A Case Report of Familial Cyclic Neutropenia

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Department of Hematology-Oncology, Institute of Medical Science, University of Tokyo, Tokyo 108, *Department of Internal Medicine, Shyutoh General Hospital, Yanai 747, †Department of Pathology, Iwate Medical University, School of Medicine, Morioka 020, ‡Department of Internal Medicine, Yamaguchi University, School of Medicine, Ube 755, and § Yamaguchi University School of Allied Health Science, Ube 755

INOUE, T., TANI, K., TAJIRI, M., ISHIDA, Y., SEGUCHI, M., TANAKA, H., ASANO, S., KANEKO, T. and MATSUMOTO, N. A Case Report of Familial Cyclic Neutropenia. Tohoku J. Exp. Med., 1992, 167 (2), 107-113 — A 34-year-old female with cyclic neutropenia is reported. Family studies showed that her three sons and her mother were also involved. Oscillations in the blood neutrophil counts were almost regular, with a periodicity of 21 days. Numbers of colony-forming unit-granulocyte macrophage (CFU-GM) formed from the bone marrow cells of normal volunteers co-cultured with the patient’s serum or mononuclear cell-conditioned medium (MNC-CM) were examined. Her serum prepared during the neutropenic phase inhibited the growth of CFU-GM, while her MNC-CM stimulated it. Human granulocyte colony-stimulating factor (hG-CSF) level in her serum was persistently high, with the peak occurring during the neutrophenic phase. These results suggest that some inhibitory factors in the serum may be pathophysiologically important for cyclic neutropenia. To control infections, a pharmacological dose of hG-CSF was administered for 7 days around the early neutropenic phase. Her peripheral neutrophil counts oscillated from 1,200/mm³ to 17,000/mm³ with G-CSF, and from 150/mm³ to 1,800/mm³ without G-CSF.

Cyclic neutropenia (CN) is a rare disorder characterized by regular periodic oscillation of peripheral neutrophil counts. More than 150 cases have been reported since its initial description in 1910 (Leale 1910). The mode of autosomal
dominant inheritance was suggested in previous reports (Dale and Hammond 1988). The cycle time in most patients is 21 days (Dale and Hammond 1988; Lange 1983). Neutropenia is usually associated with such clinical symptoms as malaise, fever, aphthous stomatitis and the skin infections. Pathophysiological studies in patients and in grey collie dogs (Lund et al. 1967), in which a very similar disorder is found, indicate that the cause of cycling could be a defect in the regulation of hematopoietic stem cells. In this communication we describe one Japanese familial CN case with an autosomal dominant trait in which serum inhibitory factor(s) may have played an important physiological role.

CASE REPORT

The propositus, a 34-year-old female, was referred to our hospital, Shyutoh General Hospital, in January 1987, with the chief complaints being fever and hoarseness. She had had recurrent episodes of pyrexia since her childhood, diphtheria at the age of three, and bronchopneumonia at the age of twenty-eight. On admission, her white blood cell count was 1,900/mm³ with 76/mm³ neutrophils, red blood cell count was 397 × 10⁴/mm³, and platelet count was 16.7 × 10⁴/mm³. Bone marrow examination showed normal cellularity with reduced granulopoiesis. Physical examination revealed pyrexia and a laryngeal ulcer, and chest x-ray showed bronchopneumonia. She was started on antibiotics and her white blood cell count returned to 4,000/mm³, with 1,980/mm³ neutrophils one week after admission. However, her serial peripheral blood cell count demonstrated 21-day cyclic neutropenia (Fig. 1). The monocyte count was slightly elevated during the neutropenic phase.

Three typical clinical pictures led to the diagnosis of cyclic neutropenia with 21-day cycles. The patient's three sons, 8-, 6-, and 3-year-old, all had had recurrent episodes of infection and had been diagnosed as CN because of their oscillating neutrophil counts. Furthermore, her mother, who was then 64 years old, was also found to have CN by subsequent blood examination. She, however, had had no history of severe infections or repeated pyrexia.

