In Vivo Activation of Human Neutrophil Functions by Administration of Recombinant Human Granulocyte Colony-Stimulating Factor in Patients With Malignant Lymphoma

By Akimichi Ohhsaka, Seiichi Kitagawa, Shinobu Sakamoto, Yasusada Miura, Naoki Takanashi, Fumimaro Takaku, and Masaki Saito

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) was administered (50 to 800 µg/m²) once daily as a half-hour intravenous (IV) infusion for 14 days to seven patients with malignant lymphoma. In all patients, administration of rhG-CSF not only ameliorated the decrease in absolute neutrophil count after the cytotoxic chemotherapy but also enhanced superoxide (O₂⁻) release in neutrophils stimulated by N-formyl-methionyl-leucyl-phenylalanine (FMLP). The priming effect of rhG-CSF on neutrophil O₂⁻ release was rapid (evident within 6.5 hours) and sustained at least for 24 hours after a single IV administration of rhG-CSF. The responsiveness to further in vitro challenge of rhG-CSF was lost or reduced in neutrophils isolated after rhG-CSF treatment, indicating that neutrophils already primed in vivo by rhG-CSF are desensitized to this factor. In contrast to the results obtained with FMLP, when phorbol myristate acetate (PMA) was used as stimulus, no consistent enhancement of O₂⁻ release was observed, suggesting that rhG-CSF modulates the signal transduction pathways linked to FMLP receptors rather than increases the components of the O₂⁻-producing enzyme complexes. Administration of rhG-CSF also rapidly (evident within 15 minutes) caused an increase in expression of neutrophil C3bi-receptors that was sustained for at least 24 hours after a single IV administration of rhG-CSF. Pharmacokinetic study of rhG-CSF showed a half-life (t½) of 114 min. These findings show that rhG-CSF is a potent activator for neutrophil functions both in vivo and in vitro.

MATERIALS AND METHODS

Patients. Seven adult patients with malignant lymphoma were studied. Their characteristics are shown in Table I. All patients had a good performance status. Three patients received previous chemotherapy. Three patients had bone marrow (BM) involvement; one of them was in a leukemic state. Informed consent was obtained from all patients.

Administration of rhG-CSF. Highly purified rhG-CSF produced by E.coli was supplied by Kirin Brewery (Tokyo, Japan) in collaboration with Amgen Biologicals (Thousand Oaks, CA). Specific activity of purified rhG-CSF (an apparent molecular weight [mol wt] of 18,800) was approximately 1 x 10⁶ U/mg protein when assayed by colony formation of human BM cells. Endotoxin contamination of purified protein was <1 ng/mg protein. rhG-CSF (50 to 800 µg/m² body surface) was diluted in 100 mL 5% glucose in water, and was infused IV in 30 minutes once daily. The treatment consisted of daily administration of rhG-CSF for 14 days, beginning 72 hours after chemotherapy with cytotoxic drugs. The same preparation of rhG-CSF was also used for in vitro experiments with human neutrophils. The cytotoxic drugs used for treatment of malignant lymphoma are shown in Table 1. Each patient received two consecutive courses of the same cytotoxic drugs with an interval of 4 to 5 weeks. Five patients received rhG-CSF after the first course of chemotherapy, and two patients (patients 3 and 5) received rhG-CSF after the second course.

Preparation of neutrophils. PB was obtained from the patients immediately before, during (at 6.5 hours and on day 2 or 3), and after (day 15) treatment with rhG-CSF. Neutrophils were prepared as described previously with dextran sedimentation and centrifuga-
Patients 2, 4, and 5 had BM involvement by lymphoma cells, and patient 5 was in a leukemic state. Patients 2, 4, and 7 had previous chemotherapy. Patients 3 and 5 were treated with rhG-CSF after the second course of cytotoxic chemotherapy, and the other patients were treated with rhG-CSF after the initial course.

Abbreviations: HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; CY, cyclophosphamide; ADM, doxorubicin; VCR, vincristine; PSL, prednisolone; VP, VP-16, etoposide; MTX, mitoxantrone; PCZ, procarbazine; MTX, methotrexate; and ACM, aclacinomycin.

