Serum Albumin Is a Significant Intermediate in Cholesterol Transfer between Cells and Lipoproteins†

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ABSTRACT: The function of albumin in the movement of cholesterol into and out of non-cholesterol-loaded fibroblasts has been investigated. Cholesterol efflux from cholesterol-labeled normal human skin fibroblasts to fatty acid-free human serum albumin (HSA) is biphasic with a rapid first phase that plateaus at about 15 min followed by a nearly linear phase up to 90 min, the longest incubation in this study. Saturation of efflux is observed at about 10 mg of albumin/mL. Efflux is specific to albumin since other molecules, such as ovalbumin or gelatin, do not induce efflux. The ability of HSA to induce cellular cholesterol efflux is low compared to reconstituted discoidal lipoprotein A-I (LpA-I). HSA at 2 mg/mL produces a rate of cholesterol efflux similar to that of LpA-I at 45 μg of protein/mL; however, these concentrations are within the physiological range for both HSA and apolipoprotein A-I (apoA-I). The efflux to the medium containing both LpA-I and HSA is greater than that to each of them alone but does not show complete additivity, indicating a competition between HSA and LpA-I. The HSA-mediated cholesterol movement is bidirectional as demonstrated by the transfer of cholesterol from HSA−(^{3}H)-cholesterol complexes to fibroblasts; moreover, the HSA-mediated transfer is much faster than that from cholesterol-containing LpA-I (0.8 versus 0.2 pmol (μg of cell protein)$^{-1}$ (90 min)$^{-1}$. However, the presence of either low-density lipoprotein (LDL) or LpA-I in the incubation medium significantly inhibits the transfer of cholesterol from HSA−(^{3}H)-cholesterol complexes to fibroblasts, thus allowing the bidirectional transfer of cholesterol between HSA and cells to possibly operate as a net efflux. In conclusion, albumin plays a significant role in cholesterol transfer between cells and lipoproteins.

Previous studies have suggested the possible involvement of albumin in cholesterol metabolism and in the process of atherosclerosis. For example, a positive correlation was reported between the plasma total cholesterol/albumin ratio and the plasma total cholesterol/HDL[1] ratio (Nanji, 1983) or between serum albumin and total serum cholesterol (Gillum & Makuc, 1992; Gillum, 1993). Relatively low serum albumin has been suggested as a predictor of increased incidence and mortality for coronary heart disease (Phillips et al., 1989; Gillum & Makuc, 1992; Gillum, 1993).

Albumin contains six hydrophobic domains and is able to interact with a wide variety of hydrophobic ligands. The major ligands are fatty acids, which are otherwise insoluble in circulating plasma. Each albumin molecule has been reported to be able to bind up to six molecules of fatty acids [reviewed by Carter and He (1990) and Spector (1986)] as well as long-, medium-, and short-chain monoacylglycerol (Wang et al., 1993). In addition, albumin is also able to bind bile acids (Brock, 1976; Roda et al., 1982), steroid hormones, and some hydrophobic drugs, such as digitoxin (Brock, 1976). The albumin receptors on the surface of the liver cells take up fatty acids and other albumin-bound substrates (Weisiger et al., 1981; Forker & Luxon, 1981). There is no doubt that albumin is an important multifunctional transport protein in plasma.

Albumin might also play a role in the transport of lipid and cholesterol in plasma. An early report of Fielding and Moser (1982) showed that removal of albumin from plasma by affinity chromatography reduced by more than 50% the ability of plasma to release cholesterol from normal cultured fibroblasts. The study of Mendel and Kunitake (1988) showed a concentration-dependent stimulation of cholesterol efflux from the same cells by bovine serum albumin (BSA). Incubation of cholesterol-enriched Fu5AH rat hepatoma or GM 3468 human skin fibroblasts with BSA could significantly reduce the cell cholesterol content (Johnson et al., 1991). The function of albumin in lipid metabolism has been clearly demonstrated in analbuminemic patients and in nephrotic syndrome patients, as well as in Nagase analbuminemic rats (Cohen et al., 1980; Baldo-Enzi et al., 1987).

However, in spite of the above evidence for the participation of albumin in the metabolism of lipid and cholesterol, this protein has not been considered as a specific and significant contributor to cellular cholesterol movement and is in fact frequently used as a saturating carrier protein in such studies. This ignorance of the role of albumin in cholesterol efflux has been reinforced by recent characterization of high-affinity acceptors for cellular cholesterol which have been obtained using methods which could not register the participation of a bulk carrier such as albumin to this process (Castro & Fielding, 1988; Huang et al., 1994). We demonstrate here that, even in short-term incubations, albumin specifically mediates a bidirectional movement of

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[1] Abbreviations: apoA-I, apolipoprotein A-I; HSA, essentially fatty acid free human serum albumin; BSA, essentially fatty acid-free bovine serum albumin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; LpA-I, reconstituted high-density lipoprotein-like particles that containing only apolipoprotein A-I, NAR, Nagase analbuminemic rat; UC, unesterified cholesterol.

