Interaction of plasma-derived lipid transfer protein with macrophages in culture

Richard E. Morton

Department of Brain and Vascular Research, Research Institute of the Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44106

Abstract This study investigates the ability of human plasma-derived lipid transfer protein to facilitate lipid transfer to and from intact viable cells in culture. Mouse peritoneal macrophages or J774 macrophages were preincubated with acetylated low density lipoprotein and [^{3}H]oleate/albumin to promote the intracellular synthesis and accumulation of cholesteryl [^{3}H]oleate and ^{3}H-labeled triglyceride. The addition of partially purified lipid transfer protein to cultures of lipid-loaded macrophages resulted in a time and concentration-dependent transfer of radiolabeled cholesteryl ester and triglyceride from macrophages to the medium. At 48 hr, lipid transfer protein facilitated the net transfer of 16 and 11% of cellular cholesteryl ester and triglyceride radioactivity, respectively, to the medium; transfer in the absence of the lipid transfer protein was <2%. The transfer of cholesteryl ester radioactivity was accompanied by a similar decrease in cellular cholesteryl ester mass indicating a net transfer event. Lipid transfer from cells was not dependent on the presence of a lipoprotein acceptor in the medium; however, low and high density lipoproteins present at 200 μg cholesterol/ml did significantly stimulate the transfer protein-facilitated efflux of these lipids. Lipid transfer protein did not appear capable of transferring radiolabeled lipid from low density or high density lipoprotein to macrophages. Radiolabeled cholesteryl ester and triglyceride transferred from cells to the medium by lipid transfer protein were associated with large molecular weight ($>2 \times 10^6$) components in the medium with an average density greater than 1.21 g/ml; these lipids were not associated with lipid transfer protein itself. However, these radiolabeled lipids were readily incorporated into low or high density lipoproteins when these lipoproteins were added to the medium either during or after its incubation with cells. It is concluded that lipid transfer protein can facilitate the net efflux of cholesteryl esters from intact, living macrophages. These studies suggest a novel and potentially antiatherogenic role for lipid transfer protein. —**Morton, R. E.** Interaction of plasma-derived lipid transfer protein with macrophages in culture. *J. Lipid Res.* 1988. **29:** 1367–1377.

Supplementary key words high density lipoproteins • atherogenesis • cholesteryl esters • triglyceride

Several investigators have purified a protein from human lipoprotein-deficient plasma that is responsible for the majority of the cholesteryl ester and triglyceride transfer activity in this plasma fraction (1, 2). This protein, designated LTP, is characterized as a hydrophobic glycoprotein of ≅74,000 molecular weight. LTP facilitates the transfer of cholesteryl ester, triglyceride, and phospholipid, as well as retinyl ester and cholesteryl ether, but not unesterified cholesterol, between lipoprotein particles (3). Although the transfer mechanism is incompletely understood, it appears that the physical binding of LTP to the lipoprotein surface, perhaps through interaction with phospholipid head groups (4), is an essential event in the transfer process (5).

Consistent with the aforementioned interaction of LTP with lipoproteins through surface phospholipids, it appears that LTP can utilize a wide variety of phospholipid-containing membrane surfaces as substrate. For example, in addition to the well-recognized transfer of apolar lipids among very low, low, and high density lipoproteins (6–8), LTP also catalyzes the transfer of lipids between lecithin-cholesterol liposomes and low density lipoproteins (3), between lipoproteins and Intralipid (9), and between isolated rough and smooth endoplasmic reticulum (10). Furthermore, Stein et al. (11) have shown that partially purified LTP can facilitate the transfer of cholesteryl ester, which is associated with the extracellular matrix of cultured smooth muscle cells, to the cell culture medium. More recently, these authors have demonstrated that partially purified LTP can promote the efflux of cholesteryl ester from "disintegrating" (i.e., chemically fixed and permeabilized) smooth muscle cells and macrophages to the culture medium (12).

The results described above collectively suggest that LTP may also interact with intact viable cells. Indeed, Granot, Tabas, and Tall (13) recently demonstrated that LTP can promote the transfer of cholesteryl ester from high density lipoprotein into cultured hepatocytes and

Abbreviations: LTP, lipid transfer protein; BSA, bovine serum albumin; DMEM, Dulbecco's Modified Eagle's Medium; LDL, low density lipoprotein; HDL, high density lipoprotein; PBS, phosphate-buffered saline.

smooth muscle cells. In light of the central role of the macrophage in lipid metabolism in the atherosclerotic lesion, we have presently examined the capacity of LTP to promote lipid transfer to and from cultured cholesteryl ester-loaded macrophages.

EXPERIMENTAL PROCEDURES

Materials

[1α,2α(n)-^{3}H]cholesterol (44 Ci/mmol) and [9,10(n)-^{3}H]oleic acid (4.8 Ci/mmol) were purchased from Amersham Corp. (Arlington Heights, IL). [^{3}H]Cholesteryl oleyl ether was synthesized from radiolabeled cholesterol and oleyl alcohol as described by Halperin and Gatt (14). Precoated thin-layer chromatography plates (SG-60) were obtained from E. Merck (Darmstadt, Germany). Fatty acid-free BSA (A-6003) and fraction V BSA (A-7906), cholesteryl oleate, triolein, and reagents for enzymatic cholesterol and DNA assays were from Sigma Chemical Co. (St. Louis, MO). Tissue culture plates were from Costar (Cambridge, MA). DMEM (Cat. #430-1600), penicillin-streptomycin solution, and fetal calf serum were obtained from Gibco (Grand Island, NY). Fixed *Staphylococcus aureus* cells (Pansorbin) were purchased from Calbiochem (La Jolla, CA).

Preparation of LTP and antisera

Partially and highly purified LTP were isolated from lipoprotein-deficient human plasma (15) as previously described (3). Lipid transfer activity was assayed by a standardized assay using radiolabeled LDL as donor and unlabeled HDL as acceptor (3, 7). The partially purified preparation (CM-cellulose fraction), which was routinely enriched 700- to 900-fold in transfer specific activity, was used for most studies. Highly purified LTP (Bio-Gel fraction, ref. 3) was prepared from this fraction and its activity was stabilized by the addition of BSA (1 mg/ml). SDS-polyacrylamide gel electrophoresis (16) of the Bio-Gel fraction under reduced conditions demonstrated two proteins of M_r = 72,400 and 57,500, in a relative abundance of $\cong$1:2, based on Coomassie blue staining, respectively. The 72,400 M_r band co-migrated in this gel system with homogeneous LTP isolated by the emulsion method of Hesler, Swenson, and Tall (1). To investigate the possibility that the lipid transfer activity of the Bio-Gel fraction measured with macrophages was due to the 57,500 M_r component instead of LTP, we also investigated the ability of LTP-deficient CM-cellulose fractions to promote these transfers. LTP-deficient CM-cellulose fractions were prepared by passing 4 ml of the CM-cellulose fraction through a 1.5-ml column of LDL-Sepharose as previously described (5). The resulting sample contained greatly reduced LTP (measured by a lipoprotein-lipoprotein assay) but contained essentially all of the 57,500 M_r protein as judged by the intensity of this band on Western blots (see antibody preparation below).

