Effects of Activation on Lipid and Lipoprotein Metabolism in Murine Macrophages

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The effects of activation on lipid and lipoprotein metabolism were examined in resident murine macrophages, inflammatory cells elicited by thiglyglycate, primed cells elicited by pyran copolymer, and activated cells elicited by Corynebacterium parvum. Low density lipoprotein receptors were reduced by 70%, while scavenger receptors were reduced 60% in activated cells. Basal cholesteryl ester and triglyceride synthesis were increased fourfold in activated cells, whereas the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase was high in resident cells and progressively declined by >60% in activated cells. Activities of neutral cholesteryl esterase and neutral triglyceride lipase were increased two- to fourfold in inflammatory, primed, and activated macrophages. These results demonstrate the diverse changes in lipid and lipoprotein metabolism that occur with activation and emphasize how the behavior of macrophages in atherosclerotic lesions can be altered by activation. (Arteriosclerosis 10:8–16, January/February 1990)

Atherosclerotic lesions are composed of prominent lipid-laden foam cells, which appear to be predominantly derived from macrophages.1,2,3 The metabolic pathways leading to lipid accumulation in macrophages have been intensively investigated. Although macrophages express low density lipoprotein (LDL) receptors, transport of LDL does not lead to lipid accumulation in macrophages because the LDL receptor is down-regulated in response to an increase in cellular cholesterol.4,5 Nevertheless, several lipoproteins, including cholesterol-rich, β-migrating very low density lipoprotein (β-VLDL), normal VLDL, VLDL from hypertriglyceridemic subjects, and chylomicron remnants, are recognized by the LDL receptor and cause lipid accumulation in macrophages.6-15 Some evidence16-17 suggests that lipoprotein lipase secretion by macrophages facilitates the lipid uptake of some of these triglyceride-rich lipoproteins.16,17 In addition, macrophages possess a scavenger receptor that recognizes a variety of chemically or biologically modified lipoproteins.4,5,20,21 In contrast to LDL receptors, cellular cholesterol content does not affect the expression of scavenger receptors.4,5 Once bound to receptors on the macrophage cell surface, lipoproteins are rapidly endocytosed and delivered to lysosomes where the protein and lipid moieties are hydrolyzed.4,22 The free cholesterol released is either re-esterified for storage by the action of acyl coenzyme A: cholesterol acytranferase (ACAT) or excreted from the cell.22,23 Finally, the lipids stored within the cell are continuously hydrolyzed by the action of cytoplasmic lipases.24,25 Since macrophages are immunocompetent cells that can exist in vivo within a spectrum of functional states of activation, which range from unstimulated or resident cells that display low levels of metabolic and phagocytic activities to fully activated cells that display new biochemical features,26,27 it would not be surprising for activation to be associated with changes in lipid homeostatic pathways. Indeed, a number of alterations have been observed to occur in macrophage lipid and lipoprotein metabolism with activation. Exposure of human monocyte-macrophages to conditioned media from activated lymphocytes has been reported to decrease LDL and scavenger receptor activities.28,29 In addition, the secretion of apoprotein E by murine macrophages is suppressed by activation with several different agents.30,31 We have reported that the secretion of lipoprotein lipase is increased in inflammatory murine macrophages and markedly suppressed in fully activated cells.32 Given these alterations, the present studies were undertaken to assess the effects of activation on lipid and lipoprotein metabolism in murine macrophages.

Methods

Macrophages

Macrophages were obtained from Swiss Webster mice (25 to 32 g) by peritoneal lavage with 10 ml of phosphate-buffered saline (PBS) pH 7.4 as described previously.32 Unstimulated or resident macrophages were obtained by peritoneal lavage without prior treatment of the mice. Inflammatory macrophages were obtained 3 to 4 days...
after the intraperitoneal injection of 2 ml of thioglycolate broth (Difco Laboratories, Detroit, MI); primed macrophages, 5 days after the injection of 100 μg of pyran copolymer (a gift of R.A. Corrano and Adria Laboratories, Plain City, OH); and fully activated macrophages, 7 days after the injection of 700 μg of *Corynebacterium parvum* (Burroughs Wellcome, Research Triangle Park, NC). The peritoneal exudate cells were plated in 12-well multiwells at a concentration of 2 x 10^6 cells/well (inflammatory cells were plated at 1 x 10^6 cells/well) in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). After 2 hours, the cells were washed three times with DMEM, then maintained overnight in DMEM/FBS. As judged by light microscopy and phagocytosis of latex particles or antibody-coated red blood cells (RBCs), more than 99% of the attached cells in each group were macrophages.

