Macrophage Colony-stimulating Factor (M-CSF) Prevents Infectious Death Induced by Chemotherapy in Mice, While Granulocyte-CSF Does Not

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To clarify the effect of granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF/CSF-1) on chemotherapy-induced infection, we estimated the effect of those CSFs on a mouse model under severe myelosuppression. First, we established an animal model in which 48.9% (22/45) of C3H/Hej mice died of sepsis related to severe myelosuppression after intraperitoneal administration of a single dose (9 mg/kg) of mitomycin C (MMC). G-CSF or M-CSF was administered to this model on various administration schedules after chemotherapy, and the effect of those CSFs on survival rates, peripheral blood granulocyte counts, expression of adhesion molecules (CD11a, CD11b, CD18) on granulocytes and granulocyte function (phagocytosis and superoxide anion production) were examined. In all G-CSF administration groups, peripheral blood granulocyte counts were increased, but improvements in expression of adhesion molecules such as CD11a and CD18, and granulocyte function were less marked and survival rates were not improved. Meanwhile, when M-CSF was administered from 1 to 7 days after chemotherapy, granulocyte and platelet counts were increased, and moreover, expression of adhesion molecules and granulocyte function were markedly improved. Furthermore, the survival rate was significantly improved to 77.8% (28/36) compared with the MMC group (*P*<0.05). Positive rate of blood culture examination at 7 days after chemotherapy in the M group was 0%, and was significantly lower than that in the G group (40%) and the MMC group (40%) (*P*<0.05). These results demonstrated that it is important not only to increase the granulocyte counts, but also to improve granulocyte functions for preventing infection under myelosuppression after chemotherapy.

Key words: M-CSF — G-CSF — Chemotherapy — Infection — Granulocyte function

Infection related to granulocytopenia during chemotherapy is a critical issue. Dose intensity in chemotherapy has recently been a focus of attention and the importance of strategies for the prevention of infection in patients under myelosuppression is increasing.[1–8] In current clinical practice, granulocyte colony-stimulating factor (G-CSF) preparations are commonly prescribed for patients with chemotherapy-induced granulocytopenia. There are many reports which demonstrated the effectiveness of G-CSF on prevention of chemotherapy-induced infection.[9–13] Macrophage colony-stimulating factor (M-CSF/CSF-1) has also been reported to shorten the duration of neutropenia and prevent the onset of infection, although this agent slowly improves the hematopoietic cell system, including granulocyte and platelet counts impaired by chemotherapy.[14–21]

A clinically important goal is not to increase the neutrophil counts in patients after chemotherapy, but to prevent the onset of infection in these patients by administering CSF preparations. From the viewpoint of infectious disease prevention, granulocyte function is one of the most important factors, and suppression of this function may lead to serious infection. The influence of G-CSF on granulocyte functions in chemotherapy-induced neutropenia has recently been reported[22–26]; however, some reports pointed out that the preventive effect of G-CSF on infection is questionable.[27–34] In addition, there are very few studies describing the influence of M-CSF on neutrophil functions in chemotherapy-induced neutropenia. It is required to clarify the influence of these CSFs on neutrophil functions and to establish a standard CSF administration schedule to prevent infection.

In the present study, we established an animal model that showed a severe myelosuppression pattern closely resembling that in humans. Using this model, we examined the influence of G-CSF and M-CSF on the survival rate, granulocyte counts, platelet counts, granulocyte function (phagocytosis and superoxide anion production) and granulocyte adhesion molecules. As a result, G-CSF increased the granulocyte counts, but did not prevent infectious death. However, M-CSF markedly improved the granulocyte function, prevented infection and decreased the infection-related mortality, although this agent increased the granulocyte counts less markedly compared with G-CSF.

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MATERIALS AND METHODS

Animals Seven-week-old male C3H/Hej mice ranging from 22 to 25 g in weight (purchased from Japan Clea, Tokyo) were bred in a conventional environment and used in the present study.

Reagents An anticancer drug, mitomycin C (MMC) was supplied by Kyowa Hakko Co., Ltd. (Tokyo). Recombinant human G-CSF was supplied by Chugai Pharmaceutical Co., Ltd. (Tokyo), and recombinant human M-CSF was supplied by the Biochemical Research Laboratory, Morinaga Milk Industry Co., Ltd. (Kanagawa).

