metastases were found in the liver three years after surgery. She was treated with 6 cycles of CY 500 mg/m^2, iv, epirubicin 40 mg/m^2, iv, and 5FU 500 mg/m^2, iv. Control AHSCT cases comprised 12 cases in which autologous bone marrow transplantation (ABMT) was performed, six in which PBSCT alone was performed, and 11 in which both ABMT and PBSCT were used for the treatment of advanced recurrent breast cancer between January 1991 and November 1996. In cases of ABMT, median age was 49.6 (28−60) with recurrence in lymph node (4 cases), lung (3 cases), liver (3 cases) and chest wall (2 cases). In cases of PBSCT median age was 49.8(43−58) with recurrence in lung (3 cases), lymph node (1 case), liver (1 case) and chest wall (1 case). In cases of ABMT and PBSCT combined, median age was 50.8(28−64) with recurrence in lymph node (4 cases), lung (4 cases), liver (3 cases). Many of control cases were treated with 2−3 cycles of CY+epirubicin (CY 1000 mg/m^2, iv, and epirubicin 130 mg/m^2, iv on day 1), while some were treated with 3−4 cycles of FMV.

PBSC

The conditioning regimen for PBSC harvesting was CY+epirubicine in Cases 1and 2 of $CD34^+$ selection and all control cases. After the number of leukocytes fell to 1000/mm^3 or less, subcutaneous injection of a 75 μg daily dose of G-CSF was performed, and on days 14, 15 and 16, PBSC harvesting was carried out. In Case 3 of $CD34^+$ selection the conditioning regimen for PBSC harvest was CY 500 mg/m^2, iv, ADR 40 mg/m^2, iv, and 5FU 500 mg/m^2, iv, all on day 1, followed by subcutaneous injection of a 75 μg daily dose of G-CSF. PBSC harvesting was carried out on day 14, 15 and 16. For the harvesting, a Cobe 2991 Blood Cell Processor (Cobe Laboratories, Lakewood, CO, USA) was employed.

CD34-positive selection

The harvested PBSC were processed with an Isolex 300i™ magnetic cell separator system (Baxter, Irvine, CA, USA) using anti-CD34 monoclonal antibody and paramagnetic particles. After platelets were removed from PBSC by washing, 5% immunoglobulin preparation (Venoglobulin I®, Green Cross, Osaka, Japan) was added to a final concentration of 0.5%. PBSC were sensitized with mouse anti-human CD34 monoclonal antibody at 1mg/ml for 15 minutes at room temperature. Then buffer solution was added, and the mixture was centrifuged at 800× g for 10 minutes to remove non-reacting antibodies. This washing process was repeated twice. Next, 10 ml of washed anti-mouse sheep IgG-bound paramagnetic beads was added and rosette formation was brought about by the addition of 5% immunoglobulin preparation to a final con-

centration of 0.5%. The mixture was incubated for 30 minutes. The rosettes were separated magnetically from cells other than target cells and mixed with 20 ml of peptide solution to release target cells. After incubating for 30 minutes, the solution was harvested as the $CD34^+$ cell fraction. The harvested $CD34^+$ cells were washed twice by centrifugation for 10 minutes at 800× g, suspended in 20 ml of buffer solution, and preserved. In PBSC depleted of platelets, flow cytometry was used to determine the number of nucleated cells and the percent of $CD34^+$ cells. The viability (%) was determined, and the number of $CD34^+$ cells was calculated from the following formula:

Number of $CD34^+$ cells = number of nucleated cells × percent of $CD34^+$ cells/100 × viability (%)/100.

Also, the number of CFU-GM was determined by a conventional colony assay. In the $CD34^+$ cell fraction, the same measurements were made, and the $CD34^+$ yield (%) was calculated from:

$CD34^+$ cell number/$CD34^+$ cell number after pretreatment (%)

The CFU-GM yield (%) was calculated similarly.

HDC with AHSCT

HDC with AHSCT was conducted on a regimen of CY 2000 mg/m^2/day, iv, and thiotepa 200 mg/m^2/day, iv, on days −5, −4 and −3, and the hematopoietic stem cells were transplanted on day 0. From day 1, G-CSF 300 μg/m^2 was used until the neutrophil count rose above 2000/mm^3. When the platelet count was below 20,000/mm^3, platelet transfusion was carried out. When the hemoglobin level was below 7 g/dl, erythrocytes were transfused. Informed consent was obtained from all patients. Toxicities were examined in accordance with the criteria of the Japan Clinical Oncology Group (JCOG) [3].

