Low Density Lipoprotein Metabolism in Human Macrophages Stimulated with Microbial or Microbial-Related Products

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To determine whether stimulation of macrophages with products related to, or released as a consequence of, infectious processes could play a role in inducing the formation of foam cells, we studied the metabolism of native and acetylated low density lipoprotein (LDL) by human macrophages stimulated with lipopolysaccharide (LPS), muramyl dipeptide (MDP), polyinosinic:polycytidilic acid (Poly I:Poly C) and γ-interferon. Cholesteryl ester (CE) synthesis by macrophages stimulated with LPS, MDP and Poly I:Poly C was markedly increased when the cells were incubated with native LDL (p < 0.05). When incubated with acetylated LDL, LPS-stimulated macrophages showed a depression in CE synthesis (p < 0.05). When incubated with acetyl-LDL, macrophages stimulated with Poly I:Poly C and γ-interferon showed a significant increase (p < 0.05) in CE synthesis. The increase in CE synthesis by LPS-stimulated macrophages exposed to native LDL and by γ-interferon-stimulated macrophages exposed to acetylated LDL was paralleled by an increase in cholesterol ester mass. The increase in CE synthesis and accumulation observed in LPS-stimulated macrophages incubated with native LDL seems to be due to an increase in the receptor mediated uptake of LDL. LPS inhibited and γ-interferon activated the expression of the scavenger pathway in human macrophages. This may explain the changes observed in CE synthesis and accumulation when macrophages activated by the above stimuli were incubated with acetylated LDL. In conclusion, activation of human macrophages by some products released during, or as a consequence of, infectious processes led to an increase in CE synthesis and accumulation that may be relevant to the formation of "foam cells". (Arteriosclerosis 7:176-184, March/April 1987)

Foam cells, the hallmark of the atherosclerotic lesion, are believed to be derived from macrophages and smooth muscle cells. Recent studies of human atheromatous lesions indicated that many of the foam cells can be identified as phagocytic cells on the basis of Fc receptor activity, and it is presumed that their origin is probably the circulating monocyte. Human monocyte-derived macrophages, in contrast to mouse peritoneal macrophages, have high affinity receptors for low density lipoproteins (LDL). However, incubation of these cells with native LDL does not lead to the massive accumulation of esterified cholesterol usually seen in foam cells.

Cholesteryl ester (CE) accumulation of the degree found in foam cells may be induced when macrophages are incubated with lipoprotein particles of abnormal composition such as B-very low density lipoprotein (B-VLDL) obtained from cholesterol-fed animals or with chemically modified LDL such as acetylated, acetoacetylated, and malondialdehyde-treated LDL. Until recently, there was no evidence that chemically modified lipoproteins could be generated in vivo. Henriksen et al. have now demonstrated that LDL can be converted by incubation with cultured endothelial cells into a form that is recognized by the scavenger pathway. Other modified LDL that could potentially be formed in vivo are glucosylated LDL and malondialdehyde LDL that may be formed during platelet aggregation.

In our studies we examined a possible alternative mechanism leading to foam cell formation. We determined whether CE accumulation of the magnitude found in foam cells could be induced by metabolic stimulation of human macrophages. We chose as stimulating agents microbial or microbial-related products that may have biological relevance: lipopolysaccharide (LPS), a glycolipid present in the outer membrane of all Gram-negative bacteria; muramyl dipeptide (MDP), a copy of a fragment of M. tuberculosis peptidoglycan; polyinosinic:polycytidilic acid (Poly I:Poly C), an inducer of α-interferon, a product released during viral infections; and γ-interferon, a biological response modifier released by stimulated lymphocytes.
Methods

