Cholesterol efflux from cells to immunopurified subfractions of human high density lipoprotein: LP-AI and LP-AI/AII

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Abstract Using immunoaffinity chromatography, we separated human high density lipoprotein (HDL) into two subfractions: LP-AI, in which all particles contain apolipoprotein A-I (apoA-I) but no apoA-II, and LP-AI/AII, in which all particles contain both apoA-I and apoA-II. To compare LP-AI and LP-AI/AII as acceptors of cell cholesterol, the isolated subfractions were diluted to 50 μg phospholipid/ml, and then incubated with monolayer cultures of cells in which whole-cell and lysosomal cholesterol has been labeled with ^{14}C and ^{3}H, respectively. We used three cell types (Fu5AH rat hepatoma cells, normal human skin fibroblasts, and rabbit aortic smooth muscle cells). When these cells were prepared to contain normal physiological quantities of cholesterol (20–35 μg/mg protein), LP-AI and LP-AI/AII were nearly equally efficient in promoting efflux of both whole-cell and lysosomal cholesterol. For whole-cell cholesterol, the rate constants for efflux to LP-AI and LP-AI/AII were: 0.050/h and 0.053/h, respectively, with Fu5AH cells; 0.0063/h and 0.0074/h with GM3468 human skin fibroblasts; and 0.0076/h and 0.0079/h with rabbit aortic smooth muscle cells. When cholesterol in hepatoma cells or fibroblasts was elevated two- to threefold above normal, there was still not difference in efflux of whole-cell cholesterol to LP-AI and LP-AI/AII. In long-term incubations, the net depletion of cholesterol mass from cholesterol-enriched cells was either identical with the two HDL subfractions, or somewhat greater with LP-AI/AII. The present results suggest that with several types of mammalian cells: *1*) LP-AI and LP-AI/AII function equally well in removing cholesterol that originates in either the plasma membrane or lysosomes; and *2*) efflux to both fractions is likely to be governed primarily by the unmediated diffusion of cholesterol between the plasma membrane and the lipoprotein particles.—**Johnson, W. J., E. P. C. Kilsdonk, A. van Tol, M. C. Phillips, and G. H. Rothblat.** Cholesterol efflux from cells to immunopurified subfractions of human high density lipoprotein: LP-AI and LP-AI/AII. *J. Lipid Res.* 1991. **32:** 1993–2000.

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The major portion of cholesterol in nucleated cells of mammals is found in the plasma membrane, although significant amounts also are found in intracellular membranes and lipid storage droplets (1). In most extrahepatic cells, there is little or no oxidative degradation of cholesterol, and sterol homeostasis requires that excess cholesterol be removed and transported to the liver. These removal and transport processes collectively are termed reverse cholesterol transport, and are mediated by plasma lipoproteins (reviewed in ref. 2). The initial removal of sterol from cells involves the transfer of cholesterol from the plasma membrane and possibly from intracellular membranes to particles within the high density lipoprotein (HDL) fraction of blood plasma and interstitial fluid (3–5).

Using immobilized antibodies to the major apolipoproteins of HDL (apoA-I and apoA-II), plasma HDL can be separated by immunoaffinity chromatography into two major subfractions: LP-AI, in which all particles contain apoA-I but no apoA-II, and LP-AI/AII, in which all particles contain both apoA-I and apoA-II (6). By nondenaturing two-dimensional gel electrophoresis, it has been shown that plasma HDL contains minor components that migrate to the pre-beta position on agarose gel electrophoresis and that contain apoA-I and apoD, but no apoA-II (7). These pre-beta particles should contribute to the LP-AI fraction of HDL.

In studies on the efflux of cell cholesterol to immunopurified subfractions of HDL, Barbaras et al. (8) found that LP-AI can efficiently remove excess cholesterol from cholesterol-enriched OB1771 mouse adipocytes,

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; r[^{3}H-CO]LDL, low density lipoprotein reconstituted with [^{3}H]cholesteryl oleate; apoA-I, apolipoprotein A-I; LP-AI, immunopurified HDL fraction containing apoA-I, but no apoA-II; LP-AI/AII, immunopurified HDL fraction containing both apoA-I and apoA-II; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; BSA, bovine serum albumin; FC, free (unesterified) cholesterol; MEM, Eagle's minimum essential medium; ACAT, acyl-coenzyme A:cholesterol acyltransferase; LCAT, lecithin:cholesterol acyltransferase.

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whereas LP-AI/AII has little or no effect on cell sterol levels. Similarly, Castro and Fielding (9) have reported that when fibroblasts are incubated with human plasma, the primary acceptors of cell cholesterol are pre-beta HDL particles, which contain apoA-I, but no apoA-II. These observations suggest that particles within the HDL fraction that contain apoA-I, but no apoA-II, may have critical functions in the removal of sterol from cells and in the initiation of reverse cholesterol transport. It has been proposed that LP-AI induces the mobilization and removal of cell cholesterol through interaction with HDL-specific binding sites on the adipocyte surface, and that LP-AI/AII, which also binds to HDL-specific sites on cells, antagonizes this process (8, 10).