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cellular cholesterol with low affinity but high capacity. By virtue of its high concentration in plasma, albumin contributes to a significant proportion of cholesterol efflux. Furthermore, we show that albumin, like HDL, promotes a multidirectional transfer of cholesterol between cells and extracellular lipoproteins.

MATERIALS AND METHODS

Materials. Unlabeled cholesterol (+99% grade), gelatin, ovalbumin, and bovine or human serum albumin products have been purchased from Sigma Chemical Co. (St. Louis, MO). 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) has been obtained from Avanti Polar Lipids (Birmingham, AL) and guanidine hydrochloride from Bethesda Research Laboratories (Bethesda, MD). 1-2*n*-^{3}H-Cholesterol (specific activity of 52 Ci/mmol) was from Du Pont Canada Inc. (Mississauga, Ontario). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, and penicillin−streptomycin used for cell culture were purchased from Gibco (Grand Island, NY). All other reagents were analytical grade.

Cell Culture. Normal human skin fibroblasts at the 9th passage were purchased from Clonetics Inc. The cells were maintained in DMEM supplemented with 10% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.4 mM L-glutamine in an atmosphere of 5% CO_2 and 95% air, 37 °C. Cells used in this study were between 16th and 22nd passages.

Cholesterol Efflux from Cells. Four days before the experiment, human skin fibroblasts were seeded in Falcon 12-well tissue culture plates at a density of 5.5×10^4 cells/well and cultured for 48 h in the maintenance culture condition to reach approximately 70% confluence. The cell monolayers were then washed twice with phosphate-buffered saline (PBS), pH 7.4, containing 2 mg/mL essentially fatty acid-free bovine serum albumin (BSA) and incubated for 5 min at 37 °C in each washing, and twice with PBS alone. The labeling medium was made by drying 1-2*n*-^{3}H-cholesterol (20 μCi/well) under nitrogen and then resolubilizing in ethanol (less than 0.1% of the final medium volume). FBS at 5% of total volume was mixed with the ^{3}H-cholesterol−ethanol solution, then added to the final volume of DMEM containing complete supplements. The labeling medium (1 mL/well) was added to the washed cells, which were then cultured at 37 °C for 48 h before the efflux study.

For efflux studies, the ^{3}H-cholesterol-labeled cells were washed twice with DMEM containing 2 mg/mL BSA and twice with DMEM alone. The washed cells were then incubated at 37 °C with DMEM in the presence or absence of different cholesterol acceptors, using an orbital shaker (1.5 cycles/s). At the indicated time intervals, an aliquot of incubation medium (50 μl) was taken and mixed with 100 μL of PBS containing 2 mg/mL BSA. The mixtures were centrifuged at 10 000 rpm for 5 min to remove any possibly detached cells, and 100 μl of the supernatant was used for radioactivity counting. At the end of the incubation, cells were washed twice with PBS containing 2 mg/mL BSA and twice with PBS alone and lysed with 0.5 mL of 0.1 N NaOH. Aliquots were used for radioactivity counting and protein concentrations assayed by the Lowry method.

Incorporation of Cholesterol into Essentially Fatty Acid-Free Human Serum Albumin by Sonication. The incorporation of cholesterol into essentially fatty acid-free human serum albumin (HSA) was done basically as described by Jonas et al. (1989) for the labeling of reconstituted HDL-like particles. Cold cholesterol at a molar ratio of albumin/cholesterol of 1/2.5 was mixed with ^{3}H-cholesterol and dried under nitrogen. Then cold acetone (100 μL) was used to dissolve the cholesterol and added drop-wise during vortexing into a HSA solution (10 mg/ml in a recombination buffer containing 10 mM Tris-HCl, 1 mM NaN_3, 150 mM NaCl, and 0.01% EDTA). After evaporation of the acetone under nitrogen, the mixture was sonicated at an energy output of 40 W, 100% duty cycle (DC) for 1 min, incubated at 37 °C for 1.5 h, and sonicated 3×1 min again at 90% DC. The nonincorporated cholesterol was removed first by passage through a 0.45-μm syringe-tip filter, and then by size-exclusion chromatography on a Superose 12 column (50×2.5 cm) running at a flow rate of 0.25 mL/min. The fractions corresponding to the HSA peak were collected and concentrated with a Centriprep-30 concentrator (Amicon Inc., Beverly, MA). The specific activity of the final solution was 2.01×10^8 cpm/μmol of cholesterol or 4.81×10^6 cpm/μmol of HSA.