Isolation of Monocytes

Monocytes were isolated from leukapheresis specimens by countergradient centrifugal elutriation as previously described. Briefly, leukapheresis specimens were passed over standard Ficoll-Hypaque gradients to produce an unfractionated mononuclear leukocyte suspension. These leukocyte cell preparations were then suspended in elutriation medium (RPMI 1640, Flow Laboratories, Virginia, Australia) with 2 g/100 ml of clinical grade human albumin (Cutter Laboratories, Berkeley, California) with an initial medium flow rate of 5 ± 0.4 ml/minute. The elutriation rotors speed was maintained at 2,020 ± 10 rpm, and the centrifuge temperature was held at 18° C. Elutriation medium leaving the elutriator rotor was collected in 50-ml aliquots in polypropylene conical centrifuge tubes (Corning Glass Works, Corning, New York), and the cells in each aliquot were sized by a Coulter H4 channelizer system (Coulter Electronics, Hialeah, Florida). The flow rate of medium was gradually increased until equal numbers of lymphocytes and monocytes were seen to be leaving the separation chamber (as determined by Coulter channelizer analysis), and the 50-ml aliquot with the above described 50:50 lymphocyte/monocyte ratio became the penultimate aliquot of the isolation procedure. Purified monocytes left in the rotor were then collected by stopping the rotor while maintaining constant medium flow rate. An average of 5 hours of time elapsed from the start of leukapheresis until the final isolation of purified monocytes. All medium used was documented to be endotoxin-free (<0.1 ng/ml of endotoxin, by limulus assay) and no antibiotics were added to any stage of the monocyte isolation procedure. The leukapheresis specimens used for the separation of monocytes as described above were obtained by leukapheresis of normal volunteers for 2 hours on a Celltrifuge II apparatus. Donors with abnormalities on physical or laboratory examinations were excluded as previously described.

Monocyte Identification Procedures

The purity of the monocyte preparations obtained by elutriation was confirmed by morphology on Wright's-stained cytocentrifuge preparations, by nonspecific esterase staining and by the ability to ingest latex particles as previously described. Viability was determined by trypan blue exclusion. The average purity of the monocytes utilized in this study by Wright's staining was 93%; by esterase staining, 92%; and by latex ingestion, 93%. The average viability of the cells used was 99%. The average number of monocytes obtained per donor was 650 million.

Transformation of Monocytes into Macrophages

Monocytes isolated as described above were suspended in a specially formulated serum-free medium (SFM) to a final concentration of 1 × 10^6 cells/ml. The medium was prepared using Iscoe's modified Dulbecco's medium (IMDM) supplemented with human serum albumin (fatty acid free, 4 mg/ml), cholesterol (>90% pure, 20 µg/ml), L-α-phosphatidylcholine (80 µg/ml), and human transferrin (98% pure, 1 µg/ml), all from Sigma Chemical Company (St. Louis, Missouri); human insulin (0.128 U/ml, Eli Lilly and Company, Indianapolis, Indiana); ferrous chloride (7 × 10^-11 M, Fisher Scientific Company, Fairlawn, New Jersey); and β-mercaptoethanol (10^-7 M, Eastman Kodak, Rochester, New York) as previously described.

One ml of the above cell suspension was plated in 35 mm culture dishes and incubated at 37° C in a 5% CO2 incubator for 2 to 3 hours to allow the monocytes to adhere to the plastic. After this time the medium was removed and replaced with medium containing 30% (vol/vol) of whole human serum (Whittaker, M.A., Bioproducts, Walkersville, Maryland). The cells were further incubated for 6 to 8 days and the medium was changed every 3 days. The protein content of the macrophages was, on the average, three times higher than that of monocytes, thus the pooled human serum used was not toxic for the cells. After maturation of monocytes into macrophages, SFM containing no cholesterol was used to perform all the experiments.

Evaluation of Viability, Functional Integrity, and State of Activation of Macrophages

Human macrophages were stimulated with LPS, derived from E. coli (055:BS) (Difco Laboratories, Detroit, Michigan); muramyl dipeptide (MDP) (Calbiochem-Behring, San Diego, California); Poly I:Poly C, sodium salt (Sigma Chemical Company, St. Louis, Missouri); and γ-interferon (Immunomodulator Laboratories, Stafford, Texas) at concentrations ranging from 1 ng to 400 µg/ml for the first three stimuli and at concentrations varying from 10 to 3000 U/ml for γ-interferon. Viability of the cells after exposure to the various stimuli was assessed directly by trypan blue dye exclusion and indirectly by determining the protein content (Lowry's method) of each culture dish at the end of the experiment and after adequate washing. After incubation with the various stimuli at their highest concentrations, 90% to 94% of the macrophages excluded trypan blue. Their protein content was, on average, 112 ± 9.1 (unstimulated); 119 ± 5.5 (LPS-stimulated); 114 ± 6.8 (MDP-stimulated); 109 ± 2 (Poly I:Poly C-stimulated) and 111 ± 2.1 µg/10^6 cells (γ-interferon-stimulated) (mean ± sd).