In the present studies, we prepared LP-AI and LP-AI/AII by accepted procedures and compared the two subfractions as acceptors of cholesterol derived from serveral types of cells. We chose cell types that are established tissue culture models for studies on sterol movement between HDL and cells. The cell-labeling procedures permitted examination of the efflux of both plasma membrane and LDL-derived lysosomal cholesterol. In addition, we investigated the ability of the subfractions to produce net depletion of cell cholesterol mass, and possible changes in efflux induced by the enrichment of cells with cholesterol.

MATERIALS AND METHODS

Materials

Commercial sources of chemicals, supplies, solvents, and isotopic compounds were as described previously (11). Human low density lipoprotein (LDL, d 1.019–1.063 g/ml), HDL_3 (d 1.125–1.21 g/ml), and lipoprotein-deficient serum (density > 1.21 g/ml), as well as bovine delipidized serum protein and human low density lipoprotein reconstituted with [1,2-^{3}H]cholesteryl oleate (r[^{3}H-CO]LDL) were prepared as described previously (11). Compound 58-035 (an inhibitor of acyl-coenzyme A:cholesterol acyltransferase, ACAT) was a gift from Dr. John Heider, Sandoz Incorporated (Hanover, NJ).

Immunopurified subfractions of HDL (LP-AI and LP-AI/AII) were prepared from human plasma as described by Kilsdonk, Van Gent, and van Tol (12). All preparation procedures and storage were done at 4°C or on ice. The major proteins of LP-AI/AII, as determined by denaturing polyacrylamide gel electrophoresis, were apoA-I and apoA-II, whereas the major protein of LP-AI was apoA-I, with no detectable apoA-II. Neither fraction contained detectable apoE. The phospholipid-to-protein ratios in LP-AI/AII and LP-AI were 0.39 and 0.46 (w/w), respectively. The corresponding free cholesterol-to-phospholipid ratios were 0.30 and 0.47 (mole/mole), respectively. Complete compositional data on LP-AI and LP-AI/AII are provided by Kilsdonk et al. (12). Prior to experiments, the fractions were dialyzed exhaustively against phosphate-buffered saline and then against 30–50 volumes of MEM tissue culture medium (containing 14 mM HEPES buffer, pH 7.4, and 50 μg/ml of gentamicin). After dialysis, the fractions were sterilized by filtration (0.45 μm pore size), and then assayed for phospholipid content (13). To prepare efflux media, the fractions were diluted to a concentration of 50 μg phospholipid/ml (ca. 120 μg protein/ml). This concentration approximates the level of HDL in mammalian interstitial fluid (14), and is within a range used previously to demonstrate dramatic differences between LP-AI and LP-AI/AII in the ability to remove cholesterol from OB1771 mouse adipocytes (8). Typically, 1–2 weeks elapsed between the drawing of blood and the use of immunopurified fractions in experiments.

Plasma from female donors was found to contain high concentrations of both LP-AI and LP-AI/AII, whereas male donor plasma typically contained very little LP-AI. To ensure well-controlled comparisons with adequate amounts of both subfractions, all experiments were conducted with subfractions obtained from female donors. In a given experiment, LP-AI and LP-AI/AII were from a single collection of plasma obtained from one donor. For both LP-AI and LP-AI/AII, there appear to be only minor compositional differences between male- and female-derived materials (15, 16). Thus, it seems unlikely that the results of the present studies were biased significantly by using only subfractions from female donors.

The cell lines used in these experiments were Fu5AH rat hepatoma cells, GM3468A human skin fibroblasts, GM0637 human fibroblasts, and rabbit aortic smooth muscle cells (grown from an explant in this laboratory). All cells were grown in monolayer culture. The growth media for routine propagation of cells were 5% bovine calf serum/95% MEM (for Fu5AH cells) and 10% fetal bovine serum/90% MEM (for fibroblasts and smooth muscle cells). The Fu5AH cell was chosen for initial comparisons between subfractions because of this cell's ability to release cholesterol rapidly from its plasma membrane (2). This property allows incubation times to be kept relatively short. In addition, it increases the likelihood that the efflux of internal sterol will be rate-limited by intracellular transport rather than desorption from the cell surface, thus improving the reliability of using efflux of internal sterol as a measure for delivery of the sterol to the plasma membrane.

Methods

Unless otherwise indicated, all work with cells was performed at 37°C. In a given experiment, cells were prepared in one of two ways.