Drug administration schedule As shown in Fig. 1, a single dose of MMC (9 mg/kg) was intraperitoneally injected into C3H/Hej mice (MMC group). For the administration of CSF preparations, G-CSF (3 μg/mouse, subcutaneous injection) or M-CSF (3 μg/mouse, intravenous injection) were administered to these mice after chemotherapy. The doses of G-CSF and M-CSF were determined according to Maeda et al.[35] and Sakurai et al.[36] In the MMC group, we established the G group in which G-CSF was administered for 7 days starting from the day following chemotherapy, the M group in which M-CSF was administered for 7 days and the MG concurrent administration group in which M-CSF and G-CSF were concurrently administered for 7 days. In addition, we established the Nadir G group in which G-CSF was administered for 7 days starting from 8 days after chemotherapy, the M-G group in which the Nadir G was combined with treatment in the M group and the G-G group in which G-CSF was administered for 14 days starting from the day following chemotherapy.
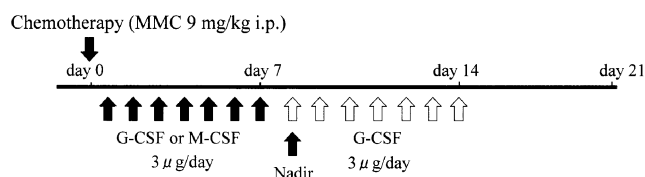

Fig. 1. Drug administration schedule. A single dose of MMC (9 mg/kg) was intraperitoneally injected (MMC group). With respect to the administration of CSF preparations, G-CSF (3 μg/mouse, subcutaneous injection) or M-CSF (3 μg/mouse, intravenous injection) was administered to these mice after chemotherapy. MMC group: administration of an anticancer drug alone, G group: G-CSF was administered for 7 days starting from the day following chemotherapy, M group: M-CSF was administered for 7 days starting from the day following chemotherapy, Nadir G group: G-CSF was administered for 7 days starting from 8 days after chemotherapy, MG concurrent administration group: M-CSF and G-CSF were concurrently administered for 7 days starting from the day following chemotherapy, M-G group: Nadir G was combined in the M group, G-G group: G-CSF was administered for 14 days starting from the day following chemotherapy, and the control group: no anticancer drug was administered.

Measurement of leukocytes, granulocytes and platelet counts Twenty microliters of heparinized blood was collected through the tail vein of the mice. Blood specimens were diluted using an AD-270 automatic diluting device (Sysmex, Inc., Kobe). Leukocyte and platelet counts were measured using an F-800 automatic blood cell counter (Sysmex, Inc.) every 3 days after chemotherapy. The F-800 automatic blood cell counter cannot differentiate mature granulocytes from immature myeloid cells or monocytes. Therefore, the granulocyte count was calculated from the percentage of the granulocyte fraction, which was calculated by flow cytometry and the above leukocyte count. In calculating granulocyte fraction by flow cytometry, the following antibodies were used: an FITC-labeled anti-Gr-1 monoclonal antibody (MAb) (Cedarlane Laboratories, Ltd., Ontario, Canada) to measure Gr-1 on the mature granulocyte surface, and PE-labeled anti-Mac-1 MAb (Oxford Biotechnology, Ltd., Oxfordshire, UK) to measure Mac-1 on the monocyte surface, as described by Lagasse and Weissman[37] and Holmes et al.[38]

Surface marker analysis To measure CD11a, CD11b and CD18 adhesion molecules on the granulocyte surface, the following antibodies were used: an FITC-labeled anti-CD11a and anti-CD11b MAb (Immunotech, Inc., Marseille, France) and a PE-labeled anti-CD18 MAb (Immunotech, Inc.). CD11a, CD11b and CD18 expression on granulocytes was analyzed with FACS Calibur (Becton Dickinson, Inc., San Jose, CA). To evaluate the expressions of CD11a, CD11b and CD18 quantitatively, the mean fluorescence intensity channel number (MFI) was obtained using FACScan consort 30 software.

Granulocyte function analysis Granulocyte phagocytosis was determined, as described by Takano et al.[39] Briefly, 2 μl of latex beads (Polysciences, Inc., Warrington, PA) measuring 2 μm in particle diameter were labeled with Fluoresbrite, added to 100 μl of whole blood and incubated at 37°C for 60 min. After the reactions were stopped, hemolysis was induced and blood specimens were centrifuged at 1400 rpm for 5 min at 4°C and washed with PBS twice. Thereafter, the MFI of fluorescent beads was analyzed with FACS Calibur.

The superoxide anion production of the granulocytes was measured, as described by Emmendorffer et al.[40] Briefly, 1.25 μl of 20 μg/ml phorbol 12-myristate 13-acetate (PMA; Sigma, Inc., St. Louis, MO) and 2 μl of 10 μg/ml dihydrorhodamine (DHR 123; Sigma, Inc.) were added to 100 μl of blood and reacted at 37°C for 15 min. After the reactions were stopped, hemolysis was induced and blood specimens were centrifuged at 1000 rpm for 5 min at 4°C, then washed with PBS twice. Thereafter, differences in MFI between PMA-treated specimens and untreated specimens were analyzed with FACS Calibur.

Blood culture sampling We observed the general state of

mice in detail every 6 h. When we judged that the mice were just before death, we took blood samples for culture. This judgment was made when the mice manifested severe sweating, piloerection, coldness, inactivity, weakness and so on. Indeed, all these mice died within 24 h after the sampling. In order to establish the normal microbial flora of the mice, we took samples from the pharynx, feces and blood of mice at the start of the experiment.

