Potent Cholesterol-Lowering Effect by Human Granulocyte-Macrophage Colony-Stimulating Factor in Rabbits

Possible Implications of Enhancement of Macrophage Functions and an Increase in mRNA for VLDL Receptor

Toshiyuki Ishibashi, Keiko Yokoyama, Joji Shindo, Yoichi Hamazaki, Yuichi Endo, Tetsuo Sato, Sadao Takahashi, Yutaka Kawarabayasi, Masashi Shiomi, Tokuo Yamamoto, Yukio Maruyama

Abstract The mechanism by which granulocyte-macrophage colony-stimulating factor (GM-CSF) lowers plasma cholesterol levels is not well understood. We tested recombinant human GM-CSF (rhGM-CSF) on plasma cholesterol and triglycerides in rabbits and attempted to determine the mechanisms of the cholesterol-lowering effect. rhGM-CSF (20 μg · kg⁻¹ · d⁻¹) was administered to normal and cholesterol-fed rabbits for 2 weeks and to Watanabe heritable hyperlipidemic (WHHL) rabbits for 1 week. The administration of rhGM-CSF markedly lowered cholesterol and triglycerides, an effect that persisted in normal and cholesterol-fed rabbits even after termination of treatment. The cholesterol-lowering effect of rhGM-CSF was also observed in WHHL rabbits. rhGM-CSF was capable of stimulating granulocyte-macrophage colony formation in vitro in rabbits with an effect comparable to that in humans. Northern blot analysis with rabbit very-low-density-lipoprotein (VLDL) receptor cDNA revealed that rhGM-CSF increased the levels of VLDL receptor mRNA in muscle of rabbits after only 1.5 hours of treatment compared with control (2.6-fold), with the 1.5-fold increase following a 5-day administration. No changes in the levels of LDL receptor mRNA in liver, spleen, and bone marrow were observed in the treated rabbits. These findings suggest that the cholesterol-lowering effect of rhGM-CSF may be mediated by enhancement of macrophage functions in lipid metabolism and the increase in mRNA for VLDL receptor in rabbits. (Atheroscler. Thromb. 1994;14:1534-1541.)

Key Words • granulocyte-macrophage colony-stimulating factor • cholesterol • triglycerides • macrophages • VLDL receptor

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the proliferation and maturation of myeloid and monocytic lineages in vitro and in vivo. This substance has been used clinically to treat patients with hematologic and oncologic disorders. Besides the effects of GM-CSF on hematopoietic cells, various other actions unrelated to hematopoeisis have been reported, including decreases in plasma cholesterol levels in patients who were receiving human GM-CSF for hematologic disorders. These observations suggest that this molecule might play an important role in lipid metabolism.

Another molecule, macrophage colony-stimulating factor (M-CSF), reduces the levels of cholesterol in humans and rabbits in vivo. The mechanisms by which M-CSF lowers plasma cholesterol levels have been extensively studied. M-CSF stimulates the secretion of lipoprotein lipase and apolipoprotein (apo) E from macrophages and also promotes the uptake and degradation of acetylated LDL, cholesterol esterification, and cholesterol efflux in macrophages. M-CSF also increases net hydrolysis of cholesteryl ester in monocyte-derived macrophages.

Both GM-CSF and M-CSF enhance the proliferation and maturation of monocytic/macrophage lineage and have a wide variety of biological effects on various types of cells. To test the hypothesis that the mode(s) of actions of the two molecules on plasma lipid content might be mediated by pathways other than activating macrophages, we administered recombinant human GM-CSF (rhGM-CSF) to normal, cholesterol-fed, and Watanabe heritable hyperlipidemic (WHHL) rabbits to analyze the effect of GM-CSF on the metabolism of lipoproteins. In this article we describe a dramatic decrease in plasma cholesterol and fat levels in rabbits treated with rhGM-CSF. This reduction was accompanied by an increase in the levels of mRNA for very-low-density lipoprotein (VLDL) receptor in muscles. The levels of the LDL receptor mRNA in livers of the treated rabbits were unchanged. In addition to enhancing macrophage functions, the cholesterol-lowering effect of GM-CSF may be partially mediated by the VLDL receptor pathway.
Cultured in IMDM supplemented with 1% methylcellulose, Animals

4x10⁶ U/mg protein in the standard mouse colony assay by the Morinaga Milk Industry Co. The specific activity was measured directly into 40 U/mL preservative-free heparin and stored at -80°C in the Institute for Experimental Animals, Kobe University School of Medicine. Each rabbit was individually caged and maintained at the Experimental Animal Center of Fukushima Medical College.

