Receptor-mediated Uptake of Remnant Lipoproteins by Cholesterol-loaded Human Monocyte-macrophages*

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Normal human monocyte-macrophages were cholesterol-loaded, and the rates of uptake and degradation of several lipoproteins were measured and compared to rates in control cells. Receptor activities for \(^{125}\)I-\(\beta\)-very low density lipoprotein (\(\beta\)-VLDL), \(^{125}\)I-human low density lipoprotein, and \(^{125}\)I-human chylomicrons were down-regulated in cholesterol-loaded cells; however, the rate of uptake and degradation of \(^{125}\)I-human chylomicron remnants was unchanged from control cells. Cholesterol-loaded alveolar macrophages from a Watanabe heritable hyperlipidemic rabbit, which lack low density lipoprotein receptors, showed receptor down-regulation for \(^{125}\)I-\(\beta\)-VLDL but not for \(^{125}\)I-human chylomicron remnants. In addition to chylomicron remnants, apo-E-phospholipid complexes competed for \(^{125}\)I-chylomicron remnant uptake, but apo-A-I-phospholipid complexes did not. Chylomicrons competed for lipoprotein uptake in control cells but were not recognized under conditions of cholesterol loading. Chylomicron remnants and \(\beta\)-VLDL were equally effective in competing for \(^{125}\)I-\(\beta\)-VLDL and \(^{125}\)I-chylomicron remnant uptake in cholesterol-loaded macrophages. When normal human monocyte-macrophages were incubated in serum supplemented with chylomicron remnants, the cholesteryl ester content increased 4-fold over cells incubated in serum with low density lipoprotein added. We conclude: 1) specific lipoprotein receptor activity persists in cholesterol-loaded cells; 2) this receptor activity recognizes lipoproteins (at least in part) by their apo-E content; and 3) cholesteryl ester accumulation can occur in monocyte-macrophages incubated with chylomicron remnants.

Dietary cholesterol is transported in the blood by chylomicron remnants, which are derived from intestinal chylomicrons through the action of lipoprotein lipase (1). These remnants enter the liver by receptor-mediated endocytosis after binding to specific remnant receptors (2). \(\beta\)-Migrating very low density lipoproteins (\(\beta\)-VLDL)\(^1\), found in the plasma of cholesterol-fed animals, are considered to represent modified forms of remnant particles (3).

Macrophages derived from human monocytes (4), mouse peritoneal macrophages (5), and endothelial cells (6) have surface receptors that specifically bind \(\beta\)-VLDL. These receptors appear to be subject to feedback regulation since cells that are loaded with cholesterol have a reduced \(\beta\)-VLDL receptor activity (4, 5). Chylomicron remnant receptors, however, do not appear to be regulated. Metabolic perturbations that affect hepatic low density lipoprotein (LDL) receptors do not influence the hepatic uptake of chylomicron remnants (1, 2). Recognition sites on chylomicron remnants for cellular interactions have also been investigated. Sherrill et al. (7) have shown that high density lipoproteins containing only apolipoprotein E (apo-E) isolated from cholesteryl-fed dogs compete with \(^{125}\)I-labeled chylomicron remnants for uptake in perfused rat livers. It would appear that apo-E is also an important determinant of the affinity of \(\beta\)-VLDL for hepatic receptors (8).

We describe here a receptor-mediated process that persists in cholesteryl-loaded macrophages that recognizes apo-E-containing lipoproteins. Additionally, the uptake of chylomicron remnants by monocyte-macrophages leads to cholesteryl ester accumulation in the cell.

**EXPERIMENTAL PROCEDURES**

**Materials**

\(\text{Na}^{125}\text{I} (17 \text{ mCi/mg})\) and \([1-\text{\(^{14}\)C}]\)oleic acid (54 mCi/mmol) were obtained from Amersham and New England Nuclear, respectively. Phosphate-buffered saline and Dulbecco's modified Eagle's medium (catalogue No. 430-1600) were purchased from Grand Island Biological Co. Crystallized human albumin (catalogue No. A-8763) was purchased from Sigma. Cholesterol (catalogue No. CH-K) was obtained from Sigma. Ficoll-Hypaque was purchased from Pharmacia-Falcon (catalogue No. 3047) multiwell tissue culture plates were purchased from Van Waters and Rogers, Norwalk, CA. Nalgene Teflon perfluoroalkoxy containers were from Scientific Products, Irvine, CA. Homovanillic acid, horseradish peroxidase (type II), and cholesteryl oxidase were purchased from Sigma. Cholesterol esterase was purchased from Boehringer Mannheims. Affi-Gel 10 was purchased from Bio-Rad. Protamine sulfate (10' 4020) was purchased from Sigma. All other equipment and supplies were purchased from sources previously reported (9).

