Acetylated Low Density Lipoprotein Reduces Its Ligand Activity for the Scavenger Receptor after Interaction with Reconstituted High Density Lipoprotein*

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Complexes of apolipoprotein A-I (apoA-I) with phospholipids are known to induce cholesterol efflux from cells. In a cholesterol ester accumulation system in which gavactational macrophages were incubated with acetylated low density lipoprotein (acetyl-LDL) and either dimyristoylphosphatidylcholine complexes (DMPC/apoA-I) or native high density lipoprotein (HDL), DMPC/apoA-I exhibited a much stronger effect than native HDL in preventing cholesterol ester accumulation. The mechanism for this phenomenon was investigated in the present study. After 18 h incubation with DMPC/apoA-I in a cell-free system, acetyl-LDL was re-isolated from DMPC/apoA-I by Sephacryl S-300 gel filtration chromatography. Re-isolated acetyl-LDL exhibited an increase in its phospholipid content by 80% as well as a reduction in the electrophoretic mobility. Its endocytic degradation by macrophages was reduced by 60% when compared with control acetyl-LDL, suggesting a significant reduction in the ligand activity for the macrophage scavenger receptor. Transfer of apolipoproteins between acetyl-LDL and DMPC/apoA-I did not occur. These results indicate that transfer of DMPC from DMPC/apoA-I to acetyl-LDL weakens the ligand activity for the scavenger receptor due probably to a decrease in negative charges. This phenomenon may explain one of the anti-atherogenic functions of HDL in vivo.

Macrophage-derived foam cells characterized by massive cholesteryl ester droplets in the cytoplasm have an essential role in the progression of the early stage of atherosclerosis (1). Macrophages are known to take up chemically modified LDLs, such as acetyl-LDL and oxidized LDL, through the scavenger receptor pathway and to become foam cells in vitro (1, 2). The molecular structure of the scavenger receptor was recently demonstrated (3). It has been suggested, however, that there is another type of scavenger receptor with a different ligand specificity (4–6). Recent immunochemical studies emphasized oxidized LDL as a likely candidate for an atherogenic lipoprotein in vivo (1, 7, 8).

In contrast to LDL, HDL has been regarded as an anti-atherogenic lipoprotein (9). The mechanism for the anti-atherogenic capacity of HDL is not fully understood. At a cellular level, HDL or HDL apolipoproteins have a capacity to enhance cholesterol efflux from peripheral tissues, the first step of “reverse cholesterol transport” (10–16). At a cell-free level, its direct preventive effect on LDL from oxidative modification was suggested by recent experiments (17–19). It seems reasonable therefore to expect that HDL might protect macrophages from CE accumulation not only by promoting cholesterol efflux from cells, but also by inhibiting the production of atherogenic lipoproteins.

Modified LDLs after being produced in vivo have to meet a series of events before foam cell formation occurs; cellular uptake by the scavenger receptor, lysosomal degradation, intracellular cholesterol transport, and re-esterification by acyl-CoA cholesterol acyltransferase. However, it remains unclear whether or to what extent HDL could affect these processes. Using the experimental system in which macrophages were incubated with HDL simultaneously with atherogenic lipoprotein such as β-very low density lipoprotein (20) and acetyl-LDL (21), we observed that DMPC complexes with apoA-I (DMPC/apoA-I) could prevent macrophages from acetyl-LDL-induced CE accumulation much more strongly than HDL did (21). This mechanism was examined in the present study. The results revealed that DMPC/apoA-I interacted with acetyl-LDL and reduced its ligand activity for the scavenger receptor. The “neutralizing” effect of DMPC/apoA-I was explained by the transfer of DMPC from DMPC/apoA-I to acetyl-LDL. Since complexes of apoA-I with phospholipids (discoidal HDL) are known to occur in vivo (13, 22–24), the marked reducing effect of apoA-I phospholipid complexes on the ligand activity of the atherogenic lipoprotein might reflect a new anti-atherogenic function of HDL in vivo.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture media and reagents were obtained from Life Technologies Inc. Silica gel on aluminum sheets for TLC was obtained from Merck. Na125I and [1a,2a-3H]Cholesteryl oleoyl ether (45 Ci/mmol) were purchased from Amersham. [1-14C]Oleate (56 mCi/mmol) was purchased from DuPont New England Nuclear.