1) To examine the efflux of both whole-cell cholesterol and LDL-derived lysosomal cholesterol, cells were pre-

pared in 22-mm wells of 12-well tissue culture plates as described in Johnson et al. (11). Briefly, this involved incubation of cell monolayers for 2 days in MEM-based medium that contained 10 mg protein/ml of human lipoprotein-deficient serum, and 0.025–0.05 μCi/ml [4-^{14}C]cholesterol (dispersed with 5 μg/ml egg phosphatidylcholine and 0.1% ethanol), followed by a 5-h, 15°C pulse with r[^{3}H-CO]LDL (10 μg protein/ml), and then incubation at 37°C in efflux medium, which consisted of HEPES-buffered MEM containing HDL, an HDL subfraction, or bovine serum albumin (BSA). During the 2-day incubation with [^{14}C]cholesterol, this tracer should label all cellular pools to the same specific activity. Thus, it labels whole-cell cholesterol and is expected to be present mostly in the plasma membrane at the beginning of the efflux period (17). Previously, we have found that the efflux of whole-cell cholesterol from intact cells is representative of cholesterol desorption from the plasma membrane (18). During the 5-h incubation at 15°C, reconstituted LDL is delivered to endosomes by LDL receptor-mediated endocytosis (19). Subsequently, when cells are warmed to 37°C, the LDL is delivered rapidly to lysosomes, where it is degraded, resulting in the generation of free [^{3}H]cholesterol in lysosomes. This label becomes available for efflux only after transport to the plasma membrane.

II) To examine the efflux of whole-cell cholesterol from cells that were enriched with free cholesterol, cells were prepared as described by Johnson et al. (20). Briefly, this involved incubation of cell monolayers with human lipoprotein-deficient serum, followed by a 1-day incubation with cholesterol enrichment medium, which contained 50 μg protein/ml of native human LDL, 100 μg cholesterol/ml of cholesterol-enriched liposomes (cholesterol/egg phosphatidylcholine $\geq$ 2 mol/mol), 1 μg/ml of the ACAT inhibitor (Sandoz compound 58-035) (dispersed with 0.5% dimethylsulfoxide), and 0.05–0.1 μCi/ml [^{14}C]cholesterol (dispersed with 0.1% ethanol and 1% fetal bovine serum). Cells then were incubated with efflux medium that was supplemented with compound 58-035 (dispersed with 0.5% dimethylsulfoxide). Control (unenriched) cells were treated identically, except that the enrichment medium was replaced with medium that contained cholesterol-free liposomes and did not contain LDL. The enrichment conditions raised the content of cellular cholesterol two- to threefold above normal control levels.

At the initiation of efflux, cultures were 80–100% confluent. One-half ml of efflux medium was added to each well. HDL subfractions were used at a concentration of 50 μg phospholipid/ml. Maximum incubation times were 8 h for Fu5Ah cells and 24 h for fibroblasts and smooth muscle cells. Incubations were ended, and the cell and medium samples were analyzed as described previously (11, 21).

All incubations were performed at least in triplicate. Each value is the mean $\pm$ 1 SD of replicate determinations, unless otherwise indicated. The significance of differences was assessed by the two-tailed Student's *t*-test for unpaired observations.

RESULTS

Cholesterol efflux from Fu5AH rat hepatoma cells to LP-AI and LP-AI/AII

The efflux of whole-cell and lysosomal cholesterol from Fu5AH cells to LP-AI and LP-AI/AII is shown in **Fig. 1**. Three important parameters were monitored over an 8-h efflux period: the release of whole-cell [^{14}C]cholesterol (Fig. 1A), the lysosomal hydrolysis of LDL [^{3}H]cholesteryl oleate (Fig. 1B), and the release of the lysosomally generated free [^{3}H]cholesterol from the cells (Fig. 1C). As the data indicate, the time course of each of these processes was essentially the same with either LP-AI or LP-AI/AII in the incubation medium. The cumulative release of cholesterol tracers during the 8-h incubations was 14% for whole-cell cholesterol and 15% for lysosomal cholesterol, suggesting rapid equilibration of lysosomal cholesterol into the various whole-cell pools. In several other experiments under the same conditions, the release of whole-cell and lysosomal cholesterol to media containing BSA (2 mg/ml), but no lipoproteins, was less than 1% (data not shown). Thus, the spontaneous release of cholesterol was expected to be quite low in this experiment and should not have obscured any significant differences in efflux to the two types of HDL. In the present experiment, esterification of [^{14}C]cholesterol in media was less than 1% after 8 h (data not shown), indicating that the activity of lecithin:cholesterol acyltransferase (LCAT) in the two subfractions was minimal. Approximately 10% of the ^{3}H label in both media was esterified after 8 h. The kinetics of appearance of this tracer in the media suggested that it was due to a small amount of desorption or retroendocytosis of undegraded LDL from the cells early in the incubations (data not shown), rather than any preferential esterification of lysosomally derived cholesterol.

To determine whether the enrichment of cells with cholesterol induces a difference in the responsiveness of efflux to LP-AI and LP-AI/AII, we prepared Fu5AH cells to contain normal (control) and elevated amounts of free cholesterol. In both cases, whole-cell cholesterol was labeled with ^{14}C, and the labeling of cells and the efflux of cholesterol were examined in the presence of compound 58-035 to prevent cellular esterification of cholesterol. The levels of cholesterol in control and enriched cells were 28 and 68 μg/mg protein, respectively. Thus, the enrichment procedure elevated cell cholesterol 2.4-fold above the control level. As shown in **Fig. 2**, there was very striking

