The effects of human recombinant macrophage colony-stimulating factor (M-CSF) on the secretion of lipoprotein lipase were studied in rat alveolar macrophages. Five nanograms per milliliter M-CSF significantly enhanced lipoprotein lipase secretion (threefold), and the maximal effect (10-fold) of M-CSF on lipoprotein lipase secretion was observed at a dose of 200 ng/ml M-CSF. The effect of M-CSF was time dependent but was not manifested during the first 8 hours of incubation. After 24 hours, its effects were evident and dose dependent. On blot hybridization of macrophage RNAs with human cDNA of lipoprotein lipase, a remarkable and dose-dependent increase in mRNA level (73-fold) was found in M-CSF–treated alveolar macrophages. The secretion of lipoprotein lipase was also enhanced in human monocyte–derived macrophages (2.6-fold), whereas the secretion from either THP-1 cells, P388 cells, or J774 cells was not significantly enhanced. These results indicate that the stimulation of lipoprotein lipase secretion after M-CSF treatment was evident in rat alveolar macrophages and human monocyte–derived macrophages on the basis of both enzyme activity and mRNA level; therefore, M-CSF may be involved in lipoprotein metabolism of macrophages through modulation of the secretion of lipoprotein lipase.

(arteriosclerosis and thrombosis 1991;11:1315-1321)

Colony-stimulating factors (CSFs) have been extensively studied because of their ability to induce the growth and differentiation of hematopoietic progenitor cells. Among the four major types of CSFs identified in the murine system, granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) have recently been demonstrated to lower serum cholesterol level. These findings suggested the involvement of CSFs in plasma cholesterol metabolism. GM-CSF and M-CSF can also influence macrophage functions in the subendothelial space, where macrophages play a significant role in atherogenesis. Macrophages take up lipids, and when excessive amounts of lipids accumulate intracellularly, these cells take on a foamy appearance. Such transformed macrophages are implicated as the foam cells observed in atheromatous lesions. Lipoprotein lipase (LPL) secreted from macrophages is probably involved in this process. LPL could facilitate the uptake of LPL-modified lipoproteins by producing remnant lipoproteins.

Among CSFs, the action of M-CSF is selective for the monocyte–macrophage lineage. The cDNA coding for M-CSF has already been sequenced, and recombinant M-CSF secreted from Chinese hamster ovary cells is available for the study of its biochemical characteristics. In the present study, we examined the effects of recombinant M-CSF on the activity of LPL secreted from rat alveolar macrophages, human monocyte–derived macrophages, and THP-1, P388, and J774 cells.

Methods

Materials

Male Sprague-Dawley rats weighing 200–250 g were fed laboratory chow ad libitum. Recombinant human M-CSF of 85 kd was supplied by Morinaga Milk Industry Co., Ltd., Tokyo, Japan. The batch was endotoxin free. The M-CSF was produced in mammalian Chinese hamster ovary cells and had a specific activity of 2 × 10⁶ units/mg glycoprotein. One unit of M-CSF indicates an activity forming one macrophage colony in a standard bone marrow assay.

Cell Cultures

The rats were anesthetized, and the chest was opened. Then the lungs were briefly perfused with 10
ml 0.9% saline three times. The lavage fluid was centrifuged at 100g for 10 minutes to isolate macrophages, and the resulting pellet was suspended in RPMI-1640 medium (GIBCO Laboratories, Grand Island, N.Y.) and centrifuged again at the same speed. After resuspension of the pellet in RPMI-1640 medium containing 100 units/ml penicillin and 100 μg/ml streptomycin, the cells (5x10^6 cells/dish) were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 2 hours. The dishes were washed twice with RPMI-1640 and incubated in the same medium supplemented with 10% fetal bovine serum (Sigma Chemical Co., St. Louis, Mo.) for 2 days. On day 3, the cells were washed twice with 1 ml phosphate-buffered saline (PBS; pH 7.4), and then the culture was continued for the designated time with M-CSF.

Human mononuclear cells were isolated by Ficoll-Hypaque density centrifugation, and 2x10⁶ to 3x10⁶ cells were seeded into each well (well diameter, 22 mm) of 12-well plates with 1 ml medium. After incubation at 37°C for 2 hours, the cells were washed three times with prewarmed PBS to remove nonadherent cells, and then adherent cells were incubated with RPMI-1640 supplemented with 10% autologous serum.

