Apolipoprotein B Stimulates Formation of Monocyte-Macrophage Surface-connected Compartments and Mediates Uptake of Low Density Lipoprotein-derived Liposomes into these Compartments*

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Much of the cholesterol that accumulates in atherosclerotic plaques is found within monocyte-macrophages transforming these cells into “foam cells.” Native low density lipoprotein (LDL) does not cause foam cell formation. Treatment of LDL with cholesterol esterase converts LDL into cholesterol-rich liposomes having >90% cholesterol in unesterified form. Similar cholesterol-rich liposomes are found in early developing atherosclerotic plaques surrounding foam cells. We now show that cholesterol-rich liposomes produced from cholesterol esterase-treated LDL can cause human monocyte-macrophage foam cell formation inducing a 3–5-fold increase in macrophage cholesterol content of which >60% is esterified. Although cytochalasin D inhibited LDL liposome-induced macrophage cholesteryl ester accumulation, LDL liposomes did not enter macrophages by phagocytosis. Rather, the LDL liposomes induced and entered surface-connected compartments within the macrophages, a unique endocytic pathway in these cells that we call patocytosis. LDL liposome apoB rather than LDL liposome lipid mediated LDL liposome uptake by macrophages. This was shown by the findings that: 1) protease treatment of the LDL liposomes prevented macrophage cholesterol accumulation; 2) liposomes prepared from LDL lipid extracts did not cause macrophage cholesterol accumulation; and 3) purified apoB induced and accumulated within macrophage surface-connected compartments. Although apoB mediated the macrophage uptake of LDL liposomes, this uptake did not occur through LDL LDL receptor-related protein, or scavenger receptors. Also, LDL liposome uptake was not sensitive to treatment of macrophages with trypsin or heparinase. Cholesterol esterase-mediated transformation of LDL into cholesterol-rich liposomes is an LDL modification that: 1) stimulates uptake of LDL cholesterol by apoB-dependent endocytosis into surface-connected compartments, and 2) causes human monocyte-macrophage foam cell formation.

One feature of developing atherosclerotic plaques is accumulation of cholesterol in plaque monocyte-macrophages. Epidemiological studies have linked the development of atherosclerotic plaques with levels of low density lipoprotein (LDL), the major carrier of plasma cholesterol. Evidence suggests that macrophage cholesterol originates in part from circulating LDL that enters the blood vessel wall. However, native LDL itself fails to induce cholesterol accumulation in macrophages. Because of this finding, it has been surmised that LDL must undergo changes that increase its uptake by macrophages.

Modifications to LDL that aggregate the LDL also promote LDL accumulation by macrophages (1–8). Recently, we showed that LDL aggregated through vortexing or treatment with phospholipase C caused its uptake by a unique endocytic pathway in monocyte-macrophages (9). These aggregated LDLs induced and entered a labyrinth of surface-connected membrane bound compartments (SCC) within the macrophage. Because this endocytosis pathway results in uptake and storage of material within compartments that remain open to the extracellular space, we have named this endocytic process “patocytosis” from the Latin, patere, meaning to lie open. LDL entry into SCC does not depend on the LDL receptor. Aggregated LDL that enters SCC is mostly stored rather than degraded.

Unesterified cholesterol-rich liposomes are a prominent component of lesion lipid (10–14). These unique cholesterol-rich particles are found in early developing atherosclerotic lesions and their appearance precedes foam cell development within lesions (14–16). Previously, we showed that treatment of LDL with cholesterol esterase converts the 0.02-μm LDL particles into =0.1-μm liposomes (17). LDL-derived liposomes are similar in size and chemical composition to liposomes found in atherosclerotic lesions. Both LDL-derived liposomes and lesion liposomes show a high molar ratio of unesterified cholesterol to phospholipid (>2:1) and a high percentage (>75%) of their cholesterol is unesterified. Like aggregation of LDL, conversion of LDL into liposomes potentially exposes lipid and protein domains normally hidden in native LDL. We now report that cholesterol esterase mediated conversion of LDL into liposomes is an LDL modification that stimulates patocytosis and foam cell formation.