**Lipoproteins**

Blood was drawn from normal fasting human subjects and was mixed with a preservative to yield final concentrations of 3 mM ethylenediaminetetraacetic acid (EDTA), 10 mM raeino capric acid, 60 μM chloramphenicol, 1 mM dithionitrobenzoic acid, and 60 μg/ml gentamicin. LDL (d=1.025 to 1.055) was isolated by sequential ultracentrifugation in KBr in a 50.2 Ti rotor at 40 000 rpm for 24 hours. The LDL was washed by recentrifugation at d=1.063, was extensively dialyzed against PBS, and was sterilized by filtration. The purity of LDL and its apoprotein composition were assessed by electrophoresis on 0.5% agarose and by polyacrylamide gel electrophoresis. Acetylated LDL (AcLDL) was prepared as described previously. Lipoproteins were iodinated as described previously. Free 125I was removed by extensive dialysis against PBS. More than 99% of the lipoprotein-bound radioactivity was precipitable by 10% (wt/vol) trichloroacetic acid, and less than 5% of the radioactivity was extractable into chloroform/methanol (2:1). Specific activities of the 125I-lipoproteins ranged from 150 to 500 cpm/ng protein. Lipoprotein-deficient serum (LPDS) was prepared from fetal bovine serum by ultracentrifugation at a density of 1.215 g/ml.

**Cell-mediated Cytolysis**

Cell-mediated cytosis was assessed by the ability of macrophages to lyse Epstein-Barr virus-transformed mouse L cells (a gift of Dr. Kenneth Melmon, Stanford University, Stanford, CA). The L cells were labeled by incubation with 200 μCi of 51Cr in 100 μl PBS for 30 minutes at 37°C. The cells were washed five times with sterile saline and were suspended in 2 ml of PBS. Several concentrations of labeled L cells were incubated with macrophages in DMEM. After 24 hours, the culture medium was removed and centrifuged at 16 000 g for 30 seconds, and aliquots of the supernatant were counted. The macrophages were washed three times with DMEM, were dissolved in 0.5 ml of 0.5 N NaOH, and were counted. The percentage of cells lysed was calculated as the ratio of the counts per minute (cpm) in the culture medium (release) and in the macrophages (re-uptake) divided by the total cpm added to the walls. Cell lysis was corrected for spontaneous leakage of 51Cr from the target cells incubated in wells not containing macrophages.

**Lipoprotein Binding**

Macrophages were washed with DMEM and were then incubated in duplicate in 0.5 ml of DMEM with 2% bovine serum albumin (BSA), containing various concentrations of 125I-lipoproteins (2 to 70 μg/ml) at 37°C. Nonspecific binding was determined in parallel incubations with a 20-fold excess of unlabeled lipoproteins or with dextran sulfate (10 μg/ml). In addition, parallel incubations were carried out in wells that contained no cells. After 4 hours, the multiwells were placed on ice, the media were aspirated, and the cells were washed three times with 1 ml of ice-cold 0.15 M NaCl, 0.05 M Tris HCl, 5 mM CaCl₂, and 2 mg/ml BSA, followed by two washes with 2 ml of the above buffer without BSA. The cells were then dissolved in 1 ml of 0.5 M NaOH, an aliquot was taken for cell protein, and the radioactivity in the remainder was determined in a gamma counter. Cell numbers were determined in parallel wells incubated in the absence of radioactivity. The data obtained represent the 125I-lipoproteins that are surface bound and/or internalized and are reported as nanograms of lipoprotein bound/10^6 cells. The specific binding of each lipoprotein was transformed by Scatchard plot, and the dissociation constants (Kd) and the total binding capacities (Bo) were obtained by a best-fit linear regression.

