Increased Susceptibility to Activation and Increased Uptake of Low Density Lipoprotein by Cholesterol-Loaded Macrophages

Judith Oiknine and Michael Aviram

Inflammation is associated with macrophage activation, and this process has been shown to occur during atherogenesis. Macrophages (J774A.1) that were activated with either lipopolysaccharide (LPS), zymosan, or phorbol ester demonstrated a 30–35% increased uptake and degradation of low density lipoprotein (LDL) in comparison with nonactivated cells. This phenomenon was also shown for LDL cellular binding, and it resulted in macrophage cholesterol accumulation, as evidenced by cholesterol mass determination and flow cell cytometric analysis. Enhanced uptake of LDL was also obtained with two other types of macrophages: mouse peritoneal macrophages and human monocyte-derived macrophages. In LPS-stimulated macrophages, high density lipoprotein-mediated cholesterol efflux was not different from that shown in nonstimulated cells. Cellular cholesterol synthesis, however, was increased by 25% in the activated macrophages. Macrophage activation, measured as cellular procoagulant activity, was higher in cholesterol-loaded than in unloaded cells. On stimulation of cholesterol-loaded macrophages, cellular uptake and degradation of LDL were increased by 3.3-fold in comparison with nonstimulated cholesterol-loaded cells. Human monocyte-derived macrophages from hypercholesterolemic patients were found to contain 52% more cholesterol mass than macrophages derived from normal healthy donors. These cells demonstrated increased activation (twofold) in response to LPS stimulation and also showed 25% enhanced cellular degradation of LDL. We conclude that activation of macrophages during atherogenesis can lead to foam cell formation, and this mechanism is probably operative in hypercholesterolemic patients. (Arteriosclerosis and Thrombosis 1992;12:745–753)

KEY WORDS • macrophage activation • low density lipoproteins • cholesterol • lipopolysaccharide • hypercholesterolemia

Inflammation processes and atherogenesis are both associated with macrophage activation.1,2 Macrophages play an important role in the immune and the inflammatory body responses, and lipoprotein–antibody immune complexes have been shown to form foam cells.3,4

Monoeyte-derived macrophages can be transformed into foam cells after incubation with modified forms of low density lipoprotein (LDL), including oxidized LDL, but not with native LDL,5 which is taken up via the cholesterol-regulated LDL receptor.6 Activated macrophages secrete many products, including polypeptides, enzymes, growth factors, free radicals, and coagulation factors.7,8 Thus, under conditions of macrophage activation, these secreted substances can possibly affect the atherogenic process. Lipopolysaccharide (LPS) is a glycolipid that is present in the outer membrane of all Gram-negative bacteria, and LPS has been shown to be a potent activator of various cells, including macrophages.9 Stimulation of human monocyte-derived macrophages (HMDMs) with LPS has been shown to enhance cellular uptake of native LDL.9 In the present study, we questioned whether activation of various types of macrophages by various macrophage activators could stimulate cellular uptake of native LDL and whether this phenomenon might be dependent on cellular cholesterol content. Finally, we assessed these questions in HMDMs from hypercholesterolemic patients.

Methods

Materials

LPS lyophilized from Salmonella typhimurium, phorbol 12-myristate 13-acetate, and zymosan were purchased from Sigma Chemical Co., St. Louis, Mo.

Cells

The J774A.1 murine macrophage–like cell line was purchased from American Type Culture Collection, Rockville, Md. The cells were plated at 5 × 10^6 cells per 16-mm dish in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and were fed every 3 days.

Human mononuclear cells were isolated by density gradient centrifugation from blood drawn from fasting normolipidemic subjects (plasma cholesterol <200 mg/dl). In some experiments, cells were obtained from

From the Lipid Research Laboratory, Rambam Medical Center, and The Bruce Rappaport Faculty of Medicine, Technion and Rappaport Institute for Research in the Medical Sciences, Haifa, Israel.

