

# Mobilization of Bone Marrow Cells by G-CSF Rescues Mice from Cisplatin-Induced Renal Failure, and M-CSF Enhances the Effects of G-CSF

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Cisplatin, which is a broadly used anticancer drug, is widely known to induce acute renal failure as a result of renal tubular injury. This article examines whether G-CSF and/or M-CSF rescues mice from renal failure induced by cisplatin. BALB/c mice received intraperitoneal injections with or without G-CSF and/or M-CSF for 5 d (from day -5 to day -1). The day after the last injection of G-CSF and/or M-CSF (day 0), the mice received an intraperitoneal injection of cisplatin. When pretreated with G-CSF or G-CSF + M-CSF, the mice showed longer survival and lower serum creatinine and blood urea nitrogen levels than mice that had been received injections of M-CSF or saline. Histologically, pretreatment with G-CSF or G-CSF + M-CSF attenuated the damage to renal tubules induced by cisplatin. BALB/c mice that had received a transplant of bone marrow cells of enhanced green fluorescent protein (EGFP)-transgenic mice ([EGFP→BALB/c] mice) were treated with or without G-CSF and/or M-CSF, followed by injection of cisplatin as well as above. [EGFP→BALB/c] mice that were treated with G-CSF or G-CSF + M-CSF showed a significantly higher number of EGFP<sup>+</sup> tubular epithelial cells in the kidney than mice that were treated with only M-CSF or saline. These results suggest that bone marrow cells mobilized by G-CSF accelerate the improvement in renal functions and prevent the renal tubular injury induced by cisplatin and that M-CSF enhances the effects of G-CSF.

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Cisplatin, an anticancer drug, is broadly used for the therapy of cancers such as ovarian, head and neck carcinomas, and germ cell tumors (1). It has been reported that cisplatin induces injury to renal tubular epithelial cells (RTEC), leading to renal failure (2). The cytotoxicity of cisplatin is considered to be due to several factors, including peroxidation of cell membrane (3), mitochondrial dysfunction (4), inhibition of protein synthesis (5), and DNA injury (6).

Recently, it was reported that bone marrow cells (BMC) can differentiate into not only hematopoietic tissue but also nonhematopoietic tissues (7–13). BMC have also been reported to differentiate into RTEC (7,14–16) and to repair renal tubules after ischemic injury (15,16). In the experiment of Kale *et al.* (15), Lin<sup>-</sup>Sca-1<sup>+</sup> bone marrow-derived cells were mobilized into the peripheral blood by transient renal ischemia and seemed to migrate specifically to injured regions of the renal tubule, followed by transdif-

ferentiation into RTEC. These data suggest that mobilized BMC might differentiate into RTEC, resulting in recovery from ischemic injury. However, very recently, it was also reported that at least some part of the phenomenon of transdifferentiation from BMC can be attributed to cell fusion (17–20).

In 1999, Takahashi *et al.* (8) reported that GM-CSF mobilized not only hemopoietic stem cells but also endothelial progenitor cells from the bone marrow into the peripheral blood. It is also widely known that G-CSF mobilizes hemopoietic stem cells from the bone marrow into the peripheral blood, and this effect is now clinically utilized for allogeneic and syngeneic peripheral blood stem cell transplantation (21). Similar to GM-CSF, G-CSF mobilizes BMC into an ischemic heart, repairing the infarcted heart and improving the heart's function (12).

In this article, we demonstrate that G-CSF mobilizes BMC into the peripheral blood and rescues the mice from cisplatin-induced renal failure. M-CSF itself does not rescue the mice from the renal failure induced by cisplatin but enhances the effects of G-CSF.

## Materials and Methods

### Mouse Model of Cisplatin-Induced Acute Renal Failure and Cytokine Administration

BALB/c male mice at 7 to 8 wk of age were obtained from SLC (Shizuoka, Japan). The mice received an injection of G-CSF (250 µg/kg

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per d; donated by Chugai Pharmaceutical Co. Ltd., Tokyo, Japan), M-CSF (250  $\mu\text{g}/\text{kg}$  per d; donated by Kyowa Hakko Kogyo, Tokyo, Japan), or G-CSF (250  $\mu\text{g}/\text{kg}$  per d) + M-CSF (250  $\mu\text{g}/\text{kg}$  per d) into the intraperitoneal space once a day for 5 consecutive days. The day after the last injection of cytokines, single intraperitoneal injections of cisplatin (20 mg/kg body wt) were given to these mice. This dose of cisplatin induces severe renal failure in mice (22). As a control, saline was injected instead of G-CSF and/or M-CSF once a day for 5 consecutive days before the injection of cisplatin. The mice were maintained on a standard diet, and water was freely available.

### Bone Marrow Transplantation

Intrabone marrow–bone marrow transplantation (IBM-BMT) from enhanced green fluorescent protein (EGFP)-transgenic mice into BALB/c mice was carried out as described previously (23). Briefly, BALB/c mice at 7 to 8 wk of age were irradiated with a single dose at 7.5 Gy by a  $^{137}\text{Cs}$  source. One day after the irradiation, BMC were collected from the femurs and tibias of EGFP-transgenic mice (24), which were donated by Dr. Okabe (Osaka University, Osaka, Japan). The BMC from the EGFP-transgenic mice were transplanted into the tibias of the irradiated BALB/c mice ([EGFP→BALB/c] mice). EGFP-transgenic mice are derived from C56BL/6 mice. However, this method of BMT (IBM-BMT) does not readily induce graft-versus-host disease, as described previously (23). Actually, in this experiment, no mice showed any symptoms of graft-versus-host disease. One month after the BMT, [EGFP→BALB/c] mice were used for experiments after confirmation that >90% of the peripheral blood nuclear cells were derived from EGFP-transgenic mice.