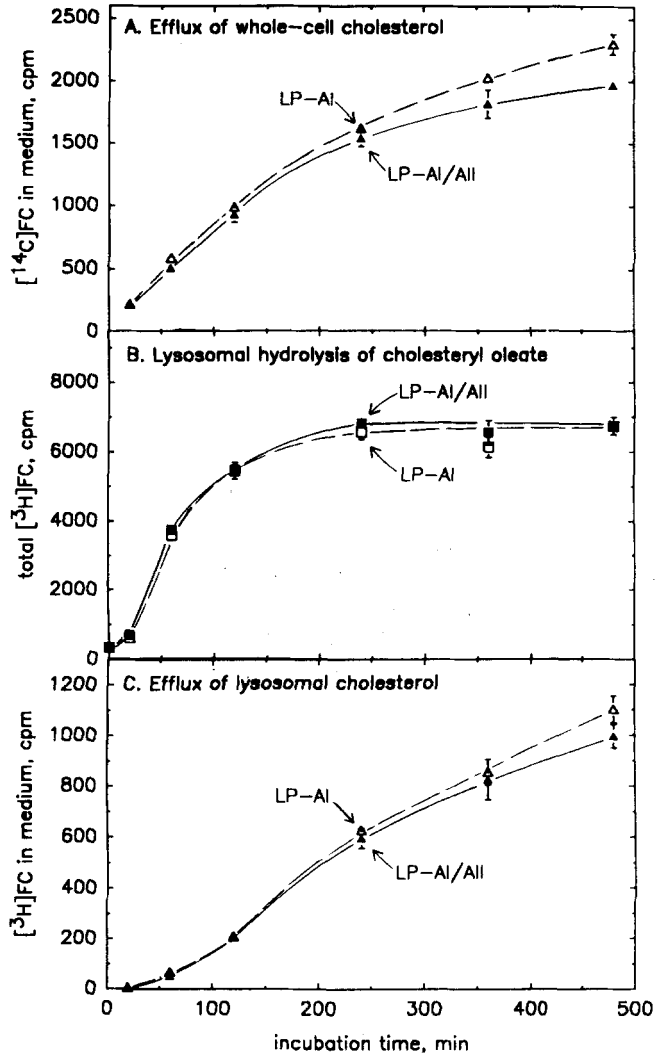

Fig. 1. Efflux of whole-cell and lysosomal cholesterol from Fu5AH rat hepatoma cells to human LP-AI and LP-AI/AII. The preparation of cells and lipoproteins was as described in Materials and Methods. The concentration of each lipoprotein was 50 μg phospholipid/ml. Initial (t = 0) cell parameters were as follows: cell protein = 0.204 ± 0.021 mg/well; total [^{14}C]cholesterol = 16001 ± 635 cpm/well (free/total = 0.958 ± 0.005); total [^{3}H]cholesterol = 9528 ± 966 cpm/well (free/total = 0.036 ± 0.005); uptake of reconstituted LDL = 684 ± 71 ng LDL protein/mg cell protein. Cell cholesterol mass was not measured in this experiment, but typically was 20–25 μg/mg protein. Panel A: Efflux of unesterified [^{14}C]cholesterol ([^{14}C]FC) to LP-AI (△) and LP-AI/AII (▲) versus time in min. Panel B: Total recovery of [^{3}H]FC from cells and medium during incubation with LP-AI (□) and LP-AI/AII (■). Panel C: Efflux of lysosomally generated [^{3}H]FC to LP-AI (△) and LP-AI/AII (▲). Each value is the mean ± 1 SD of at least three determinations. When not visible, bars indicating SD are covered by symbols.

similarity in efflux to the two forms of HDL, in both control cells (Fig. 1A) and cholesterol-enriched cells (Fig. 2B). After 8 h incubation, the cumulative release of the whole-cell tracer was approximately 17% from control cells, and 12% from enriched cells. Thus, efflux from the enriched hepatoma cells was somewhat sluggish, although it was equally efficient with the two forms of HDL. The enrichment did not induce any difference in efflux to LP-AI and LP-AI/AII.

As shown in **Table 1**, the content of cholesterol mass in sterol-enriched Fu5AH cells was changed very little by incubation for 8 h with LP-AI, LP-AI/AII, HDL_3, or BSA. There was clearly no tendency for LP-AI to produce greater depletion of cell sterol mass than either LP-AI/AII or HDL_3.

Cholesterol efflux from GM3468 human skin fibroblasts to LP-AI and LP-AI/AII

To examine the efflux of whole-cell and lysosomal cholesterol from fibroblasts, cells were prepared for