Lipoproteins and Their Modification—LDL (d = 1.019–1.063) was isolated by sequential ultracentrifugation from fresh human plasma and dialyzed against 0.15 M NaCl and 1 mM EDTA (pH 7.4) with traces of apolipoprotein B and E being removed from HDL by a heparin-agarose column (25). Acetyl-LDL was prepared as described (25).IODination of acetyl-LDL with 125I was performed as described.
described (26). Protein concentrations were determined by the BCA protein assay reagent (Pierce Chemical Co.) (14). HDL was delipidated for purification of apoA-I as described (27).

Preparation of Complexes of Apolipoprotein A-I (ApoA-I) and DMPC—Complexes of apoA-I and DMPC (DMPC/apoA-I) were prepared by the method of Jonas (28). For a standard preparation, 15 mg of DMPC, 15 mM NaCl, and 1 mM EDTA (pH 7.4) was sonicated to form small unilamellar vesicles. To this solution, 10 mg of apoA-I in 1.0 ml of the same buffer was added and equilibrated with periodic stirring for 6 h at 25 °C. The final concentrations of DMPC and apoA-I were 7.5 and 5.0 mg/ml, respectively (the molar ratio of DMPC to apoA-I being 62:1). Upon Sephacryl S-300 gel filtration chromatography, DMPC/apoA-I thus prepared was eluted as a single peak when monitored with both absorbance at 280 nm and phospholipid determination (phospholipid test B/Wako). Cella—Peritoneal macrophages were collected from nonstimulated male Wistar rats (150–300 g) with 40 ml of ice-cold phosphate-buffered saline, centrifuged at 200 x g for 5 min, and suspended in Dulbecco’s modified Eagle’s medium containing 3% bovine serum albumin, streptomycin (0.1 mg/ml), and penicillin (100 units/ml) (buffer A) (29).

Labeling of Acetyl-LDL with [3H]Cholesteryl Oleoyl Ether—In order to determine the cholesterol supply to macrophages, neutral lipid of monolayers was labeled with the nonexchangeable and nonhydrolyzable cholesteryl ester analog, [3H]cholesteryl oleoyl ether ([3H]COE) by the modified method of Roberts et al. (30). Briefly, 250 μCi of [3H]COE in acetone was mixed with 20 ml of lipoprotein-deficient serum. Acetone was evaporated to be tested. Trichloroacetic acid soluble radioactivity in the medium was determined as described previously (31).

Assay for Cholesterol Esterification—Macrophage monolayers prepared above were incubated with acetyl-LDL for 18 h in the presence of 0.1 mM [3H]oleate conjugated with bovine serum albumin (22). Cellular lipids were extracted and the radioactive cholesteryl [3H]oleate was determined as described previously (27).

Incubation and Re-isolation of Acetyl-LDL and DMPC/ApoA-I—To examine the interaction of acetyl-LDL with DMPC/apoA-I in a cell-free system, acetyl-LDL (4 mg) was incubated at 37 °C for 18 h with DMPC/apoA-I (20 mg of protein) in a total volume of 4.0 ml of 0.15 M NaCl and 1 mM EDTA (pH 7.4). The incubation mixture was applied to a column of Sephacryl S-300 (1.5 x 150 cm) and eluted with 0.15 M NaCl and 1 mM EDTA (pH 7.4) at a flow rate of 12.5 ml/h. Fractions (3.5 ml) were collected and analyzed for absorbance at 280 nm. Two control experiments were performed. First, acetyl-LDL (1 mg/ml) was incubated at 37 °C for 18 h with HDL (5 mg/ml) and re-isolated with Sephacryl S-300 gel filtration chromatography as described above. Second, acetyl-LDL (1 mg/ml) was incubated similarly with DMPC liposomes (7.5 mg of DMPC/ml). The mixture was adjusted to d = 1.015 with KBr and centrifuged at 100,000 x g for 24 h. The bottom fraction (d > 1.019) was dialyzed against 0.15 mM NaCl and 1 mM EDTA (pH 7.4).