Functional integrity of the cells after exposure to the various stimuli was assessed by evaluating their capability to phagocytize latex beads and antibody-coated red blood cells. The phagocytic index (percentage of cells capable of ingesting two or more antibody-coated red blood cells and four or more latex beads) of macrophages incubated with the highest concentrations of all stimuli studied ranged from 88% to 95%. The state of activation of LPS-, MDP-, Poly I:Poly C- and γ-interferon-stimulated monocyte-derived macrophages was determined by assaying their Fc-receptor expression and their ability to destroy IgG-coated human red blood cell targets and to release interleukin 1 and α-interferon by use of methods previously described.
Lipoprotein Isolation

Low density lipoproteins (1.019 < d < 1.063 g/ml) were isolated from plasma of normal volunteers by sequential ultracentrifugation in a preparative ultracentrifuge (Beckman Instruments, Palo Alto, California) after appropriate adjustment of the plasma density with solid KBr as previously described.19 LDL was then washed by ultracentrifugation and extensively dialyzed against 0.15 M NaCl solution containing 0.3 mM of EDTA (pH 7.4). LDL was passed through an 0.2 μm pore size to sterilize and remove aggregates.

Acetylation of LDL was performed using the protocol described by Basu et al.20 125I-labeling of LDL and acetylated LDL was performed by the McFarlane procedure as modified by Bratzler et al.21

Cholesteryl Ester Synthesis

Cholesteryl ester synthesis was measured after incubation of monocyte-derived macrophages with native LDL and acetylated LDL. After monocytes matured into macrophages as described above, each dish was extensively washed with sterile phosphate-buffered saline (PBS) and was given 1 ml of SFM containing either no additives or the stimuli to be tested. The stimuli used in our experiments were: LPS, MDP, Poly I:Poly C, and γ-interferon at concentrations ranging from 1 ng to 400 μg/ml for the first three stimuli and at concentrations varying from 10 to 3000 U/ml for γ-interferon. The cells were incubated at 37° C, 5% CO2 for 22 hours with the above products; after the incubation the medium was removed and the cells were washed with PBS. One ml of fresh SFM (no cholesterol added) containing 100 μg of native LDL or 50 μg of acetylated LDL and 0.2 nmol of 14C-oleate complexed with 2.4 mg bovine serum albumin prepared as described by Goldstein et al.22 was added to each dish. The cells were incubated for 20 hours, harvested with a rubber policeman with 6 ml of PBS, spun at 800 to 1,000 rpm for 15 minutes, and then exhaustively washed with PBS to remove any trace of medium. After the washing procedure, the cells were again centrifuged as before and the resulting cell pellet was extracted with chloroform/methanol (2:1) (vol/vol) as previously described.23 Free and total cholesterol were assayed on a gas chromatograph equipped with a hydrogen flame ionization detector. A glass column packed with 3% SP-2250 on 80/100 mesh Supelcoport was used for the chromatographic separation; its temperature was maintained at 250° C during the separation. N2 was used as the gas carrier.

For assay of total cholesterol, the chloroform extracts were evaporated to dryness and the residue was hydrolyzed by Ishikawa's method24 as previously described.19 Cholesteryl ester levels were obtained by subtracting free cholesterol from total cholesterol levels. 5α-cholestanol was used as the internal standard.

LDL Degradation Assay

The cells were processed and treated with stimuli as described for the cholesteryl ester assay. After incubation with the stimuli for 22 hours, the cells were washed and SFM containing 10 μg of 125I-labelled native or acetylated LDL with or without 250 μg of the respective unlabelled lipoproteins was added to the dishes. The cells were incubated for 20 hours and afterwards, the medium was removed and used to study degradation by the method described by Bierman, et al.25 The cells were harvested and cell protein was determined as described above. Net degradation was calculated as the difference between values obtained from identical incubations in the presence or absence of cells. Receptor-mediated degradation was calculated by subtracting the values obtained in the presence of unlabelled lipoprotein from those obtained in its absence. Nonspecific degradation was calculated by subtracting receptor-mediated degradation from net degradation.