Determination of O$_2^-$ release. O$_2^-$ was assayed spectrophotometrically by superoxide-dismutase inhibitable reduction of ferricytochrome c, and continuous assay was performed in a Hitachi 557 spectrophotometer (a double-wavelength spectrophotometer, Hitachi, Tokyo, Japan) equipped with thermostatted cuvette holder as described previously. The cell suspension in HBSS was added to a 1-mL cuvette containing 80 μmol/L ferricytochrome c with or without rhG-CSF (25 ng/mL) to obtain a final volume of 0.9975 to 0.995 mL. Final cell concentration was 5 × 10$^6$ cells/mL. The reaction mixture in a cuvette was preincubated for 10 minutes at 37°C. The cuvette was placed in a thermostatted cuvetted holder (37°C) of a spectrophotometer, and reduction of ferricytochrome c was measured at 550 nm with a reference wavelength at 540 nm. FMLP (10$^{-7}$ mol/L) or phorbol myristate acetate (PMA, 100 ng/mL) was added to the reaction mixture in a cuvette to obtain a final volume of 1 mL while the time course of cytochrome c reduction (the absorbance change at 550–540 nm) was followed on the recorder. The release of O$_2^-$ was calculated from cytochrome c reduction after FMLP and from the initial linear portion of the cytochrome c reduction for PMA.

Determination of plasma concentration of G-CSF. The plasma concentration of G-CSF was determined by a radioimmunoassay (RIA) using polyclonal rabbit anti-rhG-CSF antibody, 125I-labeled rhG-CSF, and goat anti-serum to rabbit IgG (N. Takanashi, manuscript in preparation). The sensitivity of the assay was 0.25 ng/mL, and a linear dose response was observed through 25 ng/mL in human plasma. Plasma samples were collected at indicated time points (0, 0.5, 1, 1.5, 2, 5, 2.5, 4.5, 8.5, and 12.5 hours) after rhG-CSF administration and assayed in parallel with appropriate standards for G-CSF quantitation. Experimental variability in this assay routinely averaged 5%.

Determination of C3bi-receptor expression. Heparinized blood was obtained at the indicated times (0, 0.25, 0.5, 1, 2, and 24 hours) after rhG-CSF administration, and mixed with anti-MoCl monoclonal antibody (MoAb) directly conjugated with fluorescein isothiocyanate (FITC). The cells were incubated for 30 minutes at 4°C and analyzed by cytofluorometry in an Ortho Spectrum III (Ortho Diagnostic Systems, Westwood, MA) as described previously.

Table 1. Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>rhG-CSF Administered (μg/m$^2$)</th>
<th>Cytotoxic Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53/F</td>
<td>NHL</td>
<td>50</td>
<td>CY,ADM, VCR, PSL</td>
</tr>
<tr>
<td>2</td>
<td>45/F</td>
<td>NHL</td>
<td>100</td>
<td>IFM, VP-16, MIT, PSL</td>
</tr>
<tr>
<td>3</td>
<td>77/F</td>
<td>HD</td>
<td>200</td>
<td>CY, VCR, PSL, PCZ</td>
</tr>
<tr>
<td>4</td>
<td>64/M</td>
<td>NHL</td>
<td>200</td>
<td>CY, ADM, VCR, PSL</td>
</tr>
<tr>
<td>5</td>
<td>47/F</td>
<td>NHL</td>
<td>400</td>
<td>CY, ADM, VCR, PSL, MTX</td>
</tr>
<tr>
<td>6</td>
<td>57/F</td>
<td>NHL</td>
<td>400</td>
<td>CY, ADM, VCR, PSL, MTX</td>
</tr>
<tr>
<td>7</td>
<td>79/F</td>
<td>NHL</td>
<td>800</td>
<td>CY, ADM, VCR, PSL, MTX</td>
</tr>
</tbody>
</table>

Patients 2, 4, and 5 had BM involvement by lymphoma cells, and patient 5 was in a leukemic state. Patients 2, 4, and 7 had previous chemotherapy. Patients 3 and 5 were treated with rhG-CSF after the second course of cytotoxic chemotherapy, and the other patients were treated with rhG-CSF after the initial course.