Statistical analysis With respect to the survival rate, significance was tested using the Kaplan-Meier analysis with a log-rank test. The granulocyte counts, platelet counts and MFI were expressed as the mean±standard deviation (SD) or the mean±standard error (SE). Values were compared between the groups using unpaired t test, chi-square test or repeated-measures ANOVA. $P<0.05$ was regarded as significant.

RESULTS

Preparation of a mouse myelosuppression model In preparing a myelosuppression model, we tried to adminis-

ter cyclophosphamide (225 mg/kg) and cisplatin (10 mg/kg) using C57B/6 and ICR mice. However, we could not prepare severe myelosuppression models similar to human myelosuppression in these animal experiments (data not shown).

A single dose of 7 or 8 mg/kg of MMC induced myelosuppression and a single dose of 9 or 10 mg/kg of MMC induced marked myelosuppression in male C3H/Hej mice. When a single dose of 7, 8, 9 or 10 mg/kg of MMC was administered to C3H/Hej mice, 10% (1/10), 40% (4/10), 50% (5/10) and 90% (9/10) of the animals died, respectively. Based on the findings of this preliminary study, we set the MMC dose at 9 mg/kg. In this mouse myelosuppression model, the granulocyte counts showed a nadir from 3 to 12 days after chemotherapy and recovered to the control level at 18 days after chemotherapy, while the platelet counts showed a nadir from 7 to 14 days after chemotherapy and recovered to the control level at 24 days after chemotherapy (Fig. 2, A and B). Almost half of the animals (48.9%, 22/45) died within 24 days after chemotherapy (Fig. 2C). Blood culture examination confirmed
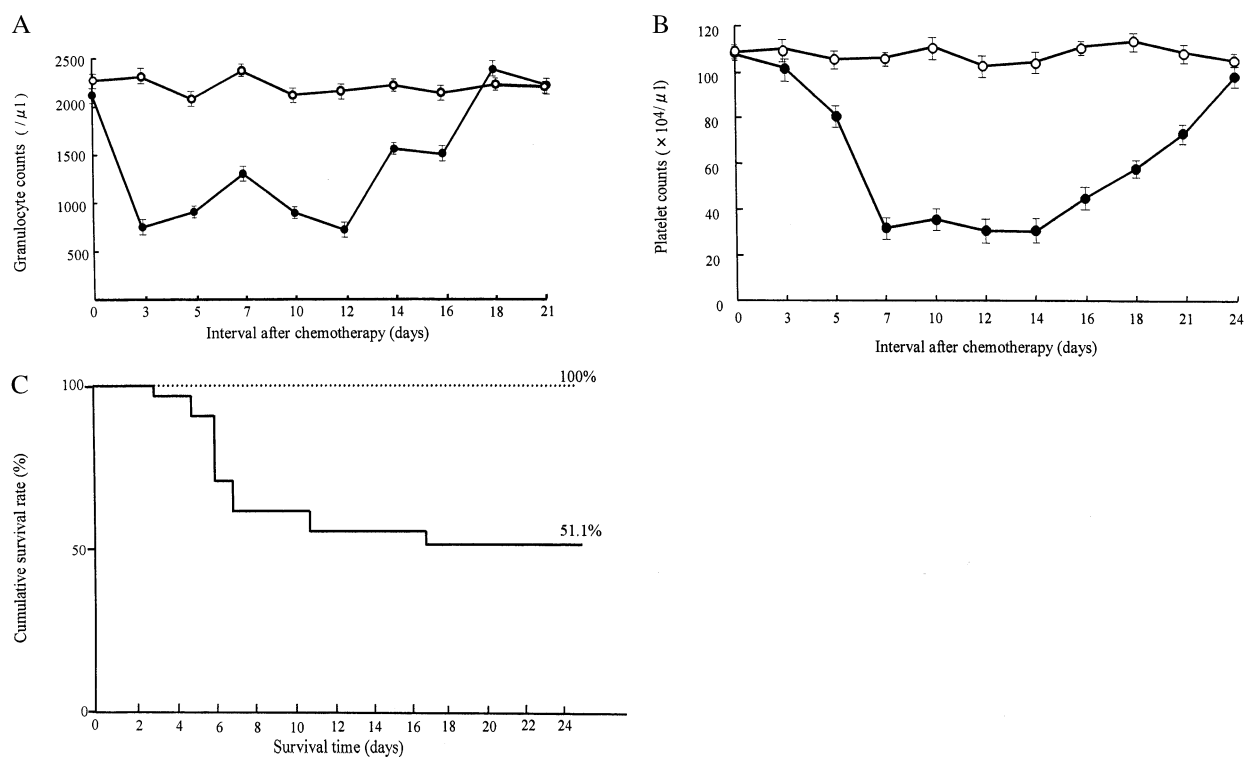