### Numbers of White Blood Cells and Neutrophils

The peripheral blood of the mice was collected using EDTA-coated tubes. The numbers of white blood cells (WBC) and neutrophils in the peripheral blood were examined using an SF-3000 autoanalyzer for the peripheral blood (Sysmex, Kobe, Japan).

### Histologic Analysis

The kidneys of the BALB/c mice were removed and fixed in 10% buffered formalin and embedded in paraffin, processed for light microscopy, and stained with hematoxylin and eosin. For detecting apoptotic cells, the TdT-mediated dUTP nick end labeling (TUNEL) method was performed using the Takara *In Situ* Apoptosis Detection Kit (Takara, Otsu, Japan). The kidneys of the [EGFP→BALB/c] mice were removed and embedded in optimal cutting temperature compound (Sakura, Tokyo, Japan) and quickly frozen in acetone cooled by dry ice. After adjustment of their horizontal planes parallel to the cutting plane, 2- $\mu\text{m}$  frozen sections were made in a cryostat.

### Antibodies

The antibodies (Ab) used in this study were as follows: rabbit polyclonal anti-pan-cytokeratin Ab (1:100; Santa Cruz Biotechnology, Santa Cruz, CA), anti-aquaporin-1 Ab (1:100; Santa Cruz Biotechnology), and R-PE–conjugated goat anti-rabbit Ab (1:50; Southern Biotechnology Associates, Birmingham, AL) for immunocytochemistry and biotin-labeled mAb (anti-CD3, anti-B220, anti-CD11b, anti-CD11c, anti-NK1.1, anti-Gr1 and anti-Ter 119; Pharmingen), PE-labeled mAb (anti-Sca-1, anti-CD34, and anti-c-kit; Pharmingen), and PE-Cy5.5–labeled avidin (Pharmingen) for flow cytometry.

### Immunocytochemistry

The specimens, which had been fixed with 4% paraformaldehyde, were stained with rabbit Ab (anti-pan-cytokeratin Ab or anti-aqua-

porin-1 Ab) and then stained with PE-labeled goat anti-rabbit Ab. The stained specimens were observed using a confocal microscope (LSM510-META, Carl Zeiss, Oberkochen, Germany; or Fluoview, Olympus, Tokyo, Japan).

### Flow Cytometry

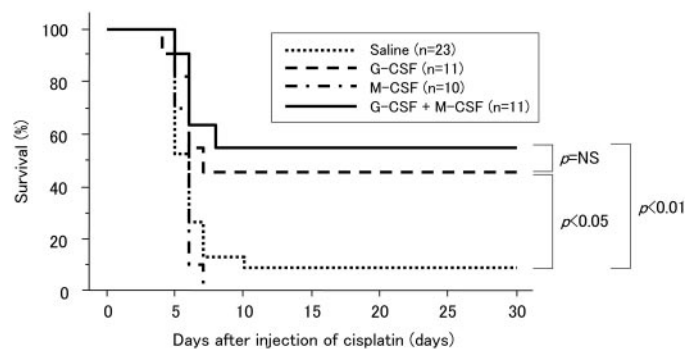
The peripheral blood was stained with (1) biotin-labeled mAb (anti-CD3, anti-B220, anti-CD11b, anti-CD11c, anti-NK1.1, anti-Gr1, and anti-Ter 119) and PE-labeled anti-Sca-1 mAb followed by staining with PE Cy5.5-labeled avidin, (2) biotin-labeled mAb (anti-CD3, anti-B220, anti-CD11b, anti-CD11c, anti-NK1.1, anti-Gr1, and anti-Ter 119) and PE-labeled anti-CD34 mAb followed by staining with PE Cy5.5-labeled avidin, and (3) biotin-labeled mAb (anti-CD3, anti-B220, anti-CD11b, anti-CD11c, anti-NK1.1, anti-Gr1, and anti-Ter 119) and PE-labeled anti-c-kit mAb followed by staining with PE Cy5.5-labeled avidin, followed by hemolysis with BD PharM Lyse (BD Bioscience Pharmingen). The samples were analyzed by a flow cytometer, BD LSR (BD Bioscience Pharmingen). Absolute numbers of lineage<sup>−</sup> (Lin<sup>−</sup>) CD34<sup>+</sup> cells, Lin<sup>−</sup> c-kit<sup>+</sup> cells, and Lin<sup>−</sup> Sca-1<sup>+</sup> cells were calculated with percentage of each fraction and number of WBC.

### Measurement of Blood Urea Nitrogen and Serum Creatinine Levels

Serum was obtained from the mice 2 to 4 d after injection of cisplatin. Blood urea nitrogen (BUN) and creatinine levels of the serum were measured using an autoanalyzer (Hitachi 7150 auto-analyzer; Hitachi, Tokyo, Japan).