MATERIALS and METHODS

Informed consent was obtained from the patient and from normal healthy volunteers. A study of peripheral blood and bone marrow cells was performed according to the safety guidelines of the Yamaguchi University School of Medicine.

Preparation of mononuclear cell-conditioned medium

The patient's blood, obtained from the antecubital vein, was drawn into a heparinized syringe every 3 to 4 days. Mononuclear cells (MNC) and serum were obtained by standard methods.

Mononuclear cell-conditioned medium (MNC-CM) was prepared by incubating 1 × 10⁶/ml of MNC in α-minimum essential medium supplemented with 10% fetal calf serum (FCS) for 48 hr. The culture supernatant obtained after centrifugation (1,500 rpm for 10 min) was used as MNC-CM in the following studies. Both MNC-CM and serum were kept frozen at −20°C until use.

CFU-GM assay and co-culture study

Bone marrow cells from normal healthy volunteers were drawn into heparinized syringes and diluted with α-medium. Granulocyte-macrophage colony-forming unit (CFU-GM) assay using bone marrow MNC was performed according to a previously de-
scribed method (Inoue et al 1984). Normal bone marrow cells (2 × 10^6/ml) were co-cultured with and without the patient's serum or MNC-CM at final concentrations of 10%. Human placental conditioned medium was used as the source of colony stimulating activity. Colonies of more than 40 cells were counted on day 7. The data were obtained from the averaged results in 5 dishes.

Recombinant human G-CSF administration and measurement of endogenous G-CSF in serum

Recombinant human granulocyte colony-stimulating factor (G-CSF) was provided by Chugai Pharmaceutical (Tokyo). Serum G-CSF at various stages was assayed by enzyme immunoassay as described previously (Motojima et al. 1989).

RESULTS

The oscillations in the patient's circulating total white cells, neutrophils, and monocytes are shown in Fig. 1. The oscillation of the blood neutrophil count was almost regular, with a periodicity of 21 days. Monocyte counts were slightly elevated during the neutropenic phase, but no other blood cell element & showed oscillation. The mode of autosomal dominant inheritance in this family was speculated from their family tree (Fig. 2). The co-culture studies of normal CFU-GM with the patient's MNC-CM or serum are shown in Table 1. During the neutropenic phase, her serum moderately inhibited the growth of normal CFU-GM, while her MNC-CM stimulated this growth. The serum G-CSF levels of this patient were consistently higher during the neutropenic than during the neutrophilic phase as reported previously (Watari et al. 1989) (Table 1).

Recombinant human G-CSF was administered to the patient subcutaneously at a dose of 2 μg/kg/day for 7 days during the early neutropenic phase. Neutrophil counts were thus maintained at levels as high as 1,240/mm³ during the

Fig. 1. Oscillation of peripheral white cell counts.
Neutrophil counts oscillate with a periodicity of 21 days in the propositus, a 34-year-old female. • •, total white blood cells; ○ o, neutrophils; ▲ ▲, monocytes.
Since the first report of CN in an infant by Leale in 1910 (Leale 1910), more than 150 cases have been reported (Lange et al. 1981; Nakamura and Tomisawa 1975; Morley et al. 1967; Krace et al. 1982; Arai et al. 1988). In about one third of these cases, family histories showed an autosomal dominant inheritance (Dale and Hammond 1988; Nakamura and Tomisawa 1975; Morley et al. 1967; Arai et al. 1988). Clinical pictures varied from recurrent infections to the absence of symptoms (Wright et al. 1981). In the present case, the propositus, her three children, and her mother were found to have CN, and the mode of autosomal dominant inheritance in this family is most likely. The propositus and her

neutropenic phase (Fig. 3).

