Clin Exp Immunol 1993; 91:308-313

Effects of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor on respiratory burst activity of neutrophils in patients with myelodysplastic syndromes

A. OHSAKA*†, S. KITAGAWA*, A. YUO‡, K. MOTOYOSHI*, S. FURUSAWA§, Y. MIURA†, F. TAKAKU‡ & M. SAITO*

Division of Haemopoiesis, Institute of Haematology and †Department of Medicine, Jichi Medical School, Tochigi, ‡Clinical Research Institute, National Medical Centre, Tokyo and §Third Department of Internal Medicine, Dokkyo University School of Medicine, Tochigi, Japan

(Accepted for publication 9 September 1992)

SUMMARY

The superoxide (O_2^-)-releasing capacity in response to N-formyl-methionyl-leucyl-phenylalanine (FMLP) and the priming effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF) and granulocyte-macrophage colony-stimulating factor (rhGM-CSF) on FMLP-induced O_2^- release were investigated in neutrophils from 14 patients with myelodysplastic syndromes (MDS). The O_2^--releasing capacity in MDS neutrophils varied from patient to patient. As compared with normal neutrophils, the O_2^--releasing capacity in MDS neutrophils was increased in 9/14 patients, normal in three patients and decreased in two patients. There was no close relationship between the O_2^--releasing capacity and the peripheral blood neutrophil count or the plasma concentration of C-reactive protein. The priming of neutrophils by rhG-CSF was not observed in five patients, whereas rhGM-CSF primed neutrophils from all patients. The priming effect of rhGM-CSF was consistently greater than that of rhG-CSF in each patient. The intravenous administration of rhG-CSF (300 μg/body) to two MDS patients showed an increase in the peripheral blood neutrophil count and enhancement of neutrophil O_2^- release. These findings demonstrate that the neutrophil O_2^--releasing capacity in MDS varies from patient to patient and is not always impaired, and that rhGM-CSF is able to prime neutrophils which never respond to rhG-CSF.

Keywords G-CSF GM-CSF myelodysplastic syndromes neutrophils superoxide release

INTRODUCTION

The primary myelodysplastic syndromes (MDS) consist of heterogeneous bone marrow disorders characterized by refractory cytopenia, ineffective and dysplastic haematopoiesis, and an increased risk for transformation to overt leukaemia. Neutropenia and in particular agranulocytosis favour bacterial infections, and mortality due to infections is exceedingly high [1]. In MDS patients, not only neutropenia but also defective neutrophil functions may be responsible for susceptibility to bacterial infections [2–5].

The clinical trials of haematopoietic growth factors, including recombinant human granulocyte colony-stimulating factor (rhG-CSF) and granulocyte-macrophage colony-stimulating factor (rhGM-CSF), on MDS patients revealed some improvement in the peripheral blood neutrophil count [6–10]. Another important function of rhG-CSF and rhGM-CSF is to activate mature neutrophil functions, including superoxide (O_2^-) release, phagocytosis and antibody-dependent cell-mediated cytotoxicity [11–14]. If rhG-CSF and rhGM-CSF could potentiate neutrophil functions in MDS patients, both factors would still be clinically useful for the treatment of MDS even if the production of neutrophils is not sufficiently stimulated. We have reported previously that the impaired neutrophil functions in some, but not all, MDS patients are restored by rhG-CSF *in vitro* [5], and that rhGM-CSF is a more potent activator of neutrophils than rhG-CSF when neutrophils from healthy adult donors [15] or from patients with aplastic anaemia [16] are employed. We have also reported that rhG-CSF is active *in vivo* and neutrophils are activated by administration of rhG-CSF in patients with malignant lymphoma [17].

In the present study, we investigated the effects of rhG-CSF, in comparison with rhGM-CSF, on O_2^- release in MDS neutrophils stimulated by N-formyl-methionyl-leucyl-phenylalanine (FMLP). The results show that (i) the O_2^--releasing

A.O. Present address: Department of Internal Medicine, Division of Haematology, Hitachi General Hospital, Ibaraki, Japan.

