Table 1.

	Patient #1 S	maxR	%	Patient #2 S	maxR	%	Patient #3 S	maxR	%
Paraprotein levels (g/dL)	6	0.8	87	3	0.3	90	3.3	1.6	51.5
Bone marrow plasmacytosis (%)	60	11	82	82	30	63	30	10	66
β2-microglobulin (mg/L)	10.4	3.2	69	8	4.9	39	3.82	1.73	55

S= starting value; MaxR= the best response; %= percentage of response.

to WHO criteria). The dose of thalidomide was reduced to 100 mg/day in 2 patients because of constipation and a transient reduction of platelets. The median time of treatment has been 5 months (range 2-9). Four of five patients (80%) have responded to the therapy – two patients with more than 70% reduction of the M-component levels, one with more than 50% and the last one with about a 30% reduction. The fifth patient had no response and stopped the therapy after two months. The interval of time necessary to obtain the best rate of response to the therapy was 50 days (range 34-64). In the three patients with the best response to the therapy a reduction in the percentage of plasma cells in bone marrow, β_2 microglobulin levels and C reactive protein was evident (Table 1). Furthermore, a good response was obtained in one patient who reduced the dose of thalidomide to 100 mg/die early in treatment. In conclusion, although our experience concerns only five cases, we confirm the results published in scientific literature[1-8] on the efficacy of thalidomide in myeloma therapy. We point out the possibility of obtaining a good objective response[9] with lower and better tolerated dose of drug. We, therefore, think it would be desirable to study the most suitable dose of thalidomide in a larger number of patients.

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Key words

Multiple myeloma, low dose thalidomide, side effects.

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Feasibility and efficacy of high-dose etoposide followed by low-dose granulocyte colony-stimulating factor as a mobilization regimen in patients with non-Hodgkin's lymphoma

The administration of high-dose (HD) etoposide (ETP) with higher doses of recombinant human (rh) granulocyte colony-stimulating factor (G-CSF) is useful for peripheral blood stem cell (PBSC) mobilization.[1-3] We report the efficacy of HD-ETP with low-dose rhG-CSF for PBSC mobilization in patients with non-Hodgkin's lymphoma (NHL).

Sir,

Twenty-three newly diagnosed patients (16 men and 7 women) with NHL were included in this study.Their median age was 51 years (range, 20-66). They were treated with 2 or 3 courses of CHOP therapy as an induction therapy. Twelve patients entered complete remission (CR), 10 patients partial remission (PR), and one patient had no change. Patients were treated with ETP 500 mg/m^2 i.v. for 2 hour daily on days 1 to 3. rhG-CSF (Kirin Brewery Co., Tokyo, Japan) was administered by s.c. injection at a dose of 50 μg/m^2 on the second day after completion of

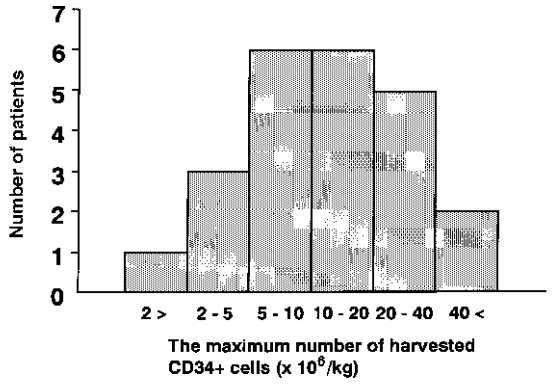

Figure 1. Distribution of the maximum number of CD34⁺ cells harvested by a single apheresis.

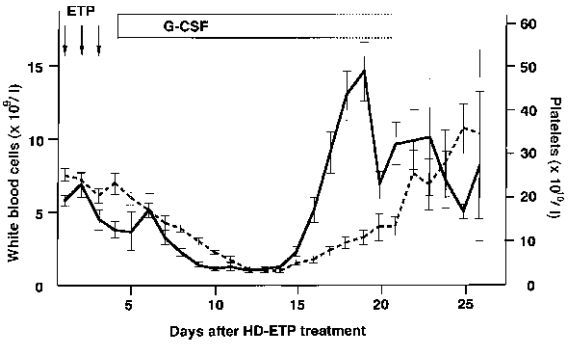

Figure 2. Changes of white blood cell counts and platelet counts after HD-ETP treatment. The black line represents the change of white blood cell counts and the dashed line represents the change of platelet counts after treatment with etoposide. Values are mean ± SD. *HD-ETP: high-dose etoposide, G-CSF: granulocyte colony-stimulating factor.*

