review

Cell cholesterol efflux: integration of old and new observations provides new insights

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Abstract **Numerous studies using a variety of cell/acceptor combinations have demonstrated differences in cholesterol efflux among cells. These studies also show that different acceptors, ranging from simple molecules like cyclodextrins to serum, stimulate efflux through a variety of mechanisms. By combining early observations with data derived from recent studies, it is now possible to formulate a model for cell cholesterol efflux which proposes that an array of different mechanisms, including aqueous diffusion, lipid-free apolipoprotein membrane microsolubilization, and SR-BI-mediated cholesterol exchange contribute to cholesterol flux. In this model the relative importance of each mechanism would be determined both by the cell type and the nature of the extracellular cholesterol acceptor.**—Rothblat, G. H., M. de la Llera-Moya, V. Atger, G. Kellner-Weibel, D. L. Williams, and M. C. Phillips. **Cell cholesterol efflux: integration of old and new observations provides new insights.** ***J. Lipid Res.*** **1999.** 40: **781–796.**

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More than 30 years ago it was established that cholesterol molecules can be readily removed from cells by exposing them to serum, and more specifically, to the α-lipoproteins (high density lipoprotein, HDL) fraction of serum (1). Glomset (2) coupled this early observation on cell cholesterol efflux to his studies on the esterification of cholesterol by lecithin:cholesterol acyltransferase (LCAT), and formulated the concept of "reverse cholesterol transport" (RCT). In the subsequent years a major research effort has been focused on developing this concept, and specifically, on the steps involved in the movement of cholesterol molecules from cells to extracellular acceptors. Even after this long period of investigation and concentrated research effort, our knowledge of the exact mechanisms involved in the efflux of cell cholesterol remains both fragmentary and controversial. As yet we do not clearly understand the role played by RCT either in cell cholesterol homeostasis or in the pathological deposition of cholesterol in the walls of blood vessels. Knowledge of the influx and subsequent metabolism of LDL cholesterol has progressed at a much more rapid pace than our understanding of HDL-mediated cholesterol efflux, the first step in RCT.

In this review we will summarize some of the insights obtained from studies on cholesterol efflux done over a number of years and then use this information to formulate a broadly based model that could serve to stimulate future investigations. This model will focus only on the steps thought to be involved in the movement of free cholesterol (FC) molecules between the plasma membrane and extracellular acceptors. The movement of cholesterol between intracellular sites and the plasma membrane will not be addressed in any detail, and the reader is referred to a number of recent reviews on this important subject (3, 4).

BI-DIRECTIONAL FLUX AND NET FLUX

Most studies on cholesterol flux have focused on the movement of FC between serum lipoproteins and cells. These studies have used in vitro tissue culture cell models to formulate mechanisms that are likely to operate in vivo. With the exception of tissues such as liver, intestine, and

Abbreviations: LCAT, lecithin cholesterol acyltransferase; RCT, reverse cholesterol transport; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; FC, free cholesterol; EC, esterified cholesterol; CE, cholesteryl ester; SUV, small unilamellar vesicle; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; apo, apolipoprotein; PLPT, phospholipid transfer protein; CETP, cholesteryl ester transfer protein; $t_{1/2}$, half-time; POPC, palmitoyl-oleoyl phosphatidylcholine; PC, phosphatidylcholine; PL, phospholipid; DMPC, dimyristoylphosphatidylcholine; SR-BI, scavenger receptor class B, type I; DRM, detergent-resistant membrane; KO, gene knockout; HS, human serum; CD, cyclodextrin; Lp, lipoprotein.

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endocrine glands in which cholesterol can be released as components of nascent lipoproteins or metabolized to bile acids or steroid hormones, the efflux of FC from cells to lipoproteins is the only mechanism for the removal of cellular cholesterol. This movement of FC molecules between cells and lipoproteins is usually a bi-directional process, with the extent and direction of net movement dependent on the ratio of influx to efflux (5–7) and determined by both cell and acceptor properties (5–7).

CHOLESTEROL ACCEPTORS

A wide variety of extracellular cholesterol acceptors have been used in cholesterol flux studies. These acceptors differ in complexity and in the extent to which they contribute to either unidirectional or bi-directional flux.

Cyclodextrins

Cyclodextrins are the simplest of the extracellular cholesterol acceptors. β-Cyclodextrins, cyclic oligosaccharides consisting of 7 β(1–4)-glucopyranose units, are water-soluble compounds with a hydrophobic cavity capable of enhancing the solubility of non-polar compounds in aqueous solutions (8, 9). β-Cyclodextrins have been found to be particularly efficient in solubilizing cholesterol. The high affinity of some cyclodextrins for cholesterol has made them valuable tools for the study of cholesterol flux and for the rapid and extensive manipulation of cell cholesterol content (10). Cyclodextrins, when present at high concentrations (5–100 mm), can remove cell cholesterol at phenomenal rates (300–400%/hour) (11–13). At lower concentrations, cyclodextrins can serve as cholesterol shuttles, transporting cell membrane cholesterol to serum lipoproteins (see below) (14). Although cyclodextrins have been used most extensively when they are free of cholesterol, they can be complexed with varying amounts of cholesterol and, when so employed, participate in bi-directional cholesterol flux (10).

Phospholipid vesicles

Another type of cholesterol acceptor that has been extensively used in cholesterol flux studies is phospholipid vesicles. A wide assortment of acceptors having a general vesicular structure can be prepared, including small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), and multilamellar vesicles (MLV). These structures can be made cholesterol-free or can be prepared with varying amounts of FC. Exposure of cells to pure phospholipid vesicles results in essentially unidirectional movement of cell cholesterol to the vesicles. Thus conditions can be established in which the efflux of radiolabeled cell cholesterol closely predicts the net reduction of cell cholesterol mass (5–7). If, however, the vesicles contain FC, conditions for bi-directional flux are established and efflux of labeled cell cholesterol does not predict changes in cell cholesterol mass.

Lipid-free/lipid-poor apolipoproteins

Considerable interest has been focused recently on the ability of lipid-free or lipid-poor apolipoproteins to participate in the process of cell cholesterol efflux (3, 15, 16). Studies have demonstrated the presence of essentially lipid-free apolipoproteins in serum (17–19). In addition, small, protein-rich particles containing apoA-I, apoA-IV, or apoE are present in plasma and lymph and contribute to cholesterol efflux in vitro (20) and possibly in vivo (21, 22). In general such particles would be relatively depleted of FC, and thus could contribute to the net efflux of cell cholesterol. At this time a precise characterization of these small HDL particles has not been achieved, and attempts to re-constitute lipoproteins having the size, composition, and charge of the native particles have not been entirely successful. Thus, a spectrum of structures including native and reconstituted apolipoprotein/phospholipid complexes with preβ-mobility (23–25) and a variety of native apolipoproteins (15, 26, 27) or synthetic peptides containing amphipathic helical structures (28, 29) have been studied.