Correspondence: Akimichi Ohsaka MD, Department of Internal Medicine, Division of Haematology, Hitachi General Hospital, 2-1-1 Jonan-cho, Hitachi-shi, Ibaraki 317, Japan.

Table 1. Characteristics of patients and effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF) and granulocyte-macrophage colony-stimulating factor (rhGM-CSF) on O_2^- release in neutrophils stimulated by N-formyl-methionyl-leucyl-phenylalanine (FMLP)

Patient no.	Diagnosis	Age/sex	Neutrophils (per μl)	CRP (ng/dl)	O_2^- release (nmol/5 min per 1×10^5 cells)*		
					Untreated	rhG-CSF	rhGM-CSF
1	RA	26/M	36	39500	0·65±0·02†	0·89±0·08§	1·23±0·04§
2	RA	37/M	108	10400	0·19±0·01	0·21±0·05	0·34±0·04¶
3	RA	68/M	720	6650	0·07±0·01‡	0·20±0·01§	0·38±0·03§
4	RA	76/F	975	320	0·92±0·10†	1·57±0·21§	3·40±0·16§
5	RA	71/M	987	2240	0·10±0·02	0·11±0·01	0·35±0·06§
6	RA	30/M	1040	<30	0·96±0·11†	1·69±0·19§	3·24±0·15§
7	RA	75/F	1056	310	0·86±0·10†	1·95±0·07§	4·69±0·38§
8	RA	40/M	1643	130	0·84±0·07†	1·59±0·09§	2·13±0·05§
9	RA	78/M	1704	<30	0·52±0·08†	0·59±0·05	0·85±0·10§
10	RAEB	30/M	96	880	0·50±0·02†	0·37±0·06	0·96±0·12§
11	RAEB	63/M	1062	15300	0·65±0·01†	0·68±0·03	0·93±0·08§
12	RAEB	42/M	1530	<30	0·56±0·05†	0·74±0·06**	1·67±0·13§
13	RAEB	81/M	2050	960	0·07±0·01‡	0·12±0·02**	0·20±0·01§
14	RAEB-T	70/M	342	3000	0·36±0·01	0·72±0·06§	1·25±0·08§
Normal subjects ($n=17$)					0·24±0·12	0·66±0·26	1·28±0·54

* Neutrophils were preincubated with or without rhG-CSF (25 ng/ml) or rhGM-CSF (2 ng/ml) for 10 min at 37°C, and then stimulated with FMLP (10^{-7} M) for release of O_2^-. The data are expressed as means±s.d. of triplicate determinations.

† Significantly increased as compared with the values obtained from normal neutrophils ($P<0·01$).

‡ Significantly decreased as compared with the values obtained from normal neutrophils ($P<0·05$).

§,¶,** Significantly increased as compared with the values obtained from neutrophils preincubated without rhG-CSF or rhGM-CSF ($P<0·01$, $P<0·05$, $P<0·02$).

RA, refractory anaemia; RAEB, refractory anaemia with excess of blasts; RAEB-T, refractory anaemia with excess of blasts in transformation; CRP, C-reactive protein.

capacity in MDS neutrophils varies from patient to patient and is not always impaired; (ii) neutrophils from some MDS patients are not primed by rhG-CSF; and (iii) rhGM-CSF is able to prime neutrophils which never respond to rhG-CSF.