For the experiments with THP-1 cells, approximately 7x10⁶ cells were seeded into each well (well diameter, 22 mm) of 12-well plates with 1 ml RPMI-1640 containing 10% fetal calf serum and induced to differentiate by addition of 120 ng/ml 12-O-tetradecanoylphorbol-13-acetate. P388 and J774 cells were seeded at a cell density of 1x10⁵ cells/well (22-mm diameter) and cultured with 1 ml RPMI-1640 and Dulbecco's modified Eagle's medium containing 10% fetal calf serum, respectively. After seeding of the THP-1, P388, and J774 cells, they were cultured in the presence of the indicated amounts of M-CSF for 72 hours.

**Lipoprotein Lipase Assay**

The activity of LPL secreted from alveolar macrophages was assayed by the method of Nilsson-Ehle and Schotz. Briefly, 150 μl of the medium was mixed with 50 μl of the substrate containing 22.6 mM tritiated triolein (1.4 μCi/μmol), phosphatidylcholine at 2.5 mg/ml, bovine serum albumin at 40 mg/ml, 33% [vol/vol] rat serum, and 33% [vol/vol] glycerc in a 0.27 M tris(hydroxymethyl)aminomethane HCl (pH 8.1), and then the mixture was incubated at 37°C for 30 minutes. Lipase activity was estimated from the amount of free oleic acid that was liberated from [3H]triolcin during incubation. It is possible that the M-CSF added to the culture medium could have influenced the lipase assay. This possibility was checked by assaying the medium containing purified LPL in the presence and absence of M-CSF. We found that 300 ng/ml M-CSF did not interfere with the assay.

**Determination of Cell Protein and Cell DNA**

After the medium was collected to measure LPL activity, each monolayer was washed twice with 1 ml PBS (pH 7.4) and dissolved in 0.5 ml 0.1 M NaOH. The aliquots were used for protein measurement by the procedure of Lowry et al and for DNA determination by that of Labarca et al.

**Northern Blot Analysis**

Blot hybridization studies were performed for LPL mRNA of rat alveolar macrophages prepared as described above. Macrophages were treated with 10, 40, or 200 ng/ml M-CSF. After 72 hours' incubation with M-CSF, total RNA was prepared by extraction with LiCl-urea as described. Thirty micrograms of the total RNA sample was subjected to 1% agarose gel electrophoresis and transferred onto nylon membranes in 20x saline–sodium phosphate–EDTA buffer (SSPE) (3.6 M NaCl, 0.2 M Na₂HPO₄, and 0.002 M EDTA [pH 7.7]). The membrane was cross-linked by exposure to ultraviolet light and prehybridized at 65°C for 2 hours in the following hybridization buffer: 50% (vol/vol) formamide, 5x Denhardt's solution (1x Denhardt's solution is 0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), 5x SSPE, 0.5% sodium dodecyl sulfate, and 100 μg/ml heat-denatured salmon sperm DNA. cDNA probes coding for human LPL and chicken β-actin were radiolabeled with phosphorus-32 by nick translation (2x10⁶ cpm/μg DNA). The cDNA probes were added separately to the hybridization buffer, and hybridizations were performed at 65°C for 16 hours. The filters were washed in 2x standard saline citrate (SSC) (1x SSC is 150 mM NaCl, 10 mM sodium citrate) and 0.5% sodium dodecyl sulfate at room temperature for 15 minutes twice and subsequently in 0.8x SSC and 0.5% sodium dodecyl sulfate at 65°C for 30 minutes twice and exposed to x-ray film at -70°C.

**In Vivo Studies**

Human recombinant M-CSF in 150 mM NaCl was injected subcutaneously into five male rats at a dose of 20 μg/animal twice for 6 days. As a control, the same amount of human serum albumin was injected into another group of rats. All animals were anesthetized by administration of sodium pentobarbital (50 mg/kg body wt), and 1 ml blood was collected from the subclavian venous plexus into tubes containing EDTA (1 mg/ml blood). The plasma was used for chemical analyses. Then heparin (200 units/100 g body wt) was administered intravenously, and blood was withdrawn from the subclavian venous plexus 3 minutes after heparin administration. The plasma was separated in a refrigerated centrifuge, and two lipases, hepatic triglyceride lipase and LPL, were measured separately by addition of 1 M NaCl following the method of Nilsson-Ehle and Schotz with slight modification, as described previously. Plasma cholesterol, triglyceride, and phospholipid were measured by enzymatic methods.
Results

Secretion of Lipoprotein Lipase

Figure 1 shows changes in the amounts of protein and DNA in cells cultured with various doses of M-CSF. The addition of at least 40 ng/ml M-CSF to macrophages increased cell protein in a dose-dependent manner. However, no change in cell DNA was observed. The effect of M-CSF on LPL secretion is shown in Figure 2. LPL activity was expressed as nanomoles free fatty acid per hour per nanogram cell DNA. The effect was evident at a concentration as low as 5 ng/ml and was maximal at a dose of 200 ng/ml.