Antisera to LTP were prepared in rabbits as follows. Highly purified LTP was combined with sufficient rabbit serum albumin to give a 0.1 mg/ml albumin solution, and then concentrated 10-fold by ultrafiltration (YM-10 membrane, Amicon Corp., Danvers, MA). An emulsion containing complete Freund's adjuvant and antigen (2:1, v/v) was prepared by repetitive passes through a small-bore tubing connecting two syringes, and $\cong$100 μg of purified LTP was injected at multiple intramuscular sites. Boosters were given at 6–8-week intervals with one-half of the original dose in complete adjuvant. Animals were bled 7–10 days after the booster dose. The resulting antiserum was useful for quantitative immunoabsorption of all cholesteryl ester and triglyceride transfer activities from partially purified transfer protein preparations even though it also recognized the 57,500 M_r protein as measured by immunoblots.

Lipoproteins

LDL was isolated from fresh (<24 hr old) plasma by differential ultracentrifugation as the 1.019<d<1.063 g/ml fraction (17). HDL was similarly isolated from plasma as the 1.063<d<1.21 g/ml fraction. LDL was acetylated by repetitive additions of acetic anhydride (18) and then extensively dialyzed against 150 mM NaCl, 0.05% Na_2 EDTA, pH 7.4. LDL was labeled with [^{3}H]cholesteryl oleyl ether by first incorporating the ^{3}H-labeled ether lipid into HDL following the lipid dispersion technique of Morton and Zilversmit (7). [^{3}H]Ether-labeled HDL (100 μg cholesterol) was then incubated at 37°C for 6 hr with LDL (2 mg cholesterol) and partially purified LTP (500 μl). [^{3}H]Ether-labeled LDL was reisolated by ultracentrifugation.

Cell culture and assays

The mouse macrophage cell line J774A.1 was obtained from American Type Tissue Collection (ATTC TB 67) and grown in a humidified atmosphere of 5% CO_2/95% air at 37°C in an equal volume mixture of DMEM and Ham's F-12 medium containing 5% fetal calf serum. For experiments, passaged cells were plated into 24-well plates at a density of 10^5 cells/well and cultured in 10% fetal calf serum in DMEM for 4 days to achieve essentially confluent cultures. These cultures were then rinsed with 0.5 ml DMEM and incubated for the indicated time with unlabeled or labeled oleate/BSA (final oleate concentration of 0.27 mM), and the indicated amount of acetyl-LDL in DMEM. Unlabeled oleate/BSA or [^{3}H]oleate/BSA solutions were prepared as previously described (19); [^{3}H]oleate/BSA had a specific activity of 1.4×10^4 cpm/nmol. Under these conditions, cells are induced to

form lipid inclusions containing cholesteryl ester and triglyceride; the presence of radiolabeled cholesteryl ester and triglyceride in cultures reflects intracellular synthesis and accumulation of these lipids. After overnight incubation, cells were rinsed with 0.5 ml DMEM, then incubated in 0.5% essentially fatty acid-free BSA/DMEM with or without LTP and lipoprotein. The latter two solutions were dialyzed against DMEM overnight at 4°C prior to their use. At the end of a transfer experiment, the medium was removed and the cells were rinsed briefly with 0.5 ml PBS. The combined wash plus original medium was centrifuged (5 min, 6740 *g*) to remove cell debris; then an aliquot of the supernatant was immediately extracted (20). The cell layer was scraped twice in 0.5 ml PBS and the cells were pelleted by centrifugation as above. The cell pellet was stored frozen (−20°C) for assay at a later time. Cell pellets were resuspended in 1 ml PBS by probe sonication (Heat Systems Co., Plainview, NY). Aliquots were taken for DNA determination and for lipid extraction (20).

Lipid extracts of medium and cell sonicates were dried under N_2, resuspended in hexanes containing unlabeled cholesteryl oleate and triolein standards, and fractionated by thin-layer chromatography (SG-60 plates) in a developing system of hexanes–diethyl ether 70:30 (v/v). ^3H radioactivity in scrapings was quantitated by liquid scintillation counting.

Some experiments were carried out as described above but with mouse peritoneal macrophages. Resident peritoneal macrophages from unstimulated adult male mice were collected and cultured as previously described (21) except that 2×10^6 cells were plated per 16-mm diameter well and the cells were allowed to adhere overnight in DMEM containing 20% fetal calf serum prior to use in an experiment.

Chemical determinations

Lipoprotein cholesterol was determined either by a colorimetric assay (Reagent-Set, Boehringer Mannheim, Indianapolis, IN) or by the fluorometric method of Gamble et al. (22). For both cholesterol assays, calibrated human plasma was used as standard. Cellular DNA was assayed by the fluorometric method of Labarca and Paigen (23) with calf thymus DNA as standard. Protein was quantitated by a dye-binding assay (Bio-Rad, Richmond, CA) with BSA as standard.

RESULTS

In these studies, except where noted, cultured J774 macrophages were preincubated with acetyl-LDL and [^{3}H]oleate to facilitate the intracellular synthesis and accumulation of radiolabeled cholesteryl oleate and triglyceride. When these lipid-labeled macrophages were subsequently incubated for 6 hr without or with partially purified LTP and LDL, the amount of cellular cholesteryl [^{3}H]oleate present in the medium was found to increase curvilinearly with LTP concentration (**Fig. 1**). The amount of cellular [^{3}H]triglyceride present in the medium also increased in a manner parallel to that seen for cholesteryl ester. Over this LTP concentration range, there were no detectable toxic effects of LTP on the cells as judged by cell morphology at the light microscopy level, or by the recovery of cellular DNA at the end of the incubation (10.9 ± 0.7 vs. 11.2 ± 0.4 μg DNA/well, respectively, for 0 and 440 μg transfer protein). Furthermore, cell viability as monitored by protein synthesis was affected only slightly. Cellular protein synthesis, measured by [^{3}H]leucine incorporation into trichloroacetic acid-precipitable material, was 60,500 ± 2,960 cpm/μg DNA (t = 6 hr, mean ± SD, n = 3) in the absence of transfer protein, and 56,600 ± 1,260 cpm/μg DNA in the presence of 490 μg of partially purified LTP. In both cases, 8% of the synthesized protein was secreted to the medium. The possibility that LTP-induced cell damage was responsible for the presence of cellular lipids in the medium was further excluded by the observation that prior exposure of cells to a high level of LTP for 1 hr followed by incubation in media without LTP did not significantly affect the