MATERIALS AND METHODS

Reagents

Highly purified rhG-CSFs produced by *Escherichia coli* (an apparent molecular weight of 18800) and Chinese hamster ovary (CHO) cells (an apparent molecular weight of 19000) were provided by Kirin Brewery (Tokyo, Japan) and Chugai Pharmaceutical (Tokyo, Japan), respectively [18,19]. rhG-CSF produced by *E. coli* had a specific activity of 1×10^8 U/mg protein (50 U is defined as the amount required for 50% of maximal stimulation of colony formation by normal marrow cells [20]). Highly purified rhGM-CSF produced by *E. coli* (an apparent molecular weight of 14700) was provided by Schering-Plough Co. Ltd. (Osaka, Japan) [21]. rhGM-CSF had a specific activity of 1×10^8 U/mg protein (the specific activity is determined by cell-mitogenesis assay based on the incorporation of ^{3}H-thymidine by the TF-1 GM-CSF-dependent cell line and 1 U is defined as 50% of the maximal response [20]). Endotoxin contamination in each preparation of rhG-CSF or rhGM-CSF was <1 ng/mg protein. rhG-CSF produced by *E. coli* was used in the present experiments, unless otherwise indicated. Cytochrome *c* type III, FMLP and superoxide dismutase were purchased from Sigma Chemical Co. (St Louis, MO); Conray from Mallinckrodt (St. Louis, MO); and Ficoll from Pharmacia Fine Chemicals (Piscataway, NJ).

Patients

Fourteen patients with MDS were studied. Their characteristics are shown in Table 1. The diagnosis was based on analysis of the peripheral blood films, bone marrow aspirates and cytogenetic findings. MDS was classified as refractory anaemia (RA), refractory anaemia with excess of blasts (RAEB), and refractory anaemia with excess of blasts in transformation (RAEB-T) according to the French-American-British Cooperative Group (FAB) Criteria [22]. Informed consent was obtained from all patients.

Administration of rhG-CSF

rhG-CSF (300 μg per body) produced by CHO cells was diluted in 100 ml of 5% glucose in water, and was infused intravenously to two MDS patients (patients 2 and 14) in 60 min once daily. The treatment consisted of daily administration of rhG-CSF for 14 days.

Preparation of neutrophils

Neutrophils were prepared as described previously [23], using dextran sedimentation and centrifugation with Conray–Ficoll. Contaminated erythrocytes in neutrophil fractions were removed by hypotonic lysis. Neutrophil fractions were suspended in Hank's balanced salt solution (HBSS) (Nissui Seiyaku, Tokyo, Japan) and contained >95% neutrophils.

Determination of O_2^- release

O_2^- was assayed spectrophotometrically by superoxide dismutase-inhibitable reduction of ferricytochrome *c*, and the continuous assay was performed in a Hitachi 557 spectrophotometer (a double-wavelength spectrophotometer, Hitachi, Tokyo, Japan) equipped with thermostatted cuvette holder as described previously [23]. The cell suspension in HBSS was added to a 1-ml cuvette containing 80 μM ferricytochrome *c* with or without rhG-CSF (25 ng/ml) or rhGM-CSF (2 ng/ml) to obtain a final volume of 0·995 ml. Final cell concentration was $0{\cdot}5–1 \times 10^5$ cells/ml. The reaction mixture in a cuvette was preincubated for 10 min at 37°C. The cuvette was placed in a thermostatted cuvette holder (37°C) of a spectrophotometer, and the reduction of ferricytochrome *c* was measured at 550 nm with a reference wavelength at 540 nm. FMLP (10^{-7} M) 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* reduced for 5 min after the addition of FMLP. In the present experiments, we used FMLP, but not phorbol myristate acetate (PMA), as stimulus, because FMLP may be a physiological stimulus and the priming effects of rhG-CSF and rhGM-CSF on O_2^- release are not observed when PMA is used as stimulus [12,17,24].

Determination of plasma concentration of G-CSF

The plasma concentration of G-CSF was determined by a radioimmunoassay using polyclonal rabbit anti-rhG-CSF antibody, ^{125}I-labelled rhG-CSF and goat anti-serum to rabbit-IgG as described previously [17]. The sensitivity of the assay used was 0·1 ng/ml. Plasma samples were collected when the O_2^--releasing capacity was assessed.

Statistical analysis

Student's *t*-test was used to determine statistical significance.

