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Allogeneic Transplantation of CD34$^+$ Peripheral Blood Progenitor Cells for Severe Combined Immunodeficiency

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Summary. **Severe combined immunodeficiency (SCID) is a fatal congenital disorder of the immune system for which the only curative treatment is the reconstitution of the immune system by stem cell transplantation. In the case reported here we attempted the allogeneic transplantation of purified CD34$^+$ cells derived from granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood of the HLA-haploidentical father for a three-month-old female SCID patient, in whom both T and B cells were absent, but natural killer (NK) cells were present. Her father was administered with G-CSF (10 μg/kg/day) subcutaneously for four days, and a total number of 2.3×10^7 ($=4.6\times10^6$/kg of patient's body weight) CD34$^+$ cells was harvested. Purified CD34$^+$ cells were transplanted into the patient without preconditioning. A skin rash consistent with graft-versus-host disease (GVHD) was observed on day 10 after transplantation. CD3$^+$ cells appeared in the peripheral blood on day 14. These findings indicate that allogeneic transplantation of purified CD34$^+$ cells could be one useful treatment option for SCID patients, although data from a greater number of patients will be required to draw definitive conclusions.**

Key words—severe combined immunodeficiency, cytomegaloviral pneumonitis, mismatched HLA, allogeneic peripheral blood stem cell transplantation, CD34$^+$ cells purification.

INTRODUCTION

Severe combined immunodeficiency (SCID) is a fatal disease for which replacement of the immune system by allogeneic blood stem cell transplantation is the only curative treatment.[1,2] A patient with SCID can be cured when there is an HLA-identical donor. Without such a donor, however, several manipulations such as Tcell depletion are needed for bone marrow transplantation (BMT).[3,4] We here report the attempt of an allogeneic peripheral blood stem cell transplantation (PBSCT) with CD34$^+$ cell purification for treatment of a SCID patient who had no HLA-identical donor. Although it did not result in success, its clinical course might be informative for other SCID patients in similar critical condition.

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CASE REPORT

A two-month-old Japanese female patient was admitted to a local hospital because of a urinary tract infection. Laboratory findings showed lymphocytopenia and hypogammaglobulinemia, suggesting immunodeficiency. After transient improvement with antibiotic treatment, dyspnea occurred. The chest rentgenogram (Fig. 1) revealed a ground glass appearance. Thoracic computed tomography (Fig. 2) showed a bilateral ground-glass pattern. These findings suggested interstitial pneumonitis. Diagnosis of cytomegaloviral pneumonitis was made, since cytomegalovirus antigen in the peripheral blood (CMV antigenemia[5]) was positive. The respiratory condition gradually deteriorated, and the patient was transferred to our hospital for the purpose of allogeneic stem cell transplantation.

On admission, blood gas analysis showed marked hypoxemia (PaO_2 48.8 mmHg). A decreased lymphocyte count (130/μl) was found. CD3$^+$ and CD19$^+$ cells were absent, but CD56$^+$ cells were present. Other labora-

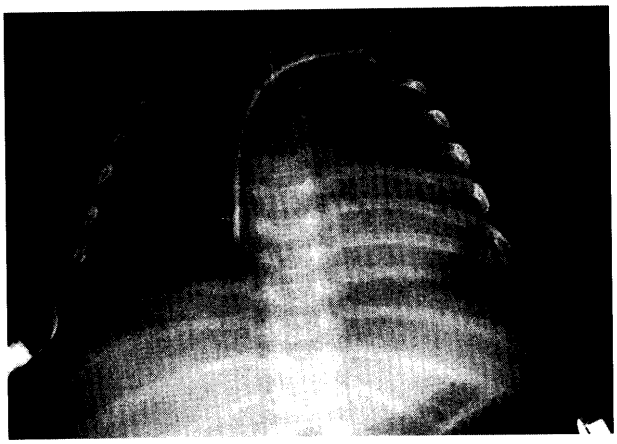

Fig. 1. Chest rentgenogram. A diffuse, hazy, ground glass appearance is revealed.