MATERIALS AND METHODS

Preparation of Lipoproteins and LDL Liposomes—Human LDL prepared with density gradient centrifugation was obtained from PerImmune (Rockville, MD) (18). LDL-derived liposomes were produced essentially as described previously (17). Briefly, LDL was sequentially treated with trypsin, soybean trypsin inhibitor, and then with cholesterol esterase to hydrolyze the cholesteryl ester core of LDL. Treatment with trypsin was found earlier to be necessary before cholesterol esterase could hydrolyze LDL cholesteryl esters. This treatment converts LDL that normally has about 25% of its cholesterol in unesterified form into unesterified cholesterol-rich liposomes that have >90% of their cholesterol in unesterified form (see Ref. 17 for the characterization of these LDL liposomes). It should be noted that trypsin treatment of LDL does not eliminate its binding to the LDL receptor (19). LDL liposomes were purified by gel filtration as described earlier with the exception of the two classes of surface-connected compartments; apoB, apolipoprotein B.
that the elution buffer was Ca^{2+} and Mg^{2+}-free Dulbecco’s phosphate-buffered saline containing 0.1% disodium EDTA. The purified LDL liposomes were sterilized by filtration through polysulfone 0.45-μm (pore-size) filters (Gelman Sciences, Ann Arbor, MI) into sterile polypropylene tubes and stored at 4 °C.

LDL liposomes were also prepared from lipid extracted from LDL in this case lipid was extracted from LDL using 2:1 chloroform/methanol as described by Folch et al. (20). Then the solvent was evaporated under a stream of nitrogen. One-mL of buffer containing 0.15 M NaCl, 50 mM Tris-HCl (pH 7.2), and 0.5 mM EDTA was added to the lipid that was then sonicated under nitrogen for 3 h at 51 °C with 50 watts power (Branson Sonifier 250, Danbury, CT). This dispersed the lipid into particles as described previously for preparation of synthetic liposomes (21). LDL liposomes were then prepared from these lipid particles as was described above for dense LDL.

In one type of experiment purified LDL liposomes (at a cholesterol concentration of 1300 nmol/ml) were further treated 2 h at 37 °C with papain (0.5 mg/ml) followed by leupeptin (0.2 mg/ml for 30 min) (both from Boehringer-Mannheim) to neutralize the papain. These protease-treated LDL liposomes were used to learn whether a protein component of the liposomes was required for macrophage uptake.

The preparation of microcrystalline cholesterol and acetylated LDL were described in Ref. 22. Rabbit ß-very low density lipoprotein was obtained from Biomedical Technology (Stoughton, MA) prepared as in Ref. 23 from plasma of New Zealand White rabbits fed a 1% cholesterol diet for 5 weeks.

**Incubation of Macrophages with LDL Liposomes and Potential Inhibitors of LDL Liposome Uptake**—Human monocyte-derived macrophages were cultured as described previously except that 2 × 10^6 monocytes/well were initially seeded into 12-well (22-mm diameter) culture plates (Plastek C, MatTek Corp., Ashland, MA) (22). Two-week-old monocyte-macrophage cultures were rinsed 3 times with RPMI 1640 medium and incubated for the indicated times at 37 °C in RPMI 1640 medium with the indicated concentrations of liposomes expressed as nanomole of total cholesterol/ml of medium.

Potential inhibitors of liposome uptake that were tested included cytochalasin D, nocodazole, polyinosinic acid (all from Sigma), C7 mouse anti-LDL receptor monoclonal antibody (purified from supernatant of cell line number 1691-CRL, American Type Culture Collection, Manassas, VA) (24), and isotype-matched control monoclonal IgG, antibody (catalog number 50330, ICN, Aurora, OH), and receptor-associated protein that inhibits lipoprotein binding to the LDL receptor-related protein (25). The role of heparan sulfate proteoglycans in LDL liposome uptake was tested by first treating macrophages for 3 h at 37 °C without or with 50 nm chlorate plus 80 units/ml heparinase I (Sigma). Macrophages were then incubated for 1 day with either 200 nmol/mL LDL liposomes or 100 μg/ml rabbit ß-very low density lipoprotein without or with 50 μM chlorate and incubated for 1 day with either 200 nmol/mL LDL liposomes or 100 μg/ml rabbit ß-very low density lipoprotein without or with 50 μM chlorate (26, 27).

In one experiment, purified apoB (PerImmune) (28) was incubated with monocyte-macrophage cultures. First, the apoB (0.5 mg), which contained detergents, was solubilized in saline. Then the detergents were removed by exhaustive dialysis against saline. The now insoluble apoB was centrifuged, resuspended in RPMI 1640 at a concentration of 1 mg/ml, dispersed by direct sonication (15 s), and then incubated 5 h with monocyte-macrophages to learn whether apoB could stimulate patocytosis. Two other insoluble proteins, collagen IV and fibrin (catalyzed by stilbamidine) were added to monocyte-macrophages and incubated for a concentration of 1 mg/ml, sonicated 15 s, and incubated with monocyte-macrophages also at a concentration of 1 mg/ml. Following incubations, macrophage cultures were analyzed for their cholesterol contents or for ultrastructural changes as described below.

