***Brief Definitive Report***

# EFFECTS OF ACTIVATION ON LIPOPROTEIN LIPASE SECRETION BY MACROPHAGES

## Evidence for Autoregulation

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Lipid-laden macrophages are a hallmark of atherosclerotic lesions (1). Over the last several years the mechanisms responsible for lipid accumulation in macrophages have been intensively studied. Macrophages possess receptors for low density lipoproteins (LDL), but exposure of macrophages to high concentrations of LDL does not result in lipid accumulation, due to normal homeostatic mechanisms (2). In contrast to normal LDL, chemically modified LDL is taken up by specific receptors (scavenger receptors) and causes massive cholesteryl ester accumulation in macrophages (2). Also, massive cholesteryl ester and/or triglyceride accumulation occurs when macrophages are exposed to a variety of different triglyceride-rich lipoproteins (2). Besides the receptor-mediated uptake of lipoproteins, macrophages can accumulate lipids through the actions of the secreted enzyme lipoprotein lipase (LPL) (3). A significant portion of the lipid accumulating in macrophages following exposure to triglyceride-rich lipoproteins appears to be due to LPL (4).

Macrophages exist in a variety of functional states in vivo, ranging from unstimulated resident cells, to inflammatory cells, to fully activated cells. The state of activation of macrophages can be characterized by a number of biochemical markers (5). Of the secretory products affected by activation, it is interesting that the secretion of apolipoprotein E by macrophages is markedly suppressed in cells activated by endotoxin, pyran copolymer, *Corynebacterium parvum* or Bacillus Calmette-Guerin (6, 7). The present studies were undertaken to examine the effects of activation on the ability of macrophages to secrete LPL.

## Materials and Methods

*Mouse Peritoneal Macrophage Collection and Cell Culture.* Mouse peritoneal macrophages were obtained in different states of activation as follows. Resident unstimulated macrophages were obtained from Swiss-Webster mice by peritoneal lavage with 10 ml of PBS. Inflammatory macrophages were obtained from mice that had been injected 1–3 d previously with 2 ml of thioglycolate medium (Difco Laboratories, Detroit, MI). Primed macrophages were obtained by injection of 100 μg of pyran copolymer (a gift of R. A. Corrano and Adria Laboratories, Plain City, OH) 5 d before the mice were killed (8).

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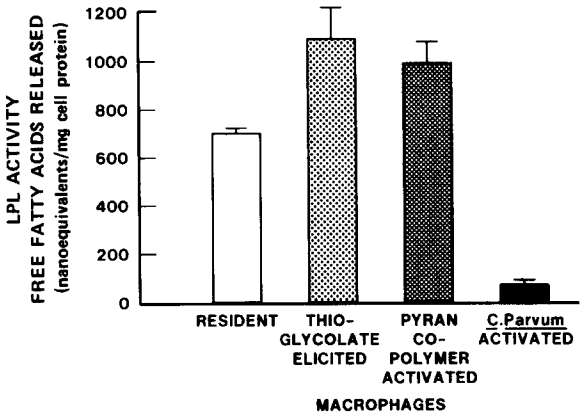


FIGURE 1. LPL secretion by macrophages at different states of activation. Mouse resident peritoneal macrophages and macrophages elicited by thioglycolate, pyran copolymer, and *C. parvum* were obtained as described in Materials and Methods. Secreted LPL was measured after a 6-h incubation of the cells in DMEM/BSA.

Fully activated macrophages were obtained by injection of 700 μg of *C. parvum* (Burroughs Wellcome Co., Research Triangle Park, NC) 7 d before harvest (8). The peritoneal exudative cells were plated and then washed 2 h later to remove the nonattached cells. >99% of the attached cells in each experimental group were macrophages as judged by light microscopy and phagocytosis of latex particles or antibody-coated RBCs. LPL activity was assayed either immediately or on the following day (similar results were obtained). The cells were maintained in DMEM with 10% FCS for up to 1 wk. TA1 adipocytes (a kind gift of G. Ringold, Stanford Univ.) were plated in Basal Medium Eagle containing 10% FCS. When the cells reached confluence, they were treated with 12.5 mM indomethacin to induce lipid storage and terminal differentiation to adipocytes.