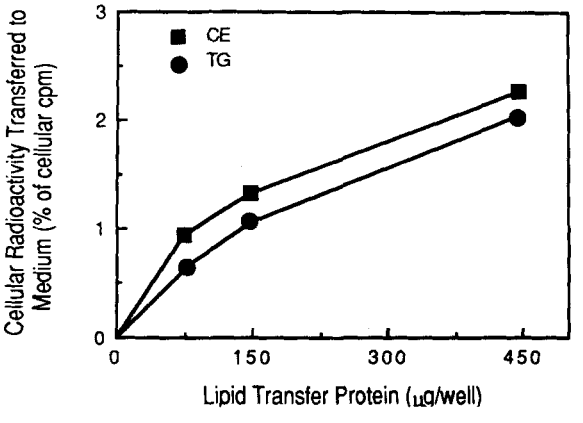

Fig. 1. Dependence of cholesteryl ester and triglyceride transfer from cells on transfer protein concentration. J774 macrophages were labeled with [^{3}H]cholesteryl ester and [^{3}H]triglyceride by preincubation of the cells in the presence of acetyl-LDL (50 μg cholesterol), [^{3}H]oleate/BSA, and DMEM in a total volume of 0.5 ml. After 16 hr, the medium was removed and the cell layer was rinsed with DMEM. Cells were subsequently incubated for 6 hr in 0.5% BSA/DMEM containing the indicated amount of partially purified LTP and LDL (2 μg cholesterol). The cholesteryl ester (■) and triglyceride (●) radioactivities in the medium were determined as described in Experimental Procedures and are presented as percentages of the cellular radioactive lipid pool. Medium radioactivity in the absence of LTP (cholesteryl ester, 0.3%, triglyceride, 0.5%) has been subtracted from the data shown. On the average, cells contained 121,700 cpm of [^{3}H]cholesteryl ester and 84,500 cpm of [^{3}H]triglyceride per well. Each point is the average of duplicate wells. These results are representative of two experiments.

amount of radiolabeled lipids present in the medium relative to that observed for nonexposed control cells.

Highly purified LTP (Bio-Gel fraction, ref. 3) was as effective in lipid transfer from cells as the partially purified fraction (CM-cellulose fraction, ref. 3). When highly purified (20 μg protein) and partially purified (366 μg protein) LTP fractions, which were equivalent in lipid transfer activity as measured by a standard LDL-to-HDL transfer assay (3), were incubated with macrophages, 2,000 ± 362 and 2,180 ± 248 cpm of cellular cholesteryl ester was transferred to the medium, respectively (t = 6 hr, mean ± SE, n = 5). Since highly purified LTP contains two proteins (LTP and a 57,500 M_r component), we also assayed the capacity of LDL Sepharose-treated, partially purified LTP to promote lipid transfer from macrophages. This treatment selectively absorbs LTP without removing the 57,500 M_r component as assessed by immunoblots (see Experimental Procedures for details). Two treated fractions containing 13 and 53% of their original LTP activity (measured by a lipoprotein-lipoprotein assay) promoted cholesteryl ester transfer from macrophages at rates that were 16 and 59%, respectively, of that observed for untreated, partially purified LTP. Collectively, these studies strongly suggest that the capacity of the partially purified preparation to facilitate lipid transfer from cells is due to the LTP previously isolated and characterized by us (3) and others (1, 2). These results also demonstrate that other components in partially purified LTP are not required for the observed lipid transfer from cells.

The mechanism of lipid transfer from macrophages to the medium was assessed by evaluating the effect of cellular perturbation on transfer. As shown in **Table 1**, the inhibition of cellular energy metabolism by NaF, of protein synthesis by cycloheximide, or the hydrolysis of cell surface proteins with trypsin (24) had little effect on the transfer of radiolabeled lipid to the medium.

TABLE 1. Effect of perturbing cellular metabolism on the transfer of cholesteryl ester to the medium

Addition to or Pre-treatment of Cells	Transfer of Cholesteryl [^{3}H]Oleate to Medium (% of control)
None	100
NaF (20 mM)	102.1 ± 13.9
Cycloheximide (0.35 mM)	131.5 ± 16.5
Trypsin (250 μg/ml)[a]	102.3 ± 33.8
Trypsin[a] + Cycloheximide	85.7 ± 7.3

J774 macrophages were loaded with lipids by pre-incubation with acetyl-LDL (50 μg cholesterol) in the presence of [^{3}H]oleate/BSA. Radiolabeled cells were subsequently incubated with partially purified LTP (141 μg protein) in the presence or absence of the indicated compound or pretreatment in a final volume of 0.5 ml. After 6 hr, media were removed and analyzed for their cholesteryl [^{3}H]oleate content as described in Experimental Procedures. NaF and cycloheximide additions had little effect on medium cholesteryl [^{3}H]oleate radioactivity observed in the absence of LTP; trypsin treatment raised these blank values by only 27%. Values are reported as percent of control values (LTP alone) which were calculated after subtracting the appropriate blank value from each sample. Results are the average (mean ± SD) of values from two experiments, each determined in duplicate.

[a]The cell layer was pretreated with trypsin (250 μg/ml) at 37°C; after 1 hr, cells were rinsed with media containing 10% serum, with media alone, and then incubated as described above.

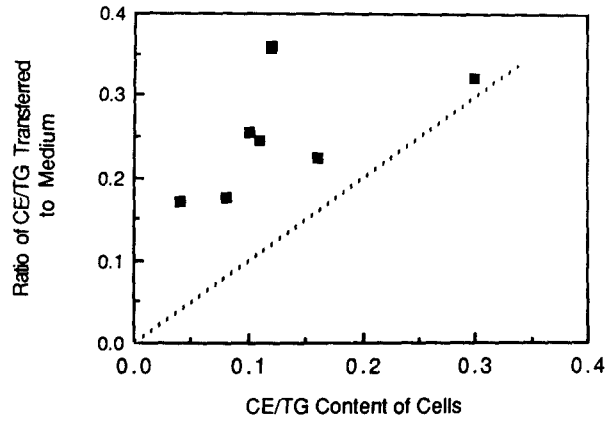

Fig. 2. Effect of variable cell loading with cholesteryl ester and triglyceride on lipid transfer from cells. J774 macrophages were incubated with various amounts of acetyl-LDL (5–50 μg cholesterol/ml) in the presence of [^{3}H]oleate/BSA for 16 hr to facilitate the intracellular accumulation of variable levels of cholesteryl ester while maintaining a constant level of cellular triglyceride. Lipid-loaded cells were then incubated with or without partially purified LTP (186 μg protein) and 0.5% BSA/DMEM in a total volume of 0.5 ml. After 6 hr, the medium was removed and the content of cholesteryl [^{3}H]oleate and [^{3}H]triglyceride was determined as described in Experimental Procedures. Data are presented as the ratio of cholesteryl ester (CE)/triglyceride (TG) transferred out of the cells by LTP as a function of the ratio of these lipids in the cells. Dashed line is line of identity. Each point is the average of duplicate wells.

The extent of protein-mediated lipid transfer from macrophages was not greatly affected by the degree to which cells were loaded with lipid or by the ratio of cholesteryl ester to triglyceride in the cells. Altering the amount of acetyl-LDL added to the preincubation media ([^{3}H]oleate/BSA concentration constant) resulted in variable loading of cells with cholesteryl ester, whereas triglyceride loading was essentially constant. Over the acetyl-LDL range tested (5–50 μg cholesterol/ml), the resulting cellular ratio of cholesteryl ester to triglyceride varied by a factor of 6. Despite this, the ratio of cholesteryl ester to triglyceride transferred varied only approximately twofold over this range; the ratio of cholesteryl ester to triglyceride transferred always exceeded the cellular ratio of these lipids (**Fig. 2**). The increase in the transfer ratio of cholesteryl ester to triglyceride largely reflected an increase in cholesteryl ester transfer as the cellular content of this lipid increased; triglyceride transfer was constant. Thus, under these conditions, cholesteryl ester was preferentially transferred from cells, consistent with the lipid specificity demonstrated for LTP with lipoproteins and liposome substrates (3, 7). This apparent specificity for cholesteryl ester further supports the conclusion that the protein-facilitated transfer of lipids to the medium is

not mediated by cell lysis, since this mechanism would yield a medium lipid composition equivalent to that in the cells.

