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**MORPHOLOGIC CHANGES OF
NEUTROPHILS IN MYELODYSPLASTIC
SYNDROME TREATED WITH
RECOMBINANT HUMAN GRANULOCYTE
COLONY-STIMULATING FACTOR**

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Two cases of myelodysplastic syndrome (MDS) were treated with recombinant human granulocyte colony-stimulating factor (rhG-CSF). In both cases, an increase of peripheral neutrophil counts was noted with a peak within 12 hr after the rhG-CSF administration. Neutrophils with ring shaped or hypersegmented nuclei were noted in the peripheral blood during the treatment, and they disappeared promptly after discontinuation of the therapy. The results indicate that the rhG-CSF might have mobilizing and differentiating effects on neutrophils derived from the MDS clone.

Key words: Myelodysplastic syndrome — Neutrophil — Granulocyte colony-stimulating factor

Proliferation and differentiation of hematopoietic progenitors are regulated by specific glycoproteins, e.g., erythropoietin, colony-stimulating factors (CSFs) and interleukin-3 (IL-3).¹⁾ Recently, the genes of these stimulating factors have been isolated and cloned, and some of the recombinant forms of these factors are now available for treatment applications.²⁾ Among them, recombinant human granulocyte colony-stimulating factors (rhG-CSF) have been purified and shown to have granulocytic effects.³⁻⁷⁾ In this study, an attempt to elucidate the physiological action

of rhG-CSF *in vivo* was made in two cases of myelodysplastic syndrome (MDS).

Case 1 was a 33-year-old male who had a three and a half year history of hemorrhagic tendency. Blood tests revealed a white blood cell (WBC) count of 1.0×10^9 /liter, a hemoglobin (Hb) level of 73 g/liter, and a platelet count of 76.8×10^9 /liter. A bone marrow aspirate revealed a normocellular marrow with marked dyserythropoiesis and 3.4% atypical myeloblasts. Chromosome analysis of the bone marrow cells showed a pseudodiploid karyotype, i.e., 46,XY, -1, +der(1)(1pter→1q42::1q11→1q42::1q11→1q42::1q11→1qter). A diagnosis of myelodysplastic syndrome (MDS)-refractory anemia (RA) was made, based on the FAB criteria. Intravenous infusion of the rhG-CSF was started on December 15th, 1987 ($50 \mu\text{g}/\text{m}^2$ for 7 days and then $100 \mu\text{g}/\text{m}^2$ for 17 days).

Case 2 was a 28-year-old male who complained of palpitation and stomatitis and was admitted on October 19, 1987. On admission, blood examination revealed a WBC count of 3.3×10^9 /liter, an Hb level of 54 g/liter, and a platelet count of 30.1×10^9 /liter. A bone marrow examination showed a nuclear cell count of 254.0×10^9 /liter with 4.2% blasts and dyserythropoiesis. Cytogenetic study of the bone marrow cells revealed a karyotype of 44,XY, -18, -20, del(5)(q13q34), t(19;21)(p12;q11). The diagnosis of MDS-RA was made. Treatment with rhG-CSF was instituted on December 17, 1987 ($50 \mu\text{g}/\text{m}^2$ for 7 days).

Recombinant human G-CSF (rhG-CSF) used in this study was kindly provided by Kirin Brewery Co., Ltd. (Tokyo). The rhG-CSF dissolved in 100 ml of 5% glucose solution were administered intravenously over a period of 30 min. Written, informed consent was obtained from both patients.

During the rhG-CSF treatment, no prominent side effects were noted, though one patient complained of a "bone compression" feeling. The WBC counts of these patients before and after the rhG-CSF treatment are

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Table I. Hematologic Findings in Myelodysplastic Syndrome Treated with Recombinant Human Granulocyte Colony-stimulating Factor

	Case 1				Case 2			
	Day 1		Day 7		Day 1		Day 7	
	Before	After	Before	After	Before	After	Before	After
White blood cell count ($\times 10^9$ /liter)	1.1	2.4	1.7	2.4	1.8	6.8	3.8	14.1
Granulocyte count ($\times 10^6$ /liter)	341	1584	833	1584	414	548	228	11139
Neutrophils with ring-shaped nuclei (/200 WBC)	0	2	1	4	0	3	1	10
Hypersegmented neutrophils (/200 WBC)	0	4	2	4	0	2	0	4
Pseudo-Pelger cells (/200 WBC)	0	0	0	0	0	0	0	1
NAP score	340	404			160	333		

Before, before the rhG-CSF treatment; After, 8.5 hr after the rhG-CSF injection. WBC, white blood cells; NAP score, neutrophil alkaline phosphatase score (control, 270-290).