### Bone Marrow Ablation

Bone marrow ablation (BMA) was performed by irradiation. BALB/c mice were irradiated at 9.5 Gy for BMA. From 1 d after irradiation, the mice received injections of G-CSF (250  $\mu\text{g}/\text{kg}$  per d) + M-CSF (250  $\mu\text{g}/\text{kg}$  per d) into the intraperitoneal space once a day for 5 consecutive days. The day after the last injection of cytokines, single intraperitoneal



**Figure 1.** Survival rates of cisplatin-administered mice that were pretreated with or without G-CSF and/or M-CSF. BALB/c mice received an injection of G-CSF (250  $\mu\text{g}/\text{kg}$  per d), M-CSF (250  $\mu\text{g}/\text{kg}$  per d), G-CSF (250  $\mu\text{g}/\text{kg}$  per d) + M-CSF (250  $\mu\text{g}/\text{kg}$  per d), or saline (as a control of cytokines) into the intraperitoneal space once a day for 5 consecutive days. The day after the last injection of cytokines, single intraperitoneal injections of cisplatin (20 mg/kg body wt) were given to these mice. The mice were maintained on a standard diet, and water was freely available. Mice were observed to determine survival rate ( $n = 23, 10, 11,$  and  $11$  for saline pretreatment group, M-CSF pretreatment group, G-CSF pretreatment group, and G-CSF + M-CSF pretreatment group).

injections of cisplatin (20 mg/kg body wt) were given to these mice. As a control of cytokine injection, saline was injected instead of G-CSF + M-CSF once a day for 5 consecutive days before the injection of cisplatin. Serum was collected from the mice 4 d after the injection of cisplatin, followed by measurement of BUN.

### Platinum Uptake by the Kidneys

BALB/c mice, which had been pretreated with or without G-CSF (250  $\mu$ g/kg) and/or M-CSF (250  $\mu$ g/kg) for 5 consecutive days, were killed 4 d after the injection of cisplatin, and the kidneys were collected. The kidneys of mice are too light to be measured individually, and we therefore asked the NAC Co. Ltd. (Tokyo, Japan) to measure the platinum concentrations of four kidneys (from two mice).

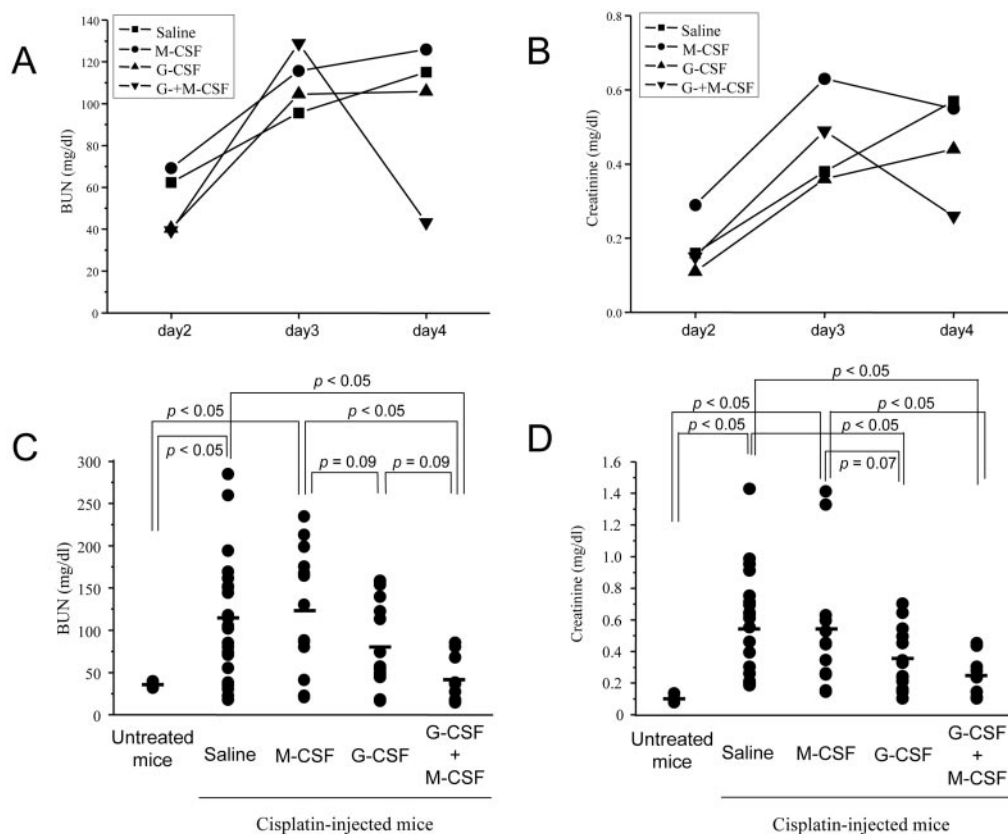
### Statistical Analyses

The results are represented as mean  $\pm$  SD. The significance of the data was determined by a two-tailed *t* test, except for the significance of survival rate. The significance of survival rate was computed with a log-rank test.  $P < 0.05$  was significant.