Purification of ApoA-I and Preparation of Reconstituted Discoidal LpA-I Particles Containing ^{3}H-Cholesterol. ApoA-I was prepared from delipidated HDL separated from normolipidemic subjects by sequential flotation ultracentrifugation (Schumaker & Puppione, 1986), and purified by anion-exchange chromatography on a Q-Sephacryl S-200 column as described previously (Brewer et al., 1986). Reconstituted discoidal HDL-like particles were made by sodium cholate dispersion (Sparks et al., 1992) starting from a mixture with the molar ratio of POPC/apoA-I/UC of 80:1:4. Hydrated Bio-Beads (1 g of Bio-Beads/2 mg of cholate) were used to remove sodium cholate. The characterization of particle size was as described by Williams et al. (1991). Protein concentration of the LpA-I was determined by the Lowry method, while phospholipid and cholesterol concentrations were assayed with enzymatic kits (Boehringer Mannheim GmbH, Mannheim, W. Germany). The specific activity of the reisolated LpA-I particles was 3.34×10^8 cpm/μmol of cholesterol or 9.64×10^8 cpm/μmol of apoA-I.

Transfer of Cholesterol from HSA−Cholesterol Complexes to Fibroblasts. Cells were seeded at 5.5×10^4 cells/well 4 days before the transfer experiment. After 48 h, the cells were washed as mentioned above for efflux experiments and changed to a medium containing 5% FBS and other supplements with the exception of label for another 48 h. The cells were then washed twice with DMEM containing 2 mg/mL BSA and twice with DMEM alone. Transfer was started by adding to the cell monolayers the medium containing either ^{3}H-cholesterol−LpA-I at a protein concentration of 45 μg/mL or HSA−(^{3}H)-cholesterol complexes at a protein concentration of 2 mg/mL. The incubation was carried out at 37 °C with gentle shaking as described for the cholesterol efflux study. At 2, 5, 15, 30, 60, and 90 min of incubation, medium was removed and the cells were washed twice with PBS containing 0.2% BSA and twice with PBS alone. Cells were then solubilized in 0.5 mL of 0.1 N NaOH, and aliquots were used for radioactivity counting and for protein assay. Cholesterol transfer was calculated as percent of original medium radioactivity transferred into cells and as cholesterol mass delivered to the cells.

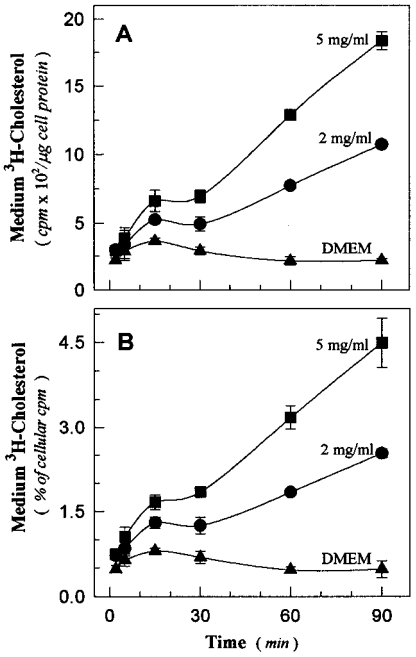

FIGURE 1: Time course of (^{3}H)-cellular cholesterol efflux to HSA. Human skin fibroblasts were labeled with (^{3}H)-cholesterol (20 μCi/well) in medium containing 5% FBS for 48 h. For the efflux study, the cells were washed twice with DMEM containing 2 mg/mL BSA and twice with DMEM alone and then were incubated with DMEM containing HSA at concentrations of 2 or 5 mg/mL, respectively. DMEM without HSA was used as control. Aliquots of medium were taken at 2, 5, 15, 30, 60, and 90 min or determination of radioactivity. Panel A shows the efflux expressed as medium radioactivity per microgram of cell protein ($n = 4$). Panel B shows the efflux expressed as percentage of (^{3}H)-cholesterol in the medium relative to total cellular radioactivity ($n = 4$).

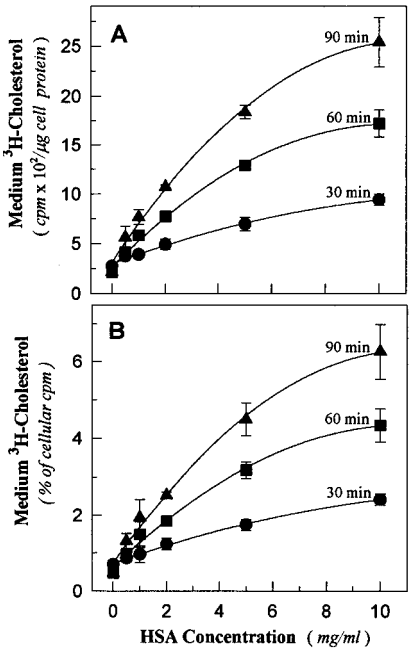

FIGURE 2: Cellular cholesterol efflux as a function of HSA concentration. Human skin fibroblasts were labeled as described in Figure 1. After washing, cells were incubated with DMEM containing 0.5, 1, 2, 5, and 10 mg/mL of HSA. DMEM without HSA was used as control. Aliquots of medium were taken at 30, 60, and 90 min respectively for determination of radioactivity. Panel A shows the efflux expressed as medium radioactivity per microgram of cell protein ($n = 4$). Panel B shows the efflux expressed as percentage of (^{3}H)-cholesterol in the medium relative to total cellular radioactivity ($n = 4$).