RESULTS

O_2^--releasing capacity in neutrophils from MDS patients

The O_2^--releasing capacity in neutrophils from MDS patients was assessed by using FMLP as stimulus, and was compared with that in neutrophils from normal controls. The data are summarized in Table 1 and Fig. 1. FMLP-induced O_2^- release in neutrophils from normal controls was $0{\cdot}24 \pm 0{\cdot}12$ nmol/5 min per 1×10^5 cells ($n = 17$). The O_2^--releasing capacity in MDS neutrophils varied from patient to patient. In 14 MDS patients studied, O_2^- release was increased in nine patients, normal in three patients, and decreased in two patients. FMLP-induced O_2^- release in neutrophils could be affected by inflammatory products and haematopoietic growth factors. Thus, the increased release of O_2^- in neutrophils from some MDS patients might reflect the underlying bacterial infections or the increased production of haematopoietic growth factors such as G-CSF. We then studied the relationship between the amount of O_2^- release and the plasma concentration of C-reactive protein (CRP), a marker of bacterial infections or inflammation, or the peripheral blood neutrophil count. As shown in Fig. 2, no close relationships were observed in these parameters. To assess further the possible role of G-CSF in increased neutrophil O_2^- release, the plasma concentration of G-CSF was measured in all patients. In all MDS patients tested, G-CSF was not detected by our assay system, indicating that the plasma concentration of G-CSF was <0·1 ng/ml.

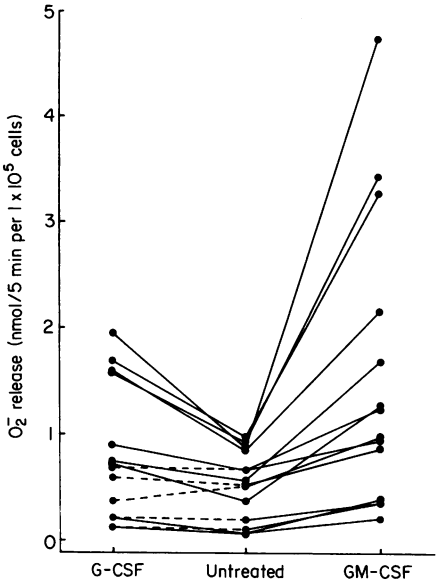

Fig. 1. Effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF) and granulocyte-macrophage colony-stimulating factor (rhGM-CSF) on O_2^- release in neutrophils stimulated by N-formyl-methionyl-leucyl-phenylalanine (FMLP). The values shown in Table 1 were plotted in this panel. A dotted line represents no significant enhancement of O_2^- release (see Table 1).

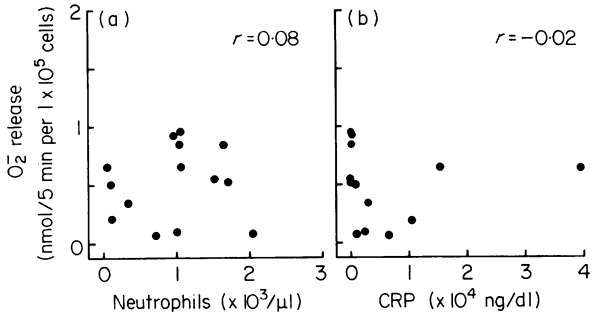

Fig. 2. Relationships between the O_2^--releasing capacity and the peripheral blood neutrophil count (a) or the plasma concentration of C-reactive protein (CRP, (b)). Release of O_2^- in neutrophils stimulated by N-formyl-methionyl-leucyl-phenylalanine (FMLP) (10^{-7} M) was assayed as described in Materials and Methods.