Analytical Methods—Lipid contents of lipoproteins were determined on a Hitachi 7450 automatic analyzer using standard enzymatic methods (33–35). Free amino groups of acetyl-LDL and LDL were determined with trinitrobenzenesulfonic acid as described (36).

RESULTS

Effect of DMPC/apoA-I on Acetyl-LDL-induced CE Accumulation in Rat Macrophages—The inhibitory effect of DMPC/apoA-I on CE accumulation induced by acetyl-LDL was compared with that of HDL. As shown in Fig. 1, the incorporation of [14C]oleate into cholesteryl [14C]oleate was inhibited by the presence of HDL in a dose-dependent manner. The extent of inhibition reached 50% at 300 μg/ml. In contrast to HDL, DMPC/apoA-I showed a much stronger inhibitory effect on CE accumulation at even lower protein concentrations. Thus, DMPC/apoA-I has a much stronger anti-atherogenic effect on macrophages than HDL when incubated simultaneously with acetyl-LDL.

Effect of DMPC/ApoA-I on Cholesterol Supply by Acetyl-LDL to Rat Macrophages—To elucidate the mechanism for the strong inhibitory effect of DMPC/apoA-I on CE accumulation, we examined the effect of DMPC/apoA-I on cellular cholesterol supply by acetyl-LDL. The neutral lipids of acetyl-LDL were labeled with the non-exchangeable and non-hydrolyzable cholesteryl ester analog, [3H]COE. Once [3H]cholesteryl ether is taken up by cells by receptor-mediated endocytosis, it accumulates in lysosome without undergoing enzymatic conversion to FC (37). When macrophages were incubated with [3H]COE-labeled acetyl-LDL, cellular accumulation of [3H]COE increased with time (Fig. 2). Under the identical conditions, the simultaneous presence of HDL did not interfere with cell association of [3H]COE (Fig. 2), suggesting that HDL does not affect cellular cholesterol supply by acetyl-LDL. Thus, the inhibitory effect of HDL on intracellular CE accumulation (Fig. 1) could be explained by enhancement of cholesterol efflux from macrophages. In contrast to HDL, however, the presence of DMPC/apoA-I inhibited [3H]COE accumulation in macrophages by 70%, indicating that DMPC/apoA-I did interfere with cellular cholesterol supply, which might well explain its strong inhibitory effect on acetyl-LDL-induced CE accumulation (Fig. 1).

Effect of DMPC/apoA-I on Endocytic Degradation of [125I]-Labeled Acetyl-LDL—The result in Fig. 2 suggests that DMPC/apoA-I might inhibit the endocytic pathway of acetyl-LDL. To test this notion, macrophages were incubated with [125I]-labeled acetyl-LDL in the presence of DMPC/apoA-I. As shown in Fig. 3, degradation of [125I]-labeled acetyl-LDL was significantly inhibited by DMPC/apoA-I. This finding together with that of Fig. 2 strongly suggests that the interaction of DMPC/apoA-I with acetyl-LDL might occur in the medium, leading to decreased recognition of acetyl-LDL by the macrophage scavenger receptor.

