Acta Med. Nagasaki 35 : 63−66

In Vitro Study of the Independent and Combined Effects of Recombinant Human GM-CSF and G-CSF on Normal Bone Marrow Granulocytes : GM-CSF Enhances the Growth Effect but Suppresses the Terminal Maturation-inducing Effect of G-CSF

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Received for publication, February 27, 1990

SUMMARY : We studied the independent and combined effects of recombinant human granulocyte colony-stimularting factor (rhG-CSF) and recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) on the growth and maturation of neutrophilic granulocytes. Absolute granulocyte numbers, cellular composition, and neutrophil alkailne phosphatase (NAP) activity were examined after normal nonphagocytic bone marrow cells were cultured for seven days with rhG-CSF, rhGM-CSF, and a combination of both growth factors. The numbers of band and segmented forms produced in the cultures containing rhGM-CSF were significantly lower than in control cultures or those with rhG-CSF. When both rhGM-CSF and rhG-CSF were in the culture total granulocyte production was highest, but the increment of band and segmented forms produced by rhG-CSF alone was reduced. Neutrophil alkaline phosphatase (NAP), an enzyme marking terminal maturation, was increased by rhG-CSF alone, but not by rhGM-CSF alone. Cultures containing both CSFs showed significantly lowered NAP activity compared to those containing rhG-CSF alone. The decrement in NAP activity was proportionate to the amount of rhGM-CSF added. These results indicate that terminal maturation-inducing effect is a property of rhG-CSF but not of rhGM-CSF. In the presence of both GM-CSF and G-CSF, the growth of granulocytes is maximumly stimulated but the terminal maturation-inducing effect of rhG-CSF is suppressed.

INTRODUCTION

Three colony-stimulating factors (CSF), granulocyte-macrophage (GM)-CSF, granulocyte (G)-CSF and interleukin 3 (IL3) are involved in granulopoiesis. G-CSF induces primarily neutrophilic granulocyte colonies, while GM-CSF induces macrophage and eosinophil colonies as well. IL3[1] shows a further diversity of target cells including basophils, erythroid cells in the presence of erythropoietin and megakaryocytes. Moreover, purified human GM-CSF and G-CSF are known to stimulate the functions of nentrophils[2,3,4]. The differences in the biological effects of these CSFs on

granulocytes are poorly understood. We compared the independent and combined effects of GM-CSF and G-CSF on the growth and maturation of granulocytes, total granulocyte number, cellular composition and neutrophil alkaline phosphatase (NAP) activity after liquid culture (7 days) of normal nonphagocytic bone marrow cells.

MATERIALS AND METHODS

Bone marrow cells from 4 normal volunteers were aspirated into a heparinized syringe after obtaining appropriate informed consent. Light-density cells were separated from each sample by Ficoll-Conray gradient centrifugation (1.077 g/ul). Nonphagocytic cells were obtained by depleting the light-density cells of phagocytes using iron-particle phagocytosis as previously described[5]. One million nonphagocytic cells in 1 ml of α-medium with 20% fetal bovine serum (FBS) were incubated in Falcon plastic Petri dishes (35 mm) with or without 10% diluted recombinant CSFs for 7 days at 37°C in a humidified atmosphere of 5% CO_2. Three concentrations ($\times 1$, $\times 5$, and $\times 25$ of maximum dose) of rhG-CSF[6] and rhGM-CSF[7] were prepared by diluting recombinant materials with α-medium containing 20% FBS. The maximum dose of each CSF was equivalent to the concentration giving a maximum colony number in semisolid culture. Both adherent and nonadherent cells were completely harvested by a Pasteur pipette after scraping the dishes gently with a rubber policeman and viable cell number was determined with a hemocytometer and trypan blue as a diluent. Average volume recovery was 0.9 ml per dish. Cell viability was at least 92% by trypan blue dye exclusion. Slides for May-Grünwald-Giemsa staining and alkaline phosphatase staining were prepared from each dish by cytocentrifugation. The alkaline phosphatase stain was performed as previously described and the same scoring system of enzyme activity was used[5]. Using this system, the normal range of peripheral blood neutrophil alkaline phosphatase (NAP) score was from 158 to 295. Student's paired *t*-test was used to assess statistical significance.