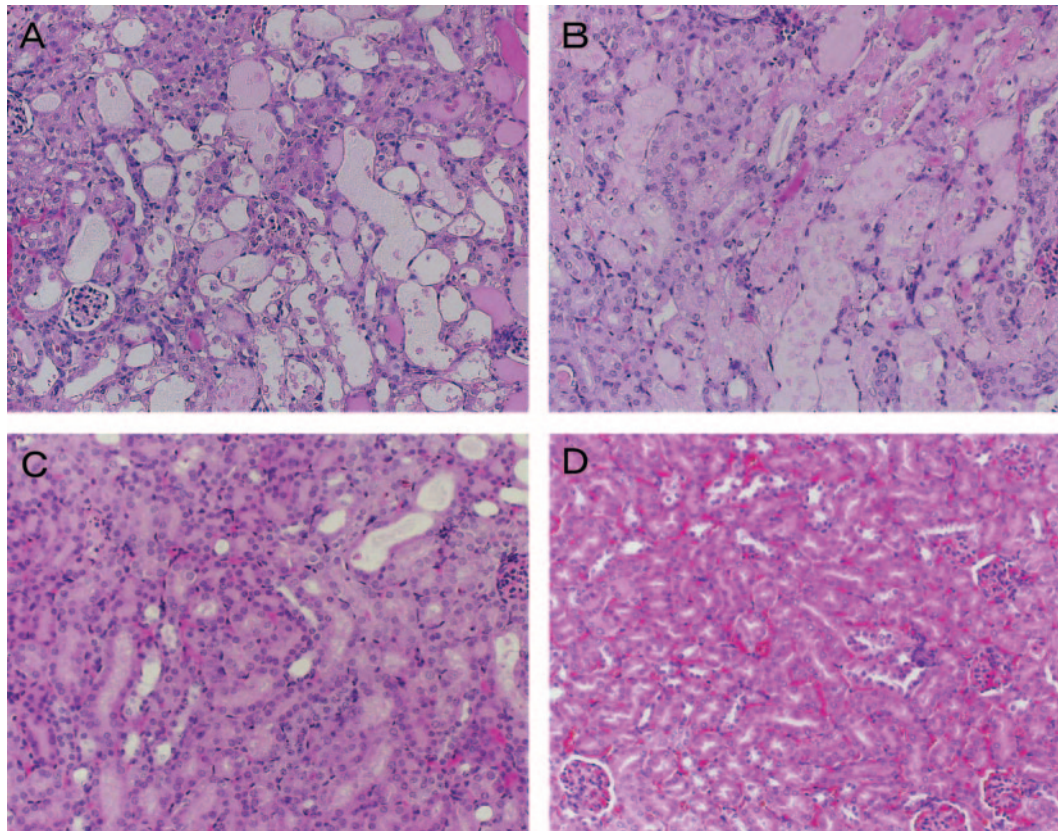
## Results

### Pretreatment with G-CSF or G-CSF + M-CSF Prolongs Survival of Cisplatin-Treated Mice

It has been reported that bone marrow stem cells help repair ischemically injured renal tubules and that G-CSF and other cytokines have the ability to mobilize bone marrow stem cells into the peripheral blood. Therefore, we examined whether pretreatment with G-CSF and/or M-CSF could prolong the survival of cisplatin-treated mice. As shown in Figure 1, treatment with G-CSF or G-CSF + M-CSF prolonged the survival of cisplatin-treated mice, whereas treatment with M-CSF or saline did not; almost all of the mice that were pretreated with only M-CSF or saline died within 10 d of the cisplatin injection. However, approximately 45% of the mice that were pretreated only with G-CSF and 55% of those that were pretreated with G-CSF + M-CSF survived up to 30 d after cisplatin injection. These results suggest that G-CSF has the ability to rescue mice from the renal tubular injury induced by cisplatin. Although M-CSF itself cannot rescue cisplatin-treated mice, it enhances the ability of G-CSF: The mice



**Figure 2.** Examination of serum creatinine and blood urea nitrogen (BUN) levels. BALB/c mice received an injection of G-CSF (250  $\mu$ g/kg per d), M-CSF (250  $\mu$ g/kg per d), G-CSF (250  $\mu$ g/kg per d) + M-CSF (250  $\mu$ g/kg per d), or saline (as a control of cytokines) into the intraperitoneal space once a day for 5 consecutive days. The day after the last injection of cytokines, single intraperitoneal injections of cisplatin (20 mg/kg body wt) were given to these mice. The mice were maintained on a standard diet, and water was freely available. From 2 to 4 d after cisplatin injection, mice were killed to examine serum creatinine and BUN levels. Mean data of BUN (A) and creatinine (B) levels of time course from day 2 to day 4 after cisplatin injection are shown. Serum BUN (C) and creatinine (D) levels of the mice 4 d after cisplatin injection are shown. “Untreated mice” means mice without cytokine and cisplatin ( $n = 5, 23, 14, 13,$  and  $10$  for untreated group, saline pretreatment group, M-CSF pretreatment group, G-CSF pretreatment group, and G-CSF + M-CSF pretreatment group).



**Figure 3.** Histologic analyses of kidneys of cisplatin-treated mice. BALB/c mice received an injection of G-CSF (250  $\mu\text{g}/\text{kg}$  per d), M-CSF (250  $\mu\text{g}/\text{kg}$  per d), G-CSF (250  $\mu\text{g}/\text{kg}$  per d) + M-CSF (250  $\mu\text{g}/\text{kg}$  per d), or saline (as a control of cytokines) into the intraperitoneal space once a day for 5 consecutive days. The day after the last injection of cytokines, single intraperitoneal injections of cisplatin (20 mg/kg body wt) were given to these mice. The mice were maintained on a standard diet, and water was freely available. Four days after cisplatin injection, the mice were killed for histologic examination of the kidneys. Morphologic changes in hematoxylin and eosin staining are shown. The figures indicate renal tubules of saline-pretreated mice (A), M-CSF-pretreated mice (B), G-CSF-pretreated mice (C), and G-CSF + M-CSF-pretreated mice (D). Representative data are shown for three independent experiments. Magnification,  $\times 50$ .

that were pretreated with G-CSF + M-CSF showed a better survival rate than the mice that were pretreated with only G-CSF, even though there was no significant difference between the groups.