Effect of rhG-CSF and rhGM-CSF on O_2^- release in neutrophils from MDS patients

Our previous studies showed that preincubation of human neutrophils with 25 ng/ml (1·3 nM) rhG-CSF or 2 ng/ml (0·14 nM) rhGM-CSF for 10 min at 37°C was sufficient for priming the cells (Table 1) [11,15]. When neutrophils from MDS patients were preincubated with rhG-CSF (25 ng/ml) for 10 min at 37°C, significant enhancement of O_2^- release stimulated by FMLP was observed in 9/14 patients and no effect was observed in five patients. On the other hand, when neutrophils from MDS patients were preincubated with rhGM-CSF (2 ng/ml) for

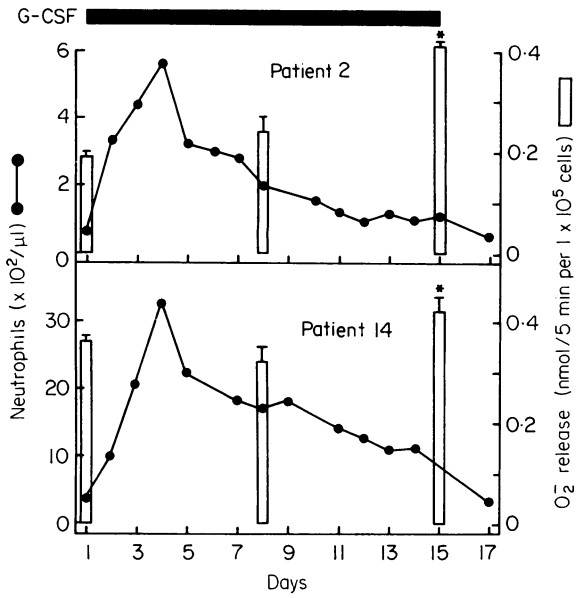

Fig. 3. Absolute neutrophil counts and the neutrophil O_2^--releasing capacity in patients treated with recombinant human granulocyte colony-stimulating factor (rhG-CSF). rhG-CSF (300 μg per body) was administered intravenously as described in Materials and Methods on days 1–14. Neutrophils were isolated immediately before, during (day 8) and after (day 15) the treatment with rhG-CSF, and O_2^- release stimulated by N-formyl-methionyl-leucyl-phenylalanine (FMLP) (10^{-7} M) was determined. * Significantly increased as compared with values obtained before rhG-CSF treatment ($P < 0.01$ in patient 2 and $P < 0.02$ in patient 14).

10 min at 37°C, significant enhancement of O_2^- release stimulated by FMLP was observed in all patients (Table 1 and Fig. 1). However, in four patients (patients 2, 3, 5 and 13) the amounts of O_2^- release in rhG-CSF- or rhGM-CSF-treated cells were remarkably decreased as compared with those from normal controls, suggesting that the maximal O_2^--releasing capacity in neutrophils is markedly impaired in these four patients.

Effect of rhG-CSF administration on the absolute neutrophil count and neutrophil O_2^- release in MDS patients

rhG-CSF (300 μg per body) was administered intravenously to two MDS patients (patients 2 and 14). As shown in Fig. 3, the absolute neutrophil count in the peripheral blood was increased in both patients within 24 h, reached a maximum by 4 days after rhG-CSF administration, and thereafter gradually declined. The transient increase of blasts in the peripheral blood (150/μl immediately before rhG-CSF administration and 615/μl on day 12, respectively) was observed in patient 14 who had RAEB-T.

To assess the *in vivo* activation of neutrophil function by rhG-CSF, neutrophils were isolated immediately before, during (day 8) and after (day 15) the treatment with rhG-CSF, and O_2^- release stimulated by FMLP was determined. As shown in Fig 3, the O_2^--releasing capacity in neutrophils was maintained or potentiated during the treatment with rhG-CSF. Although in patient 2 mature neutrophils did not respond to rhG-CSF priming *in vitro* before the treatment with rhG-CSF, progenitor cells responded well to rhG-CSF administration.