Physicochemical Changes of Acetyl-LDL after Exposure to DMPC/apoA-I—To characterize the interaction of acetyl-LDL with DMPC/apoA-I, acetyl-LDL was incubated with DMPC/apoA-I in a cell-free system. After an 18-h incubation, the mixture was subjected to Sephacryl S-300 gel filtration chromatography (Fig. 4). Acetyl-LDL was eluted as a first peak and DMPC/apoA-I as a second peak (Fig. 4) which were confirmed by SDS-polyacrylamide gel electrophoresis (Fig. 5). There was no change in protein moieties of re-isolated acetyl-LDL and DMPC/apoA-I, indicating that transfer of apolipoprotein did...
not occur between acetyl-LDL and DMPC/apoA-I (Fig. 5).

Lipid analysis of re-isolated acetyl-LDL showed a 86% increase in phospholipid and a 21% decrease in total cholesterol. The corresponding increase (43%) in phospholipid and a significant increase in cholesterol were observed with re-isolated DMPC/apoA-I, but the change in cholesterol was relatively minor when compared with that of phospholipids (Table I). This indicates that DMPC was transferred from DMPC/apoA-I to acetyl-LDL. On a protein basis, the ratio of DMPC/apoA-I to acetyl-LDL was 5 in the present experiment. However, similar changes in lipid contents did occur to acetyl-LDL when the corresponding ratio was 2 or more (data not shown).

As a control experiment, acetyl-LDL was incubated with HDL and re-isolated on Sephacryl S-300 gel filtration chromatography. Lipid analysis of re-isolated acetyl-LDL showed a slight decrease in phospholipid rather than an increase, indicating that net phospholipid transfer from HDL to acetyl-LDL did not occur (Table II). As another control experiment, acetyl-LDL was incubated with DMPC liposomes and acetyl-LDL was re-isolated by ultracentrifugation. More than 50% of acetyl-LDL became insoluble aggregates, thus resulting in poor recovery of soluble acetyl-LDL (<20% of the initial amount). Lipid analysis of soluble acetyl-LDL showed a 74% increase in phospholipid (Table III), a finding similar to acetyl-LDL re-isolated after exposure to DMPC/apoA-I (86% increase) (Table I).

Acetylation of LDL is well known to increase its electrophoretic mobility (2, 38) which is critically important for recognition by the scavenger receptor. We examined therefore a change in net negative charge of re-isolated acetyl-LDL after exposure to DMPC/apoA-I. As Fig. 6 shows, acetylation of LDL markedly...
increased its electrophoretic mobility from β to near α position. In contrast, re-isolated acetyl-LDL after incubation with DMPC/apoA-I showed almost the same electrophoretic mobility with unmodified LDL, suggesting that the interaction with DMPC/apoA-I would result in a decrease in net negative charge of acetyl-LDL. Moreover, in order to examine the role of phospholipid (DMPC), acetyl-LDL incubation with DMPC liposomes showed a decreased electrophoretic mobility (Fig. 6), indicating that incorporation of DMPC into acetyl-LDL is important for the decrease in net negative charge. In contrast to DMPC/apoA-I, acetyl-LDL re-isolated after incubation with HDL showed no change in electrophoretic mobility.

To rule out the possibility that reversal of modified amino groups might occur to acetyl-LDL after exposure to DMPC/apoA-I, free amino groups were determined with trinitrobenzenesulfonic acid. Amounts of amino groups of acetyl-LDL used were 20 ± 1% of unmodified LDL, a value indistinguishable from those of acetyl-LDL re-isolated after exposure to DMPC/apoA-I (22 ± 2%). This indicates that the decrease in net negative charge that occurred to acetyl-LDL when exposed to DMPC/apoA-I is not due to detachment of acetyl groups from the modified protein.