Figure 3 shows the results of the time-course experiment after addition of M-CSF to the medium. During the first 8 hours of incubation, M-CSF had no significant effect on enzyme activity, but an increase of about twofold was observed 24 hours after the addition. After 72 hours of incubation with M-CSF, enzyme activity was increased in a dose-dependent manner.

The results from blot hybridization of RNAs from rat alveolar macrophages with cDNA for human LPL are shown in Figure 4. The cDNA probe for LPL identified a single mRNA transcript 4.0 kb long. Hybridization with the cDNA probe at this position was significantly increased and dose dependent in M-CSF-treated macrophages. Ten nanograms per milliliter M-CSF enhanced LPL mRNA level 3.6-fold; 40 ng/ml M-CSF, 6.4-fold; and 200 ng/ml M-CSF, 7.3-fold on densitometric scanning compared with the untreated macrophages. The intensities of the bands estimated by densitometry were corrected by comparison with those of actin mRNA bands.

In human monocyte-derived macrophages, LPL activity secreted into the medium was not enhanced in the early days of the culture, and thereafter it was...
FIGURE 2. Line plot showing dose (ng/ml medium) effect of macrophage colony-stimulating factor (M-CSF) on lipoprotein lipase (LPL) activity (nmol FFA/hr/μg DNA) secreted to medium with rat alveolar macrophages cultured under exactly the same conditions as described in the legend to Figure 1. After 72 hours’ incubation, medium was collected and assayed for LPL activity. Values with bars are mean±SD of four samples. FFA, free fatty acid.

FIGURE 3. Line plot showing time course (hours) of effect of macrophage colony-stimulating factor (M-CSF) on lipoprotein lipase (LPL) activity (nmol FFA/hr/μg DNA) secreted to the medium cultured with rat alveolar macrophages. Cells were cultured as described in “Methods.” After 2 days of incubation, medium was replaced with fresh medium, and cells were incubated with 0 (○), 10 (●), 20 (△), or 40 (▲) ng/ml M-CSF. Plates were harvested at the indicated time, and medium was assayed for LPL activity. Results are mean±SD of data derived from three samples. FFA, free fatty acid.

FIGURE 4. Northern blot analysis of total RNA in rat alveolar macrophages. Total RNA (30 μg) extracted from macrophages was subjected to agarose gel electrophoresis, then transferred to nylon membranes and hybridized with a phosphorus-32-labeled human lipoprotein lipase cDNA probe. Macrophages were cultured for 2 days with RPMI-1640 medium containing 10% fetal calf serum. Then the cells were incubated with the indicated concentrations of macrophage colony-stimulating factor (M-CSF) for 72 hours. In lane H, human monocyte-derived macrophages were cultured with 100 ng/ml M-CSF for 6 days. Ten micrograms total RNA extracted from human monocyte-derived macrophages was then subjected to agarose gel electrophoresis. Molecular weight markers are at right. 28S 18S, 28S and 18S subunits of rRNA, respectively; β-actin, β-actin probe.

remarkably enhanced by M-CSF as shown in Figure 5. On the ninth day of culture, the effect of M-CSF was dose related (Figure 5, inset). One hundred nanograms per milliliter M-CSF enhanced LPL activity secreted into medium 2.6-fold. LPL activities were low in THP-1, P388, and J774 cells when compared with those in either rat alveolar macrophages or human monocyte-derived macrophages, and no significant enhancement was observed by addition of M-CSF to these cell types (Table 1).

In Vivo Study
Changes in body weight and plasma lipid concentration in the experimental and control rats are shown in Table 2. After M-CSF injection, there were no significant differences in body weight or plasma lipid levels between the two groups. Although the effect of M-CSF on hepatic triglyceride lipase was not detectable, there was a slight increase in postheparin plasma LPL activity in experimental animals compared with that of controls (57.2±3.1 versus 42.0±11.6 μmol free fatty acid/hr/ml, p<0.05) (Table 2).