LTP facilitates lipid transfer between lipoproteins by an exchange reaction, i.e., equimolar amounts of core lipid are transferred between lipoproteins (25). To investigate whether this exchange mechanism also holds for the lipid transfer from cells observed above, unlabeled lipid-loaded macrophages were incubated with LDL labeled with cholesteryl oleyl ether—a nonhydrolyzable cholesteryl ester analog that can be transferred by LTP (3, 12). The addition of 0, 150, or 300 μg of partially purified LTP and radiolabeled LDL to the wells resulted in cell-associated [^{3}H]cholesteryl ether values of 160 ± 25, 120 ± 2 and 99 ± 11 cpm/μg DNA, respectively (t = 6 hr). Thus, LTP did not facilitate the transfer of cholesteryl ether from LDL to these cells. Instead, LTP appeared to elicit a dose-dependent decrease in cell-associated cholesteryl ether radioactivity relative to control wells without transfer protein. Under the conditions of this experiment, an uptake equal in magnitude to the LTP-facilitated removal of radiolabeled cholesteryl ester from these cells would have increased the cellular content of radiolabeled cholesteryl ether by 30% over that observed in cells without transfer protein; this clearly was not observed.

The inability of LTP to facilitate lipid transfer from LDL to cells was not due to the large intracellular pool of these lipids in lipid-loaded macrophages. As shown in **Table 2,** LTP failed to facilitate cholesteryl ether transfer from LDL to either non-lipid-loaded cells or cells loaded with cholesteryl ester and triglyceride by preincubation with acetyl-LDL and oleate/BSA. In both instances, the addition of LTP decreased, rather than increased, the cellular content of [^{3}H]cholesteryl ether after the 6-hr incubation. These observations were not unique to LDL, as almost identical results were obtained when HDL was the potential lipid donor. Thus, LTP does not appear to facilitate lipid transfer from LDL or HDL to macrophages regardless of whether the cells have been previously loaded with neutral lipids.

In light of the finding that the LTP does not appear to facilitate lipid transfer from lipoproteins to macrophages, it was of interest to determine whether the protein-facilitated transfer of lipids from cells to the medium was dependent on the presence of lipoproteins in the medium functioning as acceptor of the transferred lipid. As seen in **Table 3,** neither LDL nor HDL at concentrations of up to 100 μg cholesterol/ml had a significant effect on the transfer of radiolabeled cholesteryl ester from cells to the medium by LTP. However, both LDL and HDL at 200 μg cholesterol/ml did cause significant stimulation of the LTP-facilitated transfer of lipids to the medium relative to the lipoprotein-free controls (Table 3). In separate experiments, LDL or HDL (≥200 μg cholesterol/ml) added to cells in the absence of LTP elicited less than 4 and 11%, respectively, of the lipid transfer observed with cells incubated in medium containing lipoproteins plus LTP. Some preparations of HDL were capable of stimulating lipid transfer from cells at levels significantly higher than this basal value (i.e., 11%); however, pretreatment of these HDL fractions with anti-LTP immunoabsorbant eliminated this capacity. Thus, high lipoprotein concentrations appear to enhance LTP-mediated lipid transfer from macrophages; however, the presence of LDL or HDL in the medium is not required for the transfer of cholesteryl ester and triglyceride from cells.

TABLE 2. Effect of lipid loading on LTP-facilitated transfer of [^{3}H]cholesteryl oleyl ether from LDL or HDL to J774 macrophages

Cell Preincubation Condition	Additions to Experimental Media		Cellular ^{3}H (cpm/μg DNA)
	[^{3}H]Cholesteryl Ether-Labeled Lipoprotein	Lipid Transfer Protein	
Media alone	LDL	–	624 ± 7[a]
	LDL	+	330 ± 7
Ac-LDL + oleate/BSA	LDL	–	314 ± 45
	LDL	+	248 ± 2
Media alone	HDL	–	338 ± 11
	HDL	+	243 ± 27
Ac-LDL + oleate/BSA	HDL	–	249 ± 18
	HDL	+	218 ± 42

J774 macrophages were incubated with either media alone, or with media containing acetyl-LDL (50 μg cholesterol/ml) and unlabeled oleate/BSA as described in Methods. After 18 hr, these cells were rinsed twice with media alone, and then incubated with media (0.5 ml total volume) containing 20 μg cholesterol of [^{3}H]cholesteryl oleyl ether-labeled LDL (2660 cpm/μg cholesterol) or labeled HDL (5090 cpm/μg cholesterol) in the presence or absence of partially purified LTP (186 μg protein). After 6 hr, the cell layer was washed three times, scraped, washed twice more by centrifugation and resuspension, and then analyzed for the DNA and ^{3}H content. These results are representative of two similar experiments; Ac-LDL, acetyl-LDL.

[a]Mean ± SD, n = 2.

TABLE 3. Effect of plasma lipoproteins on the transfer of cholesteryl [^{3}H]oleate from J774 macrophages to the medium

Lipid Transfer Protein	Lipoprotein Added to Medium (μg cholesterol/ml)		Cholesteryl [^{3}H]Oleate in Medium (cpm ± SE, n = 3, t = 6 hr)	Fold Stimulation[a]
	LDL	HDL		
–			621 ± 45	
+			2290 ± 277	1.0
+	50		2490 ± 376	1.1
+	100		2420 ± 202	1.1
+	200		4460 ± 450	2.3
+		50	2550 ± 348	1.2
+		100	2440 ± 304	1.1
+		200	3290 ± 156	1.6

J774 macrophages were loaded with cholesteryl ester by preincubation (16 hr) with acetyl-LDL (50 μg cholesterol) in the presence of [^{3}H]oleate/BSA. Radiolabeled cells were then incubated without or with partially purified LTP (245 μg protein), 0.5% BSA/DMEM, and the indicated lipoprotein in a total well volume of 0.5 ml. After 6 hr, the medium was removed and its content of cholesteryl [^{3}H]oleate was determined as described in Experimental Procedures. On the average, wells contained 112,900 cpm cholesteryl [^{3}H]oleate (5,600 cpm/μg DNA). These data are representative of two similar experiments.

[a]Stimulation of net transfer activity relative to wells containing LTP alone. For this calculation, medium radioactivity in the absence of LTP has been subtracted from all values.