Cytokine

rhGM-CSF obtained from Escherichia coli was a gift of Hoechst Japan Co Ltd. Its specific activity was 5x10⁶ U/mg protein as determined by the bioassay of human granulocyte-macrophage colony formation. Purified human urinary macrophage colony-stimulating factor (HuM-CSF) was provided by the Morinaga Milk Industry Co. The specific activity was 4x10⁹ U/mg protein in the standard mouse colony assay system.

 Colony Assay for Hematopoietic Progenitor Cells

Human bone marrow was obtained with informed consent from healthy volunteer donors. The bone marrow was aspirated directly into 40 U/mL preservative-free heparin and centrifuged over 1.077 g/mL Ficoll-Hypaque (Pharmacia Fine Chemicals) at 40g for 20 minutes at room temperature. The interface cells were washed twice in Iscove's modification of Dulbecco's medium supplemented with 20% fetal calf serum, 100 U/mL penicillin-streptomycin, and 200 U/mL rhGM-CSF. Duplicate plates were counted for each sample. Data are expressed as mean of duplicates; mean±SD reflects five experiments.

Methods

Animals

Male New Zealand White rabbits weighing approximately 2.5 kg were purchased from Japan SLC, Inc. Female 4-month-old WHHL rabbits weighing approximately 2.5 kg were raised in the Institute for Experimental Animals, Kobe University School of Medicine. Each rabbit was individually caged and maintained at the Experimental Animal Center of Fukushima Medical College.

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GM-CSF Administration

rhGM-CSF (20 µg·kg⁻¹·d⁻¹ SC) was administered in two doses for 14 days into normal (n=6) and cholesterol-fed (n=4) rabbits. Control animals on the normal diet (n=9) received injections of an equal amount of human serum albumin (HSA). Cholesterol feeding (1% cholesterol in the normal diet; 120 g/d) was initiated simultaneously with the 14-day injection of rhGM-CSF (n=4) or HSA (n=3) and continued for 28 days. WHHL rabbits were also administered either 20 µg·kg⁻¹·d⁻¹ rhGM-CSF (n=5) or HSA (n=5) for 7 days. Blood was drawn from a central ear artery at periodic intervals for plasma lipid content assay. Day 1 was designated as the day after the initial injection.

Determination of Plasma Lipid Concentrations

Total plasma cholesterol (TC) and plasma triglycerides (TGs) were measured enzymatically. TC, LDL cholesterol (LDL-C), VLDL cholesterol (VLDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined by ultracentrifugation.

Hematologic and Serologic Measurements

Complete blood cell counts were obtained by an automated hematologic analyzer, and differential leukocyte counts were performed on slides stained with May-Grünwald-Giemsa. Serum values of total protein, albumin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and cholinesterase were estimated by an automatic analyzer.

Histological Examination of the Livers

For light microscopy, hepatic tissue treated with rhGM-CSF or HSA was fixed overnight with 10% formaldehyde, embedded in paraffin, and examined after staining with hematoxylin and eosin.

Preparation of Specimens for RNA Extraction

Normal rabbits were administered 20 µg/kg rhGM-CSF, 200 µg HSA, or equal doses of HSA as a single injection.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>rhGM-CSF (µg/kg)</th>
<th>HSA (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHHL</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>200</td>
</tr>
</tbody>
</table>

TABLE 1. Effect of rhGM-CSF on Rabbit CFU-GM-Derived Colony Formation

<table>
<thead>
<tr>
<th>No.</th>
<th>Human</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>166</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>123</td>
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<td>3</td>
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<td>4</td>
<td>98</td>
<td>126</td>
</tr>
<tr>
<td>5</td>
<td>119</td>
<td>122</td>
</tr>
</tbody>
</table>

rhGM-CSF indicates recombinant human granulocyte-macrophage colony-stimulating factor; CFU-GM, granulocyte-macrophage colony-forming unit. Bone marrow was obtained from five healthy volunteers and five normal rabbits. A total of 1x10⁶ cells were plated in 35-mm Petri dishes containing 1 mL Iscove's modification of Dulbecco's medium supplemented with 20% fetal calf serum, 100 U/mL penicillin-streptomycin, and 200 U/mL rhGM-CSF. Duplicate plates were counted for each sample. Data are expressed as mean of duplicates; mean±SD reflects five experiments.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>CFU-GM-Derived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>128.6±24.9</td>
</tr>
<tr>
<td>Rabbit</td>
<td>121.1±7.2</td>
</tr>
</tbody>
</table>