Reconstituted and native HDL

Because of the generally accepted belief that HDL participates in the first steps of reverse cholesterol transport, the use of isolated or reconstituted lipoproteins for cholesterol flux studies has focused on HDL. A variety of HDL subclasses have been studied, including HDL particles isolated either on the basis of density by centrifugation or on the basis of apolipoprotein content by immunoaffinity chromatography (30–36). In addition, the ability to incorporate a variety of apolipoproteins into phospholipid structures, and thus synthesize HDL-like particles of various sizes and compositions, has provided a powerful tool for cholesterol flux studies. Precise control of parameters such as particle size, phospholipid composition, apolipoprotein composition, particle charge, and cholesterol content has permitted detailed analysis of the relative importance of each of these parameters on cell cholesterol flux (37–40). In addition, the ability to assemble particles whose composition ranges from cholesterol-free to cholesterol-rich has been used to assess the relative movement of cholesterol under conditions of bi-directional flux (5, 41).

Whole serum

Perhaps the most complex and yet most physiological cholesterol acceptor is serum or plasma. The wide spectrum of lipoproteins and apolipoproteins present in serum is accompanied by other factors such as LCAT, phospholipid transfer protein (PLTP), and cholesteryl ester transfer protein (CETP). All these proteins play important roles in RCT, and their presence in varying relative concentrations affects the bi-directional flux of cholesterol. The bi-directional flux of cholesterol between cells and serum is particularly complex. Influx can result from the cellular uptake of both free and esterified cholesterol, and occurs by both receptor-mediated and physical/chemical exchange processes. In addition, efflux of cell cholesterol to serum also involves a number of different mechanisms, which are detailed in subsequent sections. However, in spite of the complexities of using serum or plasma for the study of cell cholesterol flux, the potential benefits are large, particularly when the data gathered from serum

studies are integrated with information gathered utilizing isolated serum components or artificial acceptors.

OVERVIEW OF OUR CURRENT UNDERSTANDING OF EFFLUX

In the following sections we have attempted to integrate a large number of observations on cell cholesterol efflux collected using a variety of cell types and the various acceptors briefly described. We believe that many of the past studies, yielding divergent data which suggested contradictory mechanisms, can be integrated into a model of cell cholesterol efflux proposing that cells can use multiple mechanisms depending on which cellular properties are best linked to the type of acceptor available.

Observation 1: Different cell types exhibit large differences in the rate of cholesterol release to phospholipid-containing acceptors

This fundamental observation was made early during the initial investigations of cholesterol efflux (42) and is illustrated by the data presented in **Fig. 1** in which the half-times ($t_{½}$) for the rate of cholesterol efflux from a variety of cells to a common acceptor are presented. In this example, $t_{½}$ values range from a few hours with the fastest cells, Fu5AH rat hepatoma, to greater than 1 day for a number of slow cells. These relative differences between cell types persist as long as the acceptor contains phospholipid even though the efflux efficiencies of acceptor differ. This is illustrated in **Fig. 2** which correlates the rate of release of cholesterol from several different cells exposed to 5% human serum to the efflux rate observed when the cells are incubated with HDL_3 (panel A) or palmitoyl-oleoyl phosphatidylcholine (POPC-SUV) (panel B) (42, 43). The excellent correlations between efflux to the different acceptors demonstrate that although the absolute rate of cholesterol efflux is modulated by the type and concentration of acceptor, the rank order of efflux efficiency among cell types is maintained as long as the acceptor contains phospholipid.

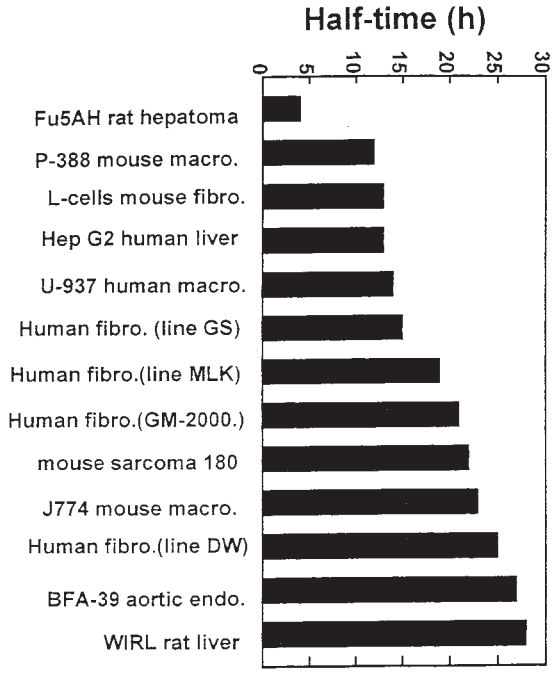

Fig. 1. Comparison of the half-times for the rate of cholesterol efflux from different cell types. Cells were prelabeled with [^{3}H]cholesterol and exposed to apoHDL/egg PC acceptor particles (1 mg/mL PC) for 6 h. Adapted from reference (42).

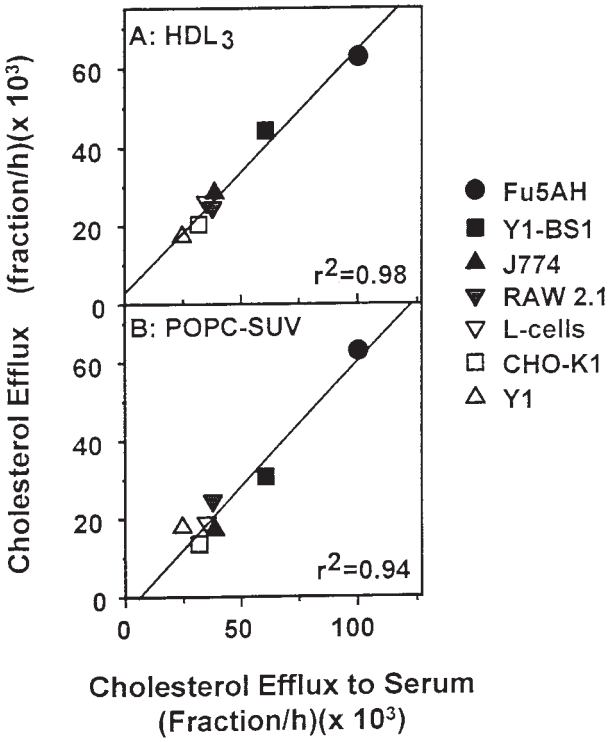

Fig. 2. Correlations between the rate of [^{3}H]cholesterol efflux from various cell types to human serum (5%), HDL_3 (75 μg protein/mL) or POPC-SUV (1 mg/mL). The rate constant for efflux was calculated from a time-course of the release of cell cholesterol during an 8-h incubation. Panel A: relationship between cholesterol efflux to 5% serum and HDL. Panel B: relationship between efflux to serum and SUV. Figure is adapted from reference 50.