The extent of triglyceride and cholesteryl ester transfer from cells was dependent on incubation time (**Fig. 3**). Cholesteryl ester transfer in the presence of LTP, and in the absence of LDL or HDL, increased almost linearly over a 48-hr period following a more rapid transfer during the initial 6 hr. There was no apparent lag in the LTP-

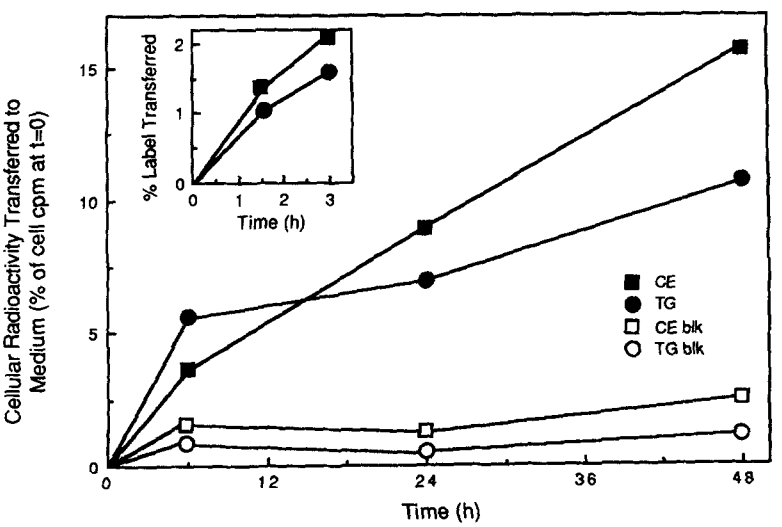

Fig. 3. Dependence of cholesteryl ester and triglyceride transfer from cells on incubation time. J774 macrophages were labeled in the triglyceride and cholesteryl ester moieties by preincubation with acetyl-LDL (25 μg cholesterol), [^{3}H]oleate/BSA, and DMEM in a total volume of 1 ml. After 16 hr, cells were rinsed and then incubated with [^{3}H]oleate/BSA plus DMEM without acetyl-LDL for an additional 24 hr to permit complete esterification of all endocytosed lipoprotein cholesterol. After the chase period, cells were rinsed with DMEM, and incubated with or without partially purified LTP (491 μg protein) in 0.5% BSA/DMEM (1 ml total medium) for the indicated time ($\leq$3 hr, inset; $\geq$6 hr, main figure). For the 48-hr point, the media were replenished at 24 hr with 0.5% BSA/DMEM ± transfer protein. Cells were processed as described in Experimental Procedures to determine the amount of cholesteryl ester (□, ■) and triglyceride (○, ●) radioactivity transferred to the medium. Data are presented as the percentage of cellular lipid radioactivity (at t = 0) transferred to medium. Open symbols represent transfer in the absence of transfer protein. Each point is the average of data from at least two wells. These data are representative of two similar experiments. For the $\geq$6-hr time points, the cells initially (t = 0 hr) contained: cholesteryl ester, 82,000 cpm, and triglyceride, 643,000 cpm per well. For the $\leq$3-hr time point (inset), cells initially contained: cholesteryl ester, 73,600 cpm, and triglyceride, 479,800 cpm per well.

stimulated transfer of lipids from cells as shown by the near linearity of shorter time points (Fig. 3 inset). At 48 hr, LTP caused an 8- to 10-fold increase in medium cholesteryl ester radioactivity over control wells without LTP. As was noted previously for 6 hr incubations, at 48 hr there was no significant difference in cell viability, as measured by morphology, between cells incubated with or without LTP, or by DNA recovery (20.2 ± 1.2 vs. 22.8 ± 2.8 μg DNA/well, respectively (n = 6)). The transfer of triglyceride to the medium followed a time course similar to that observed for cholesteryl ester during the first 6 hr, but was noticeably slower between 24 and 48 hr. This change in apparent transfer rates may be due to the 41% drop in cellular triglyceride radioactivity that occurred between the 6 and 24 hr time points in control wells; in contrast, the cholesteryl ester radioactivity in control cells remained almost constant over the assay period. At 48 hr, 15.6% of the cholesteryl ester radioactivity and 10.7% of the triglyceride radioactivity initially in the cells were transferred to the medium in the presence of LTP. In the absence of LTP, <2% of these radiolabeled lipids was recovered in the medium. In one of three time course studies, the transfer of cholesteryl ester during the 6–48 hr period was less linear than that shown in Fig. 3.

In a separate experiment, the transfers of cholesteryl ester radioactivity and cholesteryl ester mass from cells were measured concomitantly. With an amount of LTP similar to that used in Fig. 3, 16.0% of the cellular cholesteryl ester radioactivity was transferred to the medium in 48 hr (3.2 ± 0.1 vs. 19.2 ± 2.2% (mean ± SD, n = 3), without and with LTP, respectively) and this was accompanied by a 13.5% decrease in cellular cholesteryl ester mass relative to control cells (93.9 ± 4.0 vs. 81.2 ± 3.0 pmoles/μg DNA (mean ± SD, n = 3), without and with LTP, respectively). Thus, the transfer of cholesteryl ester radioactivity to the medium was associated with a similar decrement in cellular cholesteryl ester mass.

The medium from cells incubated with LTP in the absence of lipoprotein was subjected to ultracentrifugation and gel filtration to partially characterize the nature of the lipid transferred to the medium. By density gradient ultracentrifugation of the medium (**Fig. 4**), cholesteryl ester radioactivity was recovered throughout the density range of 1.08–1.26 g/ml range, with ≥50% present in densities >1.21 g/ml. The elution profile for triglyceride radioactivity was similar (data not shown). The distribution profile for LTP in the medium overlapped the profile for triglyceride and cholesteryl ester radioactivity; however, fractions containing 80% of the lipid transfer activity contained less than half of the lipid radioactivity. In three separate experiments, the peak of lipid transfer activity was distinct from the peak of triglyceride and cholesteryl ester radioactivity, suggesting that they are not associated. This conclusion was confirmed in a separate study which demonstrated that the immunoabsorption of

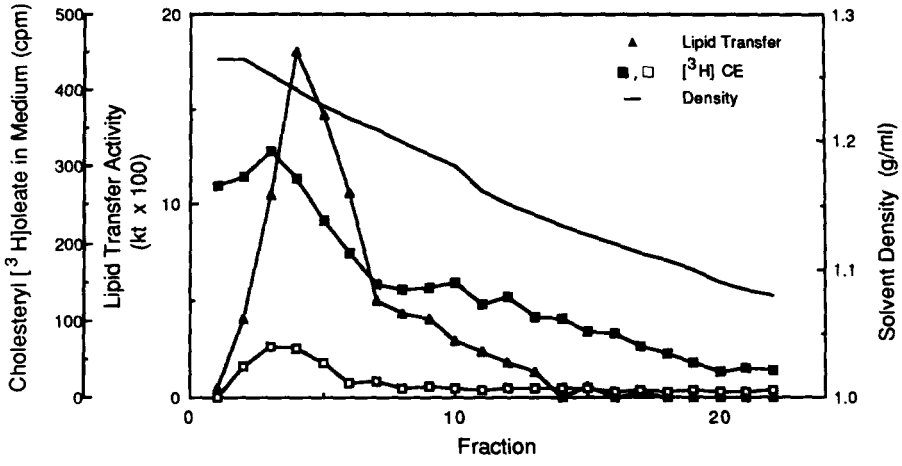

Fig. 4. Fractionation of cell medium by density gradient ultracentrifugation. Lipid-labeled J774 macrophages were incubated in 0.5% BSA/DMEM without or with partially purified LTP (450 μg protein). After 24 hr, the media from quadruplicate wells were pooled and applied to the top of a discontinuous gradient consisting of 7.2 ml each of 1.006, 1.092, 1.178, 1.264, and 1.350 g/ml NaBr solutions. Samples were centrifuged in a 60 Ti rotor at 60,000 rpm for 24 h (17°C). Fractions (1.7 ml) were removed from the bottom of the tube by a peristaltic pump (40 ml/hr). Solvent density (—) was determined by refractometry. Lipid transfer activity (▲) was measured by a standard lipoprotein-lipoprotein transfer assay (3, 7), t = 18 hr. The recovery of lipid transfer activity in the 24-hr medium sample was 104 ± 9% of that initially added to the well (t = 0), and was 91% after density gradient fractionation; no transfer activity was measured in fractions from the medium of cells incubated without LTP. Cholesteryl [^{3}H]oleate in fractions from the medium of cells incubated without (□) and with (■) LTP was determined as described in Experimental Procedures. More than 86% of the applied cholesteryl ester radioactivity was recovered after centrifugation. These data are representative of three separate experiments performed on pooled medium.