Oil-Red O Staining

The neutral lipid inclusions in human monocyte-derived macrophages were stained using oil-red O as previously described.26

Statistical Analysis

Statistical comparison of the CE synthesis, CE mass, and LDL degradation levels by stimulated and unstimulated macrophages was performed using the Wilcoxon signed rank test.27

Results

Cholesteryl ester (CE) synthesis by LPS-, MDP-, and Poly I:Poly C-stimulated human macrophages incubated...
with native LDL was significantly increased (p < 0.05) over that observed in unstimulated cells. Cholesterol ester synthesis followed dose-response kinetics to the stimuli employed (Figures 1, 2 and 3), and it was the highest with LPS at concentrations of 50 to 100 μg/ml. In contrast, γ-interferon did not affect CE synthesis by macrophages incubated with native LDL (Figure 4). When the macrophages were similarly stimulated with LPS, MDP, Poly I:Poly C and γ-interferon but incubated with acetylated LDL, the levels of CE synthesis did not parallel those observed in cells incubated with native LDL. Under these conditions, LPS at concentrations of 100 μg/ml led to a significant depression of CE synthesis (p < 0.05) (Figure 1), MDP did not significantly affect CE synthesis (Figure 2), and Poly I:Poly C and γ-interferon led to a significant increase in CE synthesis (p < 0.05) (Figures 3 and 4).

Since Poly I:Poly C is a ligand recognized by the scavenger receptor, we decided to investigate whether other ligands recognized by the same receptor were able to induce an increase in CE synthesis similar to that observed in macrophages stimulated with Poly I:Poly C. Dextran sulfate at concentrations of 5 and 15 μg/ml and fucoidin at concentrations of 10 and 50 μg/ml of medium were incubated with macrophages for 22 hours; after removal of these substances, the cells were incubated for 20 hours with acetylated LDL (50 μg/ml) and 14C-oleate/bovine serum albumin as described in Methods. No increase in CE synthesis was observed in the cells pre-incubated with either fucoidin or dextran sulfate at any of the concentrations studied (data not shown).

To assess the direct effect of the various stimuli on the basal rates of CE synthesis, we performed experiments determining CE synthesis by stimulated macrophages in the absence of lipoproteins. No effect of the stimuli on basal rates of CE synthesis was observed in LPS-, MDP-

Figure 1. CE synthesis by monocyte-derived macrophages stimulated with increasing concentrations of LPS (0.1 to 100 μg/ml), in presence of 100 μg of native LDL or 50 μg of acetylated LDL (acetyl-LDL). The data is expressed as mean ± SEM of eight different experiments. For each experiment, the monocytes used were obtained from an individual donor. Different donors were used for different experiments. The basal rate of CE synthesis in unstimulated macrophages averaged 0.354 ± 0.028 nmol of 14C-cholesteryl-oleate/mg cell protein/20 hours. In LPS-stimulated macrophages the basal rate of CE synthesis was 100 ± 8.2% of that found in unstimulated cells. The concentration of the stimuli did not affect the basal rate of CE synthesis.

Figure 2. CE synthesis by monocyte-derived macrophages stimulated with increasing concentrations of MDP (0.1 to 100 μg/ml), in presence of 100 μg of native LDL or 50 μg of acetylated LDL. The data is expressed as mean ± SEM of eight and seven different experiments, for native and acetylated LDL, respectively. The basal rate of CE synthesis in MDP-stimulated macrophages was 96 ± 8.7% of that found in unstimulated macrophages. The concentration of the stimuli did not affect the basal rate of CE synthesis.

Figure 3. CE synthesis by monocyte-derived macrophages stimulated with Poly I:Poly C, γ-interferon and MDP (0.1 to 100 μg/ml) in the presence of native LDL or acetylated LDL. The data is expressed as mean ± SEM of eight and seven different experiments, for native and acetylated LDL, respectively.