**Assay of Cholesterol Content of Monocyte-Macrophages and LDL Liposomes**—Macrophages were rinsed, harvested, and processed as described previously (29). Unesterified and esterified cholesterol contents of macrophages and LDL liposomes were determined enzymatically according to the fluorometric method of Gamble et al. (30). The mean ± S.E. (when shown) were determined from three culture wells for each data point.

**Electron Microscopy**—Extracellular and intracellular membranes were differentiated with ruthenium red according to the method of Luft (31). Monocyte-macrophage cultures were ruthenium red-stained and prepared for electron microscopy as described previously (22).

**RESULTS**

**LDL Liposomes Caused Substantial Macrophage Cholesterol Accumulation**—Conversion of LDL into liposomes with cholesterol esterase treatment greatly increased LDL uptake by human monocyte-macrophages. Native LDL did not cause much cholesterol accumulation in macrophages (Table I and Fig. 1a). Only when LDL was converted to liposomes (i.e., was treated with trypsin followed by cholesterol esterase) did LDL induce substantial cholesterol accumulation in the macrophages.

| Condition | Cholesterol ester/total cholesterol content | Macrophage cholesterol esterase | Macrophage cholesterol esterase |
|-----------|--------------------------------------------|-----------------------------|-----------------------------|
| No addition | 58 ± 1 | 65 ± 1 | 70 ± 1 | 77 ± 1 | 177 ± 1 |
| LDL | 73 | 65 ± 1 | 70 ± 1 | 77 ± 1 | 177 ± 1 |
| LDL + trypsin + STI | 72 | 65 ± 1 | 70 ± 1 | 77 ± 1 | 177 ± 1 |
| LDL + CEase | 70 | 65 ± 1 | 70 ± 1 | 77 ± 1 | 177 ± 1 |
| LDL + trypsin + STI + CEase | 8 | 65 ± 1 | 70 ± 1 | 77 ± 1 | 177 ± 1 |

Macrophages were incubated 2 days with 200 nmol of cholesterol/ml of LDL treated as indicated. 1.25 mg of LDL in 2.5 ml of 0.05 M Tris saline buffer (pH 7.2) containing 1 mm NaCl, EDFTA was treated as indicated sequentially (17) with trypsin (95 units/ml for 2 h), soybean trypsin inhibitor (STI) (0.25 mg/ml for 30 min), and cholesterol esterase (CEase) (55 units/ml for 2 h). Then, the treated LDL samples were exhaustively dialyzed against phosphate-buffered saline and added directly to macrophage cultures without isolating the treated LDL.

Macrophage cholesterol accumulation during a 2-day incubation was maximal when macrophages were incubated with LDL liposomes at a concentration of 150–200 nmol of cholesterol/ml (Fig. 1a). During such incubations, macrophage cholesterol accumulation varied but could reach levels more than 400 nmol/mg cell protein with greater than 60% of cholesterol esterified. Incubation of macrophages with LDL liposomes for up to 5 days showed that most of the increase in macrophage cholesterol content occurred during the first 2 days of incubation (Fig. 1b). The lag in total cholesterol accumulation at 1 day occurred in 2 out of 3 time course experiments. Cholesterol was esterified during the entire 5-day incubation. In another experiment not shown, the acyl-CoA:cholesterol acyltransferase inhibitor, S58-035, completely blocked esterification of cholesterol during a 3-day incubation of macrophages with LDL liposomes (200 nmol of cholesterol/ml), but did not decrease total cholesterol accumulation.

LDL liposomes induced macrophage cholesterol accumulation comparable to the levels induced by acetylated LDL and microcrystalline cholesterol (Table II). The amount of cholesteryl ester synthesized by macrophages incubated with LDL liposomes and microcrystalline cholesterol was similar although more total cholesterol accumulated in macrophages incubated with microcrystalline cholesterol. Different LDL liposome preparations induced similar levels of cholesterol accumulation when incubated with macrophages from the same culture. On the other hand, the degree of cholesterol accumulation varied with different macrophage cultures (see accompanying data for the 2-day incubation in Fig. 1a and b).