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Preparation of Specimens for RNA Extraction

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TABLE 2. Effects of rhGM-CSF on Lipid Levels in Normal Rabbits

<table>
<thead>
<tr>
<th>Pretreatment (day 0)</th>
<th>Posttreatment (day 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>Total cholesterol, mg/dL</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>LDL cholesterol, mg/dL</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>HDL cholesterol, mg/dL</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>Triglycerides, mg/dL</td>
</tr>
<tr>
<td>Pretreatment (day 0)</td>
<td>90.1±47.0</td>
</tr>
<tr>
<td>Posttreatment (day 17)</td>
<td>40.2±10.0</td>
</tr>
</tbody>
</table>

rhGM-CSF indicates recombinant human granulocyte-macrophage colony-stimulating factor; CFU-GM, granulocyte-macrophage colony-forming unit. Bone marrow was obtained from five healthy volunteers and five normal rabbits. A total of 1x10⁶ cells were plated in 35-mm Petri dishes containing 1 mL Iscove's modification of Dulbecco's medium supplemented with 20% fetal calf serum, 100 U/mL penicillin-streptomycin, and 200 U/mL rhGM-CSF. Duplicate plates were counted for each sample. Data are expressed as mean of duplicates; mean±SD reflects five experiments.

*P<.01, †P<.05 vs pretreatment.
HuM-CSF was administered intravenously to rabbits through the marginal ear vein. The rabbits were killed under CO\textsubscript{2} anesthesia after 1.5 hours of treatment. An equal dose of each compound was administered daily to normal rabbits for 5 consecutive days. These animals were also killed in the same manner 1.5 hours after the final injection. In addition, human bone marrow was aspirated from a patient with acute myelomonocytic leukemia, and human muscle was obtained from minor thoracic muscle at the time of radical mastectomy after informed consent was obtained.

**Northern Blot Analysis**

Total RNAs from liver, spleen, bone marrow, and muscle were extracted by the acid-guanidinium-thiocyanate method.\textsuperscript{22} Poly(A)\textsuperscript{+} RNA was subsequently prepared by using an oligo-d(T) cellulose column. Poly(A)\textsuperscript{+} RNA samples were electrophoresed on a formaldehyde-agarose gel (1%) and transferred to a nylon membrane (Hybond N+, Amersham) in 20\times SSPE.\textsuperscript{23} The membrane was prehybridized at 42°C for 3 hours in a buffer containing 50% formamide, 5\times SSPE, 5\times Denhardt's solution, 1% sodium dodecyl sulfate (SDS), and 200 \mu g/mL denatured salmon sperm DNA followed by hybridization with a \textsuperscript{32}P-labeled probe at 42°C for 12 hours. The membrane was washed twice with 0.1\times SSPE and 0.1% SDS for 15 minutes at 65°C and then autoradiographed with x-ray film and an intensifying screen at -70°C. The membrane was treated with boiling water containing 0.5% SDS and rehybridized. The probes used in this study were a 1.9-kb Sal I–Sma I fragment of rabbit LDL receptor cDNA,\textsuperscript{24} a 1.0-kb EcoRV–Kpn I fragment of rabbit VLDL receptor cDNA,\textsuperscript{25} a 1.5-kb Xho I fragment of the \(\alpha\)-chain of human GM-CSF receptor,\textsuperscript{26} a 2.7-kb EcoRI–Xho I fragment of the \(\beta\)-chain of human GM-CSF receptor,\textsuperscript{26} and a 1.9-kb BamHI fragment of human \(\beta\)-actin cDNA.\textsuperscript{27} Each probe was labeled with (\(\alpha\)-\textsuperscript{32}P)dCTP (3000 Ci/mmol; ICN) by random hexanucleotide priming.\textsuperscript{28} The signals of Northern blots were quantified by densitometric scanning after autoradiography. The levels of lipoprotein receptor mRNA in each experiment were normalized to \(\beta\)-actin mRNA levels. The ratio of tested sample to control \(\beta\)-actin mRNA levels. The ratio of tested sample to control was calculated.

**Statistical Analysis**

Statistical analysis was done by the paired or unpaired Student’s \(t\) test as appropriate. A level of \(P<.05\) was accepted as statistically significant.