Supported by a grant from the Rappaport Family Institute for Research in the Medical Sciences.

Address for correspondence: Prof. Michael Aviram, DSc, Lipid Research Unit, Rambam Medical Center, Haifa, Israel.

Received January 7, 1992; revision accepted March 2, 1992.
patients with familial hypercholesterolemia (FH, with plasma cholesterol >300 mg/dl).

Twenty milliliters of blood (anticoagulated with 100 units heparin/ml) was layered over 15 ml Ficoll-Paque and centrifuged at 500g for 30 minutes at 23°C. The mixed mononuclear cell band was removed by aspiration, and cells were washed twice in RPMI-1640 culture medium containing 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine. The cells were plated at 10^6 mononuclear cells per 16-mm dish (Primaria, Falcon Labware, Becton Dickinson, Oxnard, Calif.) in the same medium in the presence of 20% fetal calf serum. After 2 hours of incubation at 37°C in 5% CO_2/95% air, nonadherent cells were removed (by three washes with serum-free medium), were placed in a similar fresh medium in the presence of 20% autologous serum, and were fed twice weekly with the same medium. Macrophages were used 7-10 days after plating and contained approximately 5 x 10^5 cells per dish.

Mouse peritoneal macrophages were harvested from the peritoneal fluid of BALB/c mice (weight, 15-25 g) 4 days after intraperitoneal injection into each mouse of 3 ml 24 g/1 thioglycolate in saline. The cells (20 x 10^6 per mouse) were pooled, treated with 3 ml 8.3 g/1 NaCl (pH 7.4), and incubated for 5 minutes at 37°C to remove red blood cell contamination. The cells were washed and centrifuged three times with phosphate-buffered saline at 400g for 10 minutes and then resuspended to 10^7/l in DMEM containing 10% horse serum (heat inactivated at 56°C for 30 minutes). The cell suspension was dispensed into 16-mm plastic dishes and incubated for 2 hours. The dishes were washed once with DMEM to remove nonadherent cells, and the monolayer was then incubated with DMEM under similar conditions for 18 hours before the experiment.11

Lipoproteins

LDL was prepared from human plasma (in 4 mM EDTA) drawn from fasted normolipidemic volunteers. LDL (d=1.019-1.063 g/ml) was prepared by discontinuous density gradient ultracentrifugation in a Beckman VT 50.1 vertical rotor (Beckman Instruments, Fullerton, Calif.) as described previously.12 The lipoprotein was washed at d=1.063 g/ml and dialyzed against 150 mM NaCl, 1 mM EDTA, pH 7.4. LDL was then sterilized by filtration and was used within 2 weeks.

LDL was iodinated by the method of McFarlane as modified for lipoproteins,13 LDL was acetylated by
Cholesterol-Loaded Activated Macrophages and LDL Uptake

747

FIGURE 4. Flow cell cytometry sorting (FACS) analysis of J774A.1 macrophage-like cell line after activation and incubation with low density lipoprotein (LDL). Conditions used were as follows: J774A.1 macrophages were either not activated (1; filled space) or activated with lipopolysaccharide (200 μg/ml) followed by incubation with LDL (100 μg protein/ml) for 18 hours (2; open space). Histodiagram represents relative fluorescence intensity, using 5,000 cells in each case. Area to the right of each number represents the fluorescence intensity in each case.

repeated additions of acetic anhydride to 10 mg/ml LDL diluted 1:1 (vol/vol) with saturated ammonium acetate at 4°C. Acetic anhydride was added at a 40-fold molar excess with regard to total lysines in LDL, and the modification was confirmed by electrophoresis on cellulose acetate at pH 8.6 in barbital buffer.