**LDL Liposomes Induced Surface-connected Macrophage Surface-connected Compartments**—Experiments were carried out to determine whether LDL liposomes could induce and enter macrophage SSC as we previously reported occurs with aggregated LDL and microcrystalline cholesterol (22). Macrophages were incubated with LDL liposomes for 1 day and then processed for electron microscopy with ruthenium red, an electron dense stain that labels cellular membranes. Ruthenium red does not
penetrate the plasma membrane of aldehyde-fixed cells. Thus, in aldehyde-fixed cells, ruthenium red stains only cellular membranes that are in continuity with the extracellular space.

Fig. 2 shows that LDL liposomes induced and entered macrophage SCC that were ruthenium red stained. Ruthenium red also stained the LDL liposomes contained within the SCC. Many non-membrane bound lipid droplets accumulated in the cytoplasm of macrophages incubated with LDL liposomes reflecting esterification of LDL liposome cholesterol.

Like phagocytosis, patocytosis (the SCC endocytic process) is inhibited by cytochalasin D, an agent that disrupts actin microfilaments, but not by nocodazole, an agent that disrupts microtubules. When macrophages were incubated with LDL liposomes in the presence of cytochalasin D, LDL liposomes were observed attached to the plasma membrane but did not enter macrophages and no SCC formed (data not shown). Thus, cytochalasin D blocked uptake of LDL liposomes by macrophages, but not their binding to the macrophage surface. As a result, cytochalasin D decreased but did not eliminate macrophage cholesterol accumulation induced by LDL liposomes (Fig. 3). Some increase in macrophage unesterified cholesterol content apparently occurred due to binding of LDL liposomes to the macrophage plasma membrane. Cytochalasin D decreased

**TABLE II**

Macrophage cholesterol accumulation induced by LDL liposomes compared with other lipid particles

| Lipid particle                  | Unesterified cholesterol | Esterified cholesterol | Total cholesterol |
|--------------------------------|--------------------------|------------------------|-------------------|
| None                           | 71 ± 3                   | 4 ± 4                  | 76 ± 1            |
| Acetylated LDL                 | 126 ± 6                  | 261 ± 12               | 387 ± 18          |
| LDL liposomes (preparation 1)  | 132 ± 7                  | 188 ± 4                | 520 ± 8           |
| LDL liposomes (preparation 2)  | 141 ± 6                  | 187 ± 4                | 326 ± 10          |
| LDL liposomes (preparation 3)  | 140 ± 5                  | 188 ± 13               | 327 ± 16          |
| Microcrystalline cholesterol   | 270 ± 18                 | 189 ± 4                | 459 ± 21          |

**FIG. 1.** Effect of LDL liposome concentration and time of incubation on macrophage cholesterol accumulation. In a, macrophages were incubated for 2 days with the indicated concentrations of LDL liposomes. ▼, indicates macrophage total cholesterol content for LDL incubated at a cholesterol concentration of 200 nmol/mL. In b, macrophages from a different culture were incubated with 200 nmol/mL of LDL liposome cholesterol for the indicated number of days. After incubations, cells were rinsed and their cholesterol contents were determined as described under "Materials and Methods." TC, total cholesterol; EC, esterified cholesterol; UC, unesterified cholesterol.

**FIG. 2.** Ruthenium red staining of LDL liposome-induced surface-connected compartments. Macrophage cultures were incubated with 100 nmol/mL LDL liposome cholesterol for 1 day. Then, cells were rinsed, fixed with 2% glutaraldehyde, and then exposed to 0.15% ruthenium red during additional fixation with first fresh glutaraldehyde and then 2% osmium tetroxide. Following fixation, cells were embedded in epon plastic and thin sectioned, but not counterstained. The membranes of SCC (indicated by arrows) stained with ruthenium red. Also, note that the SCC are filled with LDL liposomes (also shown in inset) that also stained with ruthenium red. N, nucleus. Magnification is × 5000 for main photomicrograph and × 46,000 for the inset.
unesterified cholesterol accumulation by 26%. However, this inhibitor decreased esterified cholesterol accumulation to a greater extent at 88%, suggesting that uptake into SCC and possibly some additional actin-dependent process (32) was necessary for esterification of LDL liposome cholesterol.