**Results**

**General Observations**

Although normal and cholesterol-fed rabbits injected with 20 \(\mu g\cdot kg^{-1}\cdot d^{-1}\) rhGM-CSF showed approximately 10% to 20% appetite loss for the first 2 days, no reduction in voluntary intake of food was observed during the further experimental period. The body weights in rabbits treated with rhGM-CSF did not significantly differ from those of control rabbits. No irritability in behavior was observed at this dose level, nor were symptoms of appetite loss, body weight loss, or irritability observed in WHHL rabbits treated with 20 \(\mu g\cdot kg^{-1}\cdot d^{-1}\) rhGM-CSF for 7 days.

**Effects of rhGM-CSF on CFU-GM-Derived Colony Formation and Peripheral Blood Count**

rhGM-CSF stimulated human CFU-GM–derived colony formation in vitro (Table 1) with no colony formation in the absence of added rhGM-CSF as described.\textsuperscript{1,2} rhGM-CSF was added to the culture system containing rabbit bone marrow cells to assess the cross-reaction of the biological activity in vitro. The activity of rhGM-CSF on rabbit CFU-GM–derived colony formation was...
Effects of rhGM-CSF on Plasma Lipid Content

Table 2 shows the levels of plasma cholesterol and triglycerides in normal rabbits that were treated with 20 \( \mu g \cdot kg^{-1} \cdot d^{-1} \) rhGM-CSF for 2 weeks. rhGM-CSF induced a significant decrease (49±19% of control) in TC levels compared with pretreatment values \((P<.01)\), whereas HSA produced no significant change (Fig 1). The reduction was primarily attributed to a significant lowering of both LDL-C and VLDL-C levels. The percentages of reduction were 68±19% and 41±21%, respectively \((P<.01\text{ for both})\). Significant decreases in the levels of TGs \((47±27\%)\) and HDL-C \((27±21\%)\) were also observed in rabbits treated with rhGM-CSF \((P<.01\text{ and } P<.05,\text{ respectively})\). We observed that the cholesterol-lowering effect of rhGM-CSF persisted for at least 2 weeks following termination of the treatment in normal rabbits (Fig 1). The levels of TC and LDL-C were reduced by 48±29% and 59±35%, respectively, of the pretreatment values 2 weeks after the end of the GM-CSF treatment \((P<.05\text{ for both})\).

A significant reduction in TC levels was also seen in the animals fed 1% cholesterol that were also treated with 20 \( \mu g \cdot kg^{-1} \cdot d^{-1} \) rhGM-CSF (Fig 2). TC was 702±189 mg/dL in rabbits treated with rhGM-CSF 2 weeks following the end of treatment compared with 1351±154 mg/dL in controls \((P<.01)\). This lowering effect persisted for at least 2 weeks after the conclusion of the GM-CSF treatment.

GM-CSF also exhibited a cholesterol-lowering effect in WHHL rabbits (Table 3). Although the values were not statistically significant, 7-day administration of rhGM-CSF decreased the levels of plasma TC and LDL-C by 26±26% and 33±29%, respectively. This lowering effect persisted in WHHL rabbits for as long as 3 weeks after treatment. TC and LDL-C levels were reduced by 34±26% and 38±29%, respectively \((P<.05\text{ for both})\;\text{Table 3})

Effects of rhGM-CSF on Hepatic Function and Tissue

Serum values of total protein, albumin, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase were not significantly changed by the GM-CSF treatment in either normal or cholesterol-fed rabbits treated with 20 \( \mu g \cdot kg^{-1} \cdot d^{-1} \) rhGM-CSF. Although a small decrease in cholinesterase activity was observed during the treatment in the treated rabbits, the values were not significant, and they normalized 7 days after the cessation of treatment (Table 4). No alteration in cholinesterase was noticed in cholesterol-fed rabbits treated with rhGM-CSF (Table 4).

The rabbits treated with 20 \( \mu g \cdot kg^{-1} \cdot d^{-1} \) rhGM-CSF or HSA for 14 days were killed 14 days after termination of the treatment, and the livers were removed. Additional livers were obtained from rabbits treated with an equal dose of rhGM-CSF or HSA for 5 days. Histological examination of the livers revealed no significant difference between rhGM-CSF-treated and control rabbits (data not shown).