In another experiment, the LpA-I−(^{3}H)-cholesterol or HSA−(^{3}H)-cholesterol complexes used for the influx study were isolated from the incubation medium of HSA or LpA-I with (^{3}H)-cholesterol-labeled fibroblasts. The preparation procedure is as follows: Cells were seeded, washed, and labeled as for cholesterol efflux, and the cells then were incubated at 37 °C with DMEM containing either 2 mg/mL HSA or 45 μg/mL reconstituted discoidal LpA-I. The media were collected at 3 h of incubation, centrifuged at 10 000 rpm for 5 min to remove any detached cells, and then the cell-conditioned and labeled medium was added to new plates where unlabeled fibroblasts had undergone the same seeding, washing, and culture procedure. At 15 and 90 min of incubation, the medium was taken out, the cells were washed and solubilized as mentioned above, and aliquots of medium were used for radioactivity counting and protein assay.

Determination of ApoA-I and Fatty Acid Levels in Human Serum Albumin Preparations. The presence of apoA-I in different HSA preparations was assayed by both radioimmunoassay (Calabresi et al., 1993) and western blot. For western blot, 50 μg of each different HSA preparation was electrophoresed on a nondenaturing 4−20% polyacrylamide gel and stained with Coomassie blue. The proteins from a duplicate gel were transferred to a nitrocellulose membrane and immunoblotted by 4H1, a monoclonal antibody against the N-terminal 2−8 amino acids residues of apoA-I (Marcel et al., 1991). Determination of fatty acids present in different HSA preparations was carried out after extraction of lipid according to the method of Bligh and Dyer (1959), by gas chromatography using C17:0 methyl ester as internal standard. The data were expressed as total fatty acids per milligram of protein.

RESULTS

Cholesterol Efflux from Fibroblasts to Human Serum Albumin. The effect of human serum fraction V, essentially fatty acid-free, albumin (HSA) on cholesterol efflux from ^{3}H-cholesterol-labeled, non-cholesterol-loaded human skin fibroblasts was studied initially. The results indicate that this protein is able to promote a significant efflux of cellular cholesterol from fibroblasts during a 90-min incubation as illustrated in Figure 1. The efflux follows a biphasic pattern, characteristic of human skin fibroblasts, which is also observed with media containing HDL or reconstituted LpA-I (not illustrated). The initial rapid efflux reaches a peak at about 15 min and is followed by a plateau up to about 30 min. The second phase of efflux is linear for up to 90 min as observed in this study. When fibroblasts are incubated with HSA for 30, 60, or 90 min, the efflux is concentration-dependent within a range of 0.5−10 mg of protein/mL and appears to saturate at a concentration of about 10 mg/mL at both 60 and 90 min of incubation (Figure 2). The estimated EC_{50} values at 60 and 90 min of incubation are 3.87 and 2.56 mg/mL, respectively.

It should be emphasized that the ability of HSA to promote cellular cholesterol efflux is much lower than that observed from HDL or reconstituted Lp2A-I. As shown in Figure 3, HSA at a concentration of 2 mg/mL induces cholesterol efflux rates which are very close to those induced by 45 μg of protein/mL reconstituted Lp2A-I at both 60 and 90 min of incubation. The ED_{50} of efflux to Lp2A-I is in the range

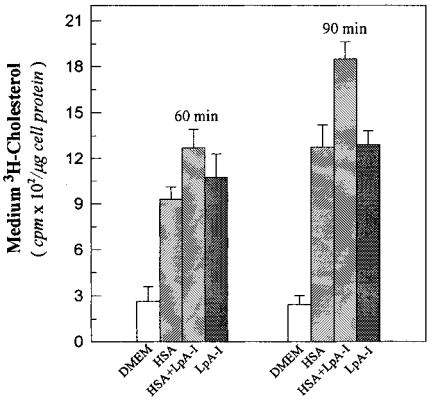

FIGURE 3: Comparison of cholesterol efflux to HSA and to LpA-I. (^{3}H)-Cholesterol-labeled human skin fibroblasts were incubated with DMEM containing either 2 mg/mL of HSA or 45 μg of protein/mL LpA-I or both. Cholesterol efflux was measured by taking medium aliquots at 60 and 90 min for radioactivity counting. The data are expressed as medium radioactivity per microgram of cell protein. *T*-test shows that the difference in efflux rates was statistically significant at 60 min ($p < 0.05$) and 90 min of incubation ($p < 0.01$) between HSA and HSA plus LpA-I and at 90 min between LpA-I and HSA plus LpA-I ($p < 0.01$).