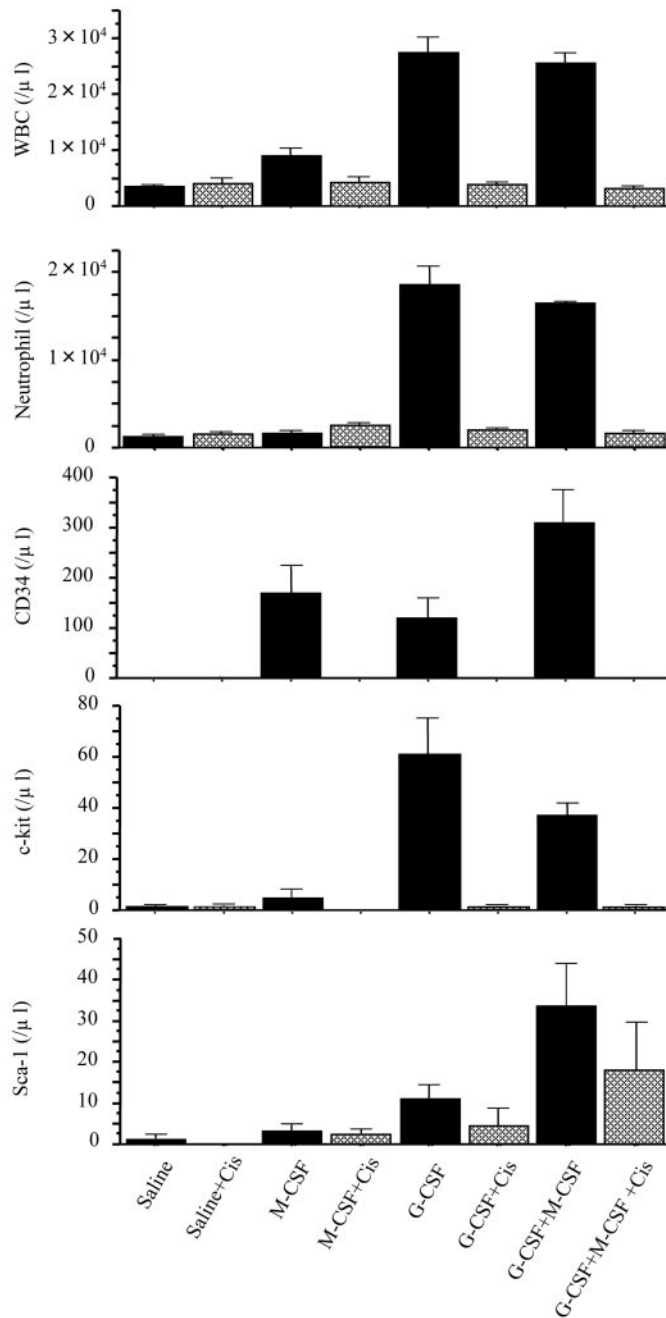
#### *Examination of Renal Functions in Cisplatin-Treated Mice*

Cisplatin causes renal tubular damage, which induces renal failure, and G-CSF or G-CSF + M-CSF rescues the cisplatin-treated mice. Therefore, it is conceivable that G-CSF or G-CSF + M-CSF protects the kidney from the injury caused by cisplatin. To clarify this question, we measured serum BUN and creatinine levels after injecting the cisplatin. As shown in Figure 2, A and B, all groups showed similar serum BUN and creatinine patterns until 3 d after cisplatin injection. Namely, 2 d after cisplatin injection, the serum BUN and creatinine levels of all groups had already increased in comparison with untreated control mice. Three days after the cisplatin injection, the serum BUN and creatinine levels of all groups had increased further. Four days after the cisplatin injection, the BUN levels of the mice that received injections of saline or M-CSF had increased further, whereas those of the mice that received injections of G-CSF remained unchanged in comparison with day 3. Surpris-

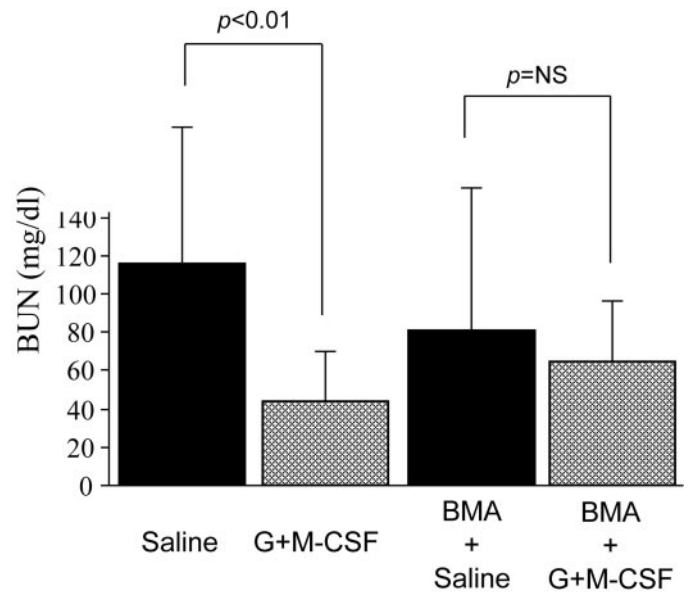
ing, the BUN and creatinine levels of the mice that received injections of G-CSF + M-CSF had decreased in comparison with day 3. We show the data from day 4 in detail in Figure 2, C and D. The mice that were pretreated with G-CSF or G-CSF + M-CSF showed significantly lower serum levels of creatinine than the mice that were treated with saline. The M-CSF-pretreated mice showed slightly lower levels of BUN than the saline-treated mice, but there was no significant difference between the M-CSF-treated mice and the saline-treated mice. The BUN levels showed a similar tendency to the serum creatinine levels, but a significant difference was seen only between the saline-treated mice and the G-CSF + M-CSF-treated mice. These results suggest that pretreatment with G-CSF and/or M-CSF improves the renal function of cisplatin-treated mice but that G-CSF is more effective than M-CSF. Moreover, the combination of G-CSF and M-CSF is more effective than the use of G-CSF or M-CSF alone.