chemotherapy and continued until PBSC collection was completed. PBSCs were harvested on 2 or 3 consecutive days during leukocyte recovery. The median number of maximum CD34⁺ cells (x10^6/kg) was 14.0 (range, 0.6-64.8) and the maximum number of CD34⁺ cells from a single apheresis was more than 5x10^6 /kg in 19 of 23 patients (Figure 1). The median day that the maximum number of CD34+ cells was harvested was 18 after HD-ETP treatment. Twenty patients did not receive red blood cell concentrates and 10 patients did not receive platelet concentrates. The changes of white blood cell counts and platelet counts after HD-ETP treatment are shown in Figure 2. The median day of neutropenia less than 1×10^9/L was 7, which is similar to that given in previous reports.[2,3] The median day that the platelet count achieved 50×10^9/L after HD-ETP treatment was 17. The platelet count decreased to less than 50×10^9/L in 22 patients. Compared to our reports on mobilization by HD-cytarabine (CA)[4], these observations suggest that HD-ETP induces more severe myelosuppression than HD-CA. Seven of 23 patients developed a fever of ≥38°C during the period of neutropenia: the fever was reduced by treatment with antibiotics. Non-hematologic adverse effects developed in nine patients. All of them were grade 1 to 2 according to the WHO toxicity scale. Neither ETP fever[1] nor mobilization-related death[5-7] was observed. In previous reports, higher doses of G-CSF (4-10 µg/kg) were used after HD-ETP.[1-3] We administered rhG-CSF at a low dose of 50 µg/m^2 (approx. 1 µg/kg) and a sufficient number of PBSC could be mobilized after 2 or 3 consecutive aphereses in 22 patients. In addition, our regimen has antitumor activity. Five of 10 patients in PR entered CR and one patient with no change entered PR. Several reports have demonstrated that salvage regimens which contain ETP are superior to HD-cyclophosphamide for mobilization.[8-10] These observations together with ours suggest that ETP is a suitable drug for PBSC mobilization and HD-ETP followed by a low dose of G-CSF is an effective and safe mobilizing regimen in patients with NHL.

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Key words

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Disappearance of factor VIII inhibitors in a severe hemophilia A neonate after steroid treatment correlated with a cytokine shift toward a T-helper 2 pattern

Factor VIII inhibitor developed in a neonate with hemophilia A after a few days of prophylactic therapy with recombinant FVIII. Interleukin-2 and interleukin-6 expression was evaluated in lymphocytes by ELISA and by mRNA *in situ* hybridization. We observed a Th1-type pattern during inhibitor production, which shifted toward a Th2 profile after steroid treatment.

Sir,

We analyzed interleukin-2 (IL-2) and interleukin-6 (IL-6) expression – as a sign of preferential T-helper 1 (Th1) or T-helper 2 (Th2) pattern – in a four-day old boy who was diagnosed as having hemophilia A (FVIII:C <1%/VWF 100%) after hemorrhagic shock due to gastrointestinal bleeding. The family history confirmed congenital hemophilia. After immediate treatment with packed red blood cells and intermediate-purity factor VIII (Hemate-P, Centeon, Marbourg, Germany) 50 U twice a day for 10 days, prophylactic administration of recombinant-FVIII (rFVIII) (Helixate, Centeon, Berkeley, CA, USA) 50 U/kg twice a week was started. Eleven days after FVIII exposure, anti-FVIII antibodies were detected by the Bethesda assay (13.5 BU). rFVIII administration was stopped and, after parents' informed consent, the patient received oral prednisone 2 mg/kg daily for three weeks, with progressive tapering to 7 mg on every other day after five weeks, and reduction of inhibitor to 1 BU.

Intron 22 inversion of the FVIII gene was excluded by polymerase chain reaction (PCR) analysis. Peripheral blood mononuclear cells (PBMC, $2x10^5$/well) were collected 5, 20 and 45 days after the appearance of the inhibitor, then incubated for 5 days in the absence or presence of rFVIII at 2 µg/mL. PBMC from a normal neonate and from a neonate with hemophilia A without inhibitor were tested as controls. IL-2 and IL-6 concentrations were measured in the supernatants by a quantitative *sandwich enzyme immunoassay* (Boehringer Mannheim, Germany) with a sensitivity >20 pg/mL for IL-2 and >10 pg/mL for IL-6. The two cytokines were also evaluated in PBMCs by mRNA *in situ* hybridization. Briefly, probes were obtained by reverse transcription-PCR as described[1] using oligonucleotide primers specific for IL-2 and IL-6. The sequences were the following:

IL-2 : 5'-ATGTACAGGATGCAACTCCTGTCTT-3'
3'-GTCAGTGTTGAGATGATGCTTTGAC-5'
IL-6: 5'-CAAATTCGGTACATCCTCGACGGC-3'
3'-CATCTGGACAGCTCTGGCTTGTTCC-5'

PCR products were reamplified using dNTPs and digoxigenin-coupled dUTP (DIG-11-dUTP) (Boehringer Mannheim, Mannheim, Germany), according to manufacturer's instructions.

In situ hybridization was performed as previously described by incubating overnight at 37°C cytospin of the PBMC with the specific probe.[2] Detection of hybridization was achieved using mouse anti-DIG monoclonal antibodies, followed by peroxidase-coupled rabbit anti-mouse and swine anti-rabbit monoclonal antibodies.

At the time the inhibitor appeared, we found high levels of IL-2 both in the supernatant and on the surface of PBMC cultured with rFVIII, by the double ELISA and *in situ* hybridization (Figures 1a and 2), whereas IL-6 was detected at very low level by the ELISA and was not visible on the surface of PBMC (Figures 1c and 2). After 45 days, when the inhibitor level had markedly reduced to 1 BU, the amount of IL-2 in the supernatant leveled off and was not visible (Figure 1b) by immunohistochemistry whereas the level of IL-6 expression was greatly enhanced (Figures 1d and 2). Cytokine level was unde-

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