**Assay of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity**

The activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis, was assayed as described previously. Macrophages were placed on ice, were washed three times with ice-cold PBS, were scraped from the dish with a rubber policeman into PBS, were centrifuged (700 g, 5 minutes, 4°C), were resuspended in 150 μl of homogenizing buffer (0.1 M KPO₄, 0.25 mM sucrose, 10 mM EDTA, 30 mM KCl, 0.25% Kyro EOB, 5 mM dithiotreitol, pH 7.4), and were disrupted by sonication. After centrifugation (1000 g, 15 minutes, 4°C), the post-nuclear homogenate supernatants were stored at −70°C until the day of the assay. Two different amounts of protein were assayed from each sample to ensure that the assay was in the linear range. Aliquots were preincubated at 37°C for 10 minutes in a final volume of 0.2 ml containing 0.1 M KPO₄, 10 mM EDTA, 5 mM dithiothreitol, 3 mM NADP, 20 mM glucose-6-phosphate, and 7 units glucose-6-phosphate dehydrogenase (pH 7.5). The assay was started by the addition of 88 μM 3-14C-HMG CoA (0.4 μCi/ml) and was stopped after 20 minutes with 30 μl of 5M HCl. An internal standard (2-14C-nevalonate, 40 000 dpm) was added, and the samples were incubated at 37°C for an additional 1 to 2 hours to allow for complete lactonization of the mevalonate. The mevalonolactone was separated by thin-layer chromatography on Whatman Linear K silica gel plates, was developed with toluene/acetone (1:1, vol/vol), was visualized with iodine, was scraped into scintillation vials, and was counted in a toluene-based scintillation cocktail. Blank values were obtained from incubations performed in the absence of added protein. Activities are expressed as nkat/mg protein.
Total Cholesterol Esterification

The incorporation of H-oleate into cholesteryl esters and triglyceride was assayed in macrophages according to previously published procedures.9,10(19) H-oleate acid was added to 50 mM of sodium oleate in 5% fatty acid-free BSA and was thoroughly vortexed. Monolayers of macrophages were incubated in triplicate with the H-oleate-albumin suspension (0.1 mM) in DMEM containing 10% LPDS for 24 hours at 37°C. The incubation was terminated by placing the multitwell on ice, aspirating the media, and washing the cells as described above for the lipoprotein binding assay. The cellular lipids were then extracted twice with 1 ml of hexane/isopropanol (3:2, vol/vol) for 30 minutes at room temperature. After the lipids were extracted, the cells were dissolved in 0.5 N NaOH, and aliquots were taken for protein determination. An internal standard containing cholesteryl-14C-oleate was added to the extracts (20 000 dpm/sample). The extracts were then evaporated to dryness under N2, were resuspended in 100 µl of chloroform, were spotted on silica gel G plates, and were developed in heptane/ethyl ether/ acetic acid (80:20:1). The plates were developed in iodine, and spots were scraped into scintillation vials and counted in 5 ml of Betaphase (WestChem, San Diego, CA) in a Beckman scintillation counter. The results are expressed as nanomoles of H-oleate incorporated into cholesteryl-H-oleate or H-triglyceride/mg cell protein.

Cellular Cholesterol Content

Cellular cholesterol content in macrophages was assayed as described previously.26 Monolayers of macrophages were washed, and the lipids were extracted as described above. After the addition of an internal standard 1,2-H-cholesterol (2.5×104 dpm) and cholesteryl-1,14C- oleate (2.5×104 dpm), cholesteryl esters and unesterified cholesterol were separated on a silicic acid/Cellite (2:1, wt/wt) column by elution with benzene and ethyl acetate, respectively. The fractions were dried under N2 and were resuspended in isopropyl alcohol. An aliquot was taken for dual-labeled liquid scintillation counting for recovery, and the remainder was assayed for cholesterol by fluorometry.38 The results are expressed as nmol/mg cell protein.