#### *Histologic Examination of Kidneys of Cisplatin-Treated Mice*

We next examined the histologic changes in the kidney after the treatment with cisplatin. The renal tubules of the mice that



**Figure 4.** Effects of G-CSF and/or M-CSF on mobilization of stem cells and effect of cisplatin on the number of stem cells in the peripheral blood. BALB/c mice received an injection of G-CSF (250  $\mu\text{g}/\text{kg}$ / per d) and/or M-CSF (250  $\mu\text{g}/\text{kg}$  per d) for 5 consecutive days. As a control, saline was injected instead of the cytokines. On the day after the last injection of cytokines, peripheral blood was obtained from the mice and the numbers of white blood cells (WBC), neutrophils,  $\text{Lin}^- \text{CD34}^+$  cells,  $\text{Lin}^- \text{Sca-1}^+$  cells, and  $\text{Lin}^- \text{c-kit}^+$  cells were examined as described in the Materials and Methods section (black bars). One day after the last injection of cytokines, cisplatin (20  $\text{mg}/\text{kg}$ ) was injected peritoneally into the mice, as described in the Materials and Methods section. Four days after cisplatin injection, the peripheral blood of the mice was obtained and numbers of WBC, neutrophils,  $\text{Lin}^- \text{CD34}^+$  cells,  $\text{Lin}^- \text{c-kit}^+$  cells and  $\text{Lin}^- \text{Sca-1}^+$  cells were examined ("Cis" in the figure, hatched bars;  $n = 4$ ).



**Figure 5.** Effect of bone marrow ablation (BMA) on renal functions. Irradiated BALB/c mice (9.5 Gy) received an injection of G-CSF + M-CSF into the intraperitoneal space once a day for 5 consecutive days. The day after the last injection of cytokines, single intraperitoneal injections of cisplatin were given to these mice, as described in the Materials and Methods section. As a control of cytokine injection, saline was injected instead of G-CSF + M-CSF once a day for 5 consecutive days before the injection of cisplatin. Serum was collected from the mice 4 d after the injection of cisplatin, followed by measurement of BUN levels ( $n = 4$ ).

were treated with saline showed severe damage (Figure 3), namely, significant numbers of RTEC had died or had detached and disappeared, whereas the renal tubules of the mice that were treated with G-CSF or G-CSF + M-CSF showed significant evidence of recovery from the renal tubular damage. The M-CSF-pretreated mice showed only slight amelioration of the renal tubular damage. To evaluate the damage to RTEC, we examined the percentages of apoptotic RTEC using the TUNEL method. The percentages of TUNEL-positive RTEC were  $12.3 \pm 2.4$ ,  $10.9 \pm 3.3$ ,  $4.5 \pm 2.2$ , and  $1.4 \pm 1.7$  in saline-, M-CSF-, G-CSF-, and G-CSF + M-CSF-treated mice, respectively. These results paralleled the changes in the serum creatinine or BUN levels. In our experiment, very few inflammatory cells were observed in the kidney even after the cisplatin injection.

#### *Mobilization of Stem Cells and/or Immature Precursor Cells by G-CSF or G-CSF + M-CSF*

Because it has been reported that G-CSF mobilizes hematopoietic precursor cells from bone marrow into the peripheral blood and that the induction of ischemia of the kidney also mobilizes precursor cells from the bone marrow (15), we attempted to ascertain whether G-CSF and/or M-CSF could mobilize precursor cells from the bone marrow into the peripheral blood in our system. As shown in Figure 4, the numbers of WBC increased after the injection of G-CSF or G-CSF + M-CSF for 5 consecutive days, but 4 d after the injection of cisplatin, the

number of WBC had decreased in all groups and there was no difference between any of the respective groups. It has been reported that CD34, c-kit, and Sca-1 are markers of stem cells and precursor cells. Therefore, we examined whether the number of Lin<sup>-</sup>CD34<sup>+</sup> cells, Lin<sup>-</sup>c-kit<sup>+</sup> cells, and Lin<sup>-</sup>Sca-1<sup>+</sup> cells increased as a result of the administration of G-CSF and/or M-CSF. In the peripheral blood of G-CSF- and/or M-CSF-treated mice, Lin<sup>-</sup>CD34<sup>+</sup> cells, Lin<sup>-</sup>c-kit<sup>+</sup> cells, and Lin<sup>-</sup>Sca-1<sup>+</sup> cells increased, whereas they decreased after cisplatin injection.

