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REGULATION OF LIPOPROTEIN LIPASE SECRETION BY MOUSE PERITONEAL MACROPHAGES

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Resident mouse peritoneal macrophages in culture spontaneously secrete lipoprotein lipase. Secretion of lipoprotein lipase was 10-fold greater in thioglycollate-elicited and 6-fold greater in mineral oil-elicited macrophages. The increase in enzyme secretion was dependent on protein synthesis and glycosylation of the newly synthesized enzyme. The addition of 1 to 500 ng per ml of lipopolysaccharide to the culture medium resulted in a dose- dependent inhibition of lipoprotein lipase secretion. Thus, secretion of macrophage lipoprotein lipase is a regulated process, and may be increased as part of the response to inflammation. This may have important implications in the atherogenic process in the arterial wall.

Human monocyte-derived macrophages (1-3), resident tissue macrophages (1,4), and J774 cells, a macrophage-like cell line (5), have all been observed to secrete lipoprotein lipase (LPL) in culture. The secretion of LPL by macrophages is increased in the presence of heparin. However, regulation of synthesis and secretion of LPL, analogous to that observed in adipose tissue, skeletal muscle and heart (6), has not been reported. In fact, the secretion of LPL by macrophages has been considered a constitutive function (1,5).

LPL-mediated hydrolysis of triglyceride-rich lipoproteins may play an important role in the atherogenic process (7). The enzyme secreted by macrophages hydrolyzes the triglyceride moiety of very low density lipoproteins (VLDL) and chylomicrons, resulting in the uptake of both the free fatty acids and triglyceride-depleted remnant particles (4,8,9). The triglyceride-poor, cholesteryl ester-rich remnant particles of chylomicrons can also be taken up by arterial smooth muscle cells, causing an increase in cholesterol content of these cells (10). LPL, therefore, may contribute to the development of macrophage- and smooth muscle cell- derived "foam cells", which are prominent features of atherosclerotic lesions.

In the present study, we report an increased synthesis and secretion of LPL in elicited mouse peritoneal macrophages, compared to resident macrophages.

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We also show the inhibition of LPL secretion from thioglycollate-elicited macrophages by lipopolysaccharide (LPS), the active component of endotoxin.

Methods

Primary cultures of mouse peritoneal macrophages were prepared as previously described (11). Elicited macrophages were obtained by injection of 2 ml of thioglycollate medium or 1 ml of mineral oil into the peritioneal cavity of mice three days prior to harvest. The cells were plated at a density of $1x10^7$ cells per 60-mm petri dish in 3 ml of α-minimal essential medium (α-MEM) supplemented with gentamycin sulfate (50 ug/ml), fungizone (0.25 ug/ml), and 10% heat inactivated fetal calf serum. After 3-4 h, non-adherent cells were removed by washing three times with α-MEM, and the cells were then maintained for 24 h in serum-free α-MEM. For each experiment, the media were removed, the cells were washed with cold phosphate-buffered saline, pH 7.4, and then harvested in 25 mM Tris-HCl, 1 mM EDTA, 20% glycerol, pH 7.4, at 4°C, with a rubber policeman. The media were centrifuged at 1000xg for 10 min to precipitate non-adherent cells, which were added to the previously harvested cells. The cells were sonicated for 12 seconds in buffer and assayed for enzymatic activities as described below.

LPL (triacylglycerol acylhydrolase, EC 3.1.1.34) was assayed in an incubation mixture of 0.8 ml, containing 0.0625 mM tri[1-^{14}C]oleoylglycerol, 0.15 M NaCl, 5 mg/ml bovine serum albumin (BSA), 50 mM Tris-HCl, pH 8.2, 3 ug/ml of apolipoprotein C-II, purified from human VLDL (12), and either cell sonicate or medium. The assay was carried out for 30 min at 30°C (5). Cholesteryl ester hydrolase (EC 3.1.1.13) was assayed similarly in 0.8 ml of assay mixture containing 0.032 mM cholesteryl [1-^{14}C]oleate, 1.25 mg/ml BSA, 50 mM potassium phosphate, pH 7.0, and either cell sonicate or medium (13). All assays were terminated by the addition of 3 ml of chloroform/methanol/benzene (1:2.4:2, v/v). [^{14}C]oleic acid was extracted by the addition of 0.1 ml of 1 M NaOH and quantified as previously described (14). Enzymatic activity was expressed as nmol of [^{14}C]oleic acid released/mg cell protein/h.