Lipoprotein Metabolism by Macrophages

LDL cellular degradation was measured after incubation of 125I-LDL (150–250 cpm/ng) with the cells for 5 hours at 37°C. The hydrolysis of LDL protein was assayed in the incubation medium by measurement of trichloroacetic acid–soluble, noniodide radioactivity. Cell-free LDL degradation was minimal and was subtracted from total degradation. The cell layer was washed three times with phosphate-buffered saline and incubated with 0.1N NaOH for 1 hour at room temperature for measurement of cellular protein content. Cholesterol esterification rate was measured as the incorporation of [3H]oleic acid into cellular cholesteryl [3H]oleate as previously described. The cells were incubated in the presence of the lipoproteins for 18 hours at 37°C. During the last 2 hours of incubation, [3H]oleate in complex with albumin (2.7 mM, 83 mmol oleate/mg albumin, 10 μCi/ml) was added to the medium. At the end of the incubation, cellular lipids were extracted with hexane/isopropanol (3:2, vol/vol), and the cholesteryl ester was separated by thin-layer chromatography (TLC) using hexane/ether/acetic acid (130:30:1.5, vol/vol/vol), scraped into vials containing 15 ml scintillation fluid, and counted in a beta scintillation counter.

Cellular contents of unesterified cholesterol, cholesteryl ester, and total cholesterol mass were determined using the ferric chloride assay. Cellular binding of LDL was analyzed at 4°C after 4 hours of LDL incubation with the cells. Cellular cholesterol efflux was determined in J774A.1 macrophages that were loaded with cholesterol by 18 hours of incubation with acetylated LDL (100 μg acetylated LDL protein/ml) and [3H]cholesterol (Amersham Corp., Arlington Heights, III.; 0.5 μCi/ml, 55 Ci/mmol, added in ethanol) for the same time of incubation. At the end of the incubation, the cells were washed three times in serum-free medium and further incubated with high density lipoprotein subfraction 3 (HDL3) (25 μg protein/ml) for 5 hours at 37°C. Radioactivity in the cells and the media (after centrifugation to remove detached cells) was measured. Macrophage cholesterol synthesis was measured in cells after 2 hours of incubation with 1,2-sodium [14C]acetate (1.25 mM, 10 μCi/ml). At the end of the incubation, the cells were washed with phosphate-buffered saline, and the radioactivity incorpo-
CONTROL +LPS 6E-3 CD 0 c 4E-3 3 CL 2E-3

FIGURE 6. Line plots showing effect of macrophage activation on low density lipoprotein (LDL) cellular binding. Panel A: Nonstimulated J774A.1 macrophages (○, control) as well as lipopolysaccharide-stimulated cells (●, 200 μg/ml LPS) were incubated for 4 hours at 4°C with increasing 125I-LDL concentrations in the absence or presence of 20-fold concentrations of nonlabeled LDL. Then the cells were extensively washed in phosphate-buffered saline and dissolved in NaOH (0.1N), and LDL-specific binding was calculated by subtraction of nonspecific from total binding. Nonspecific LDL binding ranged between 5% and 13% of total cellular binding. Panel B: Data from panel A were plotted using LDL binding on the x axis (bound) and the bound to free ratio on the y axis (free represents unbound LDL fraction). The “apparent B_m” is the point of intersection of the lines with the x axis, and the “apparent K_m” is the point of intersection with the y axis. E-3 represents 10^-3.

rated into the cholesterol moiety was determined by TLC.

Procoagulant Activity of Macrophages

The procoagulant activity (PCA) was measured using the prothrombin time assay. The supernatant (50 μl of 5×10^6 sonicated cells) that was obtained after centrifugation (1,000g for 10 minutes) was mixed with 50 μl standard human plasma and incubated for 2 minutes at 37°C. Then 50 μl 0.025 M CaCl_2 solution was added, and the time required for clot formation was determined.

Experimental Procedure

Macrophages were preincubated with LPS (200 μg/ml) for 18 hours at 37°C followed by a wash with serum-free medium before the addition of LDL (25–100 μg protein/ml) for analysis of cellular degradation of the lipoprotein, LDL binding, LDL-mediated stimulation of cellular cholesterol esterification rate, and macrophage cholesterol mass. Flow cell cytometry sorting (FACS) was used for macrophage lipid content analysis. Nile red was incubated for 5 minutes with the cells, and the fluorescence intensity was measured using an excitation wavelength of 480 nm and an emission wavelength of 610 nm.