Assay of Neutral Cholesteryl Esterase Activity

Neutral cholesteryl esterase was assayed by a modification of the method of Hajjar et al.37 Macrophages were washed three times with ice-cold PBS and were homogenized in 0.25 M sucrose, 1 mM EDTA, and 50 mM Tris HCl (pH 7.0). Neutral cholesteryl esterase activity was then assayed in the postnuclear homogenate. Substrate for the assay was prepared by adding 3.75 µCi of cholesteryl-1-14C-oleate (purified by thin-layer chromatography), 3.8 µmol of phosphatidylcholine, and 0.8 µmol of cholesteryl oleate into 8 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 2 µmol of sodium taurocholate. The solution was vortexed in a 16×100 glass tube and placed in a 40°C water bath, where it was sonicated for 45 minutes with a Branson Sonifier/Cell Disruptor model W-350 on an output setting of 5.0 (50%). The substrate was centrifuged at 3000 rpm for 15 minutes to remove metallic fragments released by the probe, and was stored under N2 at 4°C for up to 4 weeks. Aliquots of homogenate adjusted to 100 µl with buffer were mixed with 150 µl of 0.05% BSA in 100 mM KPO4, pH 7.0. After the addition of 12 µl of substrate, the assay was carried out at 37°C for 60 minutes. The assay contained final concentrations of 6 µM cholesteryl oleate, 23.7 µM phosphatidylcholine, 12.5 µM sodium taurocholate, 0.05% fatty acid-free BSA, and 85 mM potassium phosphate (pH 7.0). The reaction was stopped by addition of chloroform/methanol/ heptane (250:230:180). After the addition of borate/ carbonate buffer (0.1 M, pH 10.5), the tubes were vortexed and centrifuged, and aliquots of the upper phase were taken for liquid scintillation counting in Betaphase in a Beckman scintillation counter. The results are expressed in µkat of cholesteryl oleate hydrolyzed/mg protein.

Assay of Neutral Triglyceride Lipase Activity

Neutral triglyceride lipase was assayed in postnuclear homogenates according to the methods described by Kho et al.38 Substrate was prepared by drying down 15 mg triolein and 6.4 µCi glycerol tri-9,10(19)H-oleate under N2, dissolving it in 1 ml 100% ethanol, rapidly injecting it into 16 ml distilled water, and vortexing vigorously for 1 minute, followed by the addition of 8 ml of HEPES buffer (0.2 M, pH 6.6) and further vortexing. The cells were harvested and homogenized as described above. Aliquots of postnuclear homogenate along with BSA were incubated with substrate at 37°C for 5 to 10 minutes. The reaction was terminated and extracted as described above for the neutral cholesteryl esterase assay. The results are expressed as µkat of triglyceride hydrolyzed/mg protein.

Statistical Analyses

Statistical analyses were performed by analysis of variance and Duncan's multiple-range test to detect differences between populations. Data are expressed as means±SEM.

Results

Characterization of Macrophage Populations

The state of immunological activation of the macrophage populations was assessed by the ability of the macrophages to phagocytize antibody-coated RBCs and to lyse transformed cells. Thioglycolate-elicited (inflammatory), pyran copolymer-elicited (primed), and C. parvum-elicited (activated) macrophages all displayed greater phagocytosis of antibody-coated RBCs than did unstimulated (resident) cells (data not shown). The ability of the macrophages to lyse Epstein-Barr virus-transformed, mouse L cells was assessed to characterize the final stage of activation, cell-mediated cytolysis (Figure 1). Resident and inflammatory macrophages were unable to lyse the transformed cells, while pyran copolymer-elicited macrophages lysed some, and C. parvum-elicited macrophages lysed >90% of the cells. Therefore, based on these two functional markers,26,27 populations of macrophages that displayed characteristics that ranged from resident to inflammatory to fully activated were obtained.
Different States of Activation

When the binding curves were transformed by Scatchard analysis (Figure 2), there were no effects of activation on LDL binding to macrophages in different states of activation. AcLDL binding to macrophages was similar in all macrophages. Likewise, the binding capacity of LDL was similar in resident, inflammatory, and primed macrophages, but decreased by 70% in fully activated cells (35±5 vs. 11±2 ng LDL bound/10⁶ cells, p<0.01). Thus, there were no changes in LDL receptor expression when macrophages became inflammatory; however, a 70% fall in the number of LDL receptors occurred in fully activated macrophages.

The effects of activation on scavenger receptor expression were examined by binding isotherms of AcLDL. When the binding curves were transformed by Scatchard analysis (Figure 3), there were no effects of activation on the affinity of AcLDL for the scavenger receptor. The binding capacity of AcLDL was similar in resident, inflammatory, and primed macrophages, but was decreased by ~60% in fully activated cells (119±27 vs. 42±11 ng AcLDL bound/10⁶ cells, p<0.005). Thus, scavenger receptor expression, like the expression of LDL receptors, was relatively invariant until the final stage of activation, when there was a large decrease in the number of receptors.