Discussion
The current study demonstrated increased activities of LPL secreted from both rat alveolar macrophages and human monocyte-derived macrophages after addi-
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Figure 5. Line plot showing time course (days) of effect of macrophage colony-stimulating factor (M-CSF) on lipoprotein lipase (LPL) activity (nmol FFA/hr/μg DNA) secreted to medium cultured with human monocyte-derived macrophages. Cells were cultured with RPMI-1640 medium containing 0 (●), 30 (○), or 100 (△) ng/ml M-CSF, and medium was replaced with fresh medium every 3 days. Plates were harvested at the indicated time, and medium was assayed for LPL activity. Results are mean±SD of data derived from four samples. Inset shows dose effect of M-CSF on LPL activity secreted to the medium cultured with human monocyte-derived macrophages. Cells were cultured with RPMI-1640 medium in the absence of M-CSF for 9 days and then cultured with the indicated amounts of M-CSF for a further 24 hours. Results are the mean±SD of data derived from four samples. FFA, free fatty acid.

Table 1. Effects of Macrophage Colony-Stimulating Factor on the Activity of Lipoprotein Lipase in Various Types of Macrophages

<table>
<thead>
<tr>
<th>Cell type</th>
<th>M-CSF added (ng/ml)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Alveolar macrophages</td>
<td>196.3±12.9</td>
<td>53.0±2.9</td>
<td>399.2±26.4</td>
</tr>
<tr>
<td>Human monocytes</td>
<td>56.9±8.8</td>
<td>15.2±3.8</td>
<td>30.6±2.0</td>
</tr>
<tr>
<td>Human monocytes*</td>
<td>4.1±0.2</td>
<td>0.3±0.3</td>
<td>1.7±0.9</td>
</tr>
<tr>
<td>THP-1*</td>
<td>7.3±0.9</td>
<td>2.0±0.8</td>
<td>4.8±0.7</td>
</tr>
<tr>
<td>P388</td>
<td>21.4±1.9</td>
<td>21.5±1.2</td>
<td>22.3±1.5</td>
</tr>
</tbody>
</table>

Each value represents mean±SD.

Enzyme activities in the medium and cells that were incubated with macrophage colony-stimulating factor (M-CSF) for 72 hours were measured in quadruplicate.

*In these experiments cells were incubated with M-CSF for 9 days, and then enzyme activities in the medium and cells that were incubated with M-CSF for a further 24 hours were measured in quadruplicate.

Table 2. Effects of Macrophage Colony-Stimulating Factor on Plasma Lipids and Postheparin Plasma Lipolytic Activities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>M-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>319±10</td>
<td>320±4</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>351±17</td>
<td>343±10</td>
</tr>
<tr>
<td>Plasma triglyceride (mg/dl)</td>
<td>52±26</td>
<td>68±26</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dl)</td>
<td>64±10</td>
<td>60±9</td>
</tr>
<tr>
<td>Plasma phospholipids (mg/dl)</td>
<td>86±38</td>
<td>99±5</td>
</tr>
<tr>
<td>Postheparin lipolytic activity (μmol FFA/hr/ml)</td>
<td>42.0±11.6</td>
<td>57.2±3.1*</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic triglyceride lipase</td>
<td>67±6.23</td>
<td>70.2±3.3</td>
</tr>
</tbody>
</table>

Each value represents mean±SD.

M-CSF, macrophage colony-stimulating factor; FFA, free fatty acid.

*Significantly different at p<0.05.

M-CSF enhances LPL secretion from macrophages. The results of Northern blot analysis for LPL indicated that M-CSF primarily stimulated the synthesis of LPL mRNA and resulted in the increased secretion of this enzyme into the medium. Enhanced secretion of LPL from macrophages is suggested to be one of the hyperfunctions of mature macrophages after M-CSF treatment. M-CSF stimulates a number of functions of mature macrophages.6-24-26 It is known that resident macrophages do not proliferate in culture,27-28 although M-CSF induces proliferation and differentiation of progenitor cells. Our present study showed an increased amount of cell protein in rat alveolar macrophages after addition of M-CSF to the medium, but no significant effects on cell DNA were observed. Recently, a similar result was demonstrated in human monocyte-derived macrophages.6 These results indicate that M-CSF induces some alteration in the metabolism of mature macrophages but does not induce their proliferation.