Apolipoprotein B-mediated Uptake of LDL Liposomes into SCC—The capacity of LDL liposomes to induce cholesterol accumulation depended on its protein component. This was shown by exposing LDL liposomes to the protease, papain, then neutralizing the papain with leupeptin, and finally incubating these protease-treated LDL liposomes with macrophages. Papain treatment of the LDL liposomes significantly decreased macrophage cholesterol accumulation (Table III). If papain was neutralized with leupeptin before exposing the LDL liposomes to the papain, papain did not effectively decrease macrophage uptake of LDL liposomes. This showed that leupeptin inhibition of papain was sufficient to prevent any substantial effect of the added papain on macrophage receptors involved in uptake of the LDL liposomes. Incubation of macrophages with LDL liposomes prepared from extracted LDL lipid also did not cause any macrophage cholesterol accumulation (data not shown). This further demonstrated the importance of apoB in mediating LDL liposome uptake by macrophages.

The above results suggested that apoB, the major protein component of the LDL liposomes mediated their uptake into macrophase SCC. We directly tested this conclusion by incubating macrophages with purified apoB, a protein that is insoluble in aqueous buffers such as culture medium. Macrophages were incubated with 1 mg/ml insoluble apoB and then examined by electron microscopy for the presence of SCC. ApoB induced SCC in most macrophages and an amorphous ruthenium red-stained material (consistent with apoB protein) accumulated within the SCC (Fig. 4). On the other hand, incubation of macrophages with the same concentration of two other insoluble proteins, collagen and fibrin, did not induce SCC.

Uptake of LDL Liposomes Did Not Depend on LDL, LDL Receptor-related Protein, or Polyinosinic Acid-inhibitable Scavenger Receptors—Uptake of LDL liposome cholesterol did not depend on the LDL receptor. Cholesterol-enrichment of macrophages decreases macrophage expression of the LDL receptor (33). However, macrophage cholesterol-enrichment did not decrease cholesterol accumulation induced by LDL liposomes. When macrophages were first incubated with acetylated LDL for 2 days, their cholesterol content doubled (from 80 ± 1 to 160 ± 2 nmol of cholesterol/mg of cell protein) (Table IV). Nevertheless, a subsequent 2-day incubation with LDL lipo-
TABLE IV

| Condition       | Unesterified cholesterol | Esterified cholesterol | Total cholesterol |
|-----------------|--------------------------|------------------------|------------------|
| Condition       | nmol/mg cell protein     |                        |                  |
| 0 days          | 80 ± 5                   | 1 ± 0                  | 80 ± 5           |
| AcLDL, 2 days   | 88 ± 2                   | 72 ± 2                 | 160 ± 2          |
| RPMI, 2 days    | 73 ± 2                   | 65 ± 5                 | 138 ± 4          |
| AcLDL, 2 days; RPMI, 2 days | 199 ± 4                  | 128 ± 8                | 327 ± 5          |
| RPMI, 2 days; LDL liposomes, 2 days | 52 ± 2                   | 0 ± 1                  | 52 ± 2           |

sommes of these and other macrophages that were not cholesterol-enriched produced similar increments in macrophage cholesterol content (167 ± 4 and 170 ± 7 nmol of cholesterol/mg of cell protein, respectively). Additional findings indicated that LDL receptors were not involved in LDL liposome uptake. Incubation of LDL liposomes (200 nmol/ml) with macrophages for 2 days in the presence of 100 µg/ml of an anti-LDL receptor monoclonal antibody (C7) (24) did not decrease macrophage cholesterol accumulation. Also, methylation of apoB, which blocks apoB binding to macrophage LDL receptors (34), did not interfere with apoB capacity to induce and enter macrophage SCC.

Other potential lipoprotein receptors also did not mediate macrophage uptake of LDL liposomes. Polynosinic acid (2.5 mg/ml), a scavenger receptor inhibitor (35) failed to inhibit LDL liposome-induced macrophage cholesterol accumulation. However, polynosinic acid did inhibit by >90% acetylated LDL-induced macrophage cholesterol accumulation (both the LDL liposomes and acetylated LDL were incubated with macrophages for 2 days at a total cholesterol concentration of 200 nmol/ml). Receptor-associated protein (1 µg/ml), an inhibitor of LDL receptor-related protein and other LDL receptor family members (25), showed no effect on LDL liposome-induced macrophage cholesterol accumulation.