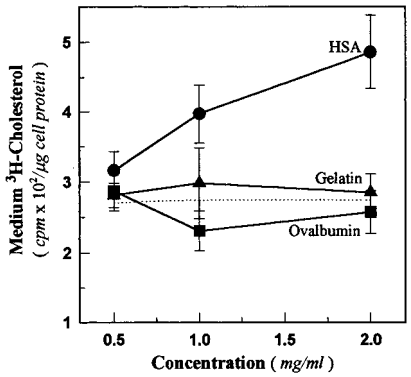

FIGURE 4: Comparison of the effects of HSA, gelatin, and ovalbumin on cellular cholesterol efflux. Human skin fibroblasts were labeled as described in Figure 1. Cellular cholesterol efflux was studied in DMEM medium containing HSA, gelatin, or ovalbumin at concentrations of 0.5, 1, or 2 mg/mL. DMEM alone was used as control (showed as the dotted line). Medium aliquots were taken at 60 min for radioactivity determination, and efflux was expressed as medium radioactivity per microgram of cell protein.

of 60−90 μg of protein/mL (Zhao and Marcel, unpublished data). The interactions of HSA and LpA-I with cholesterol coming out of cells are probably competitive since the efflux to a medium containing both HSA (2 mg/mL) and Lp2A-I (45 μg of protein/mL) is less than the sum of the efflux rates induced by each of them alone. However the efflux to a medium containing both HSA and Lp2A-I is statistically higher than that to the media with each of them alone at either 60 min ($p < 0.05$) or 90 min ($p < 0.01$) of incubation.

Specificity of Cholesterol Efflux from Fibroblasts to Human Serum Albumin. To demonstrate that this process represents a specific interaction of cholesterol with albumin, we also studied the cholesterol efflux mediated by two other molecules, ovalbumin, a non-serum albumin, and gelatin, a matrix molecule. Cholesterol efflux to a medium containing either HSA, ovalbumin, or gelatin at the same concentrations of 0.5, 1, and 2 mg/mL were compared. As shown in Figure 4, neither gelatin nor ovalbumin could replicate the stimulating effect of serum albumin on cholesterol efflux. In fact, ovalbumin has a slightly inhibitory effect on cholesterol efflux.

Since the presence of apoA-I in commercial serum albumin products has been reported previously (Fainaru & Deckelbaum, 1979; Deckelbaum et al., 1980) and since apoA-I alone is able to promote the efflux of cholesterol from cells (Forte et al., 1993; Hara & Yokoyama, 1992; Bielicki et al., 1992), the presence of apoA-I in the albumin products used in this study has been analyzed. Radioimmunoassay shows apoA-I to be present in the HSA preparation at less than 0.006 μg of apoA-I/mg of HSA. This negligible concentration of apoA-I in our HSA preparations is also corroborated by immunoblot estimation of the HSA separated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (results not illustrated). Therefore these trace amounts of apoA-I could not contribute to the efflux mediated by HSA.

The concentrations of fatty acids in regular fraction V HSA and in essentially fatty acid-free HSA were also measured and found to be 12.0 and 6.8 μg of fatty acid/mg of protein, respectively. When these different preparations of human serum albumin were compared, cholesterol efflux to regular fraction V HSA was only slightly higher than that to essentially fatty acid-free HSA, in agreement with a previous study (Chau et al., 1978). However, since immunoblot had demonstrated the presence of apoA-I in regular fraction V HSA preparation, the higher cellular cholesterol efflux to regular fraction V HSA may also reflect the contribution of the contaminating apolipoproteins.

Transfer of Cholesterol from HSA−(^{3}H)-Cholesterol Complexes or LpA-I−(^{3}H)-Cholesterol to Fibroblasts. Cholesterol transfer between cells and native HDL or reconstituted LpA-I is known to be bidirectional (Johnson et al., 1986); therefore, one should expect that in addition to promoting cellular cholesterol efflux, albumin can also deliver cholesterol to fibroblasts. To verify this possibility, we prepared HSA−(^{3}H)-cholesterol complexes by incorporation of labeled cholesterol into HSA molecules by sonication. The HSA−(^{3}H)-cholesterol complexes were purified by size exclusion chromatography. The overlap of the elution profiles of the radioactivity of ^{3}H-cholesterol and protein absorbance at 280 nm demonstrates the homogeneous distribution of cholesterol and albumin and documents the association of cholesterol with HSA (Figure 5). When the HSA−(^{3}H)-cholesterol complexes were incubated with fibroblasts (not loaded with cholesterol), the cellular ^{3}H-cholesterol content increased with time (Figure 6), demonstrating that HSA−(^{3}H)-cholesterol complexes are able to deliver their cholesterol content to cells. It is of interest that the transfer rate of cholesterol from HSA to fibroblasts is faster than that from cholesterol-containing reconstituted discoidal LpA-I. At 90 min of incubation, the percentage of cholesterol transfer from HSA−(^{3}H)-cholesterol complexes to fibroblasts was 13.8%, in contrast to 0.7% from cholesterol-containing discoidal LpA-I (Figure 6A). This represents mass transfers of 0.8 and 0.2 pmol of cholesterol/μg of cell protein, respectively, from HSA and LpA-I (Figure 6B) as calculated from their specific activities.