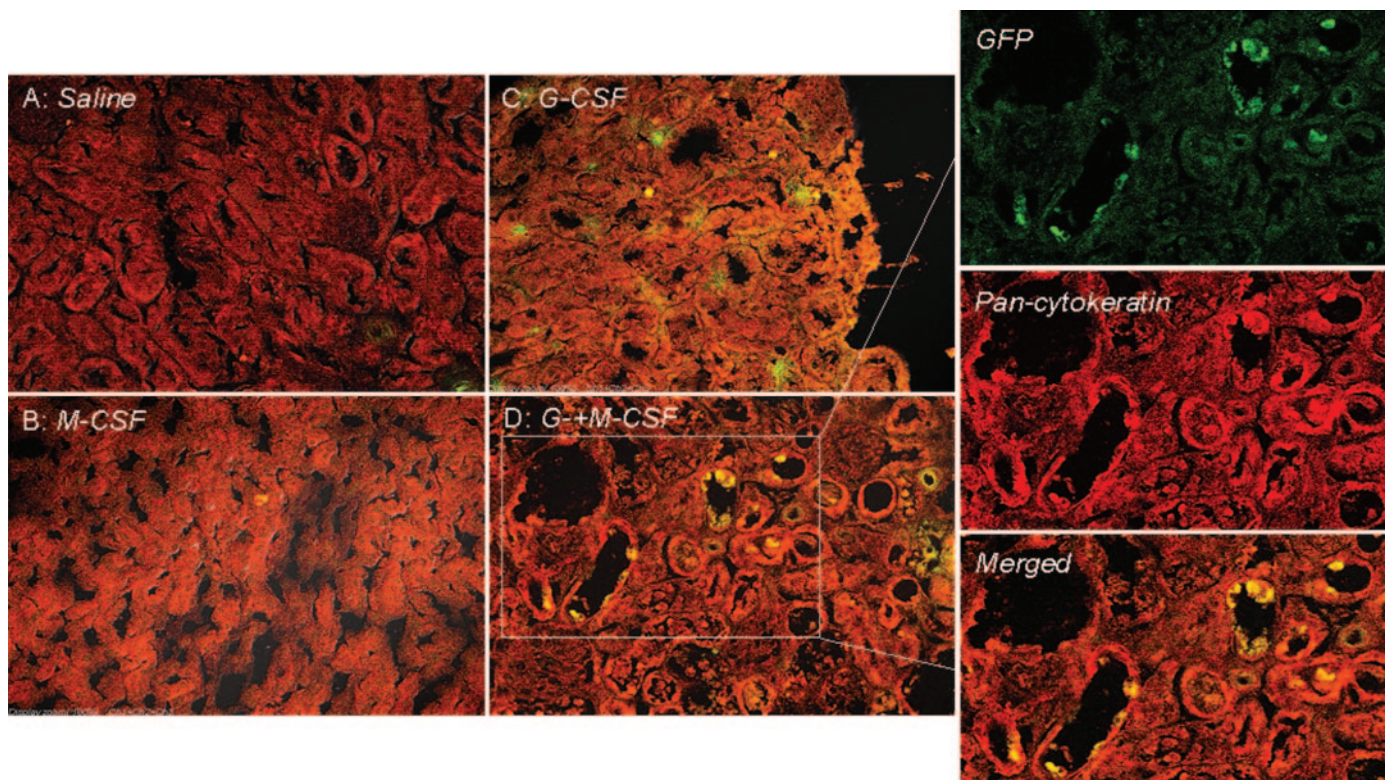
#### *BMA Prevents Improvement in Renal Functions by G-CSF and M-CSF*

Next, we examined whether BMC really contribute to the improvement in renal functions induced by G-CSF + M-CSF. As shown in Figure 5, when the bone marrow was injured by irradiation, the renal functions were not improved by the administration of G-CSF + M-CSF. This result suggests that BMC mobilized by G-CSF and G-CSF + M-CSF can be attributed to the prevention of the renal failure induced by cisplatin.

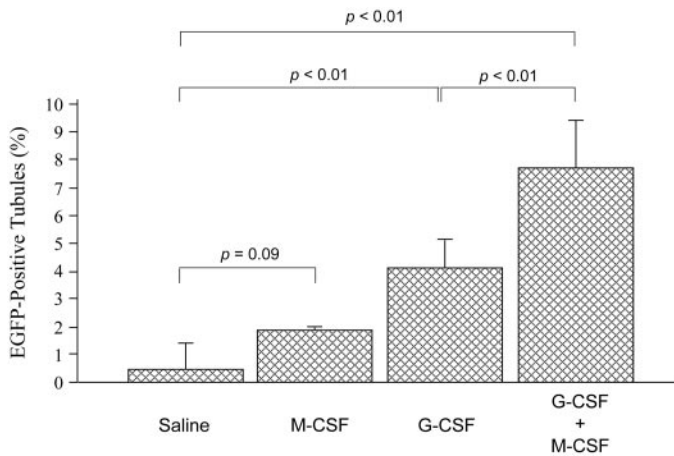
#### *Mobilized BMC by G-CSF or G-CSF + M-CSF Migrate to RTEC, Inhibiting Deterioration of Renal Functions*

It has been reported that bone marrow-derived cells develop into RTEC (7,14–16) and rescue the mice from renal failure in

the ischemia-induced model (15). Therefore, we examined whether G-CSF and/or M-CSF can mobilize the precursors of RTEC from the bone marrow into the peripheral blood. BMC from EGFP-transgenic mice were transplanted into preirradiated BALB/c mice, as described in the Materials and Methods section. One month after the BMT, we initiated the administration of G-CSF and/or M-CSF to the mice that had received bone marrow transplantation, followed by the injection of cisplatin. Four days after cisplatin injection, the mice were killed for histologic examination; frozen sections of the kidney were stained with anti-pan-cytokeratin Ab or anti-aquaporin-1 Ab followed by R-PE-conjugated goat anti-rabbit Ab, because pan-cytokeratin is a marker for epithelial cells, whereas aquaporin-1 is a marker for renal proximal tubule, which cisplatin mainly injures (25). As shown in Figures 6 and 7, there was a much greater number of EGFP<sup>+</sup> and pan-cytokeratin<sup>+</sup> cells in the renal tubules in the mice that were pretreated with G-CSF or G-CSF + M-CSF than in the mice that were pretreated with M-CSF or saline. Moreover, the cells that expressed both EGFP and pan-cytokeratin in G-CSF + M-CSF-treated mice were more than those in only G-CSF-treated mice. The pattern of the distribution of aquaporin-1<sup>+</sup>EGFP<sup>+</sup> cells was similar to that of pan-cytokeratin<sup>+</sup>EGFP<sup>+</sup> cells (data not shown). These results



**Figure 6.** Bone marrow-derived cells in renal tubules. Cisplatin was injected peritoneally into [enhanced green fluorescent protein (EGFP)→BALB/c] mice that had been pretreated with or without G-CSF and/or M-CSF. Four days after cisplatin injection, the mice were killed to obtain frozen specimens of the kidneys. Frozen sections of the kidneys were stained with anti-pan-cytokeratin (rabbit antibody [Ab]) followed by staining with PE-labeled goat anti-rabbit Ab. Therefore, in A through D, orange indicates pancytokeratin-positive cells, green indicates EGFP-positive cells, and yellow shows both pancytokeratin-positive and EGFP-positive cells. The figures indicate renal tubules of saline-pretreated mice (A), M-CSF-pretreated mice (B), G-CSF-pretreated mice (C), and G-CSF + M-CSF-pretreated mice (D). Magnification,  $\times 100$ . Representative data are shown for five independent experiments. Enlargements showing independent colors and merged colors are shown on the right side of the figure.