DISCUSSION

Our previous studies indicate that the neutrophil O_2^--releasing capacity is impaired in some MDS patients (7/11 patients studied) and the impaired functions of neutrophils from some patients are restored by rhG-CSF *in vitro* [5]. These findings were confirmed in the present experiments, and the study was extended by using rhGM-CSF. In addition, the study with two MDS patients receiving rhG-CSF administration showed that neutrophils from MDS patients were also activated *in vivo* by rhG-CSF. The restoration of impaired neutrophil functions by rhG-CSF or rhGM-CSF may be clinically important in MDS patients, since the functional impairment of neutrophils is reported to be correlated well with the susceptibility to bacterial infections in these patients [3]. It should be noted, however, that while the O_2^--releasing capacity of neutrophils from some patients was markedly depressed even after the priming with rhG-CSF or rhGM-CSF, it was increased in other patients (Table 1), consistent with the concept that MDS includes heterogeneous disorders. The increased respiratory burst activity of neutrophils has also been reported in some MDS patients [5,25,26].

The present experiments show that neutrophils from some MDS patients never respond to rhG-CSF *in vitro*; that is, FMLP-induced O_2^- release is not potentiated by rhG-CSF in five patients. One possible reason for the lack of responsiveness to rhG-CSF *in vitro* is that neutrophils may be already maximally primed *in vivo* by endogenous G-CSF. We have recently demonstrated that the responsiveness to further *in vitro* challenge of rhG-CSF is lost or reduced in neutrophils isolated after rhG-CSF administration; that is, neutrophils already primed *in vivo* by rhG-CSF are desensitized to this factor [17]. In addition, it has been reported that the serum G-CSF levels are elevated in a half of MDS patients [27]. Thus, the increased O_2^--releasing capacity and the unresponsiveness to *in vitro* rhG-CSF-priming in neutrophils from some MDS patients might be caused by the elevated serum level of endogenous G-CSF. However, in the present study we could not demonstrate the elevated serum level of G-CSF in all patients (G-CSF levels were < 0·1 ng/ml). We cannot, however, rule out the possibility that neutrophils are maximally primed by endogenous G-CSF in the bone marrow where G-CSF levels might be much higher than in the plasma.

Another possibility is that neutrophils may be primed *in vivo* by other cytokines such as GM-CSF and IL-8. In fact, we have recently demonstrated that the responsiveness to G-CSF is lost or reduced in neutrophils optimally primed *in vitro* by GM-CSF [15] or IL-8 [28]. In the present study, we could not determine the serum levels of GM-CSF and IL-8. Although there was no close relationship between the O_2^--releasing capacity and the serum level of CRP, it is possible that some inflammatory cytokines may, at least in part, be responsible for the increased neutrophil O_2^--releasing capacity and the lack of neutrophil responsiveness to rhG-CSF-priming *in vitro* [28]. A final possibility is that diminished or defective G-CSF receptors on neutrophils from some MDS patients may be responsible for reduced responsiveness to rhG-CSF-priming.

The present experiments show that the priming effect of rhGM-CSF is consistently greater than that of rhG-CSF, and that rhGM-CSF is able to prime neutrophils which never respond to rhG-CSF. As compared with rhG-CSF, rhGM-CSF

was effective at lower concentrations; that is, the optimal concentrations of rhG-CSF and rhGM-CSF produced by *E. coli* were 1·3 nM and 0·07 nM, respectively [15]. This difference could be explained by the fact that the affinity of GM-CSF receptors for non-glycosylated rhGM-CSF (kD = 0·03 nM) [29] is higher than that of G-CSF receptors for rhG-CSF (Kd = 0·55 nM) [30]. While the precise mechanisms by which rhG-CSF and rhGM-CSF prime human neutrophils remain to be determined, our recent studies show that tyrosine phosphorylation of a 42-kD protein and intracellular alkalinization are closely associated with the priming effects of rhG-CSF and rhGM-CSF and that the effects of rhGM-CSF on both functions are greater than those of rhG-CSF (Yuo, unpublished data). Thus, we suggest that the difference in maximal priming effect between rhG-CSF and rhGM-CSF is ascribed to the difference of these intracellular metabolic events. It has been recently reported that the tyrosine-phosphorylated 42-kD protein in human neutrophils is the microtubule-associated protein 2 kinase [31,32].