Levels of mRNA for GM-CSF Receptors

Five \( \mu g \) poly(A)\(^{+}\)RNA extracted from bone marrow of rabbit and the patient with acute myelomonocytic leukemia and 12 \( \mu g \) poly(A)\(^{+}\)RNA from human and rabbit muscles were applied to an agarose gel electrophoresis and hybridized with the human GM-CSF receptor \( \alpha \) and \( \beta \) chain cDNAs. Fig 3 shows the expression of GM-CSF receptor \( \beta \) chain mRNA in each sample. The mRNA for GM-CSF receptor \( \beta \) chain was detected in rabbit bone marrow, and a strong message was observed in bone marrow cells of acute myelomonocytic leukemia as a positive control.\(^{19}\) There was no

### Table 3. Effects of rhGM-CSF on Lipid Levels in WHHL Rabbits

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>LDL-C</th>
<th>VLDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>636.6±318.0</td>
<td>491.6±286.7</td>
<td>113.8±51.4</td>
<td>13.2±5.5</td>
</tr>
<tr>
<td>Day 7</td>
<td>429.8±211.6</td>
<td>274.6±69.0</td>
<td>121.4±63.2</td>
<td>16.0±2.8</td>
</tr>
<tr>
<td>Day 28</td>
<td>360.2±80.2*</td>
<td>249.0±84.2*</td>
<td>100.6±26.0</td>
<td>10.8±5.1</td>
</tr>
</tbody>
</table>

rhGM-CSF indicates recombinant human granulocyte-macrophage colony-stimulating factor; WHHL, Watanabe heritable hyperlipidemic; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and TG, triglycerides. WHHL rabbits were administered 20 \( \mu g \cdot kg^{-1} \cdot d^{-1} \) rhGM-CSF in two doses for 7 days. Data are mean±SD for five animals and are expressed as milligrams per deciliter. *P<.05 vs pretreatment.

### Table 4. Effect of rhGM-CSF on Cholinesterase Levels

<table>
<thead>
<tr>
<th>Day</th>
<th>Normal rabbits (n=6)</th>
<th>Cholesterol-fed rabbits (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>148.8±63.2</td>
<td>195.0±68.9</td>
</tr>
<tr>
<td>Day 7</td>
<td>124.0±43.5</td>
<td>184.0±139.1</td>
</tr>
<tr>
<td>Day 14</td>
<td>128.7±52.6</td>
<td>212.0±67.0</td>
</tr>
<tr>
<td>Day 21</td>
<td>148.3±63.7</td>
<td>209.0±76.0</td>
</tr>
</tbody>
</table>

rhGM-CSF indicates recombinant human granulocyte-macrophage colony-stimulating factor. rhGM-CSF \((20 \mu g \cdot kg^{-1} \cdot d^{-1})\) was administered to normal and cholesterol-fed rabbits for 14 days. Data are mean±SD and are expressed in units per liter.
FIG 3. Expression of the β-chain of granulocyte-macrophage colony-stimulating factor receptor (GM-CSF-R/β) as determined by Northern blot analysis. Five micrograms poly(A)⁺ RNA from the marrow cells of a patient with acute myelomonocytic leukemia (used as a positive control) and rabbit bone marrow and 12 μg poly(A)⁺ RNA from human and rabbit muscles were hybridized with a human GM-CSF receptor β-chain probe. Lane 1, Bone marrow cells from the patient with acute myelomonocytic leukemia; lane 2, rabbit bone marrow; lane 3, human muscle; and lane 4, rabbit muscle.

Levels of the VLDL and LDL Receptor mRNA

We performed Northern blot analyses to determine the levels of mRNA for LDL and VLDL receptors in rabbits treated with rhGM-CSF or HuM-CSF. These levels were evaluated in five pairs of normal rabbits treated with the single injection and the 5-day administration of rhGM-CSF or HSA, respectively. rhGM-CSF induced a 2.6±0.5-fold increase in the levels of the VLDL receptor mRNA in muscle after 1.5 hours of the treatment in normal compared with control rabbits (P<.01, Fig 4A); there was a 1.5±0.1-fold increase after the 5-day administration (P<.01; Fig 4B). rhGM-CSF did not alter the levels of VLDL receptor mRNA in liver following the treatments (Table 5), and no increase in the levels of the LDL receptor mRNA was observed in livers of the rabbits 1.5 hours after the single injection (Fig 5). After 5 days of GM-CSF treatment, Northern blot hybridization with the LDL receptor cDNA showed no change in the levels of mRNA in liver, spleen, or bone marrow cells (Table 5). No change in the levels of mRNA for VLDL receptor was observed in muscle (Fig 6) or liver (Table 5) either 1.5 hours after the single injection or after the 5-day administration of M-CSF in each of three pairs of normal rabbits. Table 5 summarizes the effects of rhGM-CSF and M-CSF on the levels of lipoprotein receptors in normal rabbits.