RESULTS

1. *Independent and combined effects of rhG-CSF and rhGM-CSF on the growth and maturation of granulocytes*

The number of viable cells in each sample was counted and the absolute number of each maturational compartment of granulocytes was calculated from a differential morphological analysis of May-Grünwald-Giemsa stained smears. The mean number of viable granulocytes was higher in the cultures with rhG-CSF alone than those with rhGM-CSF alone. The cultures with both CSFs together yielded more granulocytes than those with rhG-CSF alone, although the difference was not statistically significant (**Table 1**). Comparison of cellular composition of the cultures containing one or both CSFs with that containing neither disclosed that the major impact of rhGM-CSF alone was on production of myeloblasts, promyelocytes and myelocytes, while that of rhG-CSF alone was on production of metamyelocytes, band and segmented forms (**Fig. 1**). The number of myeloblasts was increased by rhGM-CSF alone but not by rhG-CSF alone. The cultures containing both CSFs at the highest concentrations did not show

Table 1. Mean Viable Cell Number ($\times 10^5$)

	Total Neutrophilic Granulocytes	Band and Sedmented Forms	
Preincubation	5.24 ± 1.33	0.79 ± 0.41	
Wtihout CSF	6.81 ± 3.02	2.87 ± 2.00	*
rhGM-CSF (250pM)	13.14 ± 6.31	0.90 ± 1.09	*
rhG-CSF (500U)	18.25 ± 9.06	6.27 ± 2.91	*
rhGM-CSF (250pM) +rhG-CSF (500U)	21.94 ± 12.15	3.12 ± 1.80	

* : $p<0.05$

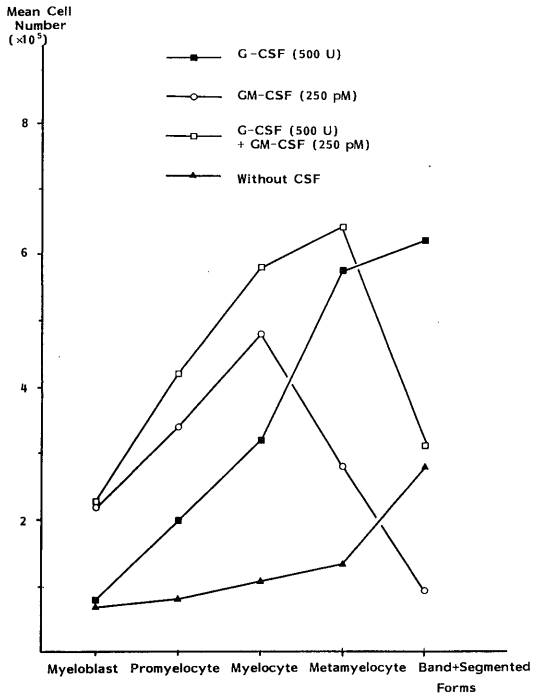

Fig. 1 Mean viable cell number of each neutrophilic stage after liquid culture for 7 days (n=4).

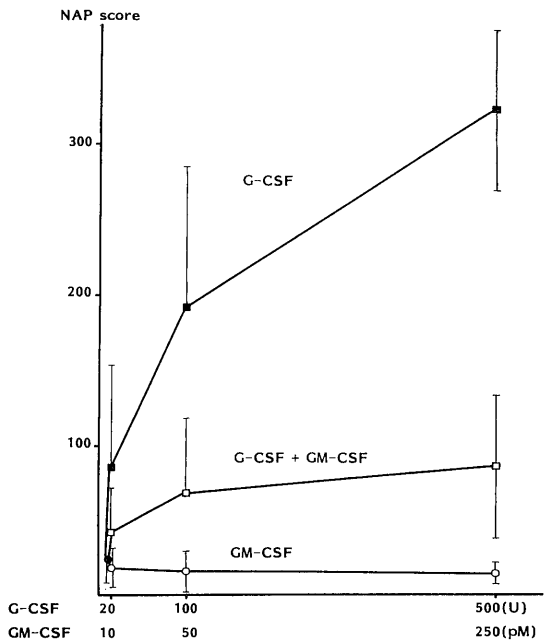

Fig. 2 Dose dependency of neutrophil alkaline phosphatse activity on rhG-CSF, rhGM-CSF and their combination. The vertical line indicate mean ± S. D. (n=4).

a further increment of myeloblasts. In the promyelocyte and myelocyte compartments, either CSF alone increased cell numbers and the culture with both CSFs showed the highest increment. No increment of band and segmented forms was observed in the cultures with rhGM-CSF alone while, with rhG-CSF alone, the increase was remarkable. The mean absolute number of band and segmented forms in the cultures with rhGM-CSF alone was significantly lower than in those with rhG-CSF alone and even that with no CSF. The cultures containing both CSFs showed only a slight increase in band and segmented forms (**Table 1**).