Statistics

The nonpaired Student’s t test was used. Results are given as mean±SD.

Results

Low Density Lipoprotein Uptake by Activated Macrophages

Macrophage activation with LPS, phorbol ester, or zymosan resulted in a 25–30% increment in cellular degradation of 125I-LDL by the J774A.1 macrophage-like cell line (Figure 1). The extent of macrophage degradation of LDL was LPS-dose dependent and reached saturation at approximately 200 μg LPS/ml (Figure 2). The LDL that was incubated with activated macrophages was not oxidized, as it did not demonstrate

FIGURE 7. Line plots showing lipoprotein competition with 125I-low density lipoprotein (LDL) for cellular degradation in nonstimulated (panel A) and lipopolysaccharide (LPS)-stimulated (panel B) macrophages. J774A.1 cells were incubated with 10 μg protein/ml of 125I-LDL in the absence or presence of increasing concentrations of unlabeled LDL or acetylated (Ac) LDL using nonstimulated cells (panel A) or LPS-stimulated (200 μg/ml) cells (panel B).
FIGURE 8. Line plots showing low density lipoprotein (LDL) degradation by activated mouse peritoneal macrophages (MPMs; panel A) and human monocyte-derived macrophages (HMDMs; panel B). Nonstimulated (○, control) or lipopolysaccharide-stimulated (200 µg/ml) (●, LPS) cells were incubated with increasing 125I-LDL concentrations. After 5 hours, 125I-LDL degradation was determined. Results are representative of three experiments, each performed in triplicate.

the formation of thiobarbituric acid-reactive substances and it migrated on cellulose acetate like native LDL (data not shown). Furthermore, the addition of 25 µM butylated hydroxytoluene to the cells for the 5-hour incubation with 125I-LDL (25 µg protein/ml) resulted in the degradation of 1.3±0.4 and 2.2±0.5 µg protein/ml in control and LPS-stimulated (100 µg/ml) cells, respectively (n=3). These results are similar to those in cells that were incubated without butylated hydroxytoluene (Figure 1), suggesting that LDL oxidation did not take place under our experimental conditions.

Low Density Lipoprotein Concentration Study and Involvement of the Low Density Lipoprotein Receptor

The LDL concentration study revealed that at all concentrations, LPS increased cellular LDL degradation (Figure 5). Unlike native LDL, oxidized LDL (25 µg protein/ml) degradation by J774A.1 macrophages was reduced from 1.70±0.31 in control cells to 0.38±0.02 µg lipoprotein protein/mg cell protein in LPS-stimulated cells. Macrophage binding of LDL was increased by twofold in LPS-stimulated cells (Figure 6A). Linearization of these data suggests that the major

**FIGURE 9.** Bar graph showing effect of macrophage-conditioned medium (MCM) from activated cells on low density lipoprotein (LDL) degradation. J774A.1 cells were preincubated with lipopolysaccharide (LPS; 300 µg/ml) or with MCM that was obtained from cells after 18 hours of incubation with 300 µg/ml LPS. Cells were then washed in serum-free medium and incubated with 125I-LDL (25 µg protein/ml) for 5 hours for LDL degradation analysis. *p<0.01 vs. control (n=3).
TABLE 2. Effect of Macrophage Cholesterol Content on Lipopolysaccharide Stimulation of Low Density Lipoprotein Cellular Degradation

<table>
<thead>
<tr>
<th>LDL concentration (µg protein/ml)</th>
<th>LDL degradation (µg protein/mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Cholesterol-loaded cells</td>
<td>0.66±0.11</td>
</tr>
<tr>
<td>Nonloaded cells</td>
<td>0.24±0.11</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Cholesterol-loaded cells</td>
<td>2.55±0.55</td>
</tr>
<tr>
<td>Nonloaded cells</td>
<td>1.04±0.10</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Cholesterol-loaded cells</td>
<td>3.65±0.65</td>
</tr>
<tr>
<td>Nonloaded cells</td>
<td>1.36±0.16</td>
</tr>
</tbody>
</table>