In another experiment, HSA or LpA-I was labeled with cholesterol by incubation for 3 h with ^{3}H-cholesterol-labeled fibroblasts as described under Materials and Methods. The cell-conditioned media were centrifuged to remove any possibly detached cells and then transferred to nonlabeled cells and incubated for 15 and 90 min in order to measure

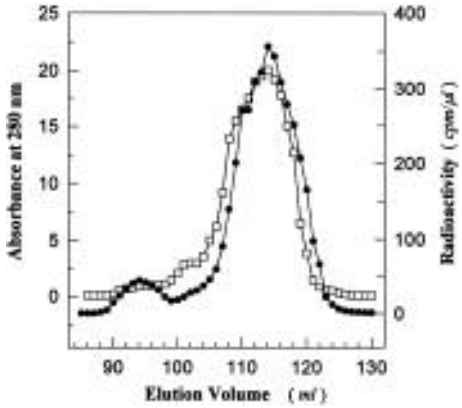

FIGURE 5: Elution profile of HSA−(^{3}H)-cholesterol complex. Cholesterol was incorporated into HSA by sonication as described under Materials and Methods. After passage through a 0.45-μm syringe-tip filter, the (^{3}H)-cholesterol-labeled HSA was purified on a Superose 12 column. Fractions collected (1.5 mL) are represented as filled circles for radioactivity and as open squares for relative absorbance at 280 nm.

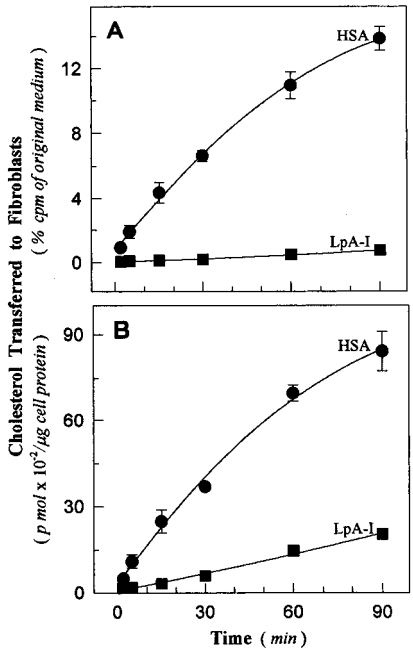

FIGURE 6: Transfer of cholesterol from HSA−(^{3}H)-cholesterol complex or from (^{3}H)-cholesterol LpA-I to fibroblasts. Fibroblasts were seeded and grown under the same conditions as in other efflux studies but without (^{3}H)-cholesterol label. The cells were then incubated with DMEM containing either HSA−(^{3}H)-cholesterol complex (4.81×10^6 cpm/μmol of protein, 2.01×10^8 cpm/μmol of cholesterol) at a concentration of 2 mg/mL or 45 mg of protein/mL discoidal reconstituted LpA-I prelabeled with (^{3}H)-cholesterol (9.64×10^8 cpm/μmol of apoA-I or 3.34×10^8 cpm/μmol of cholesterol). At the indicated times, media were taken out, cells were washed twice with PBS in the presence of 2 mg/mL BSA and twice with PBS alone. The washed cells were then lysed in 0.5 mL of 0.1 N NaOH and aliquots were used for radioactivity counting. Panel A represents the percentage of (^{3}H)-cholesterol transferred to the cells. Panel B gives the calculated mass transfer of cholesterol to the cells.

the reverse cholesterol transfer from each donor to the cells. The radioactivities associated with HSA (2 mg/mL) or with discoidal reconstituted LpA-I (45 μg/mL) after 3 h of preincubation with fibroblasts were similar (1.22×10^6 and 1.13×10^6 cpm/mL, respectively). As observed with the transfer from sonicated HSA−(^{3}H)-cholesterol complexes, cholesterol transfer from cell-conditioned HSA to fibroblasts

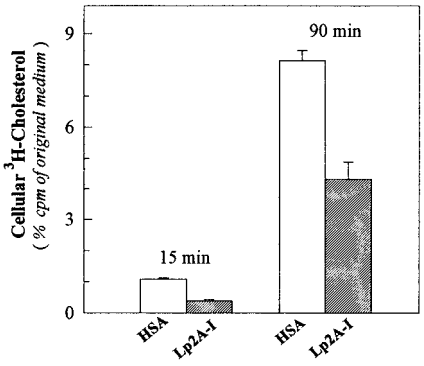

FIGURE 7: Transfer of cell-derived cholesterol from HSA or LpA-I to cells. Human skin fibroblasts were labeled with 20 μCi/well (^{3}H)-cholesterol and, after washing, were incubated with fresh DMEM containing either 2 mg/mL HSA or 45 mg/mL reconstituted discoidal LpA-I as described for Figure 1. After 3 h of incubation, the media were taken out, centrifuged to remove any detached cells, and then transferred to other 12-well plates containing unlabeled cell monolayers that had been incubated in the presence of 5% of FBS for 48 h. After 15 and 90 min of incubation at 37 °C, the media were taken out and the cells were washed and lysed with 0.1 N NaOH. The radioactivity in both media and cell lysates were counted. The data are expressed as the percentage of radioactivity transferred to cells.

was faster than that from LpA-I (1.1% versus 0.6% at 15 min and 8.1% versus 4.3% at 90 min) (Figure 7).