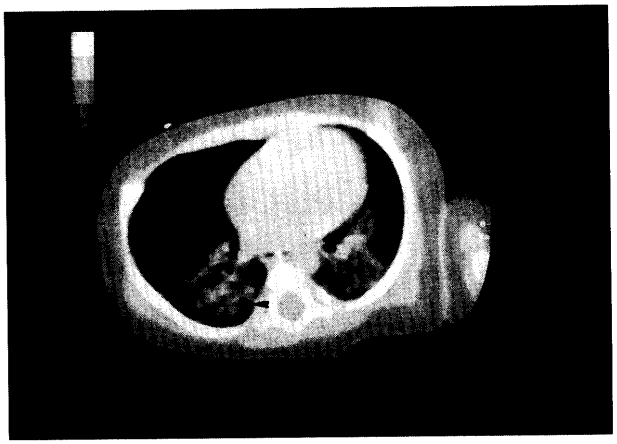

Fig. 2. Thoracic CT scan. The presence of a bilateral ground-glass pattern is found (*arrow*).

Table 1. Laboratory data (1)

pH	7.361		TP	4.5	g/dl
pCO_2	46.4	mmHg	Alb	74.8	%
pO_2	48.8	mmHg	$\alpha 1$	5.9	%
HCO_3	25.6	mmol/l	$\alpha 2$	8.7	%
BE	0.5	mmol/l	β	7.7	%
sO_2	83.5	%	γ	2.9	%
WBC	4430	/μl	BUN	4	mg/dl
RBC	305×10^4	/μl	Cre	0.3	mg/dl
Hb	8.6	g/dl	Na	128	mEq/l
Ht	26.1	%	K	3.5	mEq/l
PIt	9.2×10^4	/μl	Cl	94	mEq/l
ret	5.4	‰	Ca	6.7	mg/dl
			P	1.8	mg/dl
			GOT	184	IU/l
band	11	%	GPT	24	IU/l
seg	81	%	LDH	4780	IU/l
lym	3	%	ALP	211	IU/l
mon	4	%	γGTP	466	IU/l
eos	1	%	ChE	53	IU/l
			Amy	30	IU/l
			TB	0.4	mg/dl
CMV-IgG $<\times10$			DB	0.2	mg/dl
-IgM $<\times10$			TC	128	mg/dl
CMV-Antigenemia (+)			TG	245	mg/dl
			ferritin	2060	ng/ml
			CRP	2.0	mg/dl

Table 2. Laboratory data (2)

Peripheral blood lymphocyte surface antigens		
CD2	27.8	%
CD3	1.0	%
CD4	1.0	%
CD5	2.5	%
CD7	42.9	%
CD8	1.0	%
CD10	2.4	%
CD16	39.1	%
CD19	1.0	%
CD38	97.9	%
CD56	29.1	%
CD57	1.3	%
HLADR	31.9	%
IgG	106	mg/dl
IgA	0	mg/dl
IgM	0	mg/dl
IgE	<2.0	IU/ml
C3	65.8	mg/dl
C4	36.0	mg/dl
ConA	not done	
PHA	not done	
NK activity	not done	
ADA activity	(+)	
PNP activity	(+)	

tory data are shown in Table 1 and 2. Mechanical respiratory support with intermittent mandatory ventilation (IMV) and high frequency oscillation (HFO) were required for the respiratory failure. No HLA-identical donors were obtained among family members. PBSCT with purified $CD34^+$ cells of her haploidentical father was considered to be suitable, as rapid bone marrow recovery and the prevention of graft-versus-host disease (GVHD) were expected. After informed consent was obtained from her parents, her father was administered with granulocyte-colony stimulating factor (G-CSF) (10 μg/kg/day) subcutaneously for four days. $CD34^+$ cells were harvested with a magnetic cell separation system (Isolex 50) comprising a murine monoclonal anti-CD34 antibody (9C5) and immunomagnetic microspheres,[6] and transplanted into the patient without preconditioning. Transplanted $CD34^+$ and $CD3^+$ cells were 2.3×10^7 (4.6×10^6/kg of patient's body weight) and 3.9×10^5 (7.8×10^4/kg), respectively. The purity and the yield were about 77% and 37%, respectively. Cyclosporine A (CyA) (1.5 mg/kg/day) was administered for the prophylaxis of GVHD, but was discontinued on day 4 because of its high serum concentration (470 ng/ml) and oliguria. Erythema on the bilateral cheek appeared on day 10 and spread onto the anterior chest and the right palm. No other findings suggesting acute GVHD were recognized. Methylprednisolone was administered to control GVHD. On day 14, $CD3^+$ cells appeared in the peripheral blood. Absolute lymphocyte and $CD3^+$ cell counts were 1730/μl and 540/μl, respectively (Fig. 3). XY-chromosome cells were also detected with fluorescence *in situ* hybridization (FISH). Despite the improvement of CMV-antigenemia, the patient's respiratory condition gradually deteriorated, and she succumbed to pulmonary hemorrhage on day 23. Necropsy of the liver was permitted, and mild lymphoid cell infiltrate in Glisson sheaths and degeneration of the bile ducts were revealed. These pathological findings were consistent with that of GVHD (Fig. 4).