Fig. 2. Changes in the granulocyte (A) and platelet (B) counts after chemotherapy. Black circles represent the granulocyte (A) and platelet counts (B) in the myelosuppression model, while white circles represent those in the control group. A nadir was noted 3 to 12 days after chemotherapy in granulocytes and at 7 to 14 days after chemotherapy in platelets. The granulocyte and platelet counts were significantly decreased compared with those in the control group ($P<0.001$). The values are expressed as the mean±SE of 15 mice. C: Survival curve after chemotherapy. Almost half of the animals (48.9%, 22/45) died of sepsis related to bacterial infection. There were significant differences in survival rates between the control group and myelosuppression model ($P<0.01$, Kaplan-Meier analysis with a log-rank test). Each group consisted of 45 mice. ⋯⋯⋯control group, —— myelosuppression model.

that all these mice died of infection related to sepsis. The main causative bacteria included *E. coli*, *E. faecium*, *Klebsiella* sp., *P. mirabilis*, *E. cloacae*, *Citrobacter* sp., *Serratia* sp., and so on (Table I). The bacteria cultured from the pharynx and feces of mice at the start of the experiment were *E. coli*, *E. faecium*, *Klebsiella* sp., *P. mirabilis*, and *Citrobacter* sp. There was no great difference between the types of bacteria in the normal flora and those in the infectious state. Blood culture tests at the start of the experiment were all negative.

Effects of G-CSF and M-CSF on chemotherapy-induced myelosuppression Granulocyte counts in the G group, M group, M-G group, MG concurrent administration group, G-G group and Nadir G group were significantly increased compared with the MMC group (each $P<0.001$), and those in the G-G group were also significantly increased compared with the G group ($P<0.001$). Furthermore, the granulocyte counts in the G group, MG concurrent administration group and G-G group were sig-

Table I.   Bacteria Detected on Blood Culture Examination

E. coli	(36.4)
E. faecium	(27.3)
Klebsiella sp.	(18.2)
P. mirabilis	(18.2)
E. cloacae	(13.6)
Citrobacter sp.	(9.1)
Serratia sp.	(9.1)

Blood culture examination confirmed that all 22 dead mice died of infection-related sepsis. The main causative bacteria are listed above. Numbers in parentheses are the frequency (%) of appearance of bacteria, including duplications.

nificantly increased compared with the M group (each $P<0.005$). In addition, in the G group, G-G group, MG concurrent administration group, granulocyte counts began to increase from the nadir at 12 days after chemotherapy, in the M group at 16 days, and the Nadir G group and the MMC group at 18 days after chemotherapy (Fig. 3A).

Platelet counts in the M group, M-G group and MG concurrent administration group were significantly increased compared with the MMC group (each $P<0.001$). However, the levels in the G group, G-G group and Nadir G group were significantly decreased compared with the M group, respectively ($P<0.05$). In addition, the nadir of the platelet counts in the G-G group ($14.8\times10^4/\mu$l) was the lowest of all 8 groups. There was a significant difference in platelet recovery between the M group and G group, and between the M group and M-G group (each $P<0.05$) (Fig. 3B). Namely, the administration of M-CSF significantly increased the platelet counts, and the administration of G-CSF was associated with a delayed recovery of the platelet counts.

Survival rates after various treatments of CSFs The experiments were run three times. In each experiment, twelve mice from each of the 8 groups were treated with CSFs, and the survival rates were studied. Similar results were obtained in these three experiments, and the findings are presented as the sum of the three experiments.

In the M group, the survival rate at 24 days after chemotherapy was 77.8% (28/36), and was significantly improved compared with that in the MMC group (19/36, 52.8%), the Nadir G group (17/36, 47.2%), and the G-G group (20/36, 55.6%) (each $P<0.05$). Similarly, in the M-G group, the survival rate at 24 days after chemotherapy was 83.3% (30/36), and was significantly improved com-
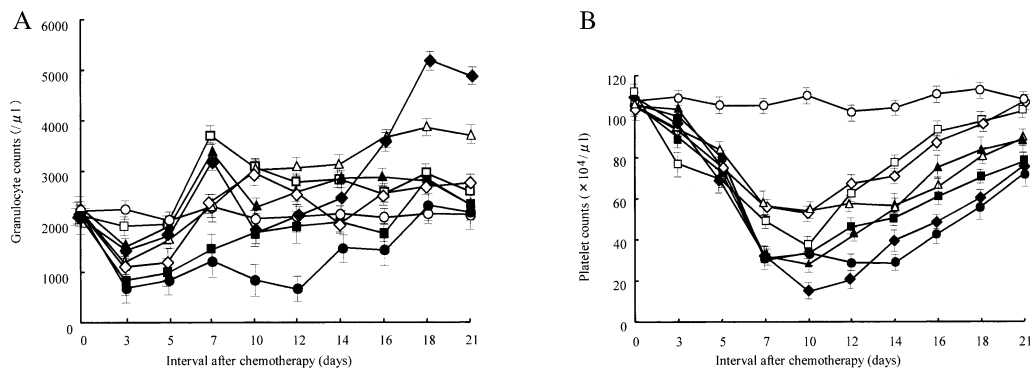