Results and Discussion

Resident mouse peritoneal macrophages spontaneously secrete LPL. About 80% of the total LPL activity (cells plus medium) was found in the medium after 24 h incubation. As shown in Figure 1A, the activity in the cells was 6.5-fold and 3.8-fold higher, in thioglycollate- and mineral oil-elicited macrophages, respectively, compared to the cellular activity in resident macrophages; the activity in the medium was increased 10-fold and 6-fold in thioglycollate- and mineral oil-elicited macrophages, respectively.

The increased synthesis and secretion of LPL by elicited macrophages is a selective process. As shown in Fig. 1B, the activity of the neutral cholesteryl ester hydrolase, a cytoplasmic enzyme believed to be responsible for the hydrolysis of cytoplasmic cholesteryl ester droplets (13), was not increased in either thioglycollate- or mineral oil-elicited macrophages. Very little neutral cholesteryl ester hydrolase activity was found in the media,

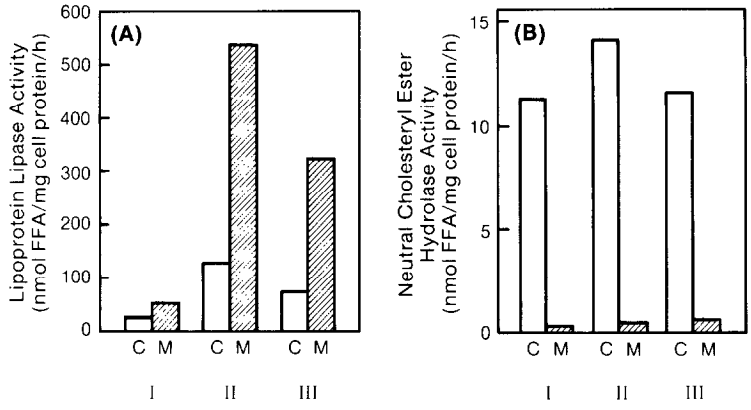

Fig. 1. The synthesis and secretion of LPL by resident, thioglycollate-elicited and mineral oil-elicited mouse peritoneal macrophages. Resident macrophages (I), thioglycollate-elicited macrophages (II), and minearal oil-elicited macrophages (III) were harvested as described under Methods. Cells were maintained in serum-free α -MEM. After 24 h, media and cells were harvested and assayed for LPL activity (panel A), and cholesteryl ester hydrolase activity (panel B). Open bars represent the mean of duplicate determinations of enzymatic activity in cell sonicates (C); hatched bars represent the mean of duplicate determinations of enzymatic activity in macrophage-conditioned media (M).

indicating that cell death and lysis were not responsible for the increased secretion of LPL.

As shown in Fig. 2, secretion of LPL into the culture medium by thioglycollate-elicited macrophages was cumulative with time. The addition of

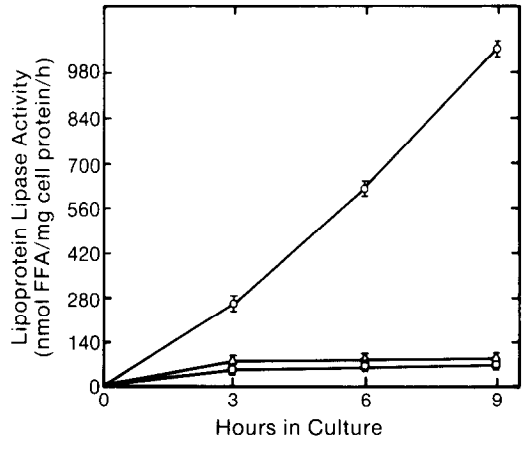

Fig. 2. The effects of cycloheximide and tunicamycin on the secretion of LPL by thioglycollate-elicited macrophages. Cells were maintained in 2 ml of serum-free α -MEM. Cycloheximide and tunicamycin were added at the start of incubation. Media without additions (○), with 1.0 ug/ml cycloheximide (△), and 5 ug/ml tunicamycin (□) were collected and replenished every 3 h. Aliquots of the media were assayed for LPL activity. The data represent the mean ± S.E. of four 60-mm dishes for control, and three 60-mm dishes each for cycloheximide and tunicamycin treated cells.