DISCUSSION

SCID is a fatal congenital disorder of the immune system. The affected patients are complicated by infections during the first six months of life, and usually suffer a terminal course within two years.[1,2] According to a World Health Organization (WHO) Scientific Group,[7] SCID is classified into two subgroups, one of which is X-linked and the other, autosomal recessive. Since our patient was female, she was categorized into the autosomal recessive type. The defect of recombinase activating gene (RAG)[8] was not examined in this case.

Early diagnosis and effective treatment such as blood stem cell transplantation are required in SCID.[9] More than 95% of SCID patients transplanted with an HLA-identical sibling donor have survived.[10] However, only about 20% of patients have an HLA identical sibling. For the other 80% of affected patients, like ours, treatment options are restricted owing to the difficulty of finding an unrelated HLA matched donor before life-threatening infections occur. T-cell depleted haploidentical marrow transplantation has been performed and about 50% of patients have been successfully treated.[3,11,12] Transplantation of $CD34^+$ cells derived from the bone marrow for SCID has also been performed.[13]

CD34 is a surface glycophosphoprotein expressed on developmentally early lymphohematopoietic stem and progenitor cells, and $CD34^+$ cells purified from marrow can reconstitute hematopoiesis.[14] Recently, allogeneic transplantation of G-CSF mobilized peripheral blood stem cells has been performed as an alternative to BMT. Allogeneic peripheral blood stem cells were reported to contain three times more $CD34^+$ cells than bone marrow[15] and to provide rapid and sustained hematopoietic and immune reconstitution without graft failure.[16,17] $CD34^+$ cells purified