**Subjects**

Normal subjects were recruited from the staff and student body at UCLA. No one received drugs that might have affected serum cholesterol levels or lipid metabolism. All had normal hematocrits, white blood cell and differential counts, serum cholesterol, and triglycerides. Informed consent was obtained in writing from each person.

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Separation of Cells

Five-hundred ml of blood were taken from adult subjects after an overnight fast, and the monocytes were separated from 300 ml of blood using counterflow centrifugation (method BB in Ref. 10).

Cell Culture

Human Macrophages—For experiments measuring lipoprotein degradation, the cells were suspended in 30% autologous serum in Dulbecco's modified Eagle's medium supplemented with NaHCO3 (24 mM), Hepes (10 mM), insulin (5 μg/ml), glucose (2 mg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and Fungizone (0.25 μg/ml), hereafter referred to as medium B. One-ml samples of the cell suspension containing 107 cells were transferred to 35-mm plastic tissue culture dishes, or 0.5-ml samples of the cell suspension containing 5 × 106 cells were transferred to 20-cm2 polyethylene wells and incubated at 37 °C in a humidified incubator with 5% CO2. For experiments measuring cell-associated radioactivity, 4 × 106 cells were suspended in 5 ml of medium B and transferred to Teflon containers to reduce adherence of the monocytes. Unless otherwise stated, the medium was aspirated and replaced with fresh medium of the same composition twice weekly. To study receptor activities in cholesterol-loaded cells, the medium was removed on day 4 and the cells were switched to 0.1% human serum albumin in medium B supplemented with 1 μl/ml 25-hydroxycholesterol and 16 μg/ml cholesterol in ethanol. Control cells received medium B supplemented with ethanol alone. After 72 h, the cells were removed and the wells were washed prior to the addition of radioactive lipoproteins.

Rabbit Alveolar Macrophages—For experiments using Watanabe heritable hyperlipidemic (WHHL) rabbit macrophages, the cells were isolated from alveolar fluid as previously described (11) but with the following modifications. Once the bronchi from the rabbit lung were dissected, the bronchial macrophages by lung lavage is an inherently nonsterile technique. In order to culture cells for several days, it was necessary to overcome contamination of the tissue fluid by bacteria as well as air-borne contamination of the tissue culture dishes, or 0.5-ml samples of the cell suspension containing 5 × 106 cells were transferred to 20-cm2 polyethylene wells and incubated at 37 °C in a humidified incubator with 5% CO2. For experiments measuring cell-associated radioactivity, 4 × 106 cells were suspended in 5 ml of medium B and transferred to Teflon containers to reduce adherence of the monocytes. Unless otherwise stated, the medium was aspirated and replaced with fresh medium of the same composition twice weekly. To study receptor activities in cholesterol-loaded cells, the medium was removed on day 4 and the cells were switched to 0.1% human serum albumin in medium B supplemented with 1 μl/ml 25-hydroxycholesterol and 16 μg/ml cholesterol in ethanol. Control cells received medium B supplemented with ethanol alone. After 72 h, the cells were removed and the wells were washed prior to the addition of radioactive lipoproteins.

Classification and Viability of Cells

The cells were classified and their viability was determined as described previously (12). Because of the media changes and the washes prior to beginning each experiment, the cells were washed five times prior to each experiment. The viability of the macrophages before the radioactive lipoproteins were added. More than 95% of the cells were viable at the end of the incubations.

Isolation and Iodination of Lipoproteins

Human LDL (d 1.019–1.063 g/ml) were prepared from sera of normal fasted subjects as described (4). Chylomicrons were isolated from the plasma of normal fasted subjects after the ingestion of a liquid meal containing 189 g of total fat and 3 g of cholesterol. Chylomicrons were also isolated from thoracic duct lymph (a generous gift from Dr. Jay Fish, University of Texas Medical Center at Galveston). The plasma and lymph chylomicrons were prepared by flotation at native density by centrifuging at 165,000 × g for 40 min (6.6 × 105 × g/min) at 15 °C in a Beckman SW 41 rotor. For the preparation of chylomicron remnants, chylomicrons isolated as described above were incubated with purified lipoprotein lipase according to the method of Matsusaka et al. (13).