To augment the secretion of LPL, human monocytes were incubated with M-CSF for at least 3–6 days.
days (Figure 5). Other functions of macrophages were also enhanced by M-CSF after a long time of incubation.\textsuperscript{6,29-31} Scavenger functions, such as acetylated LDL receptor activity, are known to be induced along with the maturation and differentiation of cells from monocytes to macrophages.\textsuperscript{32} Because M-CSF stimulates the maturation and differentiation of both human monocyte and rat alveolar macrophages, the enhanced secretion of LPL after M-CSF treatment can be understood as a result of enhanced maturation and differentiation of human monocytes. A similar time-related change was observed in the uptake of acetylated LDL after addition of M-CSF to human monocytes.\textsuperscript{6}

Recently, factors regulating the activities of LPL secreted from macrophages as well as tissue LPL have been studied. Cyclic AMP has been revealed to modify the secretion of LPL from macrophages as well as that from adipose tissue.\textsuperscript{33-35} The mode of regulation of tissue LPL and LPL secreted from macrophages is not necessarily the same. For instance, insulin increases the activity of LPL in adipose tissue\textsuperscript{36-38} but has no effect on the activity of LPL secreted from macrophages.\textsuperscript{10,33} There is species-specific regulation in the secretion of LPL. Dexa-methasone suppresses LPL secretion from mouse peritoneal macrophages,\textsuperscript{39} whereas it has the opposite effect on human monocyte-derived macrophages.\textsuperscript{39} Lipopolysaccharide also suppresses LPL secretion from macrophages.\textsuperscript{40} On the other hand, it has been reported that cholesterol-rich lipoproteins,\textsuperscript{12} hypertriglyceridemic very low density lipoproteins,\textsuperscript{13} and L-cell-conditioned medium\textsuperscript{41} enhance LPL secretion from macrophages. L-cell-conditioned medium, which stimulates the macrophage lineage, increases LPL secretion from mouse bone marrow macrophages and thioglycollate-elicited peritoneal macrophages, whereas it has no effect on LPL secretion from resident peritoneal macrophages. In the present study, recombinant human M-CSF was used instead of the conditioned medium, in which the concentration of active substances to stimulate LPL secretion was unknown. In comparison with previous reports, M-CSF remarkably increased LPL secretion from resident macrophages by about threefold to 10-fold. However, we could not demonstrate the enhanced secretion of LPL from macrophage-like cell lines such as THP-1, P388, and J774 cells. These cells secrete LPL in the absence of M-CSF to a lesser extent than do either rat alveolar macrophages or human monocyte–derived macrophages. This might minimize the effects of M-CSF on the secretion of LPL, and/or these cells might respond poorly to M-CSF although they express the cell surface receptor for M-CSF.\textsuperscript{42-44}

M-CSF injection into rats had no obvious effect on plasma lipids, including cholesterol, which has been reported to be decreased by the intravenous administration of M-CSF in both humans and rabbits.\textsuperscript{24} On the other hand, M-CSF increased LPL activity slightly in postheparin plasma ($p<0.05$). M-CSF may stimulate the secretion of LPL from tissue macrophages, as M-CSF acts toward cells of the monocyte–macrophage lineage as its target cells.

The major role of LPL is to hydrolyze plasma triglyceride and facilitate the uptake of fatty acids released after hydrolysis in tissues such as adipose tissue, muscle, and heart; however, the physiological role of LPL secreted from macrophages is not well understood. The present study showed novel regulation of LPL in macrophages. M-CSF may modulate the secretion of LPL from macrophages to supply energy as fatty acids for macrophages according to their demands. We and others have suggested that macrophage LPL hydrolyzes triglyceride in very low density lipoprotein and enhances the uptake of the very low density lipoprotein remnant by macrophages.\textsuperscript{11-14} M-CSF may also modulate this process by regulating the secretion of LPL from macrophages.

The enhancing effect of M-CSF on LPL secretion is significant, especially in vitro. Recently, we have reported enhanced cellular uptake of both LDL and acetylated LDL in human monocyte–derived macrophages after M-CSF treatment.\textsuperscript{6} Taken together, it is likely that M-CSF plays an important role in the regulation of lipoprotein metabolism in macrophages.

References

M-CSF Enhances LPL Secretion From Macrophages


KEY WORDS • lipoprotein lipase • macrophages • macrophage colony–stimulating factor
Effects of human recombinant macrophage colony-stimulating factor on the secretion of lipoprotein lipase from macrophages.

N Mori, T Gotoda, S Ishibashi, H Shimano, K Harada, T Inaba, F Takaku, Y Yazaki and N Yamada

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