Figure 4. CE synthesis by monocyte-derived macrophages incubated with LPS, MDP, Poly I:Poly C and γ-interferon but incubated with native LDL (Figure 4). When the macrophages were similarly stimulated with LPS, MDP, Poly I:Poly C and γ-interferon but incubated with acetylated LDL, the levels of CE synthesis did not parallel those observed in cells incubated with native LDL. Under these conditions, LPS at concentrations of 100 μg/ml led to a significant depression of CE synthesis (p < 0.05) (Figure 1), MDP did not significantly affect CE synthesis (Figure 2), and Poly I:Poly C and γ-interferon led to a significant increase in CE synthesis (p < 0.05) (Figures 3 and 4).

To assess the direct effect of the various stimuli on the basal rates of CE synthesis, we performed experiments determining CE synthesis by stimulated macrophages in the absence of lipoproteins. No effect of the stimuli on basal rates of CE synthesis was observed in LPS-, MDP-, γ-interferon-stimulated macrophages regardless of the concentration of the stimuli used. The basal rates of CE synthesis were 100 ± 8.2%, 96 ± 8.7%, and 99 ± 7% (mean ± SEM) respectively of those found in unstimulated cells for LPS-, MDP-, and γ-interferon-stimulated cells. In contrast, the basal rates of CE synthesis were significantly increased (p < 0.05) in cells stimulated with Poly I:Poly C (121 ± 5.5% of the values found in unstimulated cells). That increase in the basal rate of CE synthesis was, however, independent of the concentration of Poly I:Poly C. CE synthesis in macrophages stimulated with 100 ng of Poly I:Poly C was 431.1 ± 14.5 pmol 14C-cholesteryl-oleate/mg cell protein/20 hours (122 ± 4.1% of control values). Similarly, in macrophages stimulated with 200 μg/ml of Poly I:Poly C, CE synthesis was 421.3 ± 40.4 pmol 14C-cholesteryl-oleate/mg cell protein/20 hours (119 ± 11.4% of control values).

To assess whether the presence of a low concentration of β-mercaptoethanol (10^-7 M) in our serum-free medium affected CE synthesis, we determined the basal rates of CE synthesis in macrophages incubated with SFM with or without β-mercaptoethanol. The basal rates of CE synthesis in macrophages exposed to SFM containing β-mercaptoethanol were similar to those obtained in cells exposed to SFM without β-mercaptoethanol (479 ± 16 vs 457 ± 32 pmol of 14C-cholesteryl oleate/mg cell protein/20 hours, mean ± SEM, n = 8 in each group). Furthermore, β-mercaptoethanol did not magnify the responses to the various stimuli studied. The presence of β-mercaptoethanol in the medium did not alter the rates of CE synthesis in macrophages incubated with the various stimuli studied.

To determine whether the observed increase in cholesterol ester synthesis was associated with intracellular lipid
Figure 3. CE synthesis by monocyte-derived macrophages stimulated with increasing concentrations of Poly I:Poly C (0.1 to 400 μg/ml) in the presence of 100 μg of native LDL or 50 μg of acetylated LDL. Note the presence of two different scales on the ordinate axes (scale on the left for acetylated LDL and scale on the right for native LDL). The data is expressed as mean ± SEM of six and eight different experiments for native and acetyl-LDL, respectively. The basal rate of CE synthesis in Poly I:Poly C-stimulated macrophages was 121 ± 5.5% of the values found in unstimulated cells. The concentration of the stimuli did not affect the magnitude of the increase in the basal rate of CE synthesis. (See text for detailed information.)

accumulation, macrophages prestimulated as described and incubated either with normal or acetylated LDL were stained with oil-red O. In macrophages incubated with native LDL after prestimulation with LPS, MDP, and Poly I:Poly C, we observed a larger number of oil-red O-stained inclusions than in unstimulated macrophages. Cells incubated with acetylated LDL after prestimulation with Poly I:Poly C and γ-interferon had more stained inclusions than unstimulated cells (data not shown).

To determine whether these lipid inclusions were mainly constituted by cholesterol, we determined the free and esterified cholesterol content in cells that were prestimulated with LPS (100 μg/ml) and γ-interferon (1500 U/ml) and were incubated for 20 hours with 100 μg/ml of native LDL and 50 μg/ml of acetylated LDL, respectively. Cells prestimulated with LPS and incubated with native LDL accumulated significantly more (p < 0.005) esterified cholesterol than unstimulated cells exposed to the same concentration of native LDL (Figure 5). Similarly, in cells prestimulated with γ-interferon and incubated with acetylated LDL, the esterified cholesterol content of the cells was greater (p < 0.05) than that observed in unstimulated cells exposed to identical concentrations of acetylated LDL (Figure 5).