Abbreviations: HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; CY, cyclophosphamide; ADM, doxorubicin; VCR, vincristine; PSL, prednisolone; VP, VP-16, etoposide; MTX, mitoxantrone; PCZ, procarbazine; MTX, methotrexate; and ACM, aclacinomycin.

Effect of rhG-CSF administration on absolute neutrophil count. The total white blood cell (WBC) count in the PB was increased after 30-minute rhG-CSF administration, and WBCs increased in response to rhG-CSF were predominantly neutrophils. Figure 1 shows the changes of the absolute neutrophil count in the PB during 24 hours after initial administration of various doses (50 to 800 μg/m$^2$) of rhG-CSF. The absolute neutrophil count was increased in all patients within 4.5 hours and reached a maximum by 6.5 to 12.5 hours after rhG-CSF administration. Although a high dose of rhG-CSF (800 μg/m$^2$) induced a dramatic increase in the absolute neutrophil count (patient 7), a lower dose of rhG-CSF (50 μg/m$^2$) also induced a remarkable increase in the absolute neutrophil count (patient 1). There was no definite relationship between the dosage of rhG-CSF administered and an increase in the absolute neutrophil count, presumably owing to the preceding cytotoxic chemotherapy (Fig 1). Administration of rhG-CSF not only induced a rapid increase in the absolute neutrophil count but also ameliorated the decrease in the neutrophil count after the cytotoxic chemotherapy in all patients (Fig 2). The period of neutropenia (<1,000 or <500/μL) was significantly (P < .05) reduced by rhG-CSF treatment in all patients. The periods of neutropenia with <1,000 and <500/μL in the course with rhG-CSF treatment were 3.6 ± 4.6 and 1.7 ± 2.4 days, respectively, whereas those in the course without rhG-CSF treatment were 7.7 ± 6.6 and 5.4 ± 6.2 days, respectively.

Fig 1. Absolute neutrophil counts during 24 hours after initial administration of rhG-CSF. Various doses (50 to 800 μg/m$^2$) of rhG-CSF were administered IV as described in the Materials and Methods section.
The nadir of neutrophils in the course with rhG-CSF treatment was 1,226 ± 1,381/μL, whereas that in the course without rhG-CSF treatment was 492 ± 722/μL. Treatment with rhG-CSF was well-tolerated, and no patients had any side effects.

Effect of rhG-CSF administration on neutrophil \(O_2^-\) release. To assess the in vivo activation of neutrophil function by rhG-CSF, we studied release of \(O_2^-\) in human neutrophils stimulated by FMLP. PB was obtained immediately before (day 1), during (at 6.5 hours and on day 2 or 3), and after (day 15) treatment with rhG-CSF. As shown in Table 2, enhanced release of \(O_2^-\) was already detected 6.5 hours after rhG-CSF administration. Neutrophils isolated 6.5 hours after rhG-CSF administration released greater amount of \(O_2^-\) (1.2- to 2.4-fold, \(P < .02\)) with FMLP stimulation than neutrophils isolated immediately before rhG-CSF treatment in all patients tested. The representative time courses of \(O_2^-\) release stimulated by FMLP are shown in Fig 3. The priming effect was sustained for at least 24 hours after a single 30-minute IV administration of rhG-CSF, since neutrophils isolated on day 2 before a second administration of rhG-CSF released a still greater amount of \(O_2^-\) in response to FMLP than neutrophils isolated before rhG-CSF treatment (Table 2 and Fig 3). The priming effect was sustained during the entire course of rhG-CSF treatment, and FMLP-induced \(O_2^-\) release in neutrophils isolated on day 15 was 1.6- to 7.8-fold greater than that in neutrophils isolated before rhG-CSF treatment (Table 2). The priming effect was not strictly dependent on the dosage of rhG-CSF administered when assessed on the basis of magnitude of enhancement and amount of \(O_2^-\) release, and significant priming occurred even when as little as 50 μg/m² rhG-CSF was administered. In remarkable contrast to the results obtained with FMLP, the amount of \(O_2^-\) release in human neutrophils stimulated by PMA varied during the course of rhG-CSF treatment and consistent enhancement of \(O_2^-\) release was not observed (data not shown).