Chylomicron remnants were also isolated by using plasma cholesteryl ester transfer protein as described by Floren et al. (14). Affinity chromatography was used for the isolation of native chylomicron remnants from the d < 1.019 g/ml fraction of plasma from fast-fed subjects. Briefly, proteamine sulfate was covalently coupled to Affi-Gel 10 to bind chylomicron remnants in the d < 1.019 g/ml fraction, presumably based on the heparin sulfate present on the surface of the remnants (15). The chylomicron remnants used in this study had the following composition (per cent by weight of total mass): protein; 13–14%; cholesterol, 17–18%; triglyceride, 59–60%. Rabbit β-VLDL was isolated from the plasma of cholesterol-fed rabbits as previously described (4). All lipoprotein preparations were washed by second flotation at the appropriate density. Hepsolipid-apoprotein complexes were prepared as described (16).

All lipoproteins were radioiodinated according to the method of McFarlane (17) as modified by Bilheimer (18). In the case of chylomicron remnants, 6% of the total radioactivity was extracted by chloroform/methanol. Distribution of 125I among the apolipoproteins of chylomicron remnants was: apo-B, 82%; albumin, 6%; apo-E, 5%; and apo-C, 7%.

Assays

125I-Lipoprotein Degradation—Prior to the addition of radioactive lipoproteins, the cells were washed three times with 1.5 ml (cells in dishes) or 1.0 ml (cells in wells) of Dulbecco's modified Eagle's medium containing 10 mM Hepes (medium C). Unless otherwise stated, radioactive lipoproteins were added in the same medium supplemented with 24 ml NaHCO3 and 2 mg/ml glucose (medium D). To reduce the contribution of the LDL receptor to the degradation of 125I-labeled β-VLDL, 50 μg/ml unlabelled LDL were added to incubations to saturate the LDL receptor activity when β-VLDL receptor activity was being determined (4).

The proteolytic degradation of 125I-labeled lipoproteins was measured by assaying the amount of 125I–trichloroacetic acid-soluble (acid-iodide) material that was released into the culture medium as described by Goldstein and Brown (19). Corrections were made for the small amounts (<0.01% of total radioactivity added) of 125I-labeled acid-soluble material that were found in parallel incubations without cells.

Cell-associated Radioactivity—Cells were harvested from Teflon containers by vigorous pipetting of the media to dislodge any cells that were loosely attached to the container. Cells were then pelleted by centrifuging at 1000 rpm for 10 min in a Beckman J-6 centrifuge and washed by resuspension and pelleting in medium D. Aliquots of 2 × 106 cells were transferred to 1.5-ml siliconized microfuge tubes containing the lipoproteins in medium D in a total of 1.25 ml. The tubes were rotated on a Lab Quake for 4 h at room temperature. At the end of 4 h, 0.25-ml aliquots of cell binding suspension were transferred to four siliconized microfuge tubes that had been preloaded with 0.6 ml of a mixed oil layer composed of 64% Versilube F50 and 16% F650 (General Electric Co., Waterford, NY) over 0.5 ml of 12% sucrose. Tubes were centrifuged 5 min in a Brinkmann Microfuge (Brinkmann Instruments) and the sucrose and oil layers were removed with a Pasteur pipette. The bottoms of the tubes were sliced, and the pellets were counted in a γ counter.

Cholesterol and triglyceride contents were determined according to the method of Heider and Boyett (20) as modified by Feidling et al. (21). Protein was determined by the method of Lowry et al. (22). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (23) was used to evaluate the apolipoprotein composition of the lipoprotein fractions. Radial immunodiffusion (24) was used to detect the immunological reactivity of apo-E in lipoproteins.