To determine possible mechanisms that might explain the observed increase in CE synthesis, we determined degradation of native LDL and acetylated LDL by macrophages which were unstimulated or stimulated with LPS, MDP, Poly I:Poly C and γ-interferon at the concentrations that led to the highest levels of CE synthesis (Figures 1–4). In LPS-stimulated macrophages, a marked increase (p < 0.01) of LDL receptor mediated activity was found. In contrast, the expression of the scavenger receptor was inhibited (p < 0.05) (Figure 6). Since it has been reported that marked suppression of the activity of the scavenger receptor on human monocyte-derived macrophages can be induced by LPS at minimal concentrations, we determined the degradation of acetylated LDL (10 μg/ml) by macrophages stimulated by LPS at concentrations ranging from 1 ng to 100 μg/ml. Maximal inhibition was obtained only at...
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Figure 4. CE synthesis by monocyte-derived macrophages stimulated with increasing concentrations of γ-interferon (10 to 3000 U/ml), in the presence of 100 μg of native LDL or 50 μg of acetylated LDL. The data is expressed as mean ± sem of five and six different experiments, for native and acetylated LDL, respectively. The basal rate of CE synthesis in γ-interferon-stimulated macrophages was 99 ± 7% of the values found in unstimulated cells. The concentration of the stimuli did not affect the basal rate of CE synthesis.

LPS concentrations of 100 μg/ml (Figure 7). MDP- or Poly I:C-stimulated macrophages did not show any increase in the LDL receptor and scavenger receptor activities. Stimulation of macrophages with γ-interferon led to an increase in the scavenger receptor activity (p < 0.05) (Figure 6). The levels of native LDL and acetylated LDL degraded by unstimulated cells averaged 5.3 μg of LDL and 17.2 μg of acetylated LDL/mg cell protein/20 hours. Since the amount of acetylated LDL degraded during the 20-hour incubation period represents, even in unstimulated cells, approximately 20% of the amount of lipoprotein added initially, our degradation levels are underestimated, mostly in γ-interferon-stimulated cells. Our data in cells preincubated with γ-interferon therefore represents the minimal increase in the rate of degradation that could possibly be observed. Nonspecific degradation of both native and acetylated LDL by macrophages that were unstimulated or were stimulated with any of the stimuli was not significantly different.

Since β-mercaptoethanol may promote cellular activation and it seems to enhance the effects of cellular activators, particularly in cultures carried out in serum-free medium,29 we assessed the effect of β-mercaptoethanol in the degradation of native and acetylated LDL by comparing the degradation of these lipoproteins in cells containing serum-free medium with or without addition of β-mercaptoethanol.

Receptor-mediated degradation of native LDL by cells incubated with medium containing β-mercaptoethanol was enhanced (3480 ± 180 vs 1874 ± 77 ng LDL degraded/mg cell protein/20 hours, mean ± so, p < 0.05). In contrast, there was no significant difference between the degradation of acetylated LDL by cells incubated in the presence or absence of β-mercaptoethanol in the medium (15,322 ± 2033 and 16,925 ± 1720 of acetylated LDL degraded/mg cell protein/20 hours, mean ± so, respectively). The magnitude of the effect of LPS in the expression of LDL receptor activity and of LPS and γ-interferon in the expression of the scavenger receptor activity was not altered by the presence of β-mercaptoethanol in the medium.

To assess whether the 22-hour incubation of the various stimuli studied with macrophages led to activation of macrophage functions other than those related with lipid metabolism, we determined the expression of Fc-receptors and the ability of the cells to destroy IgG-coated human red blood cell targets and to release interleukin 1 and α-interferon. Macrophages activated by γ-interferon were shown to have an enhanced Fc-receptor expression (25% to 50% increase over the Fc-receptor expression observed in unstimulated cells) and an enhanced ability to destroy IgG-coated red blood cell targets (25% to 50% increase over the levels observed in unstimulated cells). Macrophages stimulated with Poly I:C were shown to have enhanced release of α-interferon (200-4000 U of α-interferon released per 10⁶ cells). Macrophages stimulated with LPS and MDP were shown to have enhanced release of interleukin 1 (20 to 100 U interleukin 1 released/10⁶ cells).