TABLE 4. Effect of LTP immunoabsorption on medium triglyceride and cholesteryl ester radioactivity

IgG Fraction Added	Medium Radioactivity (cpm/200 μl) TG	Medium Radioactivity (cpm/200 μl) CE	Medium Lipid Transfer Activity (% kt/50 μl, t = 4.5 hr)
None	1170 ± 25[a]	487 ± 3	16.1 ± 0.7
Nonimmune	878 ± 6	408 ± 4	16.0 ± 0.3
Anti-LTP	766 ± 10 (−12.8%)[b]	397 ± 16 (−2.7%)	3.9 ± 0.0 (−75.6%)

Lipid-loaded, radiolabeled J774 macrophages were incubated in the presence of partially purified LTP (265 μg protein/ml) to promote the transfer of cellular radiolabeled triglyceride and cholesteryl ester to the medium. After 24 hr, the medium was recovered, centrifuged to remove cell debris, and then aliquots (200 μl) were incubated with the IgG fraction (coupled to fixed *S. aureus* cells) from 20–25 μl of anti-LTP or nonimmune rabbit serum in a total volume of 400 μl for 2 hr. After a brief centrifugation, the supernatants were analyzed for their content of radiolabeled triglyceride and cholesteryl ester, and LTP (by an activity assay). Abbreviations: TG, triglyceride; CE, cholesteryl ester. These results are representative of two similar experiments.

[a]Mean ± SD, n = 2.

[b]Values in parentheses are the percent differences between anti-LTP-treated and nonimmune-treated samples.

the LTP in the medium resulted in only a small loss of medium cholesteryl ester and triglyceride radioactivities relative to medium treated with nonimmune sera (**Table 4**). Although recoveries of radioactivity were often low, gel filtration (Bio-Gel A-5m) analysis demonstrated that labeled triglyceride and cholesteryl ester in the medium were associated principally with components of $>2 \times 10^6$ apparent M_r.

Although the above data indicate that cellular triglyceride and cholesteryl ester that are transferred to the medium are associated with dense, large media components, it appears that this association occurs, or is stable, only in the absence of lipoproteins in the incubation medium. As shown in **Table 5**, transferred cholesteryl ester (and triglyceride, data not shown) was almost quantitatively recovered in the lipoprotein fraction when LDL or HDL was added to the medium at either low or high concentrations (i.e., nonstimulatory and stimulatory levels, see Table 3). Density gradient ultracentrifugation analysis showed that the radiolabeled lipids co-isolated with the added lipoprotein (data not shown). The association between transferred lipids and lipoproteins occurred even in the absence of cells. That is, when medium from cells that had been incubated with LTP alone was isolated and incubated with LDL or HDL, the endogenous radiolabeled lipids became associated with the added lipoprotein (**Table 6**). In the presence of both LDL and HDL, the radiolabeled lipids partitioned between these lipoproteins. This redistribution of radiolabeled lipids occurred almost as well at 0°C as at 37°C, indicating that LTP, which is present in the medium, was not involved in this lipid movement.

The transformed J774 macrophage was not unique in its ability to interact with LTP. When lipid-labeled mouse peritoneal macrophages were incubated with LTP (322 μg protein), there was a 14-fold stimulation in the medium content of cell-derived, radiolabeled cholesteryl oleate relative to control cells (9 vs. 123 pmol cholesteryl ester, t = 24 hr).

DISCUSSION

The data presented here demonstrate for the first time that the plasma-derived LTP facilitates lipid transfer from intact, viable, lipid-loaded macrophages in culture to the medium. Similar to that observed in lipoprotein-lipoprotein assays (3, 7), the LTP promoted the transfer of both

TABLE 5. Association of cholesteryl [^{3}H]oleate with lipoproteins in the medium

Lipoprotein Added to Medium	Percent of Medium Cholesteryl Ester Radioactivity Associated with the Indicated Density Fraction: d < 1.063 g/ml	d < 1.19 g/ml
None[a]		24.8 ± 4.7
HDL (30 μg/ml)[a]		89.8 ± 2.1
HDL (200 μg/ml)[a]		95.6 ± 0.3
None[b]	8.3 ± 1.0	
LDL (30 μg/ml)[b]	89.1 ± 2.8	
LDL (200 μg/ml)[b]	93.1 ± 1.4	

J774 macrophages were loaded with radiolabeled cholesteryl ester by preincubation (16 hr) with acetyl-LDL (50 μg cholesterol) in the presence of [^{3}H]oleate/BSA. Labeled cells were then incubated with partially purified LTP (300 μg/ml) in the presence or absence of lipoproteins in the medium. After 24 hr, media were removed, briefly centrifuged, and then fractionated at the indicated solvent density. Top and bottom fractions from each spin were collected and analyzed for their content of cholesteryl [^{3}H]oleate (see Experimental Procedures). Values shown are the average (mean ± SD) of duplicate determinations on medium pools from three to five wells. The results are representative of two similar experiments.

[a]Medium centrifuged at d = 1.19 g/ml.

[b]Medium centrifuged at d = 1.063 g/ml.

TABLE 6. Incubation of isolated, conditioned media with plasma lipoproteins

Addition to Isolated Media	Temperature	Percent of Medium Cholesteryl [^{3}H]Oleate Associated with the Indicated Density Fraction		
		LDL	HDL	d > 1.21
None	37°C	0.5 ± 0.3	19.1 ± 7.1	80.6 ± 6.6
LDL	37°C	71.2 ± 2.9	2.9 ± 2.5	25.9 ± 1.8
LDL + HDL	37°C	31.7 ± 7.4	58.2 ± 3.4	10.0 ± 2.6
HDL	37°C	4.3 ± 0	90.8 ± 15.5	4.9 ± 0
None	0°C		N.D.	
LDL	0°C	60.0 ± 3.1	29.4 ± 3.9	10.6 ± 2.9
LDL + HDL	0°C	36.4 ± 3.0	57.8 ± 3.2	5.8 ± 1.6
HDL	0°C	5.7 ± 1.2	79.9 ± 1.2	14.5 ± 4.8