In three pairs of WHHL rabbits, no change in the levels of LDL receptor mRNA was observed in liver, spleen, or bone marrow following the 5-day treatment.

FIG 4. Effect of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) on the levels of very-low-density lipoprotein (VLDL) receptor mRNA in normal rabbit muscle. Ten pairs of normal rabbits were killed 1.5 hours after a single injection of 20 μg/kg rhGM-CSF or human serum albumin (HSA) (A) and after the 5-day administration of 20 μg·kg⁻¹·d⁻¹ rhGM-CSF or HSA (B) (each group, five pairs). Equal doses of HSA were administered to control animals. Total RNA was isolated from muscles of the treated rabbits. Two micrograms poly(A)⁺ RNA was electrophoresed on an agarose gel (1%), transferred to a nylon membrane, and hybridized with a 32P-labeled probe for rabbit VLDL receptor. The same membrane was rehybridized with a β-actin probe. The positions of the 28S and 18S rRNA subunits are indicated. The levels of the VLDL mRNA in muscle were evaluated in five pairs of rabbits in both single-injection and 5-day-administration groups. The signals of Northern blots were quantified by densitometric scanning. The ratios of the VLDL mRNA levels were 2.6±0.5- and 1.5±0.1-fold, respectively, versus control (P<.01 for both). Representative experiments are shown. C indicates control; GM, rhGM-CSF.
of 20 \mu g \cdot kg^{-1} \cdot d^{-1} \text{ rhGM-CSF} (Table 5). However, a small but significant increase (1.4-fold) in the levels of VLDL receptor mRNA of the muscle was induced by the GM-CSF treatment (Table 5). The data from WHHL rabbits treated with rhGM-CSF were comparable to those observed in normal rabbits treated with rhGM-CSF (Table 5).

### Discussion

To determine the biological activity of rhGM-CSF in rabbits, we tested the ability of rhGM-CSF to affect rabbit CFU-GM-derived colony formation in vitro. In vitro colony assays showed that rhGM-CSF induced CFU-GM-derived colony formation with an activity comparable to that detected in humans, indicating that rhGM-CSF is capable of enhancing macrophage functions in rabbits. The numbers of circulating granulocytes and monocytes were not significantly altered by rhGM-CSF in vivo in rabbits. Similar observations using cytokine have been shown in animal models.\(^{10,30}\) In particular, the administration of human recombinant M-CSF did not induce a significant increase in the levels of circulating monocytes in rabbits, but the factor has a potent cholesterol-lowering effect in rabbits.\(^{10}\) It is well known that M-CSF modulates the homeostasis of cholesterol in vitro and in vivo.\(^{10,12-14}\) We observed that rhGM-CSF markedly decreased the levels of plasma cholesterol with no effect on the numbers of circulating granulocytes and macrophages in normal and cholesterol-fed rabbits. It also lowered the levels of cholesterol in WHHL rabbits. These findings suggest that the cholesterol-lowering effect of rhGM-CSF may be partially mediated by the enhancement of macrophage functions in lipid metabolism.\(^{10,12-14}\)

We also showed that the cholesterol-lowering effect of rhGM-CSF persisted after the cessation of the treatment in normal and cholesterol-fed rabbits. The levels of cholesterol 2 weeks after the rhGM-CSF treatment were still lower than the pretreatment values in normal rabbits (Fig 1). The sustained effect of cholesterol-lowering was clearly seen in cholesterol-fed rabbits (Fig 2). TG levels were also decreased by the administration of rhGM-CSF in normal rabbits. Shimano et al.\(^{10}\) report that M-CSF reduces cholesterol levels but does not change TG levels and that cholesterol levels begin to normalize after the end of M-CSF treatment. With respect to the mechanisms of these two molecules on the metabolism of lipoprotein, the differences in the duration of cholesterol-lowering and TG levels suggest that GM-CSF and M-CSF may act on different pathways other than activating macrophages.