RESULTS

We had previously shown (4) that incubation of macrophages for 72 h in the presence of 1 μg/ml 25-hydroxycholesterol and 16 μg/ml cholesterol markedly reduced the activities of the LDL receptor and the β-VLDL receptor. In a typical experiment using this protocol, the free cholesterol content of the cell was 37 ± 2 μg/mg of protein and the esterified cholesterol content was 39 ± 5 μg/mg of protein. We therefore used this regimen to study lipoprotein uptake in cholesterol-loaded cells. Control cells were those cells incubated with ethanol solvent alone in the medium.

Floren and Chait (25) have demonstrated that chylomicron remnants can be taken up by the LDL receptor on human monocyte-macrophages. Fig. 1 shows that in sterol-loaded alveolar macrophages from the WHHL rabbit, which lack LDL receptors, the rate of chylomicron remnant degradation was the same in both sterol-loaded and control cells. The rate
of uptake and degradation of $\beta$-VLDL, however, was reduced in cells that were sterol-loaded compared to the rate in control cells. The degradation of $^{125}$I-LDL, as expected, did not occur in WHHL macrophages. Since degradation of chylomicron remnants occurred in the absence of the LDL receptor, these results suggest that other receptor-mediated processes accounted for the hydrolysis of chylomicron remnants.

In sterol-loaded human monocyte macrophages, as shown in Fig. 2, the activities of the receptors for $\beta$-VLDL, LDL, and chylomicrons were reduced. In contrast, the rates of uptake and degradation of chylomicron remnants produced from the same chylomicrons either by hydrolysis with purified lipoprotein lipase or by incubation of the chylomicrons with post-heparin plasma were not different from the rates in control cells incubated with ethanol alone. In addition to these in vitro produced chylomicron remnants, we also used native chylomicron remnants isolated from postprandial plasma by an Affi-Gel affinity column. Fig. 3 shows an experiment comparing the abilities of chylomicron remnants produced in vitro with lipoprotein lipase (CR-LPL) and chylomicron remnants obtained from the affinity column (CR-COL) to compete for receptor-mediated degradation in sterol-loaded cells. A 25-fold excess of either unlabeled lipoprotein was equally effective in suppressing the degradation of either $^{125}$I-CR-LPL or $^{125}$I-CR-COL. In studies to follow, chylomicron remnants generated in vitro by lipoprotein lipase were used.

The degradation of $^{125}$I-chylomicron remnants as a function of concentration in cholesterol-loaded human monocyte-macrophages is shown in Fig. 4. The uptake of chylomicron remnants by these cholesterol-loaded cells appears to be a specific high capacity process that begins to saturate at approximately 500 $\mu$g/ml protein. Specific cell-associated radioactivity was also measured in sterol-loaded and control cells (Fig. 5). Human monocytes that had been preincubated with sterols demonstrated virtually no specific cell-associated radioactivity after incubation with $^{125}$I-human LDL (right panel). However, sterol-loaded and control cells had equal amounts of cell-associated radioactivity after incubation with $^{125}$I-chylomicron remnants.

It has been shown by other investigators (2, 7) that hepatic receptors for chylomicron remnants recognize a number of apo-E-containing particles. We wished to test if, in addition to chylomicron remnants, the receptor activity of sterol-loaded cells would preferentially recognize apo-E. In Fig. 6, unlabeled chylomicron remnants and synthetic phospholipid-apoprotein complexes containing either apo-E or apo-A-I were compared for their abilities to compete for $^{125}$I-chylomicron remnant degradation. Chylomicron remnants and apo-E-phospholipid complexes were equally effective in suppressing the degradation of $^{125}$I-chylomicron remnant degradation. In contrast, apo-A-I-phospholipid complexes poorly competed for this activity.

We had previously shown (4) that human thoracic duct lymph chylomicrons competed for the uptake and degradation of $\beta$-VLDL. Fig. 7 (panels A–C) shows that, in control cells, $\beta$-VLDL, chylomicron remnants, and to a lesser degree, thoracic duct lymph chylomicrons (free of immunoreactive apo-
described in the legend to Fig. 2. On day 7, the medium was removed and
the mean concentration in the presence
of excess of unlabeled human chylomicron remnants.

After 4 h of incubation at 37°C, the medium was removed and the [35S]-labeled acid-soluble content was determined. The values shown are the mean ± S.D. of quadruplicate wells.