Reduced responsiveness to further in vitro challenge of rhG-CSF in neutrophils isolated after rhG-CSF treatment. Our previous experiments showed that preincuba-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>rhG-CSF InVitro</th>
<th>InVitro Before</th>
<th>6.5 h</th>
<th>Day 2(3)*</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>1.7 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>3.5 ± 0.3</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4.3 ± 0.7</td>
<td>2.3 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>7.3 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>4.1 ± 0.6</td>
<td>ND</td>
<td>4.7 ± 0.3</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>6.0 ± 0.5</td>
<td>ND</td>
<td>5.3 ± 0.8</td>
<td>8.8 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>0.4 ± 0.1</td>
<td>ND</td>
<td>1.2 ± 0.1</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.2 ± 0.1</td>
<td>ND</td>
<td>1.2 ± 0.1</td>
<td>3.4 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>1.1 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>5.1 ± 0.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4.5 ± 0.3</td>
<td>3.0 ± 0.5</td>
<td>6.5 ± 0.4</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>4.0 ± 0.3</td>
<td>7.0 ± 0.6</td>
<td>7.5 ± 0.6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>7.1 ± 0.2</td>
<td>6.9 ± 0.9</td>
<td>6.8 ± 1.3</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>1.1 ± 0.2</td>
<td>ND</td>
<td>ND</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2.3 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>2.8 ± 0.3</td>
<td>ND</td>
<td>3.1 ± 0.4</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>5.4 ± 1.1</td>
<td>ND</td>
<td>5.2 ± 0.6</td>
<td>4.4 ± 0.4</td>
</tr>
</tbody>
</table>

Neutrophils were isolated immediately before, during (at 6.5 hours and on day 2 or 3), and after (day 15) treatment with rhG-CSF, and \(O_2^-\) release stimulated by FMLP (10⁻⁷ mol/L) was assayed with or without rhG-CSF (25 ng/mL). Data are means ± SD of triplicate determinations.

*Patients 3, 4, and 5 were tested on day 2 and patients 1, 2, and 7 were tested on day 3.

†, ‡Significantly increased as compared with values obtained before rhG-CSF treatment († \(P < .01\), ‡ \(P < .02\)).

§, ||, |||Significantly increased as compared with values obtained from neutrophils preincubated without rhG-CSF in vitro (§ \(P < .01\), || \(P < .02\), ||| \(P < .05\)).
tion of human neutrophils with 25 ng/mL rhG-CSF for 10 minutes at 37°C is sufficient for priming the cells in vitro.12 As shown in Table 2, when neutrophils isolated before rhG-CSF treatment were preincubated in vitro with 25 ng/mL rhG-CSF for 10 minutes at 37°C, significant enhancement (1.5- to 4.1-fold; P < .02) of O2− release stimulated by FMLP was observed in all patients. On the contrary, when neutrophils isolated 6.5 hours after rhG-CSF administration were preincubated in vitro with rhG-CSF, no further enhancement of O2− release was observed in any patients tested; ie, neutrophils were desensitized to rhG-CSF and further priming in vitro did not occur in neutrophils already primed in vivo. When the in vitro priming study was performed on day 2 (or 3) and day 15, a significant priming effect was observed in two of six patients tested on day 2 (or 3) and in two of five patients tested on day 15, respectively. In vitro priming by rhG-CSF on day 15 was observed in two patients receiving lower doses of rhG-CSF (50 and 100 μg/mL, respectively), whereas in vitro priming was not observed in the other patients receiving higher doses of rhG-CSF (>200 μg/mL) (Table 2). Neutrophils isolated 6.5 hours after rhG-CSF administration and primed in vitro released a significantly (P < .02) smaller amount of O2− with FMLP stimulation than did neutrophils isolated before rhG-CSF treatment and primed in vitro (patients 1 and 4 in Table 2).