Fig. 3.   Influence of G-CSF and M-CSF on changes in the granulocyte (A) and platelet (B) counts after chemotherapy. Granulocyte counts in the G group (▲), M group (◇), M-G group (△), MG concurrent administration group (□), G-G group (◆) and Nadir G group (■) were significantly increased compared with the MMC group (●) ($P<0.001$). Granulocyte counts in the G group, MG concurrent administration group and G-G group were significantly increased compared with the M group ($P<0.005$). Platelet counts in the M group, M-G group and MG concurrent administration group were significantly increased compared with the MMC group ($P<0.001$). Meanwhile, platelet counts in the G group, G-G group and Nadir G group were significantly decreased compared with the M group ($P<0.05$). Control group: (○). The values are expressed as the mean±SE of 15 mice.
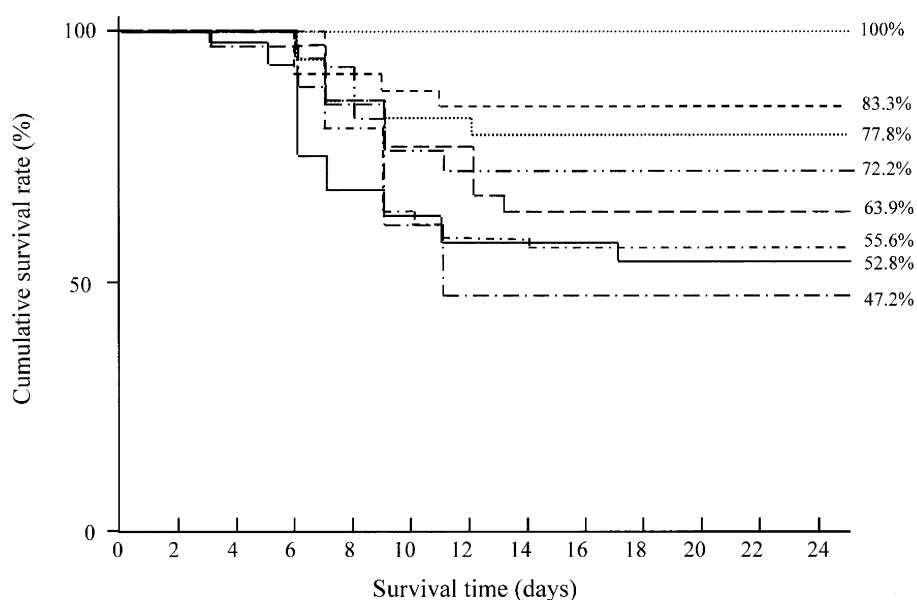
Fig. 4. Influence of G-CSF and M-CSF on survival curves after chemotherapy. Survival curves are shown as follows: control group: (⎯⎯⎯), MMC group: (⎯⎯⎯), G group: (– – – –), M group: (⎯⎯⎯), M-G group: (– – – – –), MG concurrent administration group: (–··–··–), G-G group: (–·–·–·) and Nadir G group: (–·–··–). Each group consisted of 36 mice. ∗ $P<0.05$ versus the MMC group and the Nadir G group, ∗∗ $P<0.01$ versus the MMC group and the Nadir G group.

pared with that in the MMC group, the Nadir G group, and the G-G group (each $P<0.01$). As a result, the survival rates of the groups, in which M-CSF was used, were significantly improved compared with the MMC group and the groups in which G-CSF was used. There was no improvement in the survival rate in the MG concurrent administration group, the G group, the G-G group, or the Nadir G group compared with the MMC group (Fig. 4).

Blood culture test at 7 days after chemotherapy Positive rates (%) of blood culture examination at 7 days after chemotherapy in the M group, the G group and the MMC group were 0, 40, 40%, respectively. There were significant differences between the M group and the G group and between the M group and the MMC group (each $P<0.05$) (Table II).

At the time of the blood sampling, we also took the findings of the abdominal cavity. We confirmed that there was no sign of chemical peritonitis, such as bleeding or necrosis due to MMC treatment.

Influence of G-CSF and M-CSF on the expression of adhesion molecules (CD11a, CD11b and CD18) on granulocytes Seventy-two percent of the dead mice died at 6 to 8 days after chemotherapy (Fig. 2C). Therefore, granulocyte function (adhesion molecules, phagocytosis and superoxide anion production) was examined at 7 days after chemotherapy.