**TABLE II**

| Lipid contents of acetyl-LDL re-isolated after cell-free incubation with HDL |
|--------------------------------------------------|
| Acetyl-LDL (1.0 mg/ml) was incubated at 37 °C for 18 h with HDL (5.0 mg of protein/ml) in 0.15 M NaCl and 1 mM EDTA (pH 7.4). Both particles were re-isolated by Sephacryl S-300 gel filtration chromatography (see Fig. 4) for determination of lipid contents. The abbreviations used are: TC, total cholesterol; TG, triglycerides; PL, phospholipids. Experimental errors in the lipid determination were within 5%. |
| lipid/protein weight ratio |
|--------------------------|
| TC | FC | CE | TG | PL |
| Acetyl-LDL | 1.97 | 0.47 | 1.50 | 0.47 | 1.29 |
| Re-isolated acetyl-LDL | 1.76 | 0.36 | 1.40 | 0.40 | 1.12 |
| HDL | 0.37 | 0.06 | 0.31 | 0.09 | 0.49 |
| Re-isolated HDL | 0.36 | 0.06 | 0.30 | 0.08 | 0.48 |

**TABLE III**

| Lipid contents of acetyl-LDL re-isolated after cell-free incubation with DMPC liposomes |
|--------------------------------------------------------------------------------------|
| Acetyl-LDL (1.0 mg/ml) was incubated at 37 °C for 18 h with DMPC liposomes (7.5 mg of DMPC/ml) in 0.15 M NaCl and 1 mM EDTA (pH 7.4). The mixture was adjusted to d = 1.019 with KBr and centrifuged at 100,000 × g for 24 h. Acetyl-LDL was collected from the bottom fraction and dialyzed against 0.15 M NaCl and 1 mM EDTA (pH 7.4). The abbreviations used are: TC, total cholesterol; TG, triglycerides; PL, phospholipids. Experimental errors in the lipid determination were within 5%. |
| lipid/protein weight ratio |
|--------------------------|
| TC | FC | CE | TG | PL |
| Acetyl-LDL | 1.70 | 0.43 | 1.27 | 0.46 | 1.19 |
| Re-isolated acetyl-LDL | 1.23 | 0.28 | 0.95 | 0.28 | 2.07 |

**Fig. 6.** Electrophoretic mobility of acetyl-LDL re-isolated after incubation with DMPC/apoA-I. Acetyl-LDL (1.0 mg of protein/ml) was incubated in a cell-free system with DMPC/apoA-I (5.0 mg of protein/ml or 7.5 mg of DMPC/ml), DMPC liposomes (7.5 mg of DMPC/ml), or HDL (5.0 mg of protein/ml) and re-isolated as described under "Experimental Procedures." Each sample was electrophoresed (10 μg/lane) on agarose gel and stained with Coomassie Brilliant Blue. Lane A, native LDL; lane B, HDL; lane C, acetyl-LDL; lane D, acetyl-LDL re-isolated after exposure to DMPC/apoA-I; lane E, acetyl-LDL re-isolated after exposure to DMPC liposomes; lane F, acetyl-LDL re-isolated after exposure to HDL. 

**Fig. 7.** Endocytic degradation by rat macrophages of acetyl-LDL re-isolated after exposure to DMPC/apoA-I. Macrophages (3 × 10^6) were incubated at 37 °C for 4 h with the indicated concentrations of the following 125I-labeled samples: acetyl-LDL (○), acetyl-LDL re-isolated after exposure to DMPC/apoA-I (●) or acetyl-LDL re-isolated after exposure to DMPC liposomes (▲). Trichloroacetic acid soluble radioactivity was determined as described under "Experimental Procedures." 