*Lipoprotein Lipase Assay.* Secretion of LPL was measured by incubating the macrophages for 6 h with 1 ml of DMEM containing 0.5% fatty acid–free BSA (Armour Pharmaceutical, Kankakee, IL). 5 min before the end of the incubation, 1 U of heparin (Elkins-Sinn, Cherry Hill, NJ) was added to each well to release the membrane-bound enzyme. Aliquots of medium were then immediately assayed for LPL activity by incubation at 37°C with a phospholipid-stabilized emulsion of [$^3$H]triolein containing final concentrations of 2.5 mM triolein, 2.4% BSA, and 0.2 M Tris, pH 8.5, 0.1 M NaCl, and 8% heated horse serum in a final volume of 0.25 ml. The assay was stopped by adding 3.2 ml of heptane/methanol/chloroform (180:250:230), containing 20 μg/ml oleic acid, followed by 1 ml of 0.1 M sodium borate/carbonate, pH 10.5. After the tubes were vortexed and centrifuged, the supernatants were counted in a Beckman scintillation counter (9). Activity is expressed as nanoequivalents of free fatty acids released per hour per milligram of cell protein.

*Inhibition of LPL Secretion.* After washing with DMEM, thioglycolate-elicited macrophages were incubated overnight in control DMEM/FCS, or in DMEM/FCS that had been conditioned by *C. parvum*–activated macrophages for 48 h, or in DMEM/FCS containing tumor necrosis factor (TNF) (a gift of T. White and Cetus Corp., Emeryville, CA). On the following day, the media were removed, the cells were washed with DMEM/BSA, and then incubated for 6 h in DMEM/BSA. After the addition of 1 U/ml heparin for 5 min, the amount of LPL secreted into the media was assayed as described above.

## Results

*LPL Secretion by Macrophages in Different States of Activation.* Fig. 1 shows the amounts of LPL activity secreted by the various macrophages. As reported

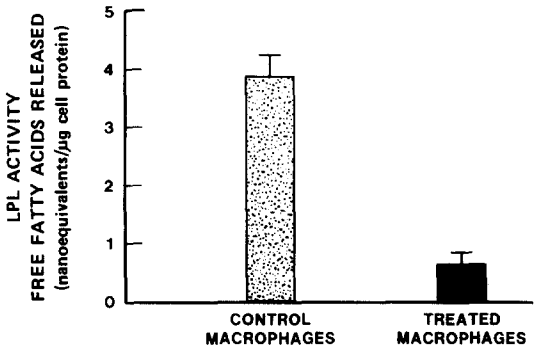


FIGURE 2. Effect of conditioned media on LPL secretion by inflammatory macrophages. After washing them with DMEM, thioglycolate-elicited macrophages were incubated overnight in control DMEM/FCS or DMEM/FCS that had been conditioned by *C. parvum*–activated macrophages for 48 h. On the following day, the media were removed; the cells were washed with DMEM/BSA, and the amount of LPL secreted into the media over 6 h was assessed.

previously (3), resident macrophages secrete significant amounts of LPL. LPL secretion was increased 20–40% in inflammatory macrophages elicited with thioglycolate and in macrophages primed with pyran copolymer. In contrast, macrophages activated with *C. parvum* secreted <10% of the LPL activity observed in resident cells. When cultured for up to 7 d, *C. parvum*–elicited macrophages continued to secrete only low levels of LPL, while resident, thioglycolate-elicited, and pyran copolymer–elicited macrophages maintained or increased their LPL secretion during this time (data not shown).