Cellular Lipid Synthesis in Macrophages in Different States of Activation

To determine if activation influenced basal cholesteryl ester or triglyceride synthesis, the incorporation of ³H-oleate into cellular lipids in the absence of added lipoproteins was assessed. As shown in Figure 4, basal cholesteryl ester synthesis was low and unchanged in resident, inflammatory, and primed macrophages, but was increased ~fourfold in fully activated (2.1±0.5 vs. 8.6±2.1 nmol oleate incorporated/mg cell protein, p<0.01). Since the rate of cholesteryl ester synthesis is dependent on the concentration of cellular free cholesterol, these results suggest that there may be an increase in the regulatory pool of cellular free cholesterol in activated macrophages. Basal triglyceride synthesis displayed a similar pattern, with unchanged rates of ³H-oleate incorporation into triglyceride in resident, inflammatory, and primed cells, and ~threefold increase (~p<0.05) in activated macrophages.

Since activation affected cholesteryl ester synthesis, the effects of activation on cholesterol synthesis were assessed by measuring the activity of HMG CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis (Figure 5). HMG CoA reductase activity was highest in resident cells and fell by 60% in inflammatory macrophages.
The present studies have documented a number of alterations in lipid and lipoprotein metabolism occurring with activation in murine macrophages. Each of the activities examined was altered at some stage of activation. Macrophages possess at least two distinct receptors that recognize lipoproteins: the LDL receptor, which recognizes apoprotein B- and E-containing lipoproteins, such as LDL, β-VLDL, and triglyceride-rich lipoproteins, and the scavenger receptor, which recognizes negatively charged particles, such as chemically modified (acetylation, acetoxylation, melondialdehyde treatment), or biologically modified cells.

**Table 1. Cellular Cholesterol Content in Macrophages in Different States of Activation**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Unesterified cholesterol</th>
<th>Cholesteryl ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resident</td>
<td>137.5±27.1</td>
<td>7.1±1.9</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>122.9±12.8</td>
<td>7.9±0.8</td>
</tr>
<tr>
<td>Primed</td>
<td>127.3±10.8</td>
<td>8.8±1.3</td>
</tr>
<tr>
<td>Activated</td>
<td>216.4±23.9</td>
<td>27.2±2.6</td>
</tr>
</tbody>
</table>

Values are given as nmol/mg cell protein and are the means±SEM of three experiments of resident macrophages, 12 of inflammatory cells, three of primed cells, and seven of activated cells.

Macrophages were obtained as described in the legend to Figure 1, and cellular cholesterol content was determined as described in the Methods section.  

**Neutral Cytoplasmic Lipases in Macrophages in Different States of Activation**

Macrophages contain a neutral cholesteryl esterase that hydrolyzes cytoplasmic cholesteryl esters. In addition, macrophages contain a neutral triglyceride lipase that can hydrolyze cytoplasmic triglyceride and which appears to differ biochemically from neutral cholesteryl esterase. When neutral cholesteryl esterase activity was measured in macrophages at different states of activation (Figure 6A), there was a two- to threefold increase in activity in inflammatory, primed, and activated cells when compared to resident macrophages. Similarly, neutral triglyceride lipase activity (Figure 6B) was increased four- to fivefold in inflammatory, primed, and activated macrophages. Thus, inflammatory, primed, and activated macrophages had increased activities of enzymes, which mobilize cytoplasmic cholesteryl esters and triglyceride.