**DISCUSSION**

The present study demonstrated that HDL and reconstituted HDL (discoidal complexes of apoA-I with DMPC) differs from HDL in its mechanism for the antiatherogenic effects on macrophages. When macrophages were incubated both with acetyl-LDL and HDL, HDL did not affect cellular cholesterol supply by acetyl-LDL (Fig. 2) but enhanced cholesterol efflux from macrophages, thereby inhibiting CE accumulation (Fig. 1). In sharp contrast, DMPC/apoA-I interacted with acetyl-LDL in the medium and decreased its ligand activity for the macrophage scavenger receptor (Fig. 7), leading to a significant weakening of its CE accumulation capacity (Figs. 1 and 8). It was reported that DMPC/apoA-I (39, 40) or other types of apo-HDL/phospholipid complexes (41, 42) could induce cholesterol efflux from cells. However, the marked protective effect of DMPC/apoA-I, free amino groups were determined with trinitrobenzenesulfonic acid. Amounts of amino groups of acetyl-LDL used were 20 ± 1% of unmodified LDL, a value indistinguishable from those of acetyl-LDL re-isolated after exposure to DMPC/apoA-I (22 ± 2%). This indicates that the decrease in net negative charge that occurred to acetyl-LDL when exposed to DMPC/apoA-I is not due to detachment of acetyl groups from the modified protein.

**Biological Changes of Acetyl-LDL after Incubation with DMPC/ApoA-I**—The ligand activity of re-isolated acetyl-LDL after exposure to DMPC/apoA-I was expected to decrease because of a decrease in net negative charge. To test this, endocytic degradation of re-isolated acetyl-LDL was compared with that of non-treated acetyl-LDL. As shown in Fig. 7, degradation of re-isolated acetyl-LDL exposed to DMPC/apoA-I was decreased by 50–60% as compared with non-treated acetyl-LDL, indicating that the ligand activity of acetyl-LDL was significantly reduced as a result of interaction with DMPC/apoA-I. Acetyl-LDL re-isolated after exposure to DMPC liposomes also decreased its ligand activity to a similar extent (Fig. 7). The capacity of re-isolated acetyl-LDL to induce CE accumulation in macrophages was also compared. Nontreated acetyl-LDL showed a marked CE accumulation, whereas acetyl-LDL re-isolated after exposure to DMPC/apoA-I or DMPC liposomes had a limited capacity for CE accumulation (around 30% of nontreated acetyl-LDL) (Fig. 8).
apo-A on CE accumulation in macrophages observed in the present study (Fig. 1) could be due largely to a decreased ligand activity of acetyl-LDL for the scavenger receptor through interaction with DMPC/apoA-I (Fig. 7).

Two major changes in physicochemical property of acetyl-LDL after incubation with DMPC/apoA-I in a cell-free system were an increase in phospholipid contents (Table I) and a decrease in net negative charge (Fig. 6). It was reported that the transfer of phospholipid from reconstituted HDL to native lipoproteins such as LDL (43, 44) and HDL (46) readily occurs. When LDL was incubated with reconstituted HDL (egg phosphatidylcholine or dipalmitylphosphatidylcholine/FC/apoA-I) in a cell-free system, phospholipids were transferred from reconstituted HDL to LDL (43, 44). Transfer of DMPC from DMPC/apoA-I to HDL was also reported (45). Thus, phospholipid transfer from reconstituted HDL to acetyl-LDL observed under the present study is not specific for acetyl-LDL, but rather a general property of reconstituted HDL.

What is most noteworthy in the present study is that the ligand activity of acetyl-LDL for the scavenger receptor was significantly reduced after exposure to DMPC/apoA-I (Fig. 7). Since it is generally accepted that an increased negative charge of the ligand is crucial for its binding to the scavenger receptor (2, 38), the decreased ligand activity of acetyl-LDL would be largely explained by a decreased net negative charge. A similar decrease in electrophoretic mobility of acetyl-LDL also occurred when incubated with DMPC liposomes alone (Fig. 6). It is likely therefore that the DMPC incorporation into acetyl-LDL per se is responsible for the decrease in net negative charge. The role of apo-A-I, however, should not be neglected. When acetyl-LDL was incubated with DMPC liposomes, more than one-half of acetyl-LDL became insoluble. A similar phenomenon was reported for LDL after interaction with phosphatidylcholine liposomes (46). Therefore, apo-A-I might help prevent acetyl-LDL particles from aggregation during interaction of acetyl-LDL with DMPC liposomes.