FIG. 3. The ability of nonradioactive human chylomicron remnants produced by lipoprotein lipase hydrolysis (CR-LPL) and nonradioactive human chylomicron remnants obtained from an affinity column (CR-COL) to inhibit the degradation of [125I]-CR-LPL (left panel) and [125I]-CR-COL (right panel) in cholesterol-loaded human monocyte-macrophages. Normal monocytes were cultured and additions were made to the medium as described in the legend to Fig. 2. On day 7, the medium was removed and the cells were washed three times with 1 ml of medium C. Then 0.5 ml of medium D was added containing 2 μg/ml [125I]-CR-LPL (202 cpm/ng of protein) or [125I]-CR-COL (186 cpm/ng of protein) alone (open bars) or in the presence of a 25-fold excess of either unlabeled CR-LPL (hatched bars) or unlabeled CR-COL (stippled bars). After 4 h of incubation at 37°C, the medium was removed and the [35S]-labeled acid-soluble content was determined. The values shown are the mean ± S.D. of quadruplicate wells.

FIG. 4. Uptake and degradation of [125I]-human chylomicron remnants in cholesterol-loaded macrophages as a function of concentration in the presence (C) or absence (O) of a 25-fold excess of unlabeled human chylomicron remnants. Normal monocytes were cultured and additions were made to the medium as described in the legend to Fig. 2. On day 7, the medium was removed and the cells were washed three times with 1 ml of medium C. Then 0.5 ml of medium D containing [125I]-chylomicron remnants (285 cpm/ng of protein) at the protein concentration shown on the abscissa was added. After 4 h of incubation at 37°C, the medium was removed and the [125I]-labeled acid-soluble content was determined.

E) all competed for the uptake of [125I]-β-VLDL (panel A), [125I]-chylomicrons (panel B), and [125I]-chylomicron remnants (panel C). In the cholesterol-loaded cells (panels D–F), there was no competition observed for [125I]-chylomicrons by any of the unlabeled ligands (panel E), indicating an absence of high affinity (specific) degradation. Whereas both unlabeled β-VLDL and chylomicron remnants were able to compete for

FIG. 5. High affinity cell-associated radioactivity in cholesterol-loaded human monocytes that were incubated with either [125I]-human chylomicron remnants or [125I]-human LDL. Normal monocytes were put into Teflon containers and cultured in 50% autologous serum in medium B. On day 4, the medium was removed and the cells were switched to 0.1% human serum albumin in medium B supplemented with 1 μg/ml 25-hydroxycholesterol and 16 μg/ml cholesterol in ethanol (right panel) or with ethanol alone (left panel). On day 7, the medium was removed and the cells were pelleted, washed, and resuspended in medium B. Two × 10^6 cells and 5 μg/ml [125I]-human chylomicron remnants (282 cpm/ng of protein) or [125I]-human LDL (185 cpm/ng of protein) were pipetted into a macrofuge tube in the presence or absence of a 50-fold excess of the corresponding unlabeled lipoprotein. After 4 h at room temperature, the contents of the tube were pipetted into a second Eppendorf tube with an oil/sucrose gradient and the tube was spun in a microfuge for 5 min. The cell pellet was then sliced from the tube and counted in a γ counter for cell-associated [125I].

FIG. 6. Abilities of unlabeled human chylomicron remnants (X), apo-A-I-phospholipid complexes (Θ), and apo-E-phospholipid complexes (Ο) to inhibit the degradation of [125I]-human chylomicron remnants in cholesterol-loaded human monocyte-macrophages. Normal monocytes were cultured and additions were made to the medium as described in the legend to Fig. 2. On day 7, the medium was removed and the cells were washed three times with 1 ml of medium C. Then 0.5 ml of medium D was added containing 2 μg/ml [125I]-human chylomicron remnants (274 cpm/ng of protein) alone or in the presence of the protein concentration of unlabeled lipoprotein shown on the abscissa. The 100% value for the degradation of the [125I]-human chylomicron remnants in the absence of competing lipoprotein was 0.312 μg degraded·4 h⁻¹·mg of protein⁻¹.
the uptake of \(^{125}\text{I}\)-\(\beta\)-VLDL (panel D) and \(^{125}\text{I}\)-chylomicron remnants (panel F), unlabeled chylomicrons were ineffective competitors in both cases (stippled bars in panels D and F). Thus, the receptor activity in cholesterol-loaded cells appears to be refractory to the apo-E-deficient chylomicrons that showed competitive ability in the control cells.