The changes in neutrophil functions observed in the present experiments might have been caused by the cytotoxic chemotherapy itself. To test this possibility, neutrophils were isolated from two patients who were not receiving rhG-CSF, and O2− release stimulated by FMLP was determined immediately before and 72 hours after cytotoxic chemotherapy. As shown in Table 3, cytotoxic chemotherapy did not cause consistent enhancement of O2− release, but rather impaired O2− release. Table 3 also shows that the responsiveness of neutrophils to rhG-CSF was not impaired by cytotoxic chemotherapy.

**Pharmacokinetic study.** The plasma concentration of G-CSF was measured by RIA. Before administration of rhG-CSF, G-CSF was not detected in all patients by this assay system, indicating that the plasma concentration of G-CSF was <0.25 ng/mL. Pharmacokinetic study of rhG-CSF performed in three patients (patients 1, 2, and 4) showed a half-life (t1/2) of 114 minutes (calculated from the initial slope), in good agreement with the t1/2 (110 minutes) obtained by a bioassay.9 It was noteworthy that administration of as little as 50 μg/m² rhG-CSF (patient 1) gave a plasma concentration of >20 ng/mL (26.8 ng/mL at 30 minutes), which continued for at least 30 minutes, a condition sufficient for maximal priming of human neutrophils in vitro.12 Administration of 200 μg/m² rhG-CSF (patient 4) gave a plasma concentration of 98.9 ng/mL at 30 minutes and 5.9 ng/mL at 12.5 hours, respectively.

**Effect of rhG-CSF administration on neutrophil C3bi-receptors.** We recently showed that pretreatment of human neutrophils with rhG-CSF in vitro increases expression of C3bi-receptors.13 We then studied the in vivo effect of rhG-CSF on neutrophil C3bi-receptors. As shown in Fig 4, administration of rhG-CSF also increased expression of C3bi-receptors on human neutrophils, as measured by binding of FITC-labeled MoAb (anti-Mo1) to this antigen. The mean fluorescence values obtained with anti-Mo1 increased after administration of rhG-CSF. The increase in binding of anti-Mo1 was rapid (evident within 15 minutes) and was sustained for at least 24 hours after a single IV administration of rhG-CSF.

**DISCUSSION**

The present study shows that rhG-CSF administration rapidly (evident within 4.5 hours) increases the absolute neutrophil count in the PB of patients with malignant lymphoma irrespective of BM involvement by lymphoma cells, and an increased level of neutrophil count is still observed 24 hours after a single 30-minute IV administration.
of rhG-CSF. In addition, rhG-CSF administration ameliorated the decrease in neutrophil count after cytotoxic chemotherapy and reduced the period of neutropenia in all patients. These findings are consistent with recent studies performed in patients with nonhematologic malignant tumors and further support the clinical usefulness of rhG-CSF in patients receiving cytotoxic chemotherapy.