Media from radiolabeled J774 macrophages that had been incubated with LTP (120 μg protein) for 24 hr were collected and centrifuged to remove cell debris. Aliquots (225 μl) were subsequently incubated with LDL (100 μg total cholesterol) and/or HDL (100 μg total cholesterol) in a total volume of 400 μl at 37°C (LTP-active) or 0°C (LTP-inactive) for 6 hr. Samples were then separated into LDL (d < 1.063 g/ml), HDL (1.063 < d < 1.21 g/ml), and d > 1.21 g/ml fractions by sequential ultracentrifugation. Fractions were analyzed for their content of radiolabeled cholesteryl ester as described in Experimental Procedures. Values are the average (mean ± SD) of duplicate determinations; N.D., not determined.

cholesteryl ester and triglyceride from cells. The extents of cholesteryl ester and triglyceride transfer were directly dependent on both the amount of LTP added and the incubation time. At high levels of LTP, cells were depleted of up to 16% of their cholesteryl ester radioactivity and 14% of their cholesteryl ester mass over a 48-hr incubation. This indicates that LTP can promote the net transfer of cholesteryl ester from cells, and strongly suggests that it may be capable of markedly reducing the cholesteryl ester content of cellular inclusions. The sterol esters in these droplets are most likely in equilibrium with membranous cholesteryl ester (26), perhaps due to the presence of intracellular neutral LTPs (27).

Whereas LTP readily promoted cholesteryl ester and triglyceride transfer out of cells, we were unable to detect the LTP-facilitated transfer of lipid (cholesteryl ether) from lipoproteins (either LDL or HDL) to J774 macrophages. This latter result confirms the finding of Granot et al. (13) with this cell line. Interestingly, however, these authors did report that LTP can promote cholesteryl ester transfer from HDL to other cell types such as Hep G2 cells and smooth muscle cells. In contrast to these results, in our studies we consistently observed that the incubation of LTP with J774 macrophages actually led to a lower cellular content of the lipoprotein-derived cholesteryl ether than that found in cells incubated with lipoproteins in the absence of transfer protein. Although the cellular accumulation of cholesteryl ether in the absence of LTP most likely reflects the uptake of intact lipoproteins (by either receptor dependent or independent mechanisms), it seems unlikely that the lower cellular content of cholesteryl ether in the presence of LTP reflects an inhibition of lipoprotein uptake, since LTP resulted in a lower cellular content of cholesteryl ether originating from either LDL or HDL, which are taken up by different receptors on the cell. Although not conclusive, the results of this study suggest that the cellular cholesteryl ether becomes available to LTP for transfer after lipoprotein uptake (and presumably degradation) by macrophages. This mechanism would explain why LTP was less effective in decreasing the cell-associated cholesteryl ether content of lipid-loaded cells compared to unloaded cells (Table 2), since the radiolabeled ether would be more extensively diluted by other endogenous LTP substrates in the foam cells. This suggestion is also consistent with the finding of Higgs et al. (28) which demonstrates that a portion of the cholesteryl ether label originally in LDL becomes cell surface-associated after receptor-dependent uptake and degradation of the lipoprotein. Thus, the use of cholesteryl ether as a cumulative marker for lipoprotein uptake may be complicated during long-term studies in vivo in animals with significant levels of plasma lipid transfer activity.

The results from this study offer several insights into the mechanism of LTP-mediated lipid transfer from macrophages. We have observed that lipid transfer is not dependent on cellular energy metabolism, active protein synthesis, or on trypsin-sensitive surface proteins. These results are consistent with the findings of Stein, Halperin, and Stein (12) which demonstrated that LTP facilitated lipid transfer from dead (formaldehyde-treated) cells. Two additional lines of evidence suggest that the interaction of LTP with cells does not involve the direct participation of plasma lipoproteins as either acceptors or donors of lipid. First, the addition of plasma LDL or HDL to the medium had no significant effect on the rate of cholesteryl ester or triglyceride transfer from cells over the 6-hr assay period except when these lipoproteins were added at high concentration. Second, LTP did not appear capable of transferring radiolabeled cholesteryl ether from LDL or HDL

to macrophages regardless of whether these cells had been previously loaded with lipids. Thus, although these studies do not rule out the possibility of lipoprotein-macrophage lipid transfers, they do demonstrate that LTP-facilitated lipid transfer from macrophages can proceed by a mechanism that is independent of plasma lipoproteins in the medium.

Although perhaps not directly involved in the transfer event, plasma LDL and HDL readily bound lipids that were transferred to the medium. Under these cell culture conditions, this association appeared to be secondary to an initial association of the transferred lipids with a large dense medium component(s). We speculate that since both LDL and HDL are present in the extracellular compartment in vivo, the relative concentration of these lipoproteins is likely to determine with which lipoproteins the cell-derived lipid associates and, therefore, to determine the fate of the transferred lipid.

The nature and origin of the medium components with which transferred lipids initially associate remain to be determined; however, the density and size of this "acceptor" suggest that it is not contaminating acetyl-LDL remaining from the preincubation period, or apolipoprotein E-phospholipid discs that are secreted by macrophages (29). This latter conclusion is further substantiated by the observation that LTP facilitated lipid transfer from peritoneal macrophages and the J774 macrophage cell line at similar rates although the synthesis of apolipoprotein E by these cells differs markedly (30). Additionally, the acceptor does not appear to be LTP itself or LTP complexed with other medium components as shown by the immunoabsorption studies.

In summary, LTP has been shown to stimulate the transfer of cholesteryl ester and triglyceride from intact viable macrophages in culture. This LTP-facilitated event is the only mechanism, to our knowledge, for the removal of intact cholesteryl esters from cells. Hence, this process may be of considerable importance in the removal of cholesteryl esters from lipid-laden cells, such as those present in the atherosclerotic lesion. A better understood mechanism for the removal of cellular sterol esters is the so-called reverse cholesterol transport mechanism in which sterol esters are hydrolyzed intracellularly and the free sterol is removed from the cell by appropriate (e.g., HDL) acceptors of the lipid (31). At present, the relative importance of the LTP and reverse cholesterol transport mechanisms under normal conditions is not known; however, there are pathological conditions under which the reverse cholesterol transport mechanism is likely to be impaired. For example, Fielding, Fielding, and Havel (32) have reported that the HDL from various hyperlipidemic individuals are ineffective acceptors of cholesterol from cells, and Randolph and Hoff (24) have shown that reverse cholesterol transport is dependent on cellular membrane proteins, and that proteases, such as those secreted by activated macrophages in the atherosclerotic lesion, severely impede cholesterol efflux from cells. Thus, it seems reasonable to speculate that, under some pathological conditions, the LTP-facilitated cholesteryl ester efflux mechanism may be the principal means of depleting cells of their accumulated sterol esters. Although the capacity of this mechanism is unknown, by extrapolation we estimate that physiologic concentrations of LTP (i.e., levels about one-sixth of the highest dose used herein) could promote the removal of more than two-thirds of the cholesteryl ester from comparably lipid-loaded cells during 40–60 days—the estimated lifespan of a tissue macrophage (33). The secretion of LTP by macrophages (34) may enhance this rate in vivo by increasing the local concentration of this protein. These studies suggest a novel and potentially anti-atherogenic role for LTP.