The clinical application of rhG-CSF and rhGM-CSF may give at least two beneficial effects: (i) increase of neutrophil count, and (ii) activation of neutrophil functions. However, not all patients with MDS respond to rhG-CSF or rhGM-CSF administration with increased number of neutrophils [7,33]. The present study demonstrates that neutrophils from most MDS patients are primed by rhG-CSF and neutrophils from all patients studied are primed by rhGM-CSF, indicating that both rhG-CSF and rhGM-CSF may be useful for potentiating the host-defence against microorganisms even in the patients who never respond to rhG-CSF or rhGM-CSF administration with increased numbers of neutrophils.

ACKNOWLEDGMENTS

This work was supported by Grants-in Aid from the Ministry of Education, Science and Culture, Japan and the Kato Memorial Trust for Nambyo Research. We thank Dr Naoki Takanashi (Special Reference Laboratories, Tokyo, Japan) for determination of plasma concentrations of G-CSF. We also thank Mrs Ikuko Suzu for excellent technical assistance.

REFERENCES

1 Beris P. Primary clonal myelodysplastic syndromes. Semin Hematol 1989; **26**:216–33.

2 Ruutu P, Ruutu T, Vuopio P, Kosunen TU, De La Chapelle A. Function of neutrophils in preleukemia. Scand J Haematol 1977; **18**:317–25.

3 Boogaerts MA, Nelissen V, Roelant C, Goossens W. Blood neutrophil function in primary myelodysplastic syndromes. Br J Haematol 1983; **55**:217–27.

4 Martin S, Baldock SC, Ghoneim ATM, Child JA. Defective neutrophil function and microbicidal mechanisms in the myelodysplastic disorders. J Clin Pathol 1983; **36**:1120–8.

5 Yuo A, Kitagawa S, Okabe T *et al.* Recombinant human granulocyte colony-stimulating factor repairs the abnormalities of neutrophils in patients with myelodysplastic syndromes and chronic myelogenous leukemia. Blood 1987; **70**:404–11.

6 Kobayashi Y, Okabe T, Ozawa K *et al.* Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor: a preliminary report. Am J Med 1989; **86**:178–82.

7 Negrin RS, Haeuber DH, Nagler A *et al.* Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. A phase I-II trials. Ann Intern Med 1989; **110**:976–84.

8 Antin JH, Smith BR, Holmes W, Rosenthal DS. Phase I/II study of recombinant human granulocyte-macrophage colony-stimulating factor in aplastic anemia and myelodysplastic syndrome. Blood 1988; **72**:705–13.

9 Vadhan-Raj S, Keating M, Le Maistre A *et al.* Effects of recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. N Engl J Med 1987; **317**:1545–52.

10 Ganser A, Volkers B, Greher J *et al.* Recombinant human granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndromes. A phase I/II trials. Blood 1989; **73**:31–7.

11 Kitagawa S, Yuo A, Souza LM, Saito M, Miura Y, Takaku F. Recombinant human granulocyte colony-stimulating factor enhances superoxide release in human granulocytes stimulated by the chemotactic peptide. Biochem Biophys Res Commun 1987; **144**:1143–6.

12 Yuo A, Kitagawa S, Ohsaka A *et al.* Recombinant human granulocyte colony-stimulating factor as an activator of human granulocytes: potentiation of responses triggered by receptor-mediated agonists and stimulation of C3bi receptor expression and adherence. Blood 1989; **74**:2144–9.

13 Weisbart RH, Golde DW, Clark SC, Wong GG, Gasson JC. Human granulocyte-macrophage colony-stimulating factor is a neutrophil activator. Nature 1985; **314**:361–3.

14 Lopez AF, Williamson DJ, Gamble JR *et al.* Recombinant human granulocyte-macrophage colony-stimulating factor stimulates *in vitro* mature human neutrophil and eosinophil function, surface receptor expression, and survival. J Clin Invest 1986; **78**:1220–8.