Another important goal of the present study was to assess the functional aspect of PB neutrophils increased in response to rhG-CSF administration. When neutrophils isolated before rhG-CSF treatment were preincubated with rhG-CSF in vitro, O$_2^-$ release stimulated by FMLP was enhanced in all patients; i.e., neutrophils were rapidly primed in vitro by rhG-CSF. The present experiments showed that neutrophils were also rapidly primed in vivo by rhG-CSF because neutrophils isolated 6.5 hours after rhG-CSF administration released a greater amount of O$_2^-$ with FMLP stimulation than did neutrophils isolated before rhG-CSF treatment. The enhanced release of O$_2^-$ stimulated by FMLP was sustained for at least 24 hours after a single 30-minute IV administration of rhG-CSF. The responsiveness to further in vitro challenge of rhG-CSF was lost or reduced in neutrophils isolated after rhG-CSF treatment, indicating that neutrophils already primed in vivo are desensitized to further in vitro challenge of rhG-CSF. These findings are consistent with our recent observation that neutrophils optimally primed in vitro by rhG-CSF do not respond to further addition of rhG-CSF. Furthermore, cytotoxic chemotherapy itself neither caused consistent enhancement of O$_2^-$ release nor impaired the responsiveness of neutrophils to rhG-CSF. These findings suggest that enhanced release of O$_2^-$ during the course of rhG-CSF treatment is caused by the specific action of rhG-CSF. Additional factors may contribute to enhancement of O$_2^-$ release in neutrophils in vivo. No definite relationship between the dosage of rhG-CSF administered and enhancement of O$_2^-$ release could be explained by the inherent variability of neutrophil responsiveness to FMLP or rhG-CSF and the fact that neutrophil functions are influenced by various substances.

In contrast to the results obtained with FMLP, when PMA was used as a stimulus, no consistent enhancement of O$_2^-$ release was observed during the course of rhG-CSF treatment. These findings are consistent with our in vitro observations that rhG-CSF enhances O$_2^-$ release stimulated by receptor-mediated agonists (FMLP and wheat germ agglutinin), but not by agonists that bypass receptors (Ca$^{2+}$ ionophore ionomycin and PMA). These findings suggest that, like in vitro priming, rhG-CSF administered in vivo modulates the signal transduction pathways linked to FMLP receptors rather than increases the components of the O$_2^-$-producing enzyme complexes, such as flavoprotein and cytochrome b$_5$. Administration of as little as 50 µg/m$^2$ rhG-CSF caused not only a significant increase in absolute neutrophil count but also a significant activation of neutrophil function. These observations were also supported by pharmacokinetic study of rhG-CSF. Administration of 50 µg/m$^2$ rhG-CSF gave a plasma concentration of >20 ng/mL G-CSF, which continued for at least 30 minutes, a condition sufficient for priming human neutrophils. Administration of higher doses (>200 µg/m$^2$) rhG-CSF did not cause much greater priming as compared with administration of 50 to 100 µg/m$^2$ rhG-CSF, although the neutrophil count was remarkably increased in patient 7 who received 800 µg/m$^2$ of rhG-CSF. The in vitro priming by rhG-CSF on day 15 was not observed in patients receiving doses of rh-G-CSF (>200 µg/m$^2$). This finding suggests that neutrophils are almost maximally primed in vivo when >200 µg/m$^2$ rhG-CSF is administered. The present experiments also showed that administration of rhG-CSF rapidly (evident within 15 minutes) increased expression of C3bi-receptors, an adhesion-related molecule on human neutrophils. Both an increased expression of C3bi-receptors and a remarkable increase in neutrophil count may predispose to neutrophil aggregation in the blood vessels, which may cause a serious side effect. The transient decrease in absolute neutrophil count observed in vivo may be, at least in part, related to an increased expression of C3bi-receptors. Indeed, we recently observed that rhG-CSF enhances neutrophil adherence to nylon fiber in vitro with concomitant increase of the expression of C3bi-receptors.

In the present experiments, we showed that rhG-CSF administration not only ameliorates the decrease in absolute neutrophil count after cytotoxic chemotherapy but also activates mature neutrophil functions without accompanying side effects. These findings further support the clinical usefulness of rhG-CSF not only in patients receiving cytotoxic chemotherapy but also in patients whose neutrophil functions are impaired.

ACKNOWLEDGMENT

We thank Ikuko Suzuki and Takashi Obata for excellent technical assistance and Kirin Brewery and Amgen Biologicals for providing rhG-CSF.
REFERENCES


From www.bloodjournal.org by guest on October 23, 2017. For personal use only.
In vivo activation of human neutrophil functions by administration of recombinant human granulocyte colony-stimulating factor in patients with malignant lymphoma

A Ohsaka, S Kitagawa, S Sakamoto, Y Miura, N Takanashi, F Takaku and M Saito