Observation 2: The efflux efficiency of sera is closely associated with HDL-phospholipid levels

This observation arises from investigations in which serum was used as the cholesterol acceptor and Fu5AH hepatoma served as the cell cholesterol donor. Two general approaches can be used to identify and compare various lipoprotein subclasses for their efflux-promoting efficiency. The first directly compares isolated lipoprotein fractions, whereas the second uses a series of serum or plasma specimens to stimulate efflux and then correlates efflux to the level of the various lipoproteins, apolipoproteins, or lipids in the specimens. The experimental approach using serum has yielded the type of data illustrated in **Fig. 3**. Specimens of human serum diluted to 5% were incubated with cholesterol-labeled Fu5AH cells, L-cells, or mouse peritoneal macrophages, and the efflux rate of the

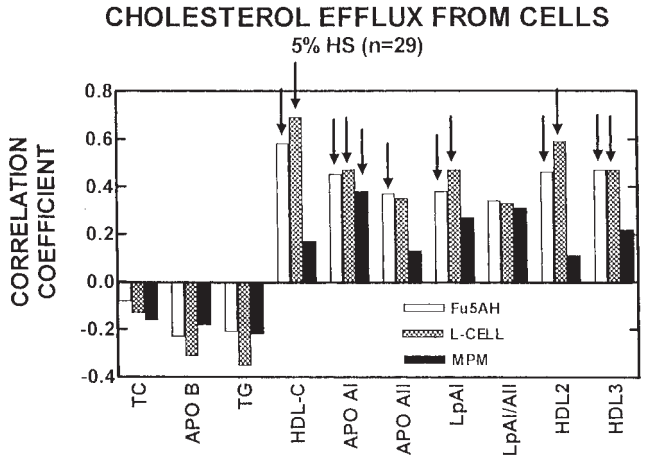

Fig. 3. Relationships between cholesterol efflux from Fu5AH cells, L-cells, and elicited mouse peritoneal macrophages and the individual lipid, apolipoprotein, and lipoprotein components of human serum. Each cell type was incubated with aliquots of specimens of human serum (5%, n = 29) and the rate of release of [^{3}H]cholesterol was correlated with the concentration of each individual component (i.e., lipid, apolipoprotein, or lipoprotein) in each specimen. The arrows indicate a significant correlation with the indicated serum component.

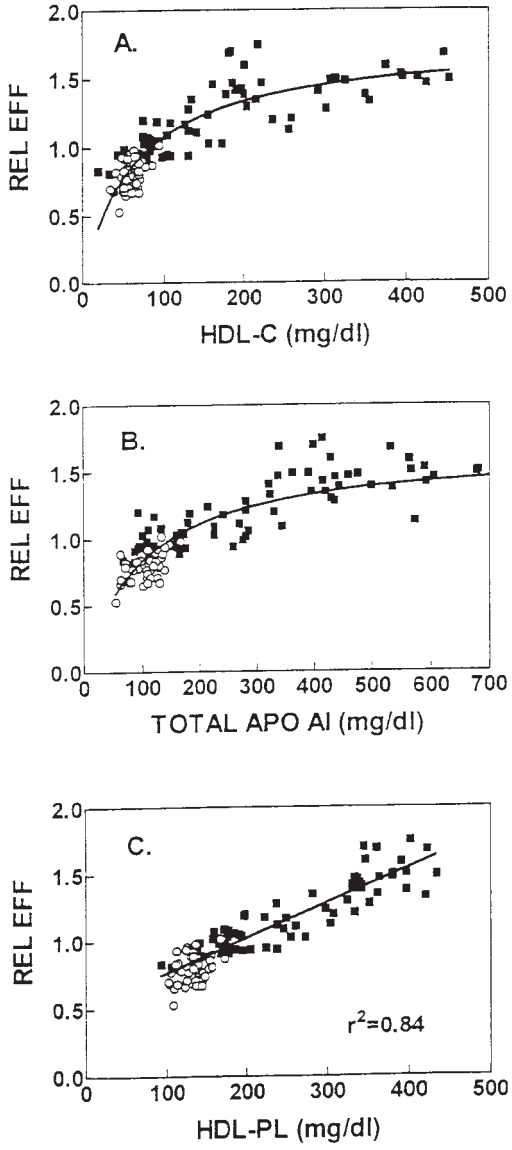

Fig. 4. Relationships between fractional cholesterol efflux from Fu5AH cells and the concentrations of HDL-cholesterol (A), total apoA-I (B), and HDL-phospholipid (C) in serum from control rats (open symbols) and rats expressing human apoA-I (closed symbols). From reference 47.

labeled cholesterol was then correlated with the level of lipoproteins and apolipoproteins in each serum. As can be seen from this figure, efflux from both Fu5AH cells and L-cells correlated with HDL. However, there is no one HDL subclass that is clearly superior (44) and the patterns of correlations between efflux and HDL subclasses appear to be different and depend on the donor cells (Fig. 3). Interestingly, efflux from macrophages correlated only with the apoA-I concentration of each serum (Fig. 3), again emphasizing the importance of cell-associated factors in cholesterol efflux.