RESULTS

CD34-positive selection

The number of the harvested $CD34^+$ cells was $3.9 \pm 2.8 \times 10^6$/kg (mean ± S.D.) (range $0.73–7.8 \times 10^6$/kg), the percent of $CD34^+$ cells was 1.9 ± 0.97% (range 0.62–3.4%), and the CFU-GM count was $8.3 \pm 5.6 \times 10^5$/kg (range $1.2–15.1 \times 10^5$/kg). The percent of $CD34^+$ cells after the separation was 81.9 ± 11.6% (range 65.7–96.4%), the $CD34^+$ yield, 71.8 ± 30.2% (range 46.0–129.6%), and the CFU-GM yield, 48.9 ± 9.1% (range 35.3–62.0%) (Table 1).

Hematological recovery

At the time of autologous peripheral blood $CD34^+$ cell transplantation, the number of nucleated cells, $CD34^+$ cells, and CFU-GM were $0.55 \pm 0.31 \times 10^7$/kg, $3.7 \pm 2.7 \times 10^6$/kg, and $31.2 \pm 17.8 \times 10^5$/kg, respectively. In the previous cases treated with AHSCT together with HDC using the same regimen, the respective numbers of nucleated cells and of CFU-GM, as hemotopoietic stem cells, were $2.30 \pm 0.85 \times 10^7$/kg and $1.73 \pm 0.89 \times 10^5$/kg in the 12 cases treated with ABMT alone, $50.0 \pm 27.3 \times 10^7$/kg and $32.4 \pm 46.6 \times 10^5$/kg in 11 ABMT+PBSCT cases, and $26.5 \pm 7.0 \times 10^7$/kg and $19.0 \pm 8.9 \times 10^5$/kg in 6 cases with PBSCT alone (Table 2). On day 9, all cases of autologous peripheral blood $CD34^+$ cell transplantation showed sufficient recovery with the leukocyte count of at least 1000/mm^3 and the neutrophil count of at

Table 1 CD34 positive selection with the Isolex 300i™ device

	Pre-selection	Post-selection
Total cell no. ($\times 10^8$/kg)	1.9 ± 0.96* (1.0–3.5)**	0.035 ± 0.03 (0.01–0.08)
$CD34^+$ %	1.9 ± 0.97 (0.62–3.4)	81.9 ± 11.6 (65.7–96.4)
CD34 cell no. ($\times 10^6$/kg)	3.9 ± 2.8 (0.73–7.8)	3.1 ± 2.8 (0.39–7.1)
CFU-GM ($\times 10^5$/kg)	8.3 ± 5.6 (1.2–15.1)	3.8 ± 2.5 (0.61–7.4)

$CD34^+$ yield (%), 71.8 ± 30.2 (46.0–129.6) CFU-GM yield (%), 48.9 ± 9.1 (35.3–62.0)
*: mean ± SD **: range

least 500/mm^3. By days 11, 13 and 16, the platelet count reached 50,000/mm^3 without platelet transfusion. Recovery was assessed by evaluating the following four post-transplantation indices; the days until the neutrophil count returned to at least 500/mm^3, the number of days with the neutrophil count below 500/mm^3, the days until the platelet count passed the 50,000/mm^3 mark, and the number of times platelet concentrate was transfused. As compared with the previous patients who received AHSCT with HDC of the same regimen, the present patients treated with autologous peripheral blood CD34$^+$ cell transplantation showed a recovery that was significantly faster than that in ABMT cases and on a par with that in PBSCT patients (Table 3). In addition, no adverse events, excluding hematological toxicity, nausea, vomiting and diarrhea, of Grade 3 or over of the JCOG toxicity criteria [3] were observed in any patient treated with autologous peripheral blood CD34$^+$ cell transplantation.

DISCUSSION

In the present study, the purity of peripheral blood CD34$^+$ cells obtained using the Isolex 300i^{TM} magnetic cell separator was 81.9 ± 11.6%, the CD34$^+$ cell yield was 71.8 ± 30.2%, the number of CFU-GM was 3.8 ± 2.5 × 10^5/kg, and the CFU-GM yield was 48.9 ± 9.1%. These results of immunomagnetic separation of CD34$^+$ cells are comparable with those obtained by Civin *et al.* [5] using bone marrow, by Alcorn *et al.* [6] with PBSC, and by Hohaus *et al.* [7] in examinations of breast cancer patients. Moreover, the present results are superior to those obtained by column-based immunoadsorption, which are reported to be from 49% to 72%. [8]

As estimated from the number of days required to regain a neutrophil count of at least 500/mm^3, the present cases of CD34$^+$ cell transplantation achieved the degree of hematological recovery following HDC not only comparable to that with PBSCT or ABMT together with PBSCT but significantly