2. *Independent and combined effects of rhG-CSF and rhGM-CSF on NAP induction*

Bone marrow light density nonphagocytic cells produced mature nentrophils without elavation of NAP activity after 7 days incubation as previously described[5]. As shown in **Fig. 2**, NAP activity rose in a dose-dependent manner when rhG-CSF was included in the culture, while rhGM-CSF did not augment NAP activity.

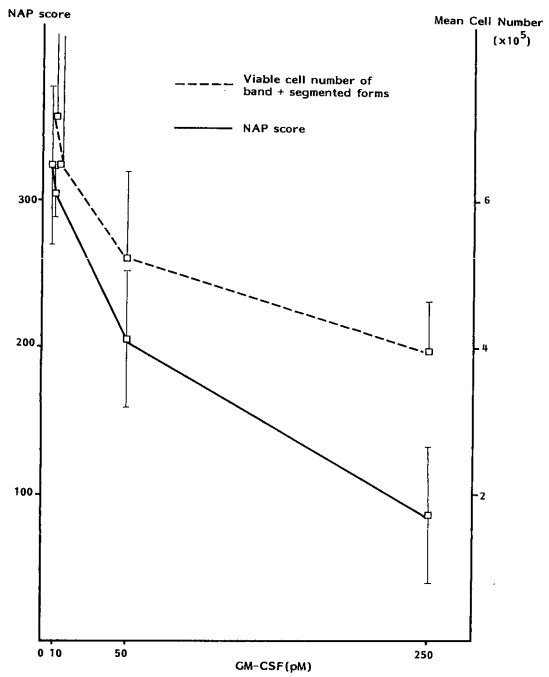

Fig. 3 Modulation by rhGM-CSF of neutrophil alkaline phosphatase score and viable cell number of band and segmented forms of the culture with rhG-CSF (500U). The vertical line indicate mean ±S. D.(n=3).

When both rhG-CSF and rhGM-CSF were added to the culture, NAP activity was siginificantly lower. When the culture was incubated in the presence of the highest dose of rhG-CSF (500U) and various doses of rhGM-CSF, NAP activity was lower proportionately to the amount of rhGM-CSF in the culture. In addition, mean viable cell number of band and segmented forms revealed a similar pattern (**Fig. 3**).

DISCUSSION

Morphological terminal maturation and NAP activity were both induced in vitro by rhG-CSF alone, while rhGM-CSF induced neither, though either CSF increased the total number of granulocytes. GM-CSF seems to play a major role in stimulating the growth of immature granulocytes but little role in inducing maturation. Recently, both CSFs were shown to increase total granulocyte production and mature neutrophils in vivo[8,9]. However, our data may not be discrepant with these studies because other unknown maturation-inducing factors may also be involved in vivo. Interestingly, rhGM-CSF seems to inhibit the maturation effect of rhG-CSF when both CSFs act simultaneously. This result is unlikely to be due to simple receptor competition since in cultures containing both CSFs the effect of rhGM-CSF on the growth of immature granulocytes was enhanced and the largest increment of total granulocyte numbers was found. Growth stimulation by rhGM-CSF seems to have priority over the maturation-inducing effect of rhG-CSF, probably through some intracellular pathway after binding receptors on the granulocyte membrane. Furher investigations on the interaction of these factors at receptor and intracellular levels will provide the best method for clinical administration of them in the near future.

AKNOWLEDGEMENT

We are grateful to Dr. Judith C. Gasson, Division of Hematology-Oncology, UCLA School of Medicine for supplying rhGM-CSF and for reviewing the manuscript and to Dr. Fumimaro Takaku, Faculty of Medicine, University of Tokyo for supplying rhG-CSF from Kirin-Amgen.

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