shown in Table I. The peak of the WBC elevation at day 1 was 2.4×10^9 /liter after 8.5 hr in case 1 and 6.8×10^9 /liter after 8.5 hr in case 2, but no particular change was noted in the platelet count or Hb level. The granulocytic count also elevated to 1584×10^6 /liter after 8.5 hr in case 1 and to 548×10^6 /liter after 8.5 hr in case 2. At day 7 of the rhG-CSF administration, the levels of WBC counts in these cases were maintained (Table I). A salient feature of the hematological examinations in these cases is an appearance of neutrophils with ring-shaped nuclei (Fig. 1), hypersegmented neutrophils and pseudo-Pelger cells. Cytochemical examination revealed those cells to contain peroxidase activity. In both cases, such neutrophils with abnormally shaped nuclei could not be detected initially by usual hematologic examinations. However, during the rhG-CSF treatment, those neutrophils were noted frequently and their incidence in granulocytic counts did not change significantly (Table I). Furthermore, those neutrophils with abnormal nuclei disappeared promptly after discontinuation of the rhG-CSF therapy. The bone marrow aspirates in these two cases during the rhG-CSF injection also showed obvious hyperseg-

mented neutrophils and pseudo-Pelger cells. At day 7, bone marrow aspirates of these cases did not show any particular change in nuclear cell count or myeloid/erythroid ratio.

Neutrophils with ring-shaped nuclei, or donut cells, have been reported in the peripheral blood of MDS patients; Langenhuijsen reported that neutrophils with ring-shaped nuclei were present in the peripheral blood obtained from 5 of 20 MDS patients.⁸⁾ Although neutrophils with this kind of morphology are also observed in myeloproliferative disorders,⁸⁾ acute erythroleukemia,⁹⁾ infectious mononucleosis¹⁰⁾ and megaloblastic anemia,¹¹⁾ neutrophils with bizarre-shaped nuclei in MDS patients are considered to derive from the MDS clone. In our two cases, neutrophils with abnormal nuclei were not recognized before the rhG-CSF treatment, but were observed during the therapy. This phenomenon might indicate that the early increase of the WBC counts and the appearance of the neutrophils with abnormal nuclei are attributable to mobilization of neutrophils from the marginal pool. On the other hand, persistence of the neutrophils with bizarre nuclei during rhG-CSF treatment might support the concept that the neutrophils had

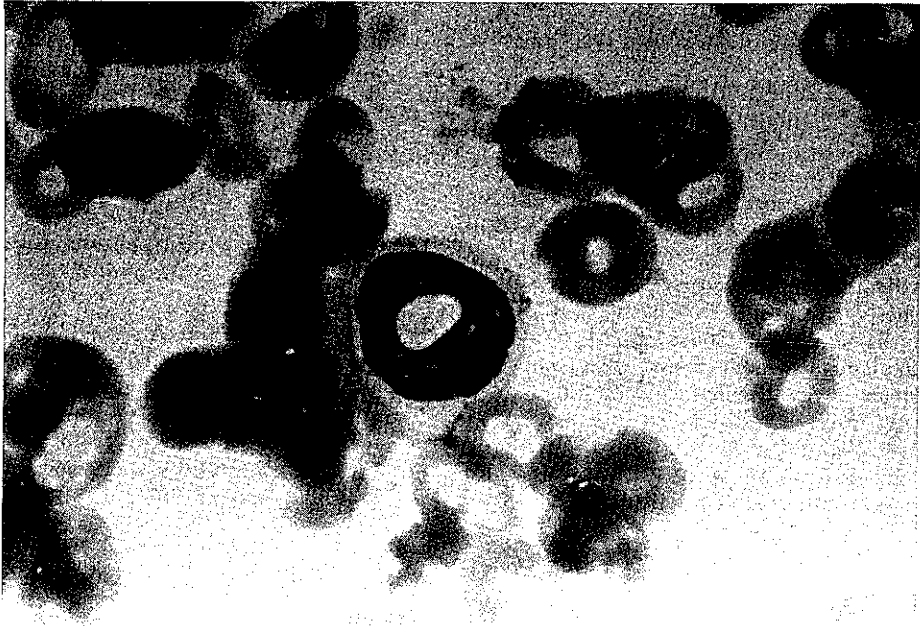


Fig. 1. Neutrophil with ring-shaped nucleus in the peripheral blood obtained from case 2 at day 1.

derived from the MDS clone and had differentiated into neutrophils with abnormal nuclear configurations. Since the presence of neutrophils with abnormal nuclei is one of the characteristics of myelodysplasia, such cells might egress into the peripheral blood. Interestingly, neutrophil alkaline phosphatase activities in the two cases were elevated (Table I) and nitro blue tetrazolium (NBT) reduction activities were within the normal range after the treatment. Though we could not check the functional activities of the neutrophils with abnormal nuclei, rhG-CSF is probably effective in enriching the functional activities of neutrophils even though they have morphological abnormalities and might derive from the MDS clone.¹²⁾

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