J774A.1 macrophages were loaded with cholesterol by incubation with 200 µg/ml acetylated low density lipoprotein (LDL) for 18 hours at 37°C. Cellular cholesterol content increased from 25±5 to 65±9 µg/mg cell protein (n=3). Cholesterol-loaded cells and nonloaded cells were activated with 200 µg/ml lipopolysaccharide for 18 hours at 37°C followed by medium removal and a further incubation in fresh medium containing increasing concentrations of 125I-LDL for 5 hours at 37°C for degradation analysis.

Effect of LPS on the cells was to increase the “apparent Bmax” by about fourfold (Figure 6B). To elucidate the nature of the macrophage receptor for LDL on activated cells, competition experiments were carried out using 10 µg protein/ml of 125I-LDL and increasing concentrations of unlabeled LDL or acetylated LDL. Figure 7 demonstrates that in LPS-stimulated (200 µg/ml) cells, 125I-LDL degradation was substantially inhibited by excess unlabeled LDL but not acetylated LDL (Figure 7B), as was also shown for nonstimulated cells (Figure 7A). Similar to the effect of LPS on J774A.1 macrophages, enhanced cellular LDL degradation was also observed when mouse peritoneal macrophages or HMDMs were used. With these two cell types, LDL degradation was increased after pretreatment with LPS by as much as 86% and 59%, respectively (Figure 8).

Cellular Cholesterol Efflux and Synthesis in Activated Macrophages

Cellular cholesterol content is determined not only by LDL-mediated cholesterol influx but also by HDL-mediated cholesterol efflux, as well as by the cellular cholesterol synthesis. LPS stimulation of macrophages did not affect HDL-mediated efflux of cholesterol from cholesterol-loaded J774A.1 cells that were prelabeled with [3H]cholesterol (Table 1). Cellular cholesterol synthesis, however, was increased in LPS-stimulated cells by 25% in comparison with control cells (Table 1).

TABLE 3. Effect of Macrophage Activation on Their Procoagulant Activity in Cholesterol-Loaded and Nonloaded Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>PCA (seconds/mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol-loaded cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>394±71</td>
</tr>
<tr>
<td>+LPS</td>
<td>208±74*</td>
</tr>
<tr>
<td>Nonloaded cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,141±412</td>
</tr>
<tr>
<td>+LPS</td>
<td>618±227*</td>
</tr>
</tbody>
</table>

J774A.1 macrophages were loaded with acetylated low density lipoprotein for 18 hours at 37°C. Then the cells were washed and incubated with lipopolysaccharide (LPS) (100 µg/ml) for 18 hours at 37°C followed by washing, sonication (twice for 20 seconds), and centrifugation. Procoagulant activity (PCA) in the supernatant was analyzed.

*p<0.01, +LPS vs. control (n=3).

Effect of Macrophage-Conditioned Medium

We then compared cellular degradation of LDL by nonactivated macrophages with that produced by cells that were activated with either LPS or conditioned medium from LPS-activated macrophages. When J774A.1 macrophages were preincubated with macrophage-conditioned medium that was obtained from LPS-activated (300 µg/ml) cells (and thus, contained both macrophage secretory products and LPS), a 43% increment in LDL degradation by macrophages was noted in comparison with a 91% increment in LDL degradation by LPS-activated cells (Figure 9).

Low Density Lipoprotein Uptake and Cell Activation in Cholesterol-Loaded Macrophages

J774A.1 macrophages were cholesterol loaded by preincubation with 200 µg/ml acetylated LDL at 37°C for 18 hours, and this resulted in a cellular cholesterol increment from 25 to 65 µg/mg cell protein. On activation of cholesterol-loaded macrophages with LPS (200 µg/ml), LDL degradation was increased by as much as threefold in comparison with activated, nonloaded cells, and this effect was LDL-concentration dependent (Table 2).