In Fig. 8, it can be seen that both \(\beta\)-VLDL and chylomicron remnants were equally effective in inhibiting the degradation of \(^{125}\text{I}\)-\(\beta\)-VLDL (panel A) and \(^{125}\text{I}\)-chylomicron remnants (panel B) in sterol-loaded cells. Thus, it would appear that the receptor activity that persists in cholesterol-loaded cells recognizes both \(\beta\)-VLDL and chylomicron remnants to the same degree.

To determine if cholesteryl esters would accumulate in monocyte-macrophages from normal subjects that were incubated with chylomicron remnants, the experiment in Fig. 9 was carried out. After incubation of cells in autologous serum with no added lipoprotein (stippled bars), the addition of LDL (open bars), or the addition of chylomicron remnants (hatched bars), the free and esterified cholesterol contents of the cells were determined. The esterified cholesterol content of cells incubated in serum supplemented with chylomicron remnants, however, was 4-fold greater than in cells incubated in the culture medium with LDL added (panel B) and constituted approximately 35% of the total content of cellular cholesterol.

**DISCUSSION**

We have demonstrated in the present study that, in cholesterol-loaded monocyte-macrophages, receptor activity exists that recognizes certain apo-E-containing lipoproteins, specifically chylomicron remnants and \(\beta\)-VLDL. Although chylomicron remnants can bind to LDL receptors in vitro, the genetic evidence presented here using macrophages from the WHHL rabbit, an animal virtually lacking LDL receptors, shows that chylomicron remnants can be taken up and degraded independently of the LDL receptor. The receptor activity in the cholesterol-loaded macrophage that recognizes chylomicron remnants is a relatively low affinity but high capacity process. In time, this receptor activity could quanti-
tatively remove lipoprotein-cholesterol from the extracellular environment, leading to intracellular accumulation of cholesterol esters. Thus, under conditions of cholesteryl loading when the cell's LDL receptor is down-regulated, chylomicron remnants could still be taken up, resulting in a further increase in cellular content of cholesteryl esters. Indeed, in our studies, normal monocyte-macrophages cultured in their own serum supplemented with chylomicron remnants showed a 4-fold greater content of cholesteryl ester than the same cells cultured in LDL-supplemented serum. After 3 weeks exposure to chylomicron remnants in 30% serum, the human monocyte macrophages contained 35% of their cholesterol in the esterified form. Foam cells in vivo are reported to contain ≥50% of their cholesterol in esterified form (26). Perhaps longer exposure is required and/or the concentration of molecules promoting cholesterol efflux may be less in the arterial wall than was the case in 30% normal human serum.

It would appear from our results that apo-E is the preferential ligand for the binding of lipoproteins to the receptor in cholesteryl-loaded macrophages. Other investigators (2, 7) have provided evidence that apo-E is the recognition site on lipoproteins for the remnant receptors on hepatocytes. The interaction of lymph chylomicrons with cells may also be governed by apo-E content. Human thoracic duct lymph chylomicrons have been shown to compete for the β-VLDL receptor on human monocyte-macrophages (4). Analysis of these lipoproteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed no detectable apo-E. However, immunoreactive apo-E was not excluded. In the current study, lymph chylomicrons were shown by Ouchterlony analysis to contain non immunologically reactive apo-E. These chylomicrons showed some ability to compete for receptor activity in control cells but not in cholesteryl-loaded cells. One explanation is that the β-VLDL receptor recognizes chylomicrons, chylomicron remnants, β-VLDL, and LDL. However, in sterol-loaded cells, β-VLDL receptor activity may be down-regulated to such an extent that its activity virtually disappears, as was the case for LDL receptor activity. Under such conditions, chylomicron remnants and β-VLDL would be processed by the chylomicron remnant receptor but chylomicrons and LDL would not.

Zilversmit (27) has proposed that postprandial lipoproteins or their remnants may be important in the development of foam cells, and therefore in the pathogenesis of atherosclerosis. Our studies have led us to conclude that foam cells are produced by the entrance of lipoprotein-cholesterol into macrophages via surface receptors which are either not regulated or poorly regulated by the cellular cholesterol content. The results presented here suggest such a mechanism and point to the potential atherogenicity of circulating lipoproteins of dietary origin.

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