The need for phospholipid in the solubilization of serum cholesterol has long been understood. Early studies of Friedman and Byers (45) demonstrated the importance of phospholipid in RCT by showing that the injection of phospholipid into animals could shift tissue cholesterol into the plasma compartment (see review by Williams, Werth, and Wolff (46)). The relationship between HDL-phospholipid and efflux was well illustrated in studies using Fu5AH cells as cholesterol donors and sera from control rats and rats transgenic for human apoA-I as cholesterol acceptors (47). In these transgenic rats, as the expression of human apoA-I increases, the resultant HDL particles become relatively depleted in phospholipid. **Figure 4** illustrates the relationship between cholesterol efflux and the level of HDL-cholesterol (panel A), serum apoA-I (panel B), and HDL-phospholipid (panel C) in sera from control rats and rats expressing differing amounts of human apoA-I. The sets of data on HDL-cholesterol (panel A) and serum apoA-I (panel B) demonstrate a curvilinear relationship, indicating that at the higher concentrations of these two components, efflux is less efficient. However, there is an excellent linear correlation between efflux from Fu5AH cells and HDL-phospholipid, consistent with the conclusion that in this cell system, HDL-phospholipid is a major determinant in cholesterol efflux (47, 48). More recent studies using similar experimental systems have confirmed the importance of HDL-phospholipid and demonstrated that apolipoprotein composition also influences acceptor efficiency, with apoA-II negatively influencing efflux (49).

Observation 3: Addition of phospholipid to serum enhances cholesterol efflux

The importance of phospholipid in modulating cholesterol efflux was further established from studies in which phospholipid was directly added to serum. When control

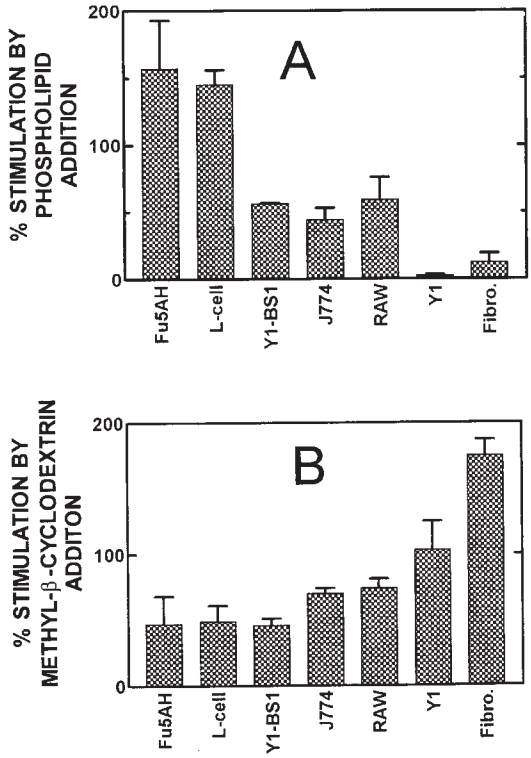

Fig. 5. Panel A: The stimulation of cholesterol efflux from various cell types produced by the supplementation of human serum with phosphatidylcholine. The method of addition of the phospholipid to the serum, the incubation conditions and the calculation of efflux values are as described in reference 43. Panel B: The stimulation of cholesterol efflux from various cell types by the addition of low concentrations of cyclodextrins to serum. Percent stimulation represents the difference in efflux rates obtained between cultures incubated with 5% human serum and with the same serum after supplementation with 0.05 mm methyl-β-cyclodextrin. Rate constants for efflux were determined from data collected during an 8-h incubation. Values are the average of 3 determinations ± SD.

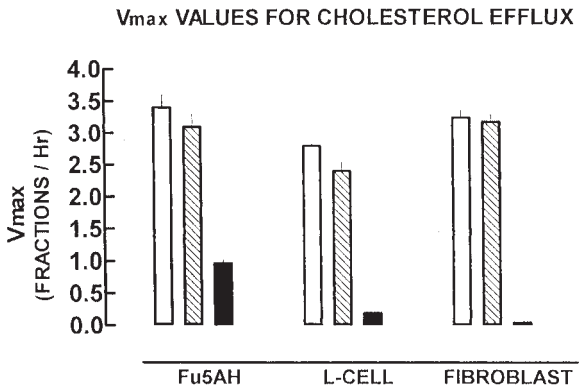

Fig. 6. V_{max} values for the rate of cholesterol efflux from Fu5AH cells, L-cells, and human skin fibroblasts. Efflux assays and V_{max} values were calculated as described in reference 11; □, methyl-β-cyclodextrin; ▨, 2-hydroxypropyl-β-cyclodextrin; ■, HDL_3.

and phospholipid-modified sera were then incubated with Fu5AH cells it was observed that the addition of the phospholipid significantly enhanced the efflux rate (43); however, the stimulation of efflux with the addition of phospholipid to serum is a cell-specific response (50). As illustrated in **Fig. 5A**, some cells, such as Fu5AH, are highly responsive, whereas other cells, such as Y-1 adrenal cells and human skin fibroblasts are resistant. Responsive cell types exhibit enhanced efflux to both phosphatidylcholine and sphingomyelin supplementation (43). Addition of these phospholipids to serum produces modifications in essentially all lipoprotein fractions (43), but efflux studies with lipoproteins isolated from PL-modified serum point to HDL as being responsible for the effect.

Observation 4: By using cyclodextrins at high concentrations it can be demonstrated that *a*) the difference in cholesterol efflux between cell types is largely lost, and *b*) cells have at least two kinetically distinct cholesterol pools

As discussed previously, and illustrated in Fig. 1, the rate of cholesterol release varies widely among cell types when the acceptor contains phospholipid. However, these differences among cells are reduced or eliminated if cyclodextrins at concentrations >5 mm are used as acceptors. **Figure 6** presents estimates of apparent V_{max} values for Fu5AH, L-cells, and fibroblasts exposed to HDL or two different cyclodextrins (11). Two points become obvious: *1*) the cell-dependent differences between cell types exposed to phospholipid-containing acceptors are not apparent when cyclodextrin acceptors are used, and *2*) the efflux-stimulating potential of cyclodextrins is far greater than that of HDL. In addition, because of the ability of cyclodextrins to remove cell cholesterol rapidly, it is possible to use these acceptors to obtain reliable estimates of the kinetics of efflux under conditions where the rate-limiting step for efflux is the movement of cholesterol molecules out of the plasma membrane. Previous attempts to study the detailed kinetics of efflux utilized high concentrations of phospholipid-containing acceptors (51) or followed the release of fluorescent analogs of cholesterol (52). Both of these approaches yielded evidence of multiple kinetic pools, but, depending on the cell-acceptor combination, estimates of these pools varied considerably in terms of pool size and $t_{½}$ (53). More recent studies on the kinetics of cholesterol efflux using high concentrations of cyclodextrins have demonstrated the presence of two kinetic pools of cholesterol in a number of cell types, and provided values demonstrating a fast pool with $t_{½}$ values of approximately 30 sec and a slow pool having $t_{½}$ ranging from 25 to 35 min (13). The size of these pools varies somewhat depending on cell type, generally falling into a range from 30% fast to 70% fast (see Fig. 13B as an example). Whether these pools represent cholesterol in the inner or outer leaflet of the membrane or are laterally separated lipid domains remains to be resolved.