It remains unclear how DMPC could decrease the total net charge of acetyl-LDL. Because DMPC itself is a neutral phospholipid, its incorporation into acetyl-LDL could not simply account for the change. In this context, LDL was reported to decrease its electrophoretic mobility when incubated with "CE microemulsions" having a monolayer of DMPC in the surface and cholesteryl linoleate in the core (47). Under the system, the incorporation of DMPC could decrease the net negative charge of LDL. If DMPC incorporation into acetyl-LDL induces a conformational change of apoB, it would, in part, explain the change of net negative charge and/or the decreased ligand activity. In this connection, the modification of lipid moiety of LDL is known to induce a conformational change of apoB and/or altered ligand activity for the LDL receptor (48-50). Depletion or significant reduction of triglycerides from LDL by hepatic lipase treatment resulted in an increased binding to the LDL receptor, under which a conformational change must have taken place in receptor binding sites of LDL because the modified LDL significantly reduced its reactivity towards the monoclonal antibody against apoB (48). Moreover, treatment of LDL with sphingomyelinase enhanced its binding capacity (49). On the other hand, treatment of LDL with cholesterol esterase reduced the binding capacity to its receptor (50). Experiments to determine whether conformational changes of apoB would take place in acetyl-LDL after exposure to DMPC/apoA-I is under investigation.

It is interesting to note that DMPC/apoA-I has a "neutralizing effect" on the ligand activity of acetyl-LDL whereas HDL does not. The lack of this effect in HDL would be due to its inability to induce net transfer of phospholipids to acetyl-LDL (Table II). Much tighter association of phospholipids to HDL particles than that of phospholipids to DMPC/apoA-I particles might prevent its phospholipids from being transferred to acetyl-LDL particles. This notion was supported by the following observations. First, after DMPC liposomes containing CE (cholesteryl linoleate) in the core portion were incubated with LDL, the liposomes became precipitated with LDL upon addition of MnCl2. Upon incorporation of apo-A-I into these liposomes, however, the co-precipitation with LDL no longer occurred (47). This indicates that the presence of CE in DMPC/apoA-I liposomes may change the particles so that their interaction with LDL is inhibited. The second observation was that transfer of phospholipids from reconstituted HDL (egg phosphatidylycholine or dipalmitylphosphatidylcholine/FC/apoA-I) to LDL was markedly inhibited by the addition of lecithin:cholesterol acyltransferase, an enzyme catalyzing the conversion of surface FC into core CE (43). This indicates that lectin:cholesterol acyltransferase-mediated CE formation in the core portion of the liposomes might inhibit the phospholipid transfer to LDL.

Interstitial fluid (22) and nascent HDL secreted from human hepatoma cells (HepG2) (23) are known to contain apoA-I/phospholipid complexes with negligible amounts of CE and FC. When macrophage foam cells were incubated with free apolipoproteins such as apo-A-I, apo-A-II, and apoE, discoidal HDL-like particles were produced from apolipoproteins and surface membrane phospholipids (13). Moreover, incubation of HDL with very LDL in the presence of free fatty acids resulted in production of discoidal particles (24). Thus, it is highly likely that discoidal HDL particles are present in an extravascular space as well as an intravascular one. In our preliminary experiment, when Cu2+-oxidized LDL was incubated with DMPC/apoA-I, the decrease in its electrophoretic mobility as well as the decrease in the ligand activity for the scavenger receptor were observed as in the case of acetyl-LDL.2 Taking these findings together, it seems reasonable to expect that neutralization of chemically modified LDL might occur in vivo through interaction with cholesterol-poor apoA-I/phospholipid complexes, and the neutralizing capacity of discoidal HDL on modified LDL might disappear in vivo along lectin:cholesterol acyltransferase-mediated maturation of HDL particles.

2 M. Sakai, A. Miyazaki, and S. Horiuchi, unpublished observations.
Interaction of Reconstituted HDL with Acetyl-LDL

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