**DISCUSSION**

Since the first report of CN in an infant by Leale in 1910 (Leale 1910), more than 150 cases have been reported (Lange et al. 1981; Nakamura and Tomisawa 1975; Morley et al. 1967; Krace et al. 1982; Arai et al. 1988). In about one third of these cases, family histories showed an autosomal dominant inheritance (Dale and Hammond 1988; Nakamura and Tomisawa 1975; Morley et al. 1967; Arai et al. 1988). Clinical pictures varied from recurrent infections to the absence of symptoms (Wright et al. 1981). In the present case, the propositus, her three children, and her mother were found to have CN, and the mode of autosomal dominant inheritance in this family is most likely. The propositus and her

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**Table 1. Transition of patient's neutrophil, effect of patient's MNC-CM and serum on normal CFU-GM, and serum G-CSF**

<table>
<thead>
<tr>
<th>Date</th>
<th>WBC* (×10⁶/m³)</th>
<th>Neutrophils (×10⁶/m³)</th>
<th>Relative growth of normal CFU-GM (%) on addition of Pt MNC-CM</th>
<th>Pt Serum</th>
<th>Serum G-CSF (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4,300</td>
<td>1,000</td>
<td>145</td>
<td>95</td>
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<td>162</td>
<td>27</td>
<td>170</td>
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<tr>
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<td>162</td>
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<td>105</td>
</tr>
<tr>
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<td>105</td>
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<td>ND</td>
<td>ND</td>
<td>60</td>
</tr>
</tbody>
</table>

*aWBC, total white blood cells; Pt, patient; ND, not done.*

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children presented with symptoms attributable to the disease itself, but her mother, who was 62 years old, had had no symptoms. The heterogeneous clinical pictures in this family could be the result of contributions by other immunological defense systems (Morley et al. 1967).

Three mechanisms have been proposed for the development of ON (Dale and Hammond 1988; Wright et al. 1989): (1) A defect or weakness of stem cell responses to normal hematopoietic regulators, (2) a defect in the humoral or cellular control mechanisms that regulate stem cell proliferation, and (3) the periodic accumulation of an inhibitor of stem cell proliferation (Dale and Hammond 1988). Krace and coworkers reported a patient who acquired ON after receiving a bone marrow transplantation for acute myelogenous leukemia from a donor who had CN. Their report suggested that CN was a disorder of dysregulated hematopoietic stem cells (Krace et al. 1982). Serial cultures of marrow cells have recently been performed and cyclical oscillations in the number of colonies in CN were reported (Wright et al. 1989). In that study, the number of CFU-GM colonies formed from samples obtained at the neutropenic phase was generally higher than the number formed from samples obtained at the neutrophilic phase. Our preliminary results on the patient’s CFU-GM colony formation showed the same tendency as that shown in previous reports, although, accidentally we were unable to determine colony numbers at the nadir (data not shown) (Eaves et al.)
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1991; Ghizzia et al. 1989; Hammond et al. 1989). We also determined the effects of the patient's MNC-CM and the patient's serum on the growth of CFU-GM obtained from normal controls. Interestingly we detected colony-stimulating and colony-inhibiting activities in MNC-CM and the serum, respectively, which were obtained during the neutropenic phase. Serum G-CSF levels almost paralleled the colony-stimulating activity of MNC-CM. As G-CSF is produced by monocytes and since the peripheral monocyte count actually increased during the neutropenic phase, the colony stimulating activity of MNC-CM appears to be, at least partially, contributed by G-CSF. Although the patient's serum contained elevated levels of G-CSF, the serum showed colony inhibitory activity during the neutropenic phase. Inflammation-induced humoral factors, including TGF-β (Cashman et al. 1990) could contribute to this finding. Another possibility is the production of soluble G-CSF receptors (Fukunaga et al. 1990) during this phase. Determination of the serum levels of these factors in CN is required to confirm this hypothesis. Oscillation of the neutrophil count could be ascribed to a balance between colony stimulatory and inhibitory factors for CFU-GM.

The beneficial effects of the pharmacological doses of recombinant human G-CSF on cyclic neutropenia have recently been reported by several investigators (Hanada and Ono 1990; Migliaccio et al. 1990; Sugimoto et al. 1990; Kurzrock et al. 1991). In the present case, G-CSF was effective in elevating granulocyte counts during the neutropenic phase. This finding thus supports the results of those investigators.

Acknowledgments

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References

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