Observation 5: Cyclodextrins at low concentrations can act as cholesterol shuttles facilitating the movement of cholesterol between plasma membranes and extracellular cholesterol acceptors

The addition of low concentrations of cyclodextrins (<1 mm) to tissue culture medium does not produce sig-

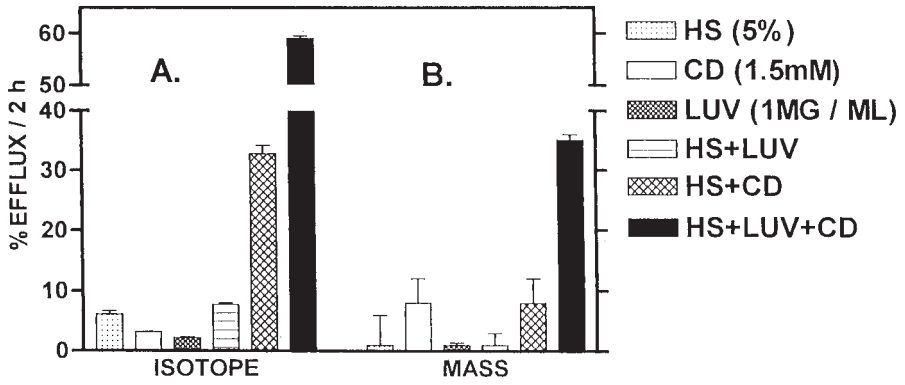

Fig. 7. Efflux of cholesterol from J774 cells exposed to combinations of human serum, LUV, and 2-hydroxypropyl-β-cyclodextrin. Isotope represents the fractional efflux of [^{3}H]cholesterol released to the indicated acceptors in a 2-h incubation. Mass is the reduction in cell FC mass during the 2-h incubation. Data are from reference 14.

nificant release of cholesterol from cells; however, if this concentration of cyclodextrin is added together with serum, the rate of cholesterol release is greater than that with serum alone (14). This is illustrated by the data presented in **Fig. 7A** showing that the fractional release of radiolabeled cholesterol from J774 cells incubated with 5% human serum is greatly enhanced after supplementation of the serum with 1 mm hydroxypropyl-β-cyclodextrin. This movement of labeled cholesterol is not accompanied by a significant change in cell cholesterol mass (Fig. 7B). However, when phospholipid vesicles are added to the serum/cyclodextrin mixture, the efflux of labeled cholesterol is accompanied by a net depletion of cell cholesterol mass (Fig. 7B). The model depicted in **Fig. 8** can explain these results. As discussed earlier, the movement of cholesterol between cells and serum lipoproteins is a bi-directional process (Fig. 8A). At low concentrations cyclodextrins can act as cholesterol shuttles, catalyzing the bi-directional movement of cholesterol molecules between the plasma membrane and lipoproteins. As both influx and efflux are equally stimulated, there is then increased turnover of radiolabeled cholesterol without a change in cell cholesterol mass (Fig. 8B). However, as illustrated in Fig. 8C, the addition of phospholipid vesicles together with cyclodextrins results in the net efflux of cell cholesterol because the phospholipid vesicles act as extracellular cholesterol

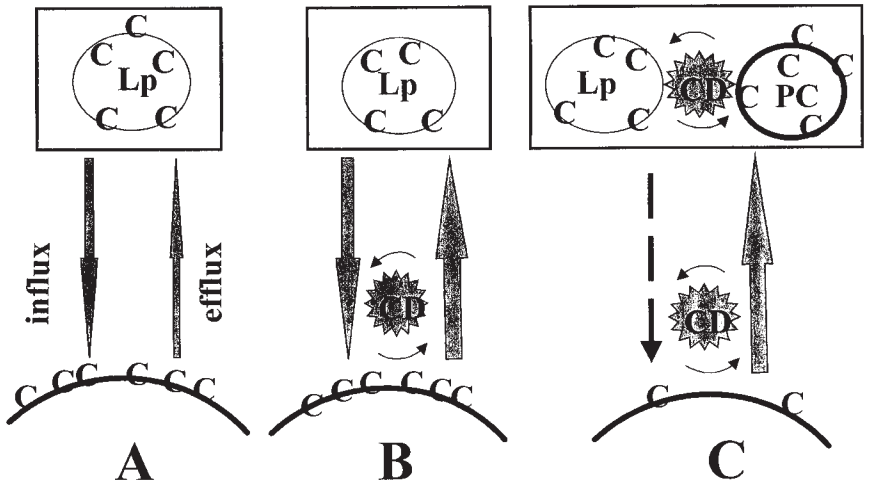

Fig. 8. The shuttle and sink model for cellular cholesterol efflux. (A) The movement of cholesterol molecules between the cell plasma membrane and a lipoprotein such as HDL is a bi-directional process with influx and efflux being separate processes. Under many conditions, FC in the membrane is in equilibrium with FC in the lipoproteins, thus there is turnover of cholesterol molecules without changes in cholesterol mass in either cell or medium compartments. When low concentrations of cyclodextrins are added to the serum (B), the rates of influx and efflux increase, but the equilibrium is not changed. Thus, cholesterol molecules turn over at a faster rate but there is no net change in cholesterol content in cells or medium. When both PL vesicles and cyclodextrins are added to the medium (C), the vesicles act as a sink for extracellular cholesterol, thus shifting the equilibrium to favor net efflux. From reference 14.

sinks. As shown in this figure, efflux rate is enhanced by the cyclodextrins, while the rate of influx is reduced because of the trapping of extracellular cholesterol in the phospholipid vesicles. The ability of low concentrations of cyclodextrins to act as cholesterol shuttles and enhance cell cholesterol efflux to serum lipoproteins is not a uniform characteristic of all cell types, as a shown in Fig. 5B. Thus, some cells are highly responsive to the presence of cyclodextrin shuttles, whereas this shuttling effect is reduced or absent with other cell types. Interestingly, those cell lines that were the most responsive to phospholipid supplementation of serum (Fig. 5A) tend to be the least responsive to the shuttling capabilities of cyclodextrins (50).