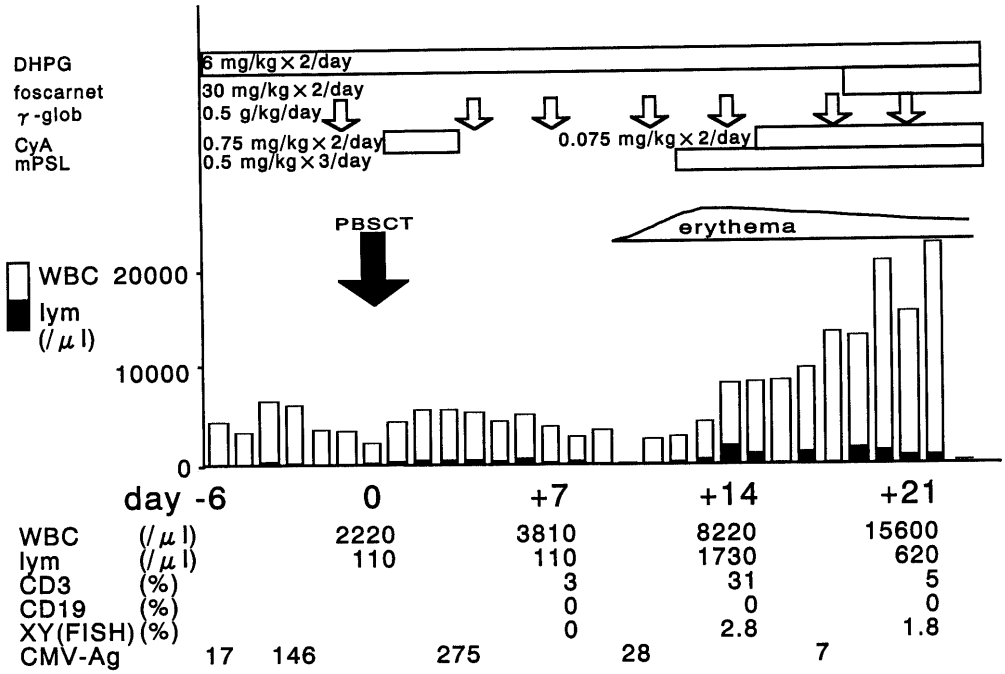

Fig. 3. Clinical course.
Counts of total leukocytes and absolute lymphocytes are shown as open and closed columns, respectively. CMV-Ag indicates the counts of CMV antigenemia-positive cells per 1.5×10^5 cells. Abbreviations: DHPG, dihydroxypropoxymethylguanine; γ-glob, γ-globulin; CyA, cyclosporine A; mPSL, methylprednisolone; PBSCT, peripheral blood stem cell transplantation.

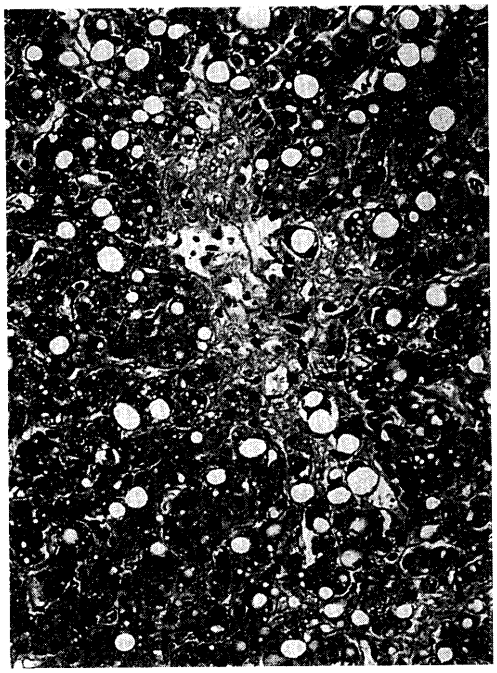

Fig. 4. Needle necropsy of the liver. Mild lymphoid cell infiltrate in Glisson sheaths and degeneration of the bile ducts are recognized (*arrow*).

from allogeneic peripheral blood stem cells have also been used clinically, and it was shown that $CD34^+$ selection could remove 2 to 4 logs of T cells[15,18,19] and that the number of obtained $CD34^+$ cells was two- to three-fold of that in grafts of conventional T cell depletion.[20] $CD34^+$ positive selection has enabled haploidentical transplantation for patients with hematologic malignancies[21] and aplastic anemia[22] who lack HLA-matched donors.

Our patient had already suffered from serious cytomegaloviral pneumonitis before the diagnosis of SCID. $CD34^+$ peripheral blood progenitor cell transplantation was considered to be suitable in this severe case for the following reasons. First, no HLA-identical donors were obtained. Second, rapid hematological and immunological reconstitution were required for the complicating severe infection. A pretransplant progressive lung infection was one of the major obstacles for BMT.[23] In our case complicated by critical interstitial pneumonitis, no conditioning chemotherapy was chosen so as to avoid drug-induced death. Engraftment was expected in view of the three following points: a skin rash consistent with that of GVHD, the increased absolute count of $CD3^+$ cells, and XY-chromosome cells detected with FISH. Partial engraftment is effective for infec-

tions in SCID,[24] and in our case the count of cytomegalovirus antigen-positive neutrophils actually decreased. After a transient increase, the number of $CD3^+$ cells gradually decreased, suggesting late rejection. The cause of the rejection was thought to be due to be the presence of NK cells, since it is known that the presence of NK cells can underscore the requirement for conditioning to achieve sustained engraftment.[25] Adequate myeloablative conditioning might be needed even in cases complicated by pretransplant life-threatening infections.

We here reported an attempt of the transplantation of purified $CD34^+$ cells derived from G-CSF mobilized peripheral blood and its clinical course. Although data from more patients will be required to draw definitive conclusions, this procedure could be one useful treatment option for SCID patients.

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