**Discussion**

The present studies have documented a number of alterations in lipid and lipoprotein metabolism occurring with activation in murine macrophages. Each of the activities examined was altered at some stage of activation. Macrophages possess at least two distinct receptors that recognize lipoproteins: the LDL receptor, which recognizes apoprotein B- and E-containing lipoproteins, such as LDL, β-VLDL, and triglyceride-rich lipoproteins, and the scavenger receptor, which recognizes negatively charged particles, such as chemically modified (acetylation, ace-toxylation, melondialdehyde treatment), or biologically modified cells.
modified (lipid peroxidation) lipoproteins. Expression of both lipoprotein receptors was invariant until the latter stages of activation, when they were decreased. Thus, the number of LDL receptors was similar in resident, thioglycolate-elicited, and pyran copolymer-elicited macrophages, but a 70% to 80% decrease was found in *C. parvum*-elicited cells. Similar to LDL receptors, the number of scavenger receptors was identical in resident, thioglycolate-elicited, and pyran copolymer-elicited macrophages and decreased by 60% in *C. parvum*-elicited cells. The mechanisms responsible for changes in lipoprotein receptor expression are unclear. Because macrophages secrete a variety of enzymes and reactive oxygen species, it is possible that the lipoproteins were modified during the experimental incubations to alter their recognition by receptors; however, this explanation seems unlikely since lipoprotein oxidation and significant modification do not appear to occur under the experimental conditions used in these studies. Furthermore, since the receptor binding studies measured both surface bound and internalized lipoproteins, it is possible that the magnitude of the decrease in lipoprotein receptor expressions was exaggerated by the increase in membrane endocytosis that occurs in activated cells, yet this does not appear sufficient to fully explain the changes. It is possible that the increase in cellular free cholesterol that occurs with activation could be responsible for the decreases in LDL receptor expression, since the number of LDL receptors is normally decreased by increases in cellular free cholesterol. However, this does not explain the fall in scavenger receptors, since their expression is not affected by cellular cholesterol. Therefore, it is likely that alterations in the number of scavenger receptors are mediated by other mechanisms.

The basal syntheses of cholesteryl esters and triglycerides were unchanged in resident, thioglycolate-elicited, and pyran copolymer-elicited macrophages, but unlike lipoprotein receptor expression, were increased three- to fourfold in *C. parvum*-elicited cells. In contradistinction, HMG CoA reductase activity (and, hence, cholesterol synthesis) progressively declined from resident to thioglycolate-elicited to pyran copolymer-elicited and to *C. parvum*-elicited macrophages until <20% of the activity was observed in these activated cells. On the one hand, the alterations in HMG CoA reductase activity and basal cholesteryl ester synthesis are consistent with increases in regulatory pools of cellular free cholesterol, since free cholesterol decreases HMG CoA reductase activity while increasing cholesteryl ester synthesis. This possibility is supported by the marked increase in the amounts of cellular-free cholesterol and cholesteryl esters found in *C. parvum*-elicited macrophages. Even though no changes were observed in the amounts of total cellular free cholesterol or cholesteryl esters in thioglycolate-elicited or pyran copolymer-elicited macrophages, it is possible that a specific regulatory pool of free cholesterol was increased in these cells. Furthermore, the fall in HMG CoA reductase activity in thioglycolate-elicited and pyran copolymer-elicited cells at a time when LDL receptor expression and cholesteryl ester synthesis are unchanged is compatible with the greater sensitivity of HMG CoA reductase to changes in cellular cholesterol. On the other hand, triglyceride synthesis is not known to be regulated by cellular cholesterol content but is affected by free fatty acid levels. This suggests that the increase in cellular triglyceride synthesis in *C. parvum*-elicited macrophages is not due to changes in cellular cholesterol but is secondary to other mechanisms, such as increases in precursors (free fatty acids) or direct stimulation of enzymatic activity. Moreover, it implies that the alterations in HMG CoA reductase activity and basal cholesteryl ester synthesis might also be secondary to specific events related to activation, which are not mediated by changes in cellular cholesterol content. Such noncholesterol-mediated changes might encompass enzymatic regulation mediated by phosphorylation cascades after the activation of protein kinase C or other kinases, or mediated by changes in regulatory proteins (possessing either inhibitory or stimulatory functions), as exemplified by the results of neutral triglyceride lipase activity.
fied by the description of the cellular inhibitory protein which regulates ACAT activity in macrophages.23