Macrophase uptake of LDL liposomes may not depend on a macrophage cell-surface protein. Exposure of macrophages to trypsin (20 µg/ml for 30 min at 37 °C) followed by soybean trypsin inhibitor (30 µg/ml for 30 min) actually increased macrophase cholesterol accumulation during a 1-day incubation with LDL liposomes (200 nmol of cholesterol/ml) and cycloheximide (20 µg/ml) added to block protein synthesis (165 ± 3 nmol/mg cell protein for sham-treated macrophages and LDL liposomes without cycloheximide compared with 211 ± 11 nmol/mg cell protein for trypsin-treated macrophages and LDL liposomes plus cycloheximide). On the other hand, as expected for a protein receptor-mediated uptake process, trypsin alone, cycloheximide alone, and trypsin plus cycloheximide treatments significantly decreased acetyl-LDL-induced (100 µg/ml) macrophase cholesterol accumulation (140 ± 1 with sham treatments, 132 ± 2 with trypsin alone, 121 ± 5 with cycloheximide alone, and 114 ± 4 nmol cholesterol/mg cell protein with combined trypsin and cycloheximide treatments).

Macrophage uptake of apoB-containing lipoproteins in some instances can be mediated by cell surface proteoglycans (26, 36–38). However, digestion of macrophages with heparinase I (80 units/ml, Sigma) followed by incubation of macrophages for 1 day with LDL liposomes (200 nmol/ml) in the presence of 50 mM chlorate (an inhibitor of proteoglycan sulfation (27)) failed to reduce macrophage cholesterol accumulation. On the other hand, as recently reported for pigeon peritoneal macrophages (26), heparinase I treatment did decrease by 70% macrophase cholesterol accumulation induced by rabbit β-very low density lipoprotein (100 µg of protein/ml) in the same experiment.

DISCUSSION

Previously we showed that microcrystalline cholesterol and aggregated LDL enter SCC that form in human monocyte-macrophages during incubation with these lipid particles (22). Uptake of the lipid particles into SCC is an actin-dependent process we call patocytosis. Patocytosis is distinct from phagocytosis in which vacuoles pinch off from the plasma membrane. We now show that liposomes derived from cholesterol esterase-treated LDL also are accumulated by macrophages through patocytosis. Uptake of LDL liposomes was predominantly mediated by the apoB component rather than the lipid component of the LDL liposomes. This was indicated by the findings that: 1) protease treatment of the LDL liposomes decreased most macrophase cholesterol accumulation; 2) LDL liposomes prepared from LDL lipid extracts caused no macrophase cholesterol accumulation; and 3) purified apoB could induce and enter macrophase SCC.

Sufficient hydrolysis of LDL cholesteryl ester may be important for the conversion of LDL into a lipid particle that can cause macrophase cholesterol accumulation. In two previous studies, it was reported that cholesterol esterase treatment of LDL did not increase and even decreased LDL uptake by macrophages. In one of these studies (39), LDL was treated with cholesterol esterase in the presence of trypsin rather than first treating LDL with trypsin and then neutralizing the trypsin with a trypsin inhibitor as in our study. In this earlier study, it is likely that there was little or no hydrolysis of LDL cholesteryl ester due to trypsin-mediated degradation of any added cholesterol esterase. While the LDL cholesteryl ester content was not monitored in this earlier study, electron microscopy showed that trypsin and cholesterol esterase treatment of LDL did not produce liposomes. This suggests that LDL cholesteryl ester hydrolysis was minimal. In the other study (40), only about 33% of LDL cholesteryl ester was hydrolyzed in contrast to the almost complete hydrolysis of LDL cholesteryl ester in our study. It is likely that the low level of cholesteryl ester hydrolysis in both these earlier studies was not sufficient to form liposomes from the LDL, and thus possibly not sufficient to permit macrophase binding of cryptic apoB domains or to create larger liposomal lipid particles with multiple copies of apoB (discussed below).

Because of the greater size of LDL liposomes compared with native LDL, more than one LDL particle should contribute to each LDL liposome. Thus, even if not greatly aggregated, LDL liposomes (like aggregated LDL) should be multivalent with respect to apoB, whereas LDL is known to have only one copy of apoB per LDL particle (41). A lipid particle with a multivalent apoB ligand may have the capacity to initiate receptor cross-linking and cell signaling pathways that could trigger uptake into SCC.

ApoB is unique among the apolipoproteins in that it is very hydrophobic, a property that accounts for its insolubility in aqueous buffer. In this regard, we have not observed SCC when macrophages were incubated, for example, with apoE, a soluble apolipoprotein that mediates LDL receptor uptake of β-very low density lipoprotein into surface-connected tubules of mouse peritoneal macrophages (42, 43). The other lipid particle that we find enters SCC is microcrystalline cholesterol, another hydrophobic material. Studies are in progress to learn whether

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2 H. S. Kruth and W-Y. Zhang, unpublished data.
ApoB Stimulates Macrophage Surface-connected Compartment Formation

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