*Inhibition of LPL Secretion by Conditioned Medium from Activated Macrophages.* Because activated macrophages secrete a number of cytokines, which act as immunoregulators, the possibility that activated macrophages displaying low LPL secretion could modulate LPL secretion by other macrophages was investigated. Macrophages elicited by thioglycolate were exposed overnight to conditioned medium from *C. parvum*–elicited macrophages. The thioglycolate-elicited macrophages were then washed, fresh medium was added, and LPL secretion was assessed. Fig. 2 shows that conditioned medium from *C. parvum*–elicited macrophages caused an 80% inhibition of LPL secretion by thioglycolate-elicited macrophages. This inhibition of LPL secretion could be observed after 30 min of exposure of inflammatory macrophages to conditioned media from *C. parvum*–elicited macrophages; maximal inhibition was seen after 6–12 h of exposure (Fig. 3*A*). The ability of conditioned media from *C. parvum*–elicited macrophages to inhibit LPL secretion by inflammatory macrophages was dose dependent (Fig. 3*B*). Half-maximal inhibition of LPL secretion was observed with 1% (vol/vol) conditioned medium.

*LPL Inhibitory Factor and TNF.* To determine whether the factor(s) that is secreted by *C. parvum*–activated macrophages and which regulates LPL secretion by macrophages was TNF/cachectin, the effects of recombinant DNA–derived TNF were compared to the effects of conditioned medium from *C. parvum*–elicited macrophages for their abilities to influence LPL secretion by inflammatory macrophages and by the stable adipogenic cell line TA1 (Table 1). In macrophages, TNF had no effect on LPL secretion, while conditioned medium

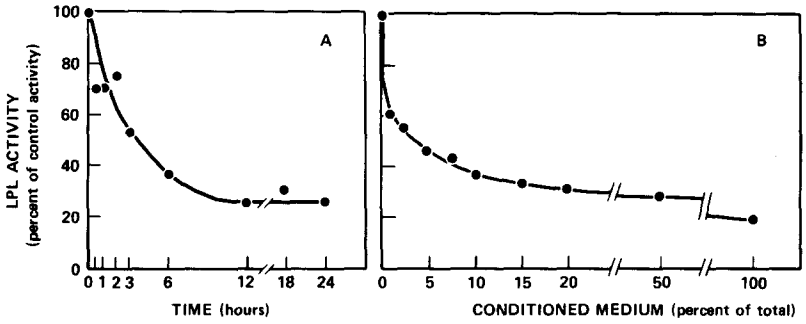


FIGURE 3. Time course (*A*) and dose response (*B*) of inhibition of LPL secretion by conditioned medium. Thioglycolate-elicited macrophages were incubated for various times (*A*) or with varying amounts (*B*) of DMEM/FCS that had been conditioned by *C. parvum*—activated macrophages for 48 h. After the incubation, the cells were washed with DMEM/BSA, and the amount of LPL secreted into the media over 6 h was determined.

TABLE I

*Regulation of LPL Secretion in Macrophages and Adipocytes*

| Treatment | LPL secretion by: | | | |
|---|---|---|---|---|
| | Inflammatory macrophages | | TA1 adipocytes | |
| | LPL activity* | Percent of control | LPL activity | Percent of control |
| Control | 0.63 ± 0.03 | 100 | 0.62 ± 0.08 | 100 |
| Conditioned medium | 0.22 ± 0.04 | 35 | 0.47 ± 0.02 | 76 |
| TNF | 0.62 ± 0.01 | 98 | 0.06 ± 0.01 | 10 |

Thioglycolate-elicited macrophages and TA1 adipocytes were incubated for 18 h with conditioned media from *C. parvum*–activated macrophages or with 100 ng/ml rTNF. After incubation, the cells were washed with DMEM/BSA and incubated with DMEM/BSA for 6 h. After the addition of 1 U/ml heparin for 5 min, the amount of LPL secreted into the media was assayed in triplicate dishes.

* LPL activity is expressed as nanoequivalents of free fatty acids released per microgram of cell protein. Results represent the mean ± SD.

from *C. parvum*–activated cells caused a 65% inhibition. In contradistinction, TNF inhibited LPL secretion by TA1 adipocytes by >90%, while conditioned medium from *C. parvum*–elicited macrophages decreased LPL secretion by 25%. In other experiments, IL-2 and IFN ($\alpha$ and $\beta$) were found to have no effects on macrophage LPL secretion (data not shown).