15 Yuo A, Kitagawa S, Ohsaka A, Saito M, Takaku F. Stimulation and priming of human neutrophils by granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor: qualitative and quantitative differences. Biochem Biophys Res Commun 1990; **171**:491–7.

16 Ohsaka A, Kitagawa S, Yuo A *et al.* Increased respiratory burst activity of neutrophils in patients with aplastic anemia: effects of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. Exp Hematol 1992; **20**:1090–3.

17 Ohsaka A, Kitagawa S, Sakamoto S *et al. In vivo* activation of human neutrophil functions by administration of recombinant human granulocyte colony-stimulating factor in patients with malignant lymphoma. Blood 1989; **74**:2743–8.

18 Souza LM, Boone TC, Gabrilove J *et al.* Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science 1986; **232**:61–5.

19 Nagata S, Tsuchiya M, Asano S *et al.* Molecular cloning and expression of cDNA for human granulocytic colony-stimulating factor. Nature 1986; **319**:415–8.

20 Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med 1992; **327**:28–35.

21 Wong GG, Witek JS, Temple PA *et al.* Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. Science 1985; **228**:810–5.

22 Bennett JM, Catovsky D, Daniel MT *et al.* Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982; **51**:189–99.

23 Ohsaka A, Saito M, Suzuki I, Miura Y, Takaku F, Kitagawa S. Phorbol myristate acetate potentiates superoxide release and membrane depolarization without affecting an increase in cytoplasmic free calcium in human granulocytes stimulated by the chemotactic peptide, lectins and the calcium ionophore. Biochem Biophys Acta 1988; **941**:19–30.

24 Yuo A, Kitagawa S, Motoyoshi K, Azuma E, Saito M, Takaku F. Rapid priming of human monocytes by human hematopoietic growth factors: granulocyte-macrophage colony-stimulating factor

(CSF), macrophage-CSF, and interleukin-3 selectively enhance superoxide release triggered by receptor-mediated agonists. Blood 1992; **79**:1553–7.

25 Itoh Y, Kuratsuji T, Aizawa S, Sai M, Ohyashiki K, Toyama K. Superoxide anion production and expression of cytochrome b558 by neutrophils are impaired in some patients with myelodysplastic syndrome. Ann Hematol 1991; **63**:270–5.

26 Komiya I, Tanoue K, Kakinuma K *et al.* Superoxide anion hyperproduction by neutrophils in a case of myelodysplastic syndrome: association with Sweet's syndrome and interstitial pneumonia. Cancer 1991; **67**:2337–41.

27 Watari K, Asano S, Shirafuji N *et al.* Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay. Blood 1989; **73**:117–22.

28 Yuo A, Kitagawa S, Kasahara T, Matsushima K, Saito M, Takaku F. Stimulation and priming of human neutrophils by interleukin-8: cooperation with tumor necrosis factor and colony-stimulating factors. Blood 1991; **78**:2708–14.

29 Cebon J, Nicola N, Ward M *et al.* Granulocyte-macrophage colony stimulating factor from human lymphocytes. The effect of glycosylation on receptor binding and biological activity. J Biol Chem 1990; **265**:4483–91.

30 Fukunaga R, Seto Y, Mizushima S, Nagata S. Three different mRNAs encoding human granulocyte colony-stimulating factor receptor. Proc Natl Acad Sci USA 1990; **87**:8702–6.

31 Okuda K, Sanghera JS, Pelech SL *et al.* Granulocyte-macrophage colony-stimulating factor, interleukin-3, and steel factor induce rapid tyrosine phosphorylation of p42 and p44 MAP kinase. Blood 1992; **79**:2880–7.

32 Raines MA, Golde DW, Daeipour M, Nel AE. Granulocyte-macrophage colony-stimulating factor activates microtubule-associated protein 2 kinase in neutrophils via a tyrosine kinase-dependent pathway. Blood 1992; **79**:3350–4.

33 Estey EH, Kurzrock R, Talpaz M *et al.* Effects of low doses of recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) in patients with myelodysplastic syndromes. Br J Haematol 1991; **77**:291–5.