Cellular PCA was measured by incubating cell homogenates with standard plasma and CaCl2 and monitoring the time required for clot formation. Increased

![Figure 10](http://atvb.ahajournals.org/) Bar graph showing effect of macrophage activation on their procoagulant activity (PCA). J774A.1 macrophages were preincubated with increasing lipopolysaccharide (LPS) concentrations for 18 hours at 37°C. The cells were then washed three times with phosphate-buffered saline followed by cell sonication (twice for 20 seconds) and centrifugation (1,000g for 10 minutes). PCA of the supernatant was then analyzed. *p<0.01 vs. “0” concentration (n=3).
hypercholesterolemic patients, increased macrophage plasminogen activator activity was found. 22 The enhanced LDL uptake effect on the cellular PCA. Similarly in hypercholesterolemic-derived cells (Table 4).

**Discussion**

Activation of cholesterol-loaded macrophages resulted in a substantial increment in the uptake of LDL, which increased cellular cholesterol accumulation. This effect was higher than the stimulatory effect on the cellular uptake of LDL that was demonstrated in activated cells that were not loaded with cholesterol. Macrophage cholesterol accumulation showed a stimulatory effect on the cellular PCA. Similarly in hypercholesterolemic rabbits, increased macrophage plasminogen activator activity was found. 22 The enhanced LDL uptake by activated cholesterol-loaded macrophages was supported by in vivo studies. LPS-stimulated HMDMs from patients with FH, which were shown in this study to be cholesterol enriched, also demonstrated an enhanced PCA and an increased LDL uptake in comparison with LPS-stimulated HMDMs from normolipidemic subjects. These results may suggest that macrophage activation under pathological conditions can be related to foam cell formation and accelerated atherosclerosis. 3,4,9

**Table 4. Procoagulant Activity and Low Density Lipoprotein Degradation by Lipopolysaccharide-Activated Human Monocyte-Derived Macrophages From Familial Hypercholesterolemic and Normolipidemic Subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>PCA (seconds/mg cell protein)</th>
<th>LDL degradation (µg/mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normolipidemic</td>
<td>755±103</td>
<td>1.50±0.05</td>
</tr>
<tr>
<td>FH</td>
<td>305±165*</td>
<td>1.88±0.06*</td>
</tr>
</tbody>
</table>

*Human monocyte-derived macrophages from normolipidemic or familial hypercholesterolemic (FH) subjects were preincubated with lipopolysaccharide (200 µg/ml) for 18 hours at 37°C followed by washing and analysis of macrophage procoagulant activity (PCA) as well as cellular low density lipoprotein (LDL; 25 µg of protein/ml) degradation. In nonstimulated cells, PCA in normolipidemic subjects and in FH patients was 1,215±190 and 570±119 sec/mg cell protein, respectively. *p<0.01 vs. normolipidemic subjects (n=4). In cholesterol-enriched macrophages, like those found in FH patients, this mechanism was indeed shown to be operative.

Modified forms of LDL, such as oxidized LDL, were shown to be taken up by the macrophage scavenger receptor. 5,6 The scavenger receptor, unlike the LDL receptor, is not regulated by the cellular cholesterol content, and thus, lipoprotein uptake via this receptor can lead to macrophage cholesterol accumulation and foam cell formation. 5,6 Macrophage activation, however, was shown to reduce scavenger receptor activity, so that LDL uptake via this latter receptor could not promote cellular cholesterol accumulation. Furthermore, the involvement of the scavenger receptor in LDL uptake by activated macrophages was ruled out, as shown by the inability of acetylated LDL (the ligand to the scavenger receptor) to compete with 125I-LDL for its cellular degradation.