The expression (MFI) of adhesion molecules such as CD11a, CD11b and CD18 on granulocytes was significantly decreased at 7 days after chemotherapy ($P<0.05$). CD11a expression levels in the M group (438.0±99.5) and the MG concurrent administration group (409.6±67.8) were significantly higher than those in the MMC group

Table II. Positive Rate (%) and Frequency (Numbers) of Appearance of Bacteria in Blood Culture Examination at 7 Days after Chemotherapy

	Positive rate (%)	Detected bacteria
M group	0	(—)
G group	40	*E. cloacae* (2)
		α-*Streptococcus* (2)
		E. coli (1)
		Corynebacterium (1)
		Staphylococcus sp. (1)
MMC group	40	*E. coli* (2)
		E. cloacae (2)
		Corynebacterium (2)
		α-*Streptococcus* (2)
		P. mirabilis (1)
		E. faecium (1)

Positive rates (%) of blood culture examination in the M group, G group and MMC group at 7 days after chemotherapy are listed above. There were significant differences in positive rate between the M group and the G group ($P<0.05$; chi-square test). The main detected bacteria are also listed above. Numbers in parentheses are the frequency of appearance of bacteria. There was no significant difference between the types of bacteria in the G group and those in the MMC group. Each group consisted of 10 mice.

(314.6±77.7) and G group (341.6±34.6) ($P<0.05$). There was no significant difference in CD11a expression between the G group and the MMC group (Fig. 5).

CD11b expression on granulocytes was significantly higher in the G group (87.6±34.6), the M group (83.5± 32.8) and the MG concurrent administration group (114.9±

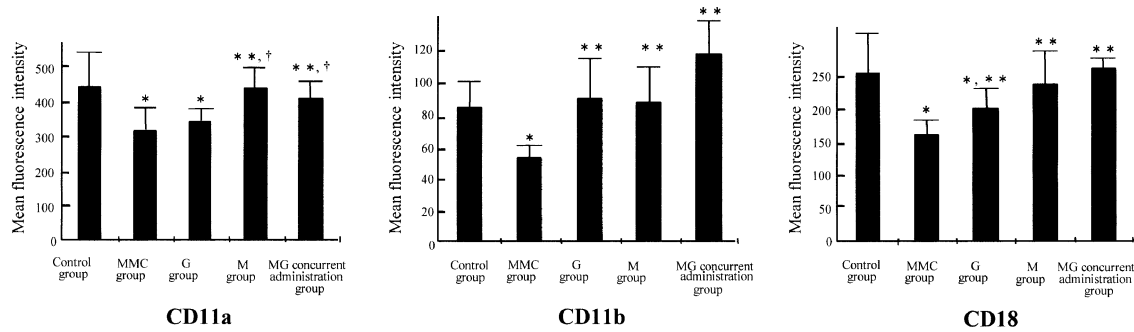**CD11a**   **CD11b**   **CD18**

Fig. 5. Influence of G-CSF and M-CSF on the expression of adhesion molecules on granulocytes after chemotherapy. The expression of CD11a, CD11b and CD18 on granulocytes was determined by flow cytometry as described in "Materials and Methods." The values are expressed as the mean±SD of 6 mice. ∗ $P<0.05$ versus the control group, ∗∗ $P<0.05$ versus the MMC group, † $P<0.05$ versus the G group.
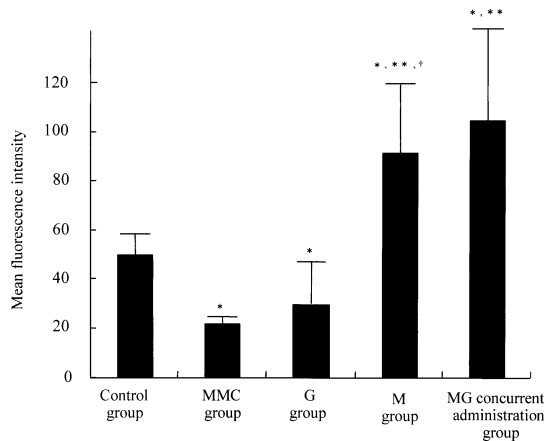