Transfer of Cholesterol from HSA−(^{3}H)-Cholesterol Complex to Fibroblasts in the Presence of LDL or Reconstituted Discoidal LpA-I. The transfer of cholesterol from HSA−(^{3}H)-cholesterol complexes to fibroblasts was studied in the presence of either LDL or reconstituted LpA-I at various concentrations up to 80 μg of protein/mL. In the study with LDL, two monoclonal antibodies, 4G3 and 5E11 (Milne et al., 1989) were added, each in 3-fold molar excess relative to LDL−apoB, in order to prevent binding of LDL to the LDL receptors of fibroblasts. As expected, in the presence of either of these lipoproteins, cholesterol transfer from HSA−(^{3}H)-cholesterol complexes to the cells decreased (Figure 8). The inhibition by LDL or LpA-I is concentration-dependent with an EC_{50} of less than 5 μg of protein/mL and a maximal inhibition at about 40 μg of protein/mL for both of these lipoproteins.

DISCUSSION

The results of this study clearly demonstrate that albumin can play a role in the transport of cholesterol between cells and lipoproteins. This is in agreement with the earlier report of Fielding and Moser (1982), which showed that removal of albumin from human plasma decreased by about 50% its ability to promote cellular cholesterol efflux. This effect is specific to serum albumin and cannot be replicated by either an avian protein such as ovalbumin or a matrix molecule like gelatin (Figure 4). There is a low level of fatty acids in the HSA preparations used in this study, and we have observed that regular fraction V HSA preparation, which contains relatively more fatty acids, promotes slightly more cellular cholesterol efflux than fatty acid-free albumin. It is therefore possible that cholesterol may be indirectly associated with albumin through interactions with some of its hydrophobic ligands. On the other hand, the presence of a trace amount of apoA-I in the essentially fatty acid-free HSA preparations used makes it unlikely that apoA-I could

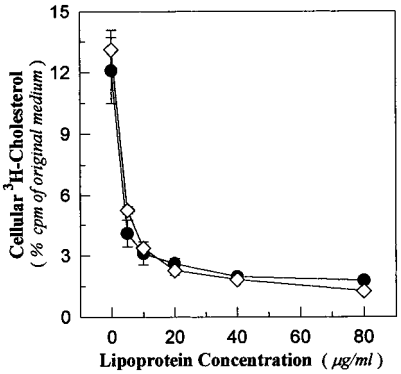

FIGURE 8: Transfer of cholesterol from HSA−(^{3}H)-cholesterol complex to fibroblasts in the presence of lipoproteins. Fibroblasts were treated as described for Figure 7 except that the incubation medium contained 5, 10, 20, 40, and 80 mg of protein/mL of either freshly separated LDL or reconstituted discoidal LpA-I which was added to the HSA−(^{3}H)-cholesterol complexes immediately before addition to the cells. For the incubation with LDL, this lipoprotein was preincubated with anti apoB mAb (4G3 and 5E11) at 3 times molar excess, 37 °C for 1.5 h before addition to the medium to prevent the binding of LDL to the LDL receptor. Media were taken out at 90 min of incubation, and cells were washed twice with PBS in the presence of 2 mg/mL BSA and twice with PBS alone. The washed cells were then lysed in 0.5 mL of 0.1 N NaOH, from which 200 μL was taken for radioactivity counting. The open lozenges represent the percentage of (^{3}H)-cholesterol transferred to the cells in the presence of LDL, while the filled circles are the transfer of (^{3}H)-cholesterol in the presence of Lp2A-I.

have contributed to the albumin-mediated efflux of cholesterol.