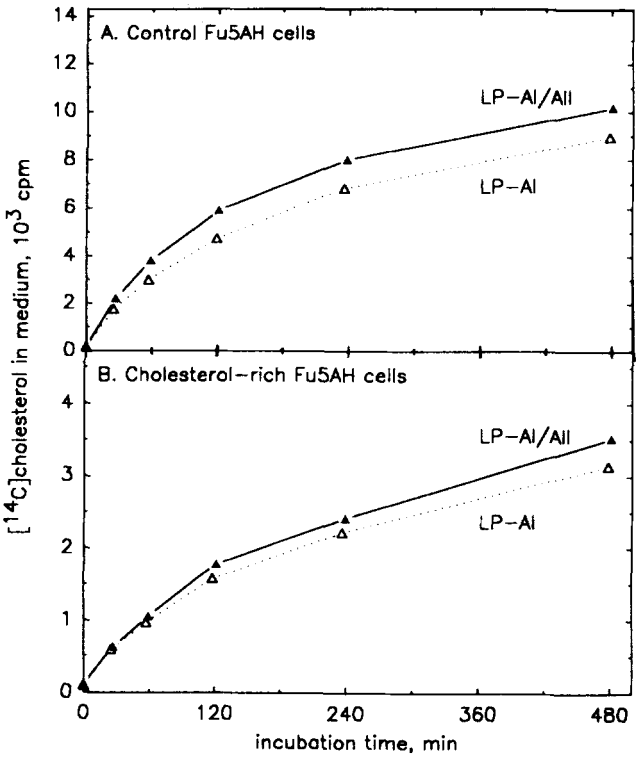

Fig. 2. Effect of enriching cells with cholesterol on the efflux of whole-cell cholesterol from Fu5AH cells to LP-AI and LP-AI/AII. Cells were prepared and labeled with [^{14}C]cholesterol as described in Materials and Methods. Efflux media contained 1 μg/ml of the ACAT inhibitor compound 58-035 (dispersed with 0.5% dimethylsulfoxide) and 50 μg phospholipid/ml of either LP-AI or LP-AI/AII. Initial cell parameters were as follows. 1) For control cells: cell protein = 0.327 ± 0.026 mg/well; free cholesterol/protein = 28.2 ± 2.1 μg/mg; total [^{14}C]cholesterol = 58098 ± 507 cpm/well (free/total = 0.984 ± 0.001). 2) For cholesterol-enriched cells: cell protein = 0.304 ± 0.028 mg/well; free cholesterol/protein = 68.4 ± 8.1 μg/mg; total [^{14}C]cholesterol = 27979 ± 814 cpm/well (free/total = 0.985 ± 0.001). Panel A: Efflux of [^{14}C]FC from control cells in the presence of LP-AI (△) and LP-AI/AII (▲) versus incubation time in min. Panel B: Efflux of [^{14}C]FC from cholesterol-enriched cells to LP-AI and LP-AI/AII. Each value is the mean of at least three determinations. All SD were quite small (maximum = 189 and 77 cpm for control and enriched cells, respectively), and therefore were not plotted.

TABLE 1. Free cholesterol content of cholesterol-enriched Fu5AH cells before and after 8-h incubation with LP-AI, LP-AI/AII, HDL_3, or bovine serum albumin (BSA)

Incubation Time	Medium	Cell Cholesterol Content
h		*μg FC/mg protein*
0		68 ± 8
8	LP-AI	60 ± 1
8	LP-AI/AII	59 ± 2
8	HDL_3	57 ± 0.3
8	BSA	64 ± 2

Enrichment conditions were as described in Fig. 2. During the 8-h efflux period, lipoproteins were used at a concentration of 50 μg phospholipid/ml, and BSA at a concentration of 100 μg/ml. BSA was not added to efflux media that contained lipoproteins. For t = 0, n = 6. For each value at t = h, n = 3. At t = 0, cell protein was 0.304 ± 0.028 mg/well; after 8 h, cell protein averaged 0.303 mg/well and varied by no more than 4% between treatment groups. Among the 8-h cell cholesterol content values, two differences were significant ($P < 0.05$): HDL_3 versus Lp-AI and BSA versus HDL_3.

experiments and incubated with lipoproteins as described above for the Fu5AH cells. Since efflux from the plasma membrane of fibroblasts is five- to tenfold slower than from the Fu5AH cell, the maximum incubation time was extended to 24 h. As shown in **Fig. 3**, the efflux of whole-cell and lysosomal cholesterol as well as the hydrolysis of LDL cholesteryl oleate in fibroblasts was the same with either LP-AI or LP-AI/AII in the incubation medium. After 24 h, the cumulative release of the whole-cell ^{14}C-labeled tracer was approximately 19%, whereas the release of the lysosomally generated ^{3}H-labeled tracer was approximately 20%.

The cholesterol enrichment procedure raised the content of cholesterol in fibroblasts to 85 μg/mg protein, approximately 2.5-fold above the normal control level in these cells. Efflux of whole-cell cholesterol from the enriched fibroblasts was virtually identical during incubations with LP-AI and LP-AI/AII (**Fig. 4**). The net depletion of cell cholesterol mass during 24 h incubation (**Table 2**) was greatest in the presence of LP-AI/AII (46%), whereas the depletion produced by LP-AI (22%) was not significantly different from that produced by BSA (32%). In this experiment, cell protein/well after 24 h was increased by approximately 23% under all three incubation conditions (legend Table 2). Thus, most of the apparent depletion of cellular cholesterol (reduced cholesterol-to-protein ratio) observed with LP-AI or BSA could be attributed to increased cell protein, rather than loss of cholesterol to the extracellular medium.