Fig. 6. Influence of G-CSF and M-CSF on granulocyte phagocytosis after chemotherapy. Granulocyte phagocytosis was determined by flow cytometry as described in "Materials and Methods." The values are expressed as the mean±SD of 6 mice. ∗ $P<0.05$ versus the control group, ∗∗ $P<0.01$ versus the MMC group, † $P<0.01$ versus the G group.
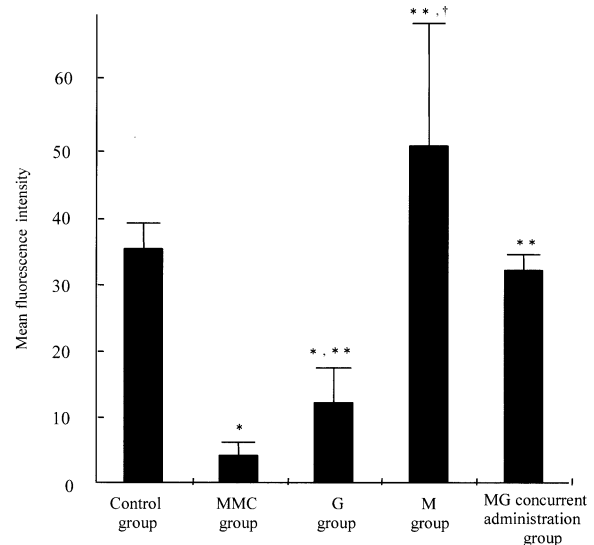

Fig. 7. Influence of G-CSF and M-CSF on the superoxide anion production of granulocytes after chemotherapy. Superoxide anion production of granulocytes was measured by flow cytometry as described in "Materials and Methods." The values are expressed as the mean±SD of 6 mice. ∗ $P<0.001$ versus the control group, ∗∗ $P<0.05$ versus the MMC group, † $P<0.05$ versus the G group.

27.4) than that in the MMC group (51.5±9.3) ($P<0.05$). There was no significant difference between the MG concurrent administration group and the M group (Fig. 5).

CD18 expression on granulocytes was significantly higher in the G group (199.8±30.0), the M group (235.8±78.2) and the MG concurrent administration group (258.9±11.8) than that in the MMC group (158.2±25.5) ($P<0.05$). In the M group and MG concurrent administration group, CD18 expression on granulocytes was similar to the control level. However, CD18 expression on granulocytes in the G group was significantly lower than that in the control group (255.0±78.5) ($P<0.05$) (Fig. 5).

In summary, G-CSF significantly increased CD11b on granulocytes to a level similar to the control level, but the levels of CD11a and CD18 were significantly lower than

the control. M-CSF significantly increased CD11a, CD11b and CD18 expression on granulocytes to levels similar to the control levels.

Influence of G-CSF and M-CSF on granulocyte phagocytosis and superoxide anion production Seven days after chemotherapy, phagocytosis and superoxide anion production of granulocytes in the MMC group were significantly decreased compared with the control group ($P<0.001$).

In the M group (90.7±30.4) and the MG concurrent administration group (103.9±45.6), MFI values of granu-

locyte phagocytosis were significantly higher than those in the MMC group (21.3±3.6), the G group (29.3±18.1) and the control group (49.7±10.2) ($P<0.01$). However, there was no significant difference between the G group and MMC group (Fig. 6).

In the G group (11.7±7.3), the MFI value of the superoxide anion production of granulocytes was significantly increased compared with that in the MMC group (3.8±1.8) ($P<0.05$), but this level was significantly lower than that of the control group (34.8±4.1) ($P<0.001$). In the M group (50.3±35.7) and MG concurrent administration group (31.6±2.5), MFI values of the superoxide anion production of granulocytes were significantly higher than those in the G group and MMC group ($P<0.05$), and these levels were similar to the control. There was no significant difference between the MG concurrent administration group and the M group (Fig. 7). Therefore, it was shown that M-CSF markedly ameliorated the chemotherapy-related impairment of granulocyte phagocytosis and superoxide anion production, essentially to the control level.

DISCUSSION

In the present study, we initially prepared a high-dose chemotherapy-induced severe myelosuppression model using mice. In this model, the pattern of myelosuppression closely resembled the pattern of severe myelosuppression in humans, and 47% of mice died of infection. The sensitivity of the mouse to G-CSF and M-CSF is very low compared with that of human. To make clear the effect of these CSFs in the mouse model, some researchers administered higher doses of CSF. According to their work, we chose suitable dose levels of G-CSF and M-CSF.[35, 36] When G-CSF was administered in this model, the granulocyte counts were significantly increased. We calculated granulocyte count by flow cytometry using a combination of antibodies (Gr-1 and Mac-1) in order to identify and purify granulocytes and monocytes, as described by Lagasse and Weissmann.[37] Moreover, Gr-1 was utilized as a marker of mature granulocytes.[38] Thus, we consider that very few immature myeloid cells or monocytes were included in the granulocyte count. When G-CSF was administered in our model, improvement in the platelet counts was significantly delayed. Similar findings were observed when G-CSF was administered during peripheral blood stem cell transplantation (PBSCT) combination chemotherapy in children,[29] and during myeloablative therapy and hematopoietic stem cell transplantation.[41] These findings are undesirable from the viewpoint of avoiding platelet transfusion and maintaining dose-intensive chemotherapy. M-CSF significantly increased the granulocyte counts, but the degree of increase was smaller than that for G-CSF in this animal model and in a clinical study in humans.[14, 18–21] In the M-G group in the present study,

improvement in the platelet counts was significantly delayed compared with that in the M group, suggesting that G-CSF delayed improvement in the platelet counts. Long-term use of G-CSF may induce hematopoietic immature progenitor cells to myeloid-committed progenitor cells, resulting in the delayed growth of megakaryocyte progenitors or platelets.[29]

In the present mouse model, the current clinical practice method, in which G-CSF is administered after chemotherapy, increased the granulocyte counts, but did not improve the survival rate. These results suggest that the granulocytes at recovery phase after G-CSF are morphologically mature but functionally immature.