The author gratefully acknowledges the technical assistance of Katherine Osborne and Diane Greene in these studies. Thanks also go to Muriel Daly for her editorial assistance and to Endre Ritly for the photographic services. This research was supported by grants HL-31272, HL-29582, and HL-36595 from the National Institutes of Health, and by a grant-in-aid from the Northeast Ohio Affiliate of the American Heart Association.

Manuscript received 4 March 1988.

REFERENCES

1. Hesler, C. B., T. L. Swenson, and A. R. Tall. 1987. Purification and characterization of a human plasma cholesteryl ester transfer protein. *J. Biol. Chem.* **262:** 2275–2282.
2. Jarnagin, A. S., W. Kohr, and C. Fielding. 1987. Isolation and specificity of a M_r 74,000 cholesteryl ester transfer protein from human plasma. *Proc. Natl. Acad. Sci. USA.* **84:** 1854–1857.
3. Morton, R. E., and D. B. Zilversmit. 1982. Purification and characterization of lipid transfer protein(s) from human lipoprotein-deficient plasma. *J. Lipid Res.* **23:** 1058–1067.
4. Pattnaik, N. M., and D. B. Zilversmit. 1979. Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles. *J. Biol. Chem.* **254:** 2782–2786.
5. Morton, R. E. 1985. Binding of plasma-derived lipid transfer protein to lipoprotein substrates. The role of binding in the lipid transfer process. *J. Biol. Chem.* **260:** 12593–12599.
6. Marcel, Y. L., C. Vezina, B. Teng, and A. Sniderman. 1980. Transfer of cholesterol esters between human high density lipoproteins and triglyceride-rich lipoproteins controlled by a plasma protein factor. *Atherosclerosis.* **35:** 127–133.
7. Morton, R. E., and D. B. Zilversmit. 1981. A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. *J. Biol. Chem.* **256:** 11992–11995.
8. Barter, P. J., G. J. Hopkins, L. Gorjatschko, and M. E. Jones. 1982. A unified model of esterified cholesterol exchanges between human plasma lipoproteins. *Atherosclerosis.* **44:** 27–40.

9. Weinberg, R. B., and A. M. Scanu. 1982. In vitro reciprocal exchange of apoproteins and nonpolar lipids between human high density lipoproteins and an artificial triglyceride-phospholipid emulsion (Intralipid). *Atherosclerosis.* **44:** 141-152.
10. Hashimoto, S., R. E. Morton, and D. B. Zilversmit. 1984. Facilitated transfer of cholesteryl ester between rough and smooth microsomal membranes by plasma lipid transfer protein. *Biochem. Biophys. Res. Commun.* **120:** 586-592.
11. Stein, Y., O. Stein, T. Olivecrona, and G. Halperin. 1985. Putative role of cholesteryl ester transfer protein in removal of cholesteryl ester from vascular interstitium, studied in a model system in cell culture. *Biochim. Biophys. Acta.* **834:** 336-345.
12. Stein, O., G. Halperin, and Y. Stein. 1986. Cholesteryl ester efflux from extracellular and cellular elements of the arterial wall: model systems in culture with cholesteryl linoleyl ether. *Arteriosclerosis.* **6:** 70-78.
13. Granot, E., I. Tabas, and A. R. Tall. 1987. Human plasma cholesteryl ester transfer protein enhances the transfer of cholesteryl ester from high density lipoproteins into cultured Hep G2 cells. *J. Biol. Chem.* **262:** 3482-3487.
14. Halperin, G., and S. Gatt. 1980. The synthesis of cholesteryl alkyl ethers. *Steroids.* **35:** 39-42.
15. Burstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11:** 583-595.
16. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250:** 4007-4021.
17. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345-1353.
18. Fraenkal-Conrat, H. 1957. Methods for investigating the essential groups for enzyme activity. *Methods Enzymol.* **4:** 247-269.
19. Goldstein, J. L., H. F. Hoff, and Y. K. Ho, S. K. Basu, and M. S. Brown. 1981. Stimulation of cholesteryl ester synthesis in macrophages by extracts of atherosclerotic human aortas and complexes of albumin/cholesterol esters. *Arteriosclerosis.* **1:** 210-226.
20. Thompson, J. N., P. Erdody, R. Brien, and T. K. Murray. 1971. Fluorometric determination of vitamin A in human blood and liver. *Biochem. Med.* **5:** 67-89.
21. Clevidence, B. A., R. E. Morton, G. West, D. M. Dusek, and H. F. Hoff. 1984. Cholesterol esterification in macrophages: stimulation by lipoproteins containing apoB isolated from human aortas. *Arteriosclerosis.* **4:** 196-207.
22. Gamble, W., M. Vaughan, H. S. Kruth, and J. Avigan. 1978. Procedure for determination of free and total cholesterol in micro- and nanogram amounts suitable for studies with cultured cells. *J. Lipid Res.* **19:** 1068-1070.
23. Labarca, C., and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* **102:** 344-352.
24. Randolph, R. K., and H. F. Hoff. 1986. Exchange and mass efflux of cholesterol in macrophages. Evidence for a common mechanism and a role for plasma membrane proteins. *J. Lipid Res.* **27:** 307-315.
25. Morton, R. E., and D. B. Zilversmit. 1983. Interrelationship of lipids transferred by the lipid-transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258:** 11751-11757.
26. Smaby, J. M., W. J. Baumann, and H. L. Brockman. 1979. Lipid structure and the behavior of cholesteryl esters in monolayer and bulk phases. *J. Lipid Res.* **20:** 789-795.
27. Wetterau, J. R., and D. B. Zilversmit. 1986. Localization of intracellular triglyceride and cholesteryl ester transfer activity in rat tissues. *Biochim. Biophys. Acta.* **875:** 610-617.
28. Higgs, D. W., D. R. van der Westhuyzen, W. Gevers, G. A. Coetzee, O. Stein, and Y. Stein. 1984. In vitro metabolism of LDL labeled with a nondegradable cholesteryl ester analogue. *Arteriosclerosis.* **4:** 208-213.
29. Basu, S. K., Y. K. Ho, M. S. Brown, D. W. Bilheimer, R. G. W. Anderson, and J. L. Goldstein. 1982. Biochemical and genetic studies of the apoprotein E secreted by mouse macrophages and human monocytes. *J. Biol. Chem.* **257:** 9788-9795.
30. Werb, Z., and J. R. Chin. 1983. Onset of apoprotein E secretion during differentiation of mouse bone marrow-derived mononuclear phagocytes. *J. Cell. Biol.* **97:** 1113-1118.
31. Fielding, C. J., and P. E. Fielding. 1982. Cholesterol transport between cells and body fluids. *Med. Clin. N. Am.* **66:** 363-373.
32. Fielding, P. E., C. J. Fielding, and R. J. Havel. 1983. Cholesterol net transport, esterification and transfer in human hyperlipidemic plasma. *J. Clin. Invest.* **71:** 449-460.
33. Fauve, R. M. 1982. Phagocytes. In Immunology. 2nd edition. J. F. Bach, editor. John Wiley & Sons, New York. 106-126.
34. Tollefson, J. H., R. Faust, J. J. Albers, and A. Chait. 1985. Secretion of a lipid transfer protein by human monocyte-derived macrophages. *J. Biol. Chem.* **260:** 5887-5890.