The studies that demonstrated the ability of cyclodextrins to act as cholesterol shuttles, and which also demonstrated that phospholipid vesicles can serve as cholesterol sinks, reinforced the hypothesis that serum lipoproteins could perform similar functions in the process of RCT (54, 55). Thus, some lipoprotein particles may participate in the initial movement of FC from the plasma membrane and then transport this cholesterol to larger lipoprotein particles that act as cholesterol sinks (14). Lipoprotein particles that function as initial cholesterol transporters would be expected to be small and have a high efficiency for acquiring cholesterol, but have a low capacity to hold cholesterol. Preβ-HDL (23, 54, 56) or small α-migrating HDL (57, 58) would have the properties necessary to be an efficient cholesterol shuttle or transporter. In contrast to the cyclodextrins that act catalytically, it is probable that physiological shuttles or transporters undergo a series of metabolic changes mediated by LCAT, CETP, and PLPT as the cell-derived cholesterol traverses the route from cell membrane to lipoprotein sink (23, 54, 56). In contrast, lipoprotein particles that serve as cholesterol sinks would be large and relatively inefficient in acquiring cholesterol directly from cells, but would have a high capacity to hold cholesterol. The chylomicrons and very low density lipoproteins (VLDL) have been shown to serve as cholesterol sinks by acquiring cell-derived cholesterol after esterification by LCAT and transfer by CETP (59).

Observation 6: Lipid-free apolipoproteins can stimulate the efflux of cellular cholesterol and phospholipid

It has been demonstrated that both apoA-I and apoA-IV can be isolated from serum or plasma in a lipid-free/lipid-poor state (17, 20, 60, 61). Investigations conducted in a number of different laboratories have shown that such apolipoproteins can participate in the efflux of cellular lipids, and this may represent initial steps in RCT. The extent to which a cell will release cholesterol or phospholipid to lipid-free apolipoproteins depends both on the cell type and on the metabolic status of the cell. Among the most responsive cells are macrophages (29, 62) whereas smooth muscle cells are among the most resistant cells (63). Thus, exposure of macrophages, CHO cells, L-cells, and fibroblasts to apoA-I, apoA-II, apoE, or apoA-IV results in the release of both cellular cholesterol and cellular phospholipid (3, 27, 29, 64). In most systems the efflux of cell cholesterol is closely paralleled by the release of phospholipid. As illustrated in **Fig. 9**, the EC_{50} values for release of these two membrane lipids from fibroblasts to either apoA-I or apoA-IV are similar (65). As a series of synthetic peptides exhibiting amphipathic helical structures have the ability to promote cellular lipid release to the same extent as native apolipoproteins (28, 29), it is obvious that specific amino acid sequences are not required for lipid efflux. Although some investigators have reported the need for multiple helices in order to obtain efflux (27, 28), other investigations have demonstrated efflux with apolipoproteins or peptides having a single helical structure (29).

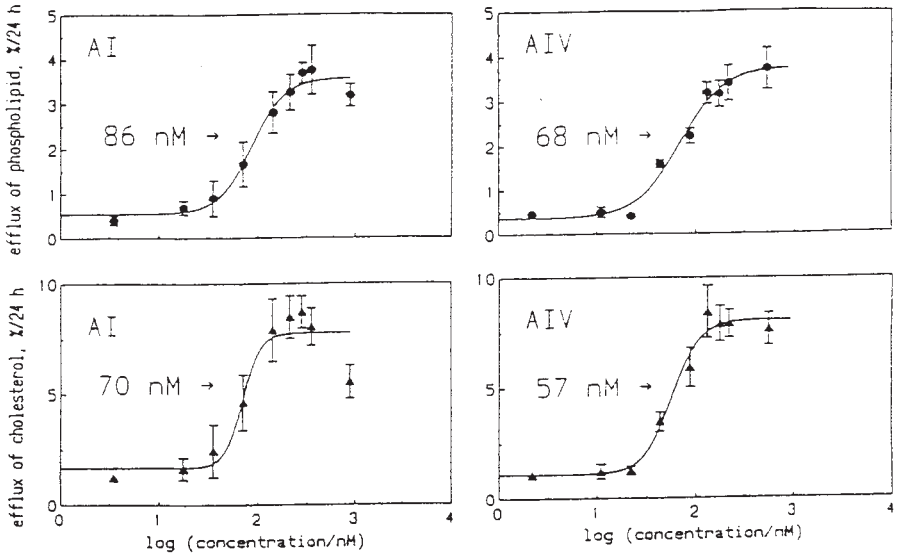

Fig. 9. Dependence of cholesterol and phospholipid efflux from cholesterol-enriched human fibroblasts on apoA-I and A-IV concentrations. Cells were labeled with [^{3}H]cholesterol and [^{32}P]phosphate, and enriched with cholesterol as described in reference 65. Increasing amounts of apolipoproteins were added to medium and incubated with cells for 24 h. Values are means ± SD, n = 3. Figure from reference 65.

Two metabolic manipulations of the cells have been shown to result in an enhancement of lipid release to lipid-poor apolipoproteins. The first treatment is the enrichment of cells with FC, and a number of different cell types have been shown to exhibit increased lipid release after cellular cholesterol loading (65). The second metabolic manipulation that enhances lipid release to lipid-free apolipoproteins is the treatment of cells with cAMP. Transformed macrophages such as J774 and RAW appear to be particularly sensitive to cAMP treatment, and efflux of cholesterol to apoA-I can be stimulated up to 5-fold above that of control cells after exposure to cAMP (66, 67). Of particular interest are recent studies demonstrating that after cAMP treatment of either RAW or J774 macrophages there is increased specific binding of lipid-free apolipoproteins to the cells which parallels the enhanced efflux of cellular lipids (**Table 1**). These results, together with a variety of other reports (3, 68–70), indicate that at least in some cell systems the efflux of cell lipids to lipid-free apolipoproteins involves the interaction of the apolipoprotein with cell membrane binding sites. Not all cells respond to cAMP exposure by up-regulating lipid efflux to apolipoproteins (67), and it is not yet definitely established whether such cells are refractive to cAMP because they cannot produce this putative receptor, or because they are constitutively expressing maximum levels of the receptor.