*Preliminary Characterization of LPL Inhibitory Factor.* Dialysis of the conditioned medium against DMEM with a 4,000 $M_r$-cutoff dialysis membrane at 4°C for 12 h resulted in almost no loss of the LPL-inhibitory effect. Heat treatment of the conditioned medium at 60°C for 30 min resulted in only a slight decrease in LPL inhibition, but when the conditioned medium was heated at 100°C for 3 min, the LPL-inhibitory effect of the medium was lost completely.

## Discussion

Macrophages obtained from several different species (mouse, rabbit, and human monocyte–derived) have been found (3) to secrete LPL. Although the secretion of LPL by macrophages probably has little impact on overall lipoprotein

metabolism, it has an important function in the catabolism of triglyceride-rich lipoproteins by macrophages. LPL contributes to the accumulation of lipids in macrophages from triglyceride-rich lipoproteins by at least two mechanisms. First, the triglycerides in lipoproteins are hydrolyzed by LPL to free fatty acids, which are then taken up and reesterified into cellular triglyceride (4). Second, following the hydrolysis of lipoprotein triglyceride by LPL, the remnant particles formed are avidly taken up by macrophages via receptor-mediated processes (4). While a physiological role of LPL in lipid and lipoprotein metabolism by macrophages has been defined, little information is available concerning the regulation of LPL secretion by macrophages. In this regard, our experiments show that the secretion of LPL by macrophages is dramatically influenced by the state of activation of the cell. Macrophages that had been elicited by thioglycolate or pyran copolymer secreted greater amounts of LPL than resident (unstimulated) macrophages. In contrast, macrophages elicited by *C. parvum* secreted little, if any, LPL. The low levels of LPL found in the media of *C. parvum*–elicited macrophages were not due to a block in the secretion of LPL, because no intracellular LPL could be detected (data not shown).

Other researchers (10) have previously reported that macrophages that had been treated with endotoxin or elicited by *C. parvum* or Bacillus Calmette-Guerin secrete a factor that inhibits LPL secretion by adipocytes. This factor has been purified and identified as TNF/cachectin (11). In our experiments, conditioned medium from macrophages elicited by *C. parvum* inhibited LPL secretion by inflammatory macrophages in a time- and dose-dependent fashion. This factor was clearly not TNF, since pure rTNF had no effect on LPL secretion by macrophages but caused almost complete inhibition of LPL secretion by cultured adipocytes. Therefore, these findings support the notion that *C. parvum*–activated macrophages secrete a mediator, distinct from TNF, that is capable of regulating the ability of surrounding macrophages to secrete LPL. In preliminary experiments, this mediator appears to be thermolabile and to have a $M_r$ >4,000.

In addition to the changes in the secretion of LPL described in this paper, activation of macrophages has been reported (6, 7, 12) to cause other alterations in macrophage lipid and lipoprotein metabolism. Conditioned medium from activated lymphocytes has been reported (12) to suppress the expression of both LDL and scavenger receptor activities in human monocyte-derived macrophages. Additionally, the synthesis and secretion of significant quantities of apolipoprotein E have been noted in resident and thioglycolate-elicited macrophages, but markedly inhibited in activated macrophages obtained from mice treated with pyran copolymer or *C. parvum* (7). Since LPL secretion is increased in pyran copolymer–elicited macrophages, these findings suggest that the secretion of LPL and apolipoprotein E are not coordinated during sequential activation. These results highlight the diverse effects of activation in lipid and lipoprotein metabolism. Furthermore, many of the changes in lipid metabolism may be caused by mediators secreted by activated macrophages.

## Summary

Lipoprotein lipase (LPL) activity was measured in the media of cultured mouse peritoneal macrophages that were isolated after the intraperitoneal injection of

inflammatory agents in order to yield a variety of states of activation. Fully activated macrophages obtained from *Corynebacterium parvum*–injected mice secreted very low levels of LPL when compared to unstimulated macrophages, while inflammatory and primed macrophages had increased LPL secretion. When inflammatory macrophages were incubated with conditioned medium obtained from fully activated macrophages, LPL secretion decreased in a time- and dose-dependent fashion. The factor(s) secreted by fully activated macrophages that inhibited LPL secretion was shown to be thermolabile and distinct from tumor necrosis factor. These results demonstrate that activation dramatically alters macrophage LPL secretion.

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