cycloheximide (1 ug/ml), an inhibitor of protein synthesis, resulted in an 83% decrease in LPL secretion at 3 h, and this inhibition was sustained for the next 6 h. Similarly, tunicamycin (5 ug/ml), an inhibitor of protein glycosylation, caused a 76% decrease in LPL secretion at 3 h, and the inhibiiton was sustained for the next 6 h. LPL secretion for the entire period was reduced by 93% by exposure to either cycloheximide or tunicamycin. Cycloheximide and tunicamycin treatment also resulted in a 94% depletion of cellular activity after 9 h (27.8 $\pm$ 10.3 and 26.6 $\pm$ 13.8 nmol FFA/ mg protein/ h in cycloheximide and tunicamycin treated cells, respectively, compared to 427.7 $\pm$ 29.3 nmol FFA/mg protein/h in untreated thioglycollate-elicited macrophages).

Medium, conditioned by exposure to thioglycollate-elicited mouse peritoneal macrophages for 36 h in the presence of endotoxin, has been found to inhibit the synthesis and secretion of LPL by 3T3-L1 adipocytes (15). However, an effect of endotoxin on the secretion of LPL by macrophages has not been reported. In the present study, we added LPS to cultures of thioglycollate-elicited macrophages and measured the LPL activity in the medium after 24 h. As shown in Fig. 3, LPS produced a concentration-dependent inhibition of LPL secretion. The secretion was inhibited 30% by 1 ng/ml of LPS, 65% by 50 ng/ml, and 68% by 500 ng/ml. LPL activity was not inhibited by LPS at these concentrations when added directly to the assay mixture. Endotoxin has been shown to induce macrophage synthesis and secretion of cachectin, a protein of Mr = 17,000; inhibition of 3T3-L1 cell LPL synthesis and secretion by the conditioned medium of endotoxin-treated macrophages is mediated by the binding of cachectin to high affinity cell surface receptors (16). Whether the inhibition of macrophage LPL synthesis and secretion observed in the present study is mediated by the binding of cachectin to such receptors on the cell surface of macrophages remains to be determined.

The present study indicates that synthesis and secretion of LPL is a regulated process that may be part of the macrophage response to inflammation. This response coincides with the thioglycollate-induced increase in secretion

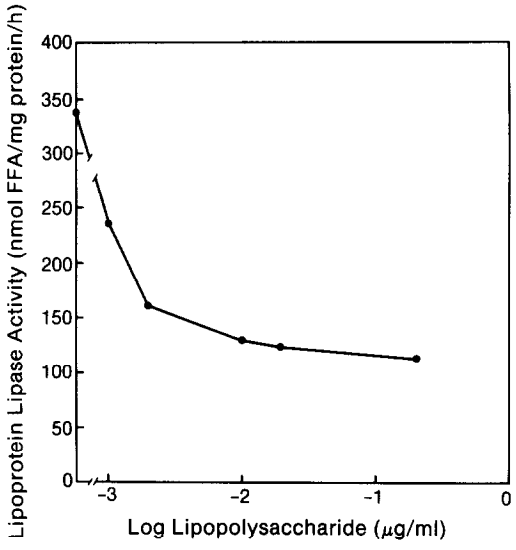

Fig. 3. The effect of lipopolysaccharide (LPS) on the secretion of LPL by thioglycollate-elicited mouse peritoneal macrophages. Cells were maintained in serum free α-MEM containing the indicated concentrations of LPS. After 24 h, the media were collected and assayed for LPL activity. Each point represents the mean of duplicate determinations of enzymatic activity from two 60-mm dishes.

of the neutral proteinases, elastase (17), collagenase (18), and plasminogen activator (19), observed in mouse peritoneal macrophages. In contrast, neutral cholesteryl ester hydrolase, a cytoplasmic enzyme, is apparently unaffected by the inflammatory process. If the macrophages residing in the subendothelial space of the arterial wall have characteristics similar to the peritoneal macrophages reported here, an inflammation-induced increase in the secretion of LPL may result in the hydrolysis of triglyceride-rich lipoproteins. The resulting remnant particles, which are enriched in cholesteryl esters, could then be taken up by macrophages and smooth muscle cells.

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