It is not unexpected that plasma albumin should be able to bind to cholesterol and appear to function as a cholesterol transporter. As discussed above, albumin is a multiligand carrier for a large variety of hydrophobic ligands, such as hormones (Brock, 1976), bile acids (Brock, 1976; Roda et al., 1982), hydrophobic drugs (Brock, 1976), and the most predominant, fatty acids and monoacylglycerol (Carter & He, 1990; Wang et al., 1993; Spector, 1986). As shown here, the binding ability of albumin for cholesterol is low: (i) the in vitro combination of HSA and cholesterol by sonication yields complexes of HSA and cholesterol with a molar ratio of approximately 20/1 and(ii) HSA at a concentration of 2 mg/mL shows an ability to release cellular cholesterol equivalent to that of LpA-I at 45 μg of protein/mL. While this makes apoA-I in LpA-I 19 times more efficient than albumin, the plasma albumin molarity is about 15-fold that of apoA-I and is therefore compatible with the concept of an albumin-mediated cholesterol efflux equivalent to that of apoA-I. Furthermore, albumin-bound cholesterol measured here in vitro may underestimate the level bound in vivo under the conditions in the blood stream where albumin is exposed to a much larger pool of exchangeable cholesterol. Nevertheless, on the basis of the in vitro-measured binding of cholesterol to albumin (20/1), we can estimate that the size of the albumin cholesterol pool may represent between 1% and 2% of the total cholesterol pool in blood (including lipoproteins and erythrocytes). In interstitial fluid, where the ratio of albumin to apoA-I is greater than that of plasma (Wong et al., 1992), where lipoprotein concentration decreases in proportion to its size, and where erythrocytes and other blood cells are excluded, the relative size of the albumin-bound cholesterol pool could be considerably greater, possibly by more than 10-fold. Thus albumin could be particularly important in mediating cholesterol equilibration between cells and lipoproteins in the interstitial fluid compartment where this process is most physiologically relevant.

This study is the first to demonstrate that albumin can mediate a multidirectional transfer of cholesterol between cells and extracellular lipoproteins. In addition to its function in promoting cellular cholesterol efflux, albumin-bound cholesterol can be transferred into cells faster than that from LpA-I (Figure 6). It is of interest that the transfer of cholesterol from HSA−(^{3}H)-cholesterol complex to cells is very sensitive to the presence of LDL or reconstituted discoidal LpA-I in the incubation medium. With only 5 μg of protein/mL, either LDL or LpA-I, the transfer of cholesterol from HSA−(^{3}H)-cholesterol complex to cells is inhibited by about 65%. This leads us to hypothesize that plasma albumin can serve as a transient carrier for cholesterol between cells and other lipoproteins. By itself, albumin may be able to promote the efflux of cholesterol from certain extrahepatic cells to form albumin−cholesterol complexes, from where the cell-derived cholesterol is transferred to lipoproteins which have higher affinity for cholesterol. Thus albumin can mediate an initial pathway for the reverse cholesterol transport which may be an alternative to that mediated by preβ-HDL (Castro & Fielding, 1988) or by γ-Lp(E) (Huang et al., 1994).

Albumin deficiency results in altered lipid and cholesterol metabolism, such as in congenital analbuminemia and in the nephrotic syndrome characterized by greatly elevated plasma levels of triacylglycerol and cholesterol (Baldo-Enzi et al., 1987; Cohen et al., 1980). The same phenomenon is also observed in the analbuminemic rat, a mutant Sprague-Dawley rat with a posttranscriptional deficiency of albumin mRNA (Nagase et al., 1979; Takahashi et al., 1983; Catanozzi et al., 1994; Van Tol et al., 1991; Joles, 1991). A positive relationship between plasma albumin and HDL cholesterol has also been reported in nephrotic syndrome (Cohen et al., 1980). Some plasma enzymes involved in lipid and cholesterol metabolism have altered activities in analbuminemia. Lecithin:cholesterol acyltransferase (LCAT) activity is increased in both analbuminemic patients and NAR (Baldo-Enzi et al., 1987; Van Tol et al., 1991), and hepatic triacylglyceride lipase is slightly decreased (Baldo-Enzi et al., 1987). However, up to now, the mechanism involved in the analbuminemia-related disorder of lipid and cholesterol metabolism is still unclear.

ApoA-I containing HDL has been reported to play a central role in cholesterol metabolism through its functions as an acceptor for cell-derived cholesterol and the subsequent esterification of the cell-derived cholesterol. However, although reduced plasma concentrations of this apolipoprotein and especially LpA-I have been reported in patients with coronary disease (Kukita et al., 1984; Puchois et al., 1987), deficiency or severe reduction of apoA-I does not increase the risk of coronary disease in mice (Li et al., 1993). Similarly, analbuminemic individuals or analbuminemic rats do not show increased risk for cardiovascular disease (Baldo-Enzi et al., 1987; Joles et al., 1991). This may be related to the compensatory synthesis of other proteins induced by deficiency of plasma albumin (Joles et al., 1991; Baldo-Enzi et al., 1987); however, the apolipoprotein profiles of analbuminemic subjects indicate that not all the apolipoproteins increase simultaneously (Joles et al., 1991; Baldo-Enzi et

al., 1987; Van Tol, 1991). The specific increase in HDL_3 apoA-I would be expected to compensate for the reduced cellular cholesterol efflux due to lack of albumin since LpA-I is well-known to be a good acceptor for cell-derived cholesterol (Mahlberg et al., 1990, 1991; DeLamatre et al., 1986; Stein et al., 1986).

In summary, we have shown that albumin is a significant mediator for cellular cholesterol movement and can contribute to the transfer of cholesterol between cells and lipoproteins, which ensures the eventual return of cell-derived cholesterol to the liver.

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