**Figure 7.** EGFP+ renal tubular epithelial cells (RTEC) increase in kidney of cytokine-treated mice with cisplatin. Cisplatin was injected peritoneally into [EGFP→BALB/c] mice that had been pretreated with or without G-CSF and/or M-CSF as described in the Materials and Methods section. Four days after cisplatin injection, the mice were killed to obtain frozen specimens of the kidneys. Frozen sections of the kidneys were stained with anti-pan-cytokeratin (rabbit Ab) followed by staining with PE-labeled goat anti-rabbit Ab. Percentages of renal tubules that contained EGFP+ RTEC per total renal tubules are shown.

suggest that G-CSF can mobilize BMC, which rescue RTEC from damage by cisplatin, into the peripheral blood followed by migration to the damaged kidneys and that M-CSF augments the effects of G-CSF.

## Discussion

Recently, bone marrow has become extremely popular as a source of most body tissues; BMC have been reported to be able to differentiate into multilineage mature cells, *i.e.*, epithelial cells of the skin, bronchus, lung and intestine, nerve cells, muscle cells (including cardiomyocytes), and hepatocytes. Very recently, it was reported that the phenomenon of transdifferentiation from BMC into other tissues can be attributed to cell fusion (but not to real transdifferentiation). In the present study, we have shown that G-CSF mobilizes BMC, which migrate to the injured RTEC, and that the epithelial cells functionally work, resulting in low creatinine and low BUN levels and an improvement in survival rates in cisplatin-treated mice. In our experiment, M-CSF thus improved renal function slightly but did not prolong the survival of the cisplatin-treated mice, although it enhanced the effect of the G-CSF.

Krause *et al.* (7,14–16) showed using BMT experiments that BMC can differentiate into RTEC in mice. It has also been demonstrated that BMC contribute to the recovery of renal tubules injured by ischemia (15,16). However, our experiment shows that G-CSF, which is already clinically used for the mobilization of hemopoietic stem cells, mobilizes BMC and rescues the cisplatin-treated mice from renal tubular failure. Until recently, it has generally been accepted that undifferentiated “stem cells” reside in the monolayer of epithelial cells in the tubular wall of the kidney and that, under selected circum-

stances, these cells may commit to a differentiation program that leads to a specialized epithelial phenotype (26). If the differentiation of the “stem cells” into RTEC is the only way to restore the injured RTEC, then cisplatin-treated mice should survive or die independent of mobilization by G-CSF.

It was reported recently that cell fusion is a major mechanism underlying organ repair (17–20), although many reports have indicated that it is due to transdifferentiation (27–29). Thus, it is controversial whether it is due to fusion or the transdifferentiation of BMC into other tissues. In this study, we attempted to elucidate this but failed. We are now in the process of elucidating the exact mechanism underlying the restoration of renal functions by pretreatment with G-CSF and/or M-CSF.

When mice received an injection of cisplatin, BMC were already mobilized into the peripheral blood. However, until 3 d after the cisplatin injection, renal function deteriorated even in the G-CSF + M-CSF-treated mice. Moreover, there was no difference in platinum uptake by the kidneys between any of the respective groups (data not shown). These results suggest that RTEC in all groups absorbed similar amounts of platinum and were injured similarly at first. However, later, bone marrow-derived cells migrated to the injured RTEC and improved the renal functions. It has been reported that G-CSF increases neutrophils and augments inflammation (30). Azoulay *et al.* (31) described how G-CSF augmented alveolar neutrophil recruitment and enhanced bleomycin-induced acute lung injury. Very recently, Tögel *et al.* (32) showed that the administration of G-CSF impairs renal function in a murine ischemic acute renal failure model. In the study, G-CSF augments the number of not only circulating progenitor cells but also neutrophils, followed by the infiltration of neutrophils into the injured kidneys, which results in the deterioration of the renal function. However, in our experiment, very few inflammatory cells existed even in the kidneys of mice that were treated with G-CSF, and the renal functions of the G-CSF-treated mice were better than those of the saline-treated control mice. Because it has been shown that neutrophils have toxic effects on various tissues (31,32), it is conceivable that the different results between Tögel *et al.* and us are attributable to the difference in the numbers between progenitor cells and neutrophils in the kidneys.

Acute renal failure based on acute renal tubular dysfunction is a common disease, and a number of strategies are used for treating acute renal tubular dysfunction (33). Hemodialysis and peritoneal dialysis are the most effective tools for treating acute renal failure because they can compensate for the loss of renal functions. However, dialysis itself cannot repair the RTEC. Some reagents, such as fosfomycin (34,35), anti-TNF (36), and antioxidants (37), have been reported to have the ability to protect RTEC from noxious substances. From our results, we suggest that the mobilization of BMC by G-CSF, *etc.*, could become a new strategy for preventing not only acute renal failure as a result of the necrosis of RTEC but also the side effects of drugs on various organs.

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