A number of different mechanisms may be involved in the release of cellular lipids to lipid-free apolipoproteins, and these mechanisms may operate simultaneously, yet independently. Studies have demonstrated that a portion of the cholesterol and phospholipid released from cells is present in the culture medium associated with the apolipoproteins in the form of small preβ-HDL-like particles (16, 71, 72) and α-migrating HDL (57). The type of HDL particle, preβ- or α-HDL, appears to be a function of the concentration of lipid-free apolipoprotein present in the medium (58). In addition to the small HDL particles, large vesicular structures containing cholesterol and phospholipid can also be recovered in the culture medium after cells are exposed to apolipoproteins (29, 72). It is likely that the small particles and large vesicles are produced by different mechanisms. Two general mechanisms have been proposed for the release of cell membrane lipids to lipid-free apolipoproteins. The first mechanism would involve the interaction of the apolipoprotein with the cell membrane where it would acquire both phospholipid and cholesterol. This process can result in the simultaneous release of both lipids (64); this process of membrane microsolubilization is the mechanism by which incompletely lipidated apoA-I molecules or preβ-HDL induce cellular cholesterol efflux. Alternatively, there is evidence for the sequential release of phospholipid followed by the acquisition by apolipoprotein/phospholipid complexes of membrane cholesterol (29, 72). Either series of events could result in the formation of the preβ-HDL-like particles that have been isolated from culture medium (15, 62). Another mechanism for apolipoprotein-mediated cell lipid efflux could involve the vesiculation of the plasma membrane followed by the release of these vesicles into the culture media, and large amorphous structures have been observed by electron microscopy in the culture medium of apoA-I-treated cells (29, 72).

TABLE 1. Effect of cAMP on lipid-free apoA-I-mediated cholesterol efflux and apoA-I binding to J774 mouse macrophages

	Control Cells	cAMP-treated Cells[a]
Efflux		
Max efflux (%/2 h)	0.20 ± 0.01	0.56 ± 0.07[c]
EC_{50} (μg apoA-I/mL)	3.4 ± 1.9	2.3 ± 1.9
Specific binding[b]		
B_{max} (ng apoA-I/mg cell protein)	53 ± 18	88 ± 13[c]
K_d (μg apoA-I/mL)	22 ± 15	11 ± 5

[a] J774 monolayers were treated with 0.3 mm CPT-cAMP for 8 h prior to incubation with lipid-free human apoA-I.
[b] Binding of ^{125}I-labeled apoA-I was conducted at 4°C for 1 h. Efflux assay at 37°C for 2 h.
[c] Control vs. cAMP-treated, $P < 0.05$.

Observation 7: The rate of cell cholesterol release and the extent of stimulation by phospholipid supplementation of serum correlates with the expression level of SR-BI protein on cells

Many of the previously discussed observations on cell cholesterol efflux can now be explained by recent observations linking cholesterol efflux to the scavenger receptor class B, type I (SR-BI). This receptor has been shown to bind HDL with high affinity (73, 74). SR-BI can also bind many other ligands, such as native LDL, modified proteins (acetylated LDL, oxidized LDL), anionic phospholipid vesicles (75), and lipid-free apolipoproteins (76). Unlike other scavenger receptors, such as those which bind and internalize the whole particle (77), SR-BI can bind HDL reversibly and mediate selective cholesteryl ester (CE) uptake (78). When a number of cell types were screened for the expression level of SR-BI, a large difference between cells was observed (79). Among all of the cells tested, Fu5AH hepatoma cells expressed the most receptor protein, while other cell lines exhibited a range of lower expression levels (79). As can be seen from **Fig. 10**, there is a direct relationship between the efflux rate of cholesterol to HDL or SUV and the level of SR-BI in several different cell lines. With all phospholipid-containing acceptors the rate of cholesterol release is most sensitive to SR-BI levels at the low concentrations of SR-BI, although the rate is not proportional to the amount of SR-BI (50). Further confirmation of the importance of SR-BI in modulating the efflux of cell cholesterol is illustrated by **Fig. 11** which shows the efflux values to serum, HDL, and SUV from vector-transfected COS-7 cells (control) and cells transiently transfected with SR-BI. SR-BI expression markedly increases efflux to each acceptor. It is noteworthy that the efflux to apolipoprotein-free phospholipid vesicles correlates with the SR-BI level even though it has been found that phosphatidylcholine or sphingomyelin liposomes cannot compete with anionic PL for binding to the receptor (75). In studies with stably transfected CHO cells or transiently transfected COS cells, it was dem-

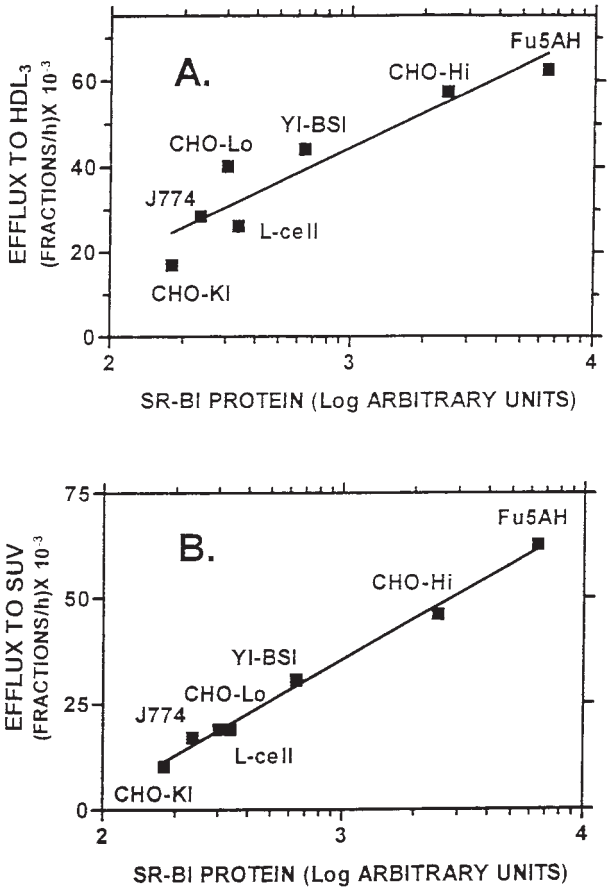

Fig. 10. Relationship between cellular cholesterol efflux rates and the expression level of SR-BI protein in different cell types. SR-BI levels were determined by Western blotting of cell membrane preparations and densitometric quantitation as described in reference 77. Panel A: Efflux of [^{3}H]cholesterol to 75 μg/mL HDL_3 protein. Panel B: Efflux of [^{3}H]cholesterol to 1 mg/mL 1-palmitoyl-2-oleoylphosphatidylcholine SUV. Data taken from references 50, 79.

onstrated that the expression of SR-BI stimulates both the efflux of cell cholesterol and influx of HDL cholesterol (**Table 2**); thus, SR-BI creates an environment in which the bi-directional flux of FC is increased (80). However, the expression of SR-BI does not increase the efflux of cell cholesterol to lipid-free apoA-I, even though the apolipoprotein is bound to the receptor (79, 80). Thus, the efflux of cholesterol to lipid-free apolipoproteins and the efflux to phospholipid-containing acceptors are two independent processes.