The administration of M-CSF for 7 days starting from the day after chemotherapy markedly improved the survival rate. All mice died of opportunistic bacterial infection with *E. coli*, *E. faecium*, *Klebsiella* sp., *P. mirabilis*, *E. cloacae*, *Citrobacter* sp., or *Serratia* sp., etc. The positive rate of blood culture after chemotherapy in the M group was significantly lower than that in the G group. These findings indicate that M-CSF decreased the infection rate. Therefore, administration of M-CSF may have enhanced the host's immunity and decreased infection-related mortality.

The initial immune response in infection is local infiltration of granulocytes at the inflammation site and this requires the following processes: rolling of granulocytes to vascular endothelial cells and subsequent sticking. It was previously demonstrated that adhesion molecules are involved in these processes.[42, 43] Therefore, we examined adhesion molecules (CD11a, CD11b and CD18) as an index of granulocyte function. High-dose chemotherapy significantly decreased the expression of adhesion molecules on granulocytes, and M-CSF significantly improved the chemotherapy-related decreases. Namely, the expression levels of CD11a, CD11b and CD18 were significantly increased to the control levels by M-CSF, but G-CSF restored only CD11b to the control level. Our data suggested that M-CSF significantly improved the chemotherapy-related decrease in the expression of adhesion molecules on granulocytes, inducing local chemotaxis of granulocytes from blood vessels, compared with G-CSF. This may be one of the reasons for the difference in mortality after high-dose chemotherapy in mice between M-CSF and G-CSF administration. Clinically, it has been shown that M-CSF improves the chemotherapy-induced decrease of expression of adhesion molecules (CD11a and CD18) on granulocytes.[44] Next, we measured phagocytosis and superoxide anion production as granulocyte functions during chemotherapy. The phagocytosis and superoxide anion production of granulocytes, that had been markedly impaired by chemotherapy, were slightly improved by G-CSF, but remained significantly lower than in the control group. The administration of M-CSF mark-

edly improved the phagocytosis and superoxide anion production of granulocytes after high-dose chemotherapy compared with the control group. It was suggested that the administration of G-CSF after chemotherapy increased the granulocyte counts and enhanced the expression of adhesion molecules to some degree, but insufficiently improved the phagocytosis and superoxide anion production of granulocytes, so that infection could occur, resulting in a poor survival rate. However, M-CSF less markedly increased the granulocyte counts than G-CSF, but enhanced the expression of adhesion molecules and markedly improved the phagocytosis and superoxide anion production of granulocytes, suggesting that M-CSF enhances the response to infection and thereby improves the survival rate. In these experiments, we administered a higher dose of CSFs to our mouse myelosuppression model compared to the human clinical dose. However, clinically, a randomized controlled trial of chemotherapy for leukemia revealed that combination therapy with M-CSF decreased the incidence of infection.[18] We also demonstrated that M-CSF reduced the incidence of febrile neutropenia by maintaining or improving superoxide anion production by granulocytes.[21] Since the M-CSF receptor, c-*fms*, is not present on granulocytes, the actions of M-CSF on granulocytes may be indirect. For example, the following action mechanism was demonstrated *in vitro*: M-CSF enhanced interleukin (IL)-8 production by monocytes in a dose-dependent manner and IL-8 increased the expression of adhesion molecules on granulocytes in healthy adults.[45] Clinically, similar findings were reported in ovarian cancer patients receiving chemotherapy.[44] A study using samples from healthy adults also indicated that M-CSF activated neutrophil function via IL-8.[46] Further,

M-CSF was suggested to have the ability to increase the number of progenitor or precursor cells for bone marrow stromal cells *in vivo*.[47] Based on these findings, M-CSF may have increased the production of some chemokines by monocytes in mice, enhancing the granulocyte function.

The present findings suggest that not only the granulocyte count, but also granulocyte function should be improved to prevent infection after high-dose chemotherapy. The present study using mice showed that the concurrent administration of M-CSF improved not only the decreased granulocyte count, but also the decreased granulocyte function. So, this treatment may be effective for preventing infectious diseases following chemotherapy. Presently, support therapy with G-CSF, which is commonly used in clinical practice to treat myelosuppression after high-dose chemotherapy, is controversial. In further clinical trials, G-CSF therapy should be compared with M-CSF therapy with respect to infection prevention after high-dose chemotherapy.

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