Cholesterol efflux from other cell lines to LP-AI and LP-AI/AII

The efflux of whole-cell and lysosomal cholesterol to LP-AI and LP-AI/AII was also examined using rabbit aortic smooth muscle cells and a second human skin fibroblast line (GM0637). With both cells, efflux of whole-cell and lysosomal cholesterol to the two forms of HDL was similar to that observed with GM3468 fibroblasts. There was no indication of preferential release of cholesterol to LP-AI (data not shown).

Rate constants for efflux of whole-cell cholesterol to LP-AI and LP-AI/AII

Estimates of k_e (the rate constant for efflux of cell cholesterol) can be obtained by analyzing time-course

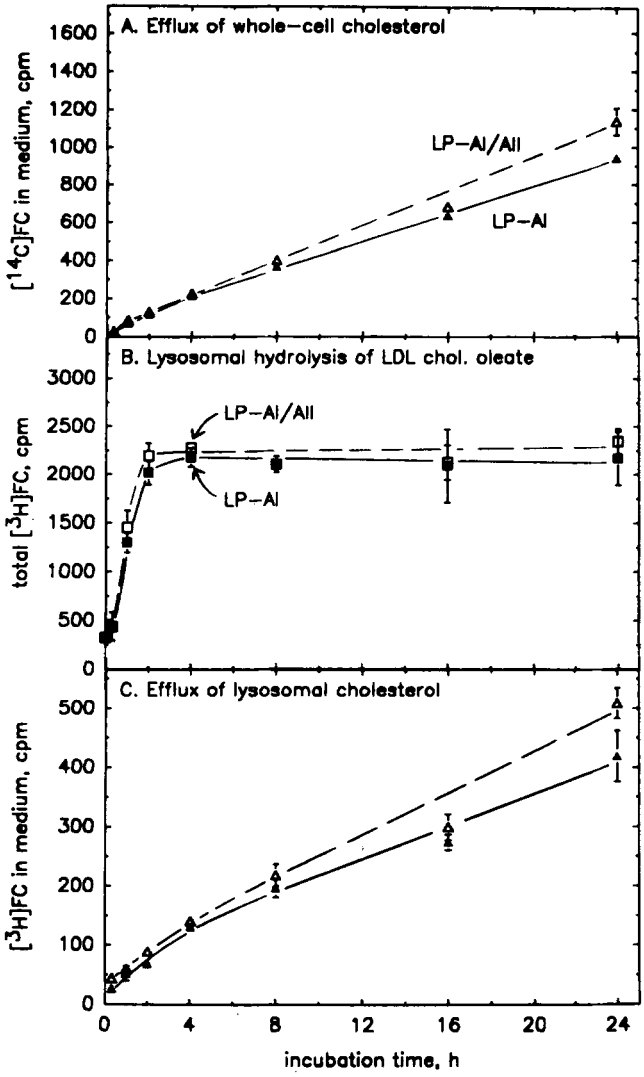

Fig. 3. Efflux of whole-cell and lysosomal cholesterol from human skin fibroblasts to LP-AI and LP-AI/AII. Conditions were as described in Fig. 1, except the maximum incubation time for efflux was 24 h. Initial cell parameters were as follows: cell protein = 40.4 ± 1.5 μg/well; free cholesterol/protein = 33.5 ± 5.8 μg/mg; total [^{14}C]cholesterol = 7012 ± 337 cpm/well (free/total = 0.996 ± 0.001); total [^{3}H]cholesterol = 3424 ± 312 cpm/well (free/total = 0.095 ± 0.003). Panel A: Efflux of whole-cell [^{14}C]free cholesterol versus incubation time with LP-AI (▲) and LP-AI/AII (△). Panel B: Total [^{3}H]free cholesterol recovered from cells and medium versus incubation time during incubation with LP-AI (■) and LP-AI/AII (□). Panel C: Efflux of lysosomally generated [^{3}H]free cholesterol to LP-AI (▲) and LP-AI/AII (△).

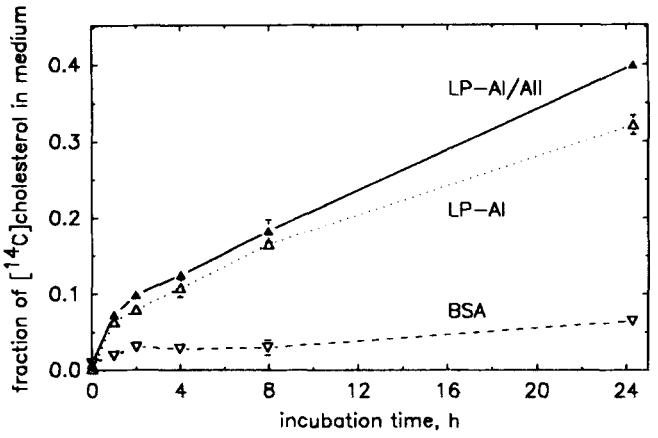

Fig. 4. Efflux of whole-cell cholesterol from cholesterol-enriched fibroblasts to LP-AI, LP-AI/AII, and BSA. The preparation of cholesterol-enriched cells and the efflux conditions were as described in Fig. 2. During incubation with BSA, its concentration was 100 μg/ml. BSA was not added to media containing LP-AI or LP-AI/AII. Initial cell parameters were as follows: protein = 36 ± 2 μg/well; free cholesterol = 85 ± 11 μg/mg protein; total [^{14}C]cholesterol = 927 ± 45 cpm/well (free/total = 0.996 ± 0.003). Control fibroblasts (not prepared in this experiment) typically contain 30 μg free cholesterol/mg protein. Data are plotted as the fractional release of [^{14}C]free cholesterol versus incubation time in h. Symbols: △, LP-AI; ▲, LP-AI/AII; ▽, BSA.