Discussion

There is increasing evidence that the foam cells present in atheromatous lesions are macrophages derived from...
Figure 6. Receptor-mediated degradation of 125I-native or acetylated LDL (10 μg/ml) by macrophages prestimulated for 22 hours with LPS (100 μg/ml), MDP (100 μg/ml), Poly I:Poly C (100 μg/ml) or γ-interferon (1500 U/ml). The values are expressed as a percentage of the degradation of 125I-native or acetylated LDL by unstimulated monocyte-derived macrophages. The data given for LPS-stimulated macrophages represents the mean ± SEM of 8 different experiments for native LDL and of 6 different experiments for acetylated LDL. The data given for MDP, Poly I:Poly C, and γ-interferon-stimulated macrophages represents the mean ± SEM of 5 different experiments for native LDL and of 6 different experiments for acetylated LDL, respectively. The levels of native LDL and acetylated LDL degraded by unstimulated cells averaged 5.3 μg of LDL and 17.2 μg of acetylated LDL/mg cell protein/20 hours. Specific activity of native LDL ranged from 293 to 580 cpm/ng of lipoprotein. Specific activity of acetylated LDL was 220 cpm/ng of lipoprotein.

Figure 7. Receptor-mediated degradation of 125I-acetylated LDL (10 μg/ml) by LPS-stimulated monocyte-derived macrophages. The data was obtained on a single experiment. Each point represents the mean of duplicate incubations. Specific activity of acetylated LDL was 220 cpm/ng of lipoprotein.

cell line with many characteristics of differentiated macrophages, accumulated CE when exposed to native LDL. The authors postulated that the J774 cell line, a tumor cell-derived line, may be metabolically activated and that this might explain the increase in intracellular CE accumulation.

Recently, Van Lenten et al.28 have shown that LPS inhibits the expression of scavenger receptor activity on human monocyte-derived macrophages but has no effect in the LDL receptor pathway activity. This activity was only seen with toxic lipid A preparations, but not with a modified nontoxic monophosphoryl lipid A derivative.

The results of our studies with LPS are partially different from those reported by the above investigators.29 In our studies, similar to what was reported by Van Lenten et al., no significant effect upon LDL receptor activity was observed with LPS concentrations up to 100 ng/ml. We have, however, carried our studies further and used higher LPS concentrations. When these higher LPS concentrations (10 μg/ml or higher) were used, we found a significant increase of CE synthesis in LPS-stimulated macrophages that were incubated with native LDL as well as a marked stimulation of the LDL receptor activity. Our results in LPS-stimulated macrophages incubated with acetylated LDL showed a similar trend to those reported by Van Lenten et al. since we found a depression in both CE synthesis and the scavenger pathway activity. However, the depression we observed was somewhat less in magnitude than that reported by others and higher concentrations of LPS were required to elicit it.

Several factors may explain the differences observed in the magnitude of the LPS effects and in the concentrations of LPS required to elicit them. These factors include the length of preincubation of macrophages with LPS, as well as the source of LPS used to stimulate the phagocytic...
cells. LPS can be extracted from different strains of bacteria by different methods that result in different degrees of LPS bioactivity. We used a preparation of LPS extracted from E. coli 055:B5, while Van Lenten et al. used LPS extracted from E. coli 0111:B4. Differences in the bioactivity of the two LPS preparations may explain the differences observed in the two studies. The differences between our experimental conditions and those employed by Van Lenten et al. may have also contributed to some of the differences observed between their results and ours. They matured their monocytes into macrophages in 30% serum for 2 days followed by LPS exposure for 3 additional days in presence of serum; we matured our monocytes for 6 to 8 days in 30% serum and exposed them to serum-free medium containing LPS for 22 hours.

In a recent study, Van Lenten et al. have shown that the inhibitory effect of LPS on scavenger receptor activity is markedly enhanced when the stimuli are complexed with LDL. Even the presence of lipoprotein-deficient serum seems to have a potentiating effect on LPS action. In our studies the stimulation with LPS was conducted in the absence of serum, and that may explain why we obtained a relatively small decrease in the scavenger receptor activity regardless of the high concentrations of LPS used.