In contrast to these patterns of changes, the activities of neutral cholesteryl esterase and neutral triglyceride lipase were lowest in resident cells and increased two- to fourfold in thioglycollate-elicited, pyran copolymer-elicited, and C. parvum-elicited macrophages. The mechanisms responsible for these changes are unclear, but are probably not due to changes in cellular lipid because cellular lipid content is not known to influence these activities. Since the major regulation of neutral cholesteryl esterase occurs via phosphorylation,24 it is tempting to speculate that the changes in its activity are due to increases in protein kinase C activity, which occur during activation.41 While a similar mechanism might regulate the activity of neutral triglyceride lipase, other mechanisms might be important since it has recently been suggested that a precursor of lysosomal acid triacylglycerol hydrolase is responsible for the neutral triglyceride lipase activity detected in macrophages.43

Given the diverse alterations in lipid and lipoprotein metabolism associated with activation, it is unlikely that a single mechanism is responsible for all the changes observed. Although changes in regulatory pools of free cholesterol could be responsible for some of these alterations, it is also possible that they occur independently. This appears to be the case for apoprotein E secretion by macrophages, which is regulated by cellular free cholesterol in resident and inflammatory cells, but not in activated macrophages.6,44 Therefore, some of the alterations observed might be due directly to specific events related to activation or might be secondary to other changes occurring simultaneously; the exact mechanisms remain to be elucidated.

Activation of macrophages has been reported by other investigators to cause changes in lipid and lipoprotein metabolism that is consistent and, in some instances, inconsistent with our findings. Using similar methods to elicit mouse macrophages, Imber et al.45 observed a decrease in mannose-specific binding in the late stages of activation but did not find any consistent changes in scavenger receptors using maleylated-BSA to examine scavenger receptor activity. It has been reported that maleylated-BSA binds not only to scavenger receptors, but also to specific maleylated-BSA receptors.46 The recognition of maleylated-BSA by two distinct receptors might have contributed to the inability of these authors to detect the decrease presently found in scavenger receptors in the late stage of activation. Fogelman et al.46 found that exposure of human monocyte-macrophages to conditioned media from concanavalin A-stimulated lymphocytes decreased the expression of LDL and scavenger receptors, as well as HMG CoA reductase activity. Moreover, activation of human monocyte-macrophages with bacterial endotoxin has been reported to suppress scavenger receptor expression without influencing LDL receptor activity or apoprotein E secretion.47 Consistent with these changes, Hirsch and Mazzone48 reported that glucocorticoids, which suppress macrophage immune functions,49 increased scavenger receptors in human monocyte-macrophages. In contrast, other investigators have reported that interferon-γ, the primary factor responsible for activating macrophages,50 increases scavenger receptors in human monocyte-macrophages.51 The reasons for the discrepancies among these studies are unclear, but some of the conflicts may be due to species differences or differences in experimental design. Nonetheless, the majority of the findings are consistent with our results and suggest that the overall trend of the changes in lipid metabolism observed with activation appears to be toward cell functions that tend simultaneously to retard lipid uptake (decreased LDL receptors, scavenger receptors, and lipoprotein lipase secretion) and cellular lipid synthesis (decreased HMG CoA reductase activity), while promoting lipid mobilization (increased activities of neutral cholesteryl esterase and neutral triglyceride lipase).

The alterations of macrophage lipid and lipoprotein metabolism that occur with activation may have relevance to the atherosclerotic process. Several studies of experimental atherosclerosis have established that, as part of the development of atherosclerotic lesions, monocytes emigrate into the arterial wall, differentiate into macrophages, and are converted to lipid-laden foam cells.1,42 The functions and the state of activation of these lipid-laden macrophages are presently unknown; however, circumstantial evidence supports the possibility that macrophages in atherosclerotic lesions may be activated to some degree. First, cultured endothelial cells,53 macrophages, and smooth muscle cells,54 as well as arteries from cholesterol-fed swine,55 reportedly secrete factors that are chemotactic for monocytes. Additionally, each of these cell types, as well as human atherosclerotic lesions,56,57 have reportedly produced platelet-derived growth factor, a potent mitogen and chemotactic factor that is capable of activating inflammatory cells.58 Second, evidence for the presence of activated cells in atherosclerotic lesions is provided by the finding of the expression of class II histocompatibility antigens, a marker of activation, on T cells59 and smooth muscle cells60 in human atherosclerotic lesions. Thus, depending on the degree and the concomitant alterations of lipid and lipoprotein metabolism which would occur with the activation of macrophages in atherosclerotic lesions, the progression and regression of atherosclerosis would be dramatically influenced.

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