data on the efflux of whole-cell cholesterol in terms of a kinetic model for tracer equilibration between two pools (20, 21). **Table 3** summarizes estimates of k_e obtained in the above-described comparison between LP-AI and LP-AI/AII. The kinetic analyses suggest that with both control and cholesterol-enriched cells, LP-AI, is slightly less efficient (by a factor of 4–15%, depending on experiment and cell type) than LP-AI/AII at promoting efflux of whole-cell cholesterol.

DISCUSSION

During incubation of cells with HDL, cholesterol is known to diffuse bidirectionally between the plasma membrane and the HDL particles (21). Net efflux is thought to occur when the plasma membrane is relatively enriched with cholesterol, or the lipoprotein is relatively depleted of cholesterol (2). In the present studies, the free cholesterol-to-phospholipid ratios of LP-AI and LP-AI/AII were 0.47 and 0.30 (mole/mole), respectively (12). Thus, LP-AI was enriched with cholesterol relative to LP-AI/AII. The above considerations predict that when LP-AI and LP-AI/AII are diluted to the same phospholipid concentration, efflux of plasma membrane cholesterol to the two acceptors ought to be similar, whereas there may be greater influx of cholesterol from LP-AI (due to its greater content of cholesterol). This would result in reduced net depletion of cell cholesterol mass during incubation with LP-AI. In the present studies, we found that when LP-AI and LP-AI/AII were diluted to the same phospholipid concentration, the two lipoproteins promoted similar efflux of whole-cell cholesterol from rat hepatoma cells, fibroblasts, and smooth muscle cells (Figs. 1–4, Table 3), and that LP-AI produced net depletions of cell cholesterol mass that were either the same as or somewhat less than the depletions produced by LP-AI/AII (Tables 1 and 2). Thus, the observed features of cholesterol movement between cells and the HDL subfractions were essentially those predicted by a model assuming unmediated diffusion of sterol between the plasma membrane and extracellular sterol carriers. This relatively simple outcome suggests that the unmediated bidirectional flux of cholesterol at the plasma membrane is likely to be the predominant factor governing sterol movement between the immunopurified HDL subfractions and the cells that were used in the present studies.

In these studies, we also found that LDL-derived lysosomal cholesterol was readily available for efflux, and that LP-AI and LP-AI/AII were equally efficient at removing the lysosomal sterol from cells (Figs. 1 and 3). These results are consistent with previous data showing that the transport of cholesterol from lysosomes to the plasma membrane is rapid and not regulated by the type of sterol acceptor in the extracellular medium (11, 22–24). In most cells lysosome-to-plasma membrane cholesterol transport is fast in comparison to efflux from the plasma membrane. Thus, it seldom is rate-limiting for the removal of lysosomal cholesterol from cells.

In previous comparisons between LP-AI and LP-AI/AII, Barbaras et al. (8) found that LP-AI is able to remove excess cholesterol from cholesterol-enriched OB1771 mouse adipocytes, whereas LP-AI/AII promotes little or no net efflux from these cells. Additional data suggest that the movement of cholesterol from these cells to LP-AI requires the presence of HDL-specific binding sites on the adipocyte surface (10). Since both LP-AI and LP-AI/AII bind with high affinity to HDL binding sites on adipocytes, it has been proposed that the specific binding of

TABLE 2. Free cholesterol content of cholesterol-enriched human skin fibroblasts before and after 24-h incubation with LP-AI, LP-AI/AII, or BSA

Incubation Time	Medium	Cell Cholesterol Content
h		*μg FC/mg protein*
0		85 ± 11
24	LP-AI	66 ± 10
24	LP-AI/AII	46 ± 3
24	BSA	58 ± 3

The preparation of cells and efflux conditions were as described in Fig. 4. For t = 0, n = 11. For each value at t = 24 h, n = 3. At t = 0, cell protein was 35.9 ± 2.1 μg/well; after 24 h, cell protein averaged 44.2 μg/well and varied by no more than 6% between treatment groups. Among the 24-h cell cholesterol content data, the following differences were significant: LP-AI versus LP-AI/AII (P = 0.03) and BSA versus LP-AI/AII (P = 0.004).