Increased LDL uptake by activated macrophages was demonstrated in the present study as well as in other studies, 3,9 but the present findings contradict a study with murine macrophages elicited by thioglycolate, in which LDL receptor activity was reduced. 26 This may be related to the different stimuli as well as to the fact that macrophage stimulation in that study was obtained by injection of the activators into the mice, whereas the LPS in our study was incubated directly with the cells, and thus, different cellular metabolic properties may have been affected. Increased LDL uptake by activated macrophages could be related (from the Scatchard plot) to increments in both the number of LDL binding sites and cholesterol-esterification rate (panel B) in human monocyte-derived macrophages from normal subjects (N) and patients with familial hypercholesterolemia (P). TC, total cholesterol; UC, unesterified cholesterol; CE, cholesteryl ester.

*p<0.01, P vs. N (n=8).
and the affinity of the lipoprotein to its receptor (increased "apparent $B_{max}$" and reduced "apparent $K_m$""). The Scatchard plot in the present study could also be interpreted as if, after LPS stimulation, two classes of binding sites for LDL appear: one with a high affinity and the other with a low affinity for LDL. The increased receptor activity in LPS-stimulated macrophages was mainly in the low-affinity binding sites, while high-affinity binding was actually almost identical to that shown in the nonstimulated cells. Because cellular cholesterol accumulation in nonactivated cells results in downregulation of the LDL receptor, it should be stated that the increased sensitivity of cholesterol-loaded macrophages to LPS and the increased cellular uptake of LDL may have resulted from factors other than cellular cholesterol content per se, such as prostaglandin formation, second-messenger activation, or other constituents that are secreted from activated cells.

Downregulation of the LDL receptor could not be demonstrated under several pathological conditions, such as lipid modifications of LDL or lipoprotein modification by platelets, in which the LDL uptake by macrophages was significantly increased. Recently, macrophage-secreted oncostatin was also shown to stimulate LDL receptor activity, and this effect was not dependent on the cellular cholesterol content.

The stimulatory effect of J774A.1 macrophage activation on LDL uptake was also shown with other stimulators and several types of macrophages and may, therefore, be the link between inflammatory and cardiovascular diseases. Enhanced cellular uptake of LDL is probably related to intracellular changes and not to macrophage-released substance(s) because macrophage-conditioned medium (medium that contained LPS) prevented less stimulation of LDL uptake than LPS alone, suggesting an inhibitory effect of macrophage-released substance(s) on the cellular uptake of LDL.

Another explanation is that because LPS binds to the macrophage membrane and is metabolized by these cells, it might be that in macrophage-conditioned medium less LPS remains in the medium. Unlike native LDL, which is taken up by the LDL receptor, macrophage uptake of modified forms of LDL (acylated LDL and oxidized LDL, which bind to the macrophage scavenger receptor) was inhibited in LPS-treated cells (in comparison with control cells), and this was related to LPS binding to the macrophage scavenger receptor.

Enhanced macrophage activation was associated with increased cellular cholesterol content both in vitro (under conditions of macrophage cholesterol loading) and in vivo (in monocytes–macrophages derived from patients with FH). These results suggest that in the atherosclerotic lesion under conditions of macrophage activation, two processes may occur: 1) induction of macrophage cholesterol accumulation as a result of the enhanced cellular uptake of LDL and 2) further cellular activation by cholesterol-enriched macrophages. These two linked phenomena (cellular activation and cholesterol accumulation) are closely interrelated and may affect each other.

Macrophages are multifunctional cells, and the interaction between their activation and the cellular uptake of LDL may contribute to acceleration of atherosclerosis. Thus, intervention by various means to inhibit macrophage activation can contribute to a reduction in the atherosclerotic risk via a reduction in LDL uptake by macrophages.

References


Increased susceptibility to activation and increased uptake of low density lipoprotein by cholesterol-loaded macrophages.
J Oiknine and M Aviram

doi: 10.1161/01.ATV.12.6.745

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/12/6/745

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/