Table 2 Summary of transfused stem cells

	Nucleated cell (× 10^7/kg)	CFU-GM (× 10^5/kg)
ABMT (n = 12)	2.30 ± 0.85	1.73 ± 0.89
ABMT + PBSCT (n = 11)	50.0 ± 27.3 *1	32.4 ± 46.6 *4
PBSCT (n = 6)	26.5 ± 7.0 *1	19.3 ± 8.9 *1
CD34$^+$ (n = 3)	0.55 ± 0.3 *1 *2 *3	31.2 ± 17.8 *1

*1: $p < 0.01$, compared with ABMT *2: $p < 0.05$, compared with ABMT + PBSCT
*3: $p < 0.01$, compared with PBSCT *4: $p < 0.05$, compared with ABMT

Table 3 Hematologic recovery indices

	ABMT	ABMT + PBSCT	PBSCT	CD34$^+$
Neutrophil >500/mm^3 (days)	13.5 (11–16)	9* (7–11)	8.5* (7–10)	9*
Neutrophil <500/mm^3 (days)	11 (8–13)	7* (4–9)	5.5* (4–10)	6* (5–6)
Platelet >50000/mm^3 (days)	28.5 (22–45)	14* (9–32)	12* (9–14)	13* (11–16)
Platelet transfusion (×10 units)	5.5 (3–16)	2* (1–7)	2* (1–3)	2* (1–2)

*: $p < 0.05$, compared with ABMT

faster than that with ABMT alone. Thus, the number of days when the neutrophil count was below 500/mm^3 after CD34$^+$ transplantation was approximately the same as when PBCST alone or both ABMT and PBSCT were used, while when ABMT was carried out alone, the length of this period was significantly longer. Again, regarding the number of days needed for the platelet count to improve to over 50,000/mm^3 after CD34$^+$ cell transplantation, both PBSCT alone and the combined use of ABMT and PBSCT resulted in almost identical recovery rates that were significantly more facilitated than with ABMT alone. The numbers of platelet transfusion were also similar. When HDC was administered, the number of the transfused nucleated cells was greatest in the combined execution of ABMT and PBSCT, and smallest in the transplantation of CD34$^+$ cells. However the number of CFU-GM was approximately equal in CD34$^+$ cell transplantation and the combined ABMT and PBSCT transplantation, whereas that in ABMT alone was the least. We believe that these findings indicate a difference in hematological recovery. With ABMT alone, the number of hematopoietic stem cells harvested was probably at its limit. It is possible to increase the count of hematopoietic stem cells by increasing the number of harvested PBSC. However, if the volume of hematopoietic stem cells transplanted exceeds 100 ml, which we have not attempted ourselves, bradycardia, hypertension, nausea, vomiting and arrhythmia have been reported to occur occasionally, depending on the total amount transfused. Furthermore, reports of chest pain and myocardial infarction also exist [9]. The transplantation of CD34$^+$ cells offers the advantage that the volume transfused is small relative to the amount of peripheral stem cells transplanted, and the above mentioned side effects are seldom encountered.

It has not been demonstrated whether CD34$^+$ cells include true hematopoietic stem cells capable of maintaining their hematopoietic properties for a long time period. Thus, with the transplantation of highly purified hematopoietic stem cell preparations, late deficiencies may occur in bone marrow engraftment patients. However, in the present cases of CD34$^+$ cell transplantation the leukocyte and platelet counts were normal, during the post-transplantation courses of 23 or 24 months indicating that there were no problems with engraftment during this period. Nevertheless, longer periods of observation are required to reach a final conclusion on these matters.

In the present study, no investigation was conducted on the purging of tumor cells. Whether the contaminating tumor cells contribute to recurrence or to advancement of the disease is still unclear. In acute infantile leukemia, genetic studies have shown that contaminating tumor cells could become the source of recurrence of cancer [10]. There are few papers on tumor cell purging by positive selection, and 1.0−4.0 log reductions of tumor cells have been reported [11]. Although purging by the negative selection of AHSCT using monoclonal antibodies is now being carried out, the monoclonal antibodies and their affinity for the tumor cells to be eliminated are of prime importance. Since, in practice, the number of contaminating tumor cells is small relative to the number of processed cells, not only is it impossible to assess the results of the elimination of the tumor cells quantitatively, but it is also difficult to obtain the complete set of the antibodies that have an affinity for the tumor cells. As a result, AHSCT is not frequently performed. In addition, the significance of the contaminating tumor cells is not clear. Because the processing is simple when the Isolex 300i^{TM} device, it is possible that, through the use of this device, the clinical significance of these contaminating cells will be clarified by prospective studies on large populations.

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