TABLE 3. Rate constants for efflux of whole-cell cholesterol to LP-AI and LP-AI/AII

Type of cell	Treatment	Cell Cholesterol	Efflux k_e, h^{-1} LP-AI/AII	Efflux k_e, h^{-1} LP-AI	Efflux LP-AI / LP-AI/AII × 100%
		μg/mg protein			
Fu5AH rat hepatoma	control	23	0.048	0.058	96 ± 23%
		not assayed	0.041	0.038	
		28	0.070	0.053	
	FC-rich	68	0.034	0.032	94%
GM3468 human fibroblasts	control	34	0.0074	0.0063	85%
	FC-rich	85	0.018	0.017	94%
Rabbit SMC	control	23	0.0079	0.0076	96%

Control and cholesterol-enriched cells were prepared as described in Materials and Methods. LP-AI and LP-AI/AII were used at a concentration of 50 μg phospholipid/ml. Each pair of values for k_e was obtained in a single experiment by fitting time-course data on the efflux of ^{14}C-labeled whole-cell cholesterol to a kinetic model for tracer equilibration between two pools (20, 21). Each time course contained data from at least six time points, with triplicate incubations performed at each time point (e.g., Fig. 1A). The percentage value (in the far right column) with an SD assigned is an average based on data from the three indicated experiments. Each of the other percentages was calculated from k_e values obtained in a single experiment. Values for k_e can be converted to half times ($t_{1/2}$) for efflux with the formula: $t_{1/2} = (1n\ 2)/(k_e)$. Abbreviations: FC, free cholesterol; SMC, smooth muscle cells; k_e, rate constant for efflux of cell cholesterol.

apoA-I (in the absence of apoA-II) stimulates the mobilization of excess cholesterol in these cells, and that the specific binding of apoA-II antagonizes this mobilization (8, 10). Two of the cells examined in the present studies (Fu5AH hepatoma cells and GM3468 fibroblasts) are known to express HDL-specific binding sites (20, 25). Thus, the lack of preferential efflux to LP-AI observed with these cells cannot be explained by the lack of HDL receptors. We speculate that the contrast between the present results and those obtained with adipocytes may be related to the unique ability of adipocytes to store very large quantities of lipid. This specialization may be accompanied by some fundamentally unique mechanism for sterol mobilization.

Recent data of Castro and Fielding (9) and Francone, Gurakar, and Fielding (26) suggest that when fibroblasts are incubated with human plasma, the initial acceptor of cholesterol that desorbs from the cells is an apoA-I-containing pre-beta lipoprotein. This lipoprotein should be a component of the LP-AI fraction of plasma. Thus, isolated LP-AI might be expected to promote greater efflux than isolated LP-AI/AII. The fact that our results are not consistent with this prediction may have a number of explanations. The simplest possibility is that pre-beta HDL, although an efficient sterol acceptor, may not be required for the rapid efflux of sterol from cells. In the absence of pre-beta HDL, other lipoproteins (such as those in LP-AI/AII) may be able to serve as efficient acceptors of the cholesterol that is continually desorbing from cell plasma membranes. This suggestion is consistent with the fact that a variety of sterol acceptors (including non-lipoprotein phospholipid vesicles and phospholipid/bile acid micelles) can support rapid efflux of cholesterol from cells (27).

Pertinent to the issue of pre-beta HDL, the procedures used for isolation of HDL subfractions in the present studies involved a preliminary gel filtration step that eliminated plasma proteins outside the particle mass range of approximately 100–700 kDa (12). This step may have resulted in low recoveries of the 71-kDA form of pre-beta HDL (7). Moreover, it is thought that pre-beta HDL are short-lived and transformed rapidly to alpha-HDL particles (7). If not regenerated in the absence of whole plasma, pre-beta HDL may disappear quickly from purified HDL subfractions. Thus, the LP-AI used in the present studies may have lacked a fully functional pre-beta HDL system. Consistent with this suggestion, we could detect very little esterification of cell-derived cholesterol in either LP-AI or LP-AI/AII (cf. Results and ref. 26). In the present studies, we did not examine the immunopurified fractions for the presence of pre-beta HDL. The postulated importance of pre-beta HDL might be addressed least equivocally by studying efflux of cell cholesterol to pure stable forms of this lipoprotein in comparison to the efflux obtained with other types of acceptors at similar particle and phospholipid concentrations. Because of the low abundance and apparent lability of naturally occurring pre-beta HDL, such experiments probably will require the reconstitution of artificial pre-beta-like particles.

The present data show that purified human LP-AI and LP-AI/AII are equally capable of removing both plasma membrane and lysosomal cholesterol from several types of mammalian cells. These results support the hypothesis that efflux of cellular cholesterol is governed largely by the unmediated diffusion of cholesterol between the plasma membrane and HDL particles. The results also are consistent with previous data that suggest that the movement

of cholesterol from lysosomes to the plasma membrane is rapid and not regulated by specific extracellular acceptors. There is a striking contrast between the present data, showing similar efflux to LP-AI and LP-AI/AII, and data reported by others, showing that LP-AI specifically stimulates efflux from sterol-rich mouse adipocytes. This contrast suggests that any unique ability of LP-AI to initiate reverse cholesterol transport is confined to a limited range of cell types. Thus far, only variants of the OB17 mouse adipocyte have demonstrated any unique responsiveness to LP-AI. Why these cells respond to LP-AI and others do not remains to be explained.

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