The effects of other microbial-related products, such as MDP or Poly I Poly C in cholesterol metabolism at the macrophage level have not been studied to our knowledge. MDP, a copy of a fragment of M. tuberculosis peptidoglycan responsible for the adjuvant activity of this bacterium, is known to increase macrophage synthesis of prostaglandin, collagenase, and fibroblast growth factors. MDP-stimulated macrophages also demonstrate increased levels of cyclic AMP. Recently, it was shown that MDP may have in vivo effects, including pyrogenesis and induction of slow-wave sleep. We have demonstrated a novel effect of MDP in lipid metabolism: MDP induces stimulation of CE synthesis by human macrophages incubated with native LDL. This increase in CE synthesis is not mediated by increased activity of the LDL receptor and might be due to an effect of the stimuli on acyl-CoA:cholesterol acyltransferase activity.

Poly I Poly C, a synthetic polynucleotide, replicates the nuclear macromolecules released after cell destruction by viruses. For this reason Poly I Poly C induces the production of α-interferon by mononuclear phagocytes. Our experiments did not allow us to determine whether the effect of Poly I Poly C in CE synthesis was direct or mediated by the release of interferon. Poly I Poly C and γ-interferon shared an identical stimulatory effect on CE synthesis by macrophages incubated with acetylated LDL. That effect seems to be secondary to an increase in the LDL scavenger pathway activity but only in γ-interferon-stimulated macrophages. In cells incubated with normal LDL, Poly I Poly C and γ-interferon do not have identical activities and only the macrophages stimulated with Poly I Poly C showed an increase in CE synthesis.

Our studies have shown that in macrophages stimulated with Poly I Poly C, the basal rates of CE synthesis are moderately increased. That increase is independent of the Poly I Poly C concentrations in the medium. This suggests that Poly I Poly C, independently of its concentration, may have the potential to mildly stimulate acyl-CoA:cholesterol acyltransferase and or neutral cholesterol hydrolase activities. That alone cannot, however, explain the increase in CE synthesis observed since a significant increase in CE synthesis was observed only in cells stimulated with high Poly I Poly C concentrations. Therefore, it appears that high concentrations of Poly I Poly C stimulate a third enzymatic system, lysosomal cholesterol esterase, in a dose-dependent manner.

Experiments designed to assess whether the effect of Poly I Poly C was related to the fact that this substance is a ligand recognized by the scavenger receptor were not conclusive. Macrophages exposed for 22 hours to fucoidin and dextran sulfate did not show an increase in CE synthesis when subsequently incubated with lipoproteins. However, these findings do not exclude the possibility that the action of Poly I Poly C derives from its property to bind to the scavenger receptor. Poly I Poly C, dextran sulfate, and fucoidin may interact with different binding sites of the scavenger receptor, and it is possible that binding to selective sites of the receptor is needed to obtain a stimulatory effect on lipid metabolism.

Current postulates concerning the role of viral infections in atherosclerosis focus on the possibility that viral infections may lead to endothelial cell damage, either directly as a consequence of the viral infection or indirectly as a consequence of the expression of Fc and or complement receptors which could allow the entrapment of immune complexes and subsequent damage by complement activation or by activated polymorphonuclear leukocytes. A possible role of bacterial infection in the pathogenesis of atherosclerosis has also been suggested by Reidy and Bowyer who demonstrated that LPS causes widespread endothelial damage. This damage appears to be more severe and persistent if LPS is administered to hypercholesterolemic animals. Our observations provide evidence suggestive of another potential mechanism by which microbial infections could play a pathogenic role in atherosclerosis, namely by increasing the uptake of LDL and increasing the intracellular synthesis and accumulation of cholesterol esters. In this respect, γ-interferon appears to differ from bacterial products in the pathway used to accumulate LDL intracellularly. LPS predominantly stimulates uptake by the LDL receptor, while interferon mainly stimulates uptake by the scavenger pathway. The relative pathological implications of these differences in the mechanisms for intracellular accumulation of cholesterol in macrophages are not clear and deserve to be studied in detail.

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References


Index Terms: LDL metabolism • LPS-stimulated human macrophages • CE synthesis • CE accumulation • γ-interferon-stimulated human macrophages
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