A cDNA for VLDL receptor was cloned by Taka-hashi et al.\(^{25}\) Although the mature VLDL receptor has a striking homology to the LDL receptor, the two receptors have distinct ligand specificities. The VLDL receptor binds apoE-containing lipoprotein, whereas the LDL receptor binds both apoE- and apoB-100-containing lipoproteins. Tissue distribution of the VLDL receptor analyzed by Northern blot showed an abundant expression of the mRNA in muscle and heart, but mRNA was barely detectable in liver.\(^{25}\) These data

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**Table 5. Comparison of the Effects of GM-CSF and M-CSF on mRNA Levels for LDL and VLDL Receptors**

<table>
<thead>
<tr>
<th></th>
<th>GM-CSF</th>
<th>M-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL receptor mRNA, liver</td>
<td>⨷</td>
<td>⨷</td>
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<tr>
<td>LDL receptor mRNA, spleen, bone marrow</td>
<td>⨷</td>
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<tr>
<td>VLDL receptor mRNA, muscle</td>
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<tr>
<td>VLDL receptor mRNA, liver</td>
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</table>

GM-CSF indicates granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; LDL, low-density lipoprotein; and VLDL, very-low-density lipoprotein.

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*Results shown in the present study.

†The effect of M-CSF on the levels of LDL receptor mRNA as described by Shimano et al.\(^{10}\)
Mediated in part by VLDL receptor in the muscle. We report that M-CSF increased the levels of mRNA for LDL receptor in bone marrow and spleen with no change in the mRNA in liver. The present study demonstrated that the levels of LDL receptor mRNA were not altered in bone marrow, spleen, and liver by the treatment of rhGM-CSF and that rhGM-CSF increased the levels of mRNA for VLDL receptor in muscle. These findings suggest that the action of GM-CSF on the levels of VLDL receptor mRNA in muscle may be mediated by another molecule(s). The mechanisms by which binding lipoprotein in muscle is metabolized and contributes to the lowering of TC and TGs in the circulation need further study.

The treatment of rhGM-CSF also decreased LDL-C levels by 38% in WHHL rabbits but did not significantly change the levels of TGs (Table 3). This observation may result from a defect in the LDL receptor in WHHL rabbit, although Northern blot analysis of VLDL receptor mRNA in WHHL rabbits revealed that the levels of the mRNA were slightly increased in the muscle by rhGM-CSF. The mutation of the LDL receptor gene in the WHHL rabbit gives rise to extremely high levels of LDL-C and TGs (Table 3 and References 24 and 31). Kita et al.\(^2\) have demonstrated that the clearance of VLDL and intermediate-density lipoprotein (IDL) is markedly delayed in WHHL rabbits. The decreased uptake of VLDL and IDL by the liver causes the accumulation of TGs in WHHL rabbits and may in turn account for the decreased clearance of TG-rich lipoproteins in WHHL rabbits treated with rhGM-CSF. Further studies are necessary to clarify the role of the VLDL receptor in the metabolism of TG-rich lipoproteins.

Moreover, the cholesterol-lowering effect of rhGM-CSF persisted even after the termination of the rhGM-CSF treatment in normal and WHHL rabbits. A possible explanation is that another molecule(s) released by the stimulation of GM-CSF may be involved. The detailed mechanism is unclear.

Finally, the cholesterol-lowering activity of GM-CSF might be due to the impairment of hepatic function.\(^6\) The present study demonstrated that the hepatic function of the treated animals was not significantly damaged during and after treatment. The 2-week administration of 20 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) day\(^{-1}\) rhGM-CSF to normal rabbits tended to decrease the levels of cholinesterase during treatment; this decrease was followed by normalization 1 week after the treatment. No change in cholinesterase activity was observed in cholesterol-fed rabbits treated with an equal dose of rhGM-CSF. However, rhGM-CSF lowered the levels of cholesterol in both normal and cholesterol-fed rabbits. Furthermore, the histology revealed no significant difference between rhGM-CSF-treated and control rabbits. Our data suggest that the impairment of hepatic function by GM-CSF may be ruled out when interpreting the cholesterol-lowering effect of GM-CSF.

The mechanisms by which GM-CSF elevates the levels of mRNA for LDL receptor in muscle remain to be elucidated. However, our experimental model may facilitate studies on the metabolism of lipoproteins mediated via the VLDL receptor.

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Potent cholesterol-lowering effect by human granulocyte-macrophage colony-stimulating factor in rabbits. Possible implications of enhancement of macrophage functions and an increase in mRNA for VLDL receptor.

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