The observation that SR-BI expression stimulates the efflux of cholesterol to PL vesicles that do not bind to the receptor provided the first evidence that binding was not the primary event that increased the flux of FC between cells and HDL. To test the importance of binding, rate of cholesterol efflux was measured using COS cells expressing either SR-BI or the closely related scavenger receptor CD36 (81). As is illustrated in **Fig. 12**, both receptors bind HDL but marked enhancement of FC efflux is obtained only with SR-BI. Additional recent experiments have demonstrated that the expression of SR-BI in COS cells results in the reorganization of lipid domains in the cell membrane as measured by an increased sensitivity of membrane cholesterol to cholesterol oxidase (80) (**Fig. 13A**) and a change in the kinetics of cholesterol efflux to cyclodextrins (Fig. 13B) (G. Weibel, unpublished observation). Thus, SR-BI expression plays an important role in FC flux, and can promote FC flux by a mechanism where cell surface binding of the acceptor is not a major component. Rather, the effect of SR-BI is more subtle and involves significant shifts in the distribution of plasma membrane cholesterol pools.

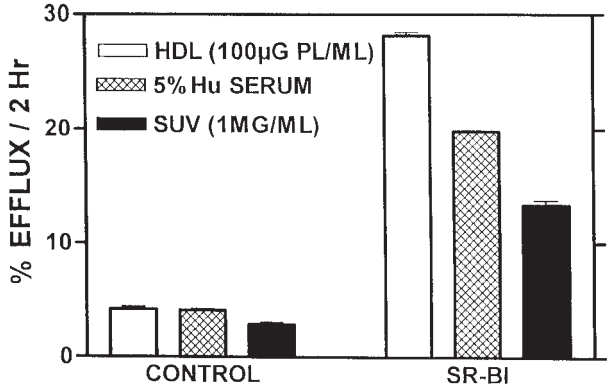

Fig. 11. Effect of SR-BI expression on cholesterol efflux to human serum, HDL, or SUV. COS-7 cells were transfected to express SR-BI or vector DNA as a control. Cells were incubated with the indicated acceptors 48 h post-transfection. Efflux of [^{3}H]cholesterol was measured after a 2-h incubation. From reference 80.

A THREE-COMPONENT MODEL FOR CELLULAR CHOLESTEROL EFFLUX

The various observations presented above can be reconciled in a hypothetical model in which it is proposed that there are three basic mechanisms involved in the efflux of cellular cholesterol (**Fig. 14**). The extent to which each of these mechanisms participates is governed by factors such as cell type, metabolic state of the cell, and nature of the extracellular acceptor. The mechanisms depicted in panels 1 and 2 operate in all cell types, whereas those illus-

TABLE 2. Effect of expression of SR-BI on the bi-directional flux of free cholesterol between cells and HDL

	Efflux	Influx	Net Flux to HDL
	μg FC/h/mg protein	*μg FC/h/mg protein*	*μg FC/h/mg protein*
CHO cells			
Control	0.8	0.5	0.3
SR-BI	1.3	0.9	0.4
COS-7 cells			
Control	0.8	2.3	−1.5
SR-BI	2.1	3.4	−1.3

Influx and efflux values were calculated from non-linear regression fits of 24 h time courses. CHO cells were incubated with 75 μg/mL HDL_3. COS-7 cells were incubated with 250 μg/mL HDL_3.

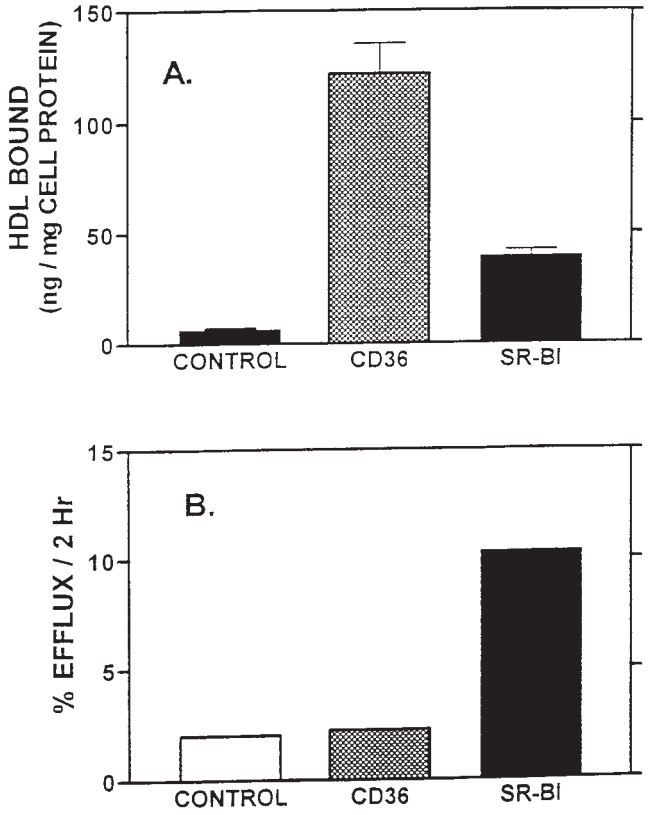

Fig. 12. Relationships between the efflux of cell cholesterol and the specific binding of HDL to SR-BI and CD36. Transfected COS-7 cells expressing SR-BI, CD36, or vector DNA were labeled with [^{3}H]cholesterol. Panel A: Efflux of cellular cholesterol to HDL (25 μg protein / mL) was measured after a 2-h incubation. Panel B: Total HDL binding and non-specific binding (plus a 20-fold excess unlabeled ligand) were measured at 4°C in parallel dishes. Specific binding was determined as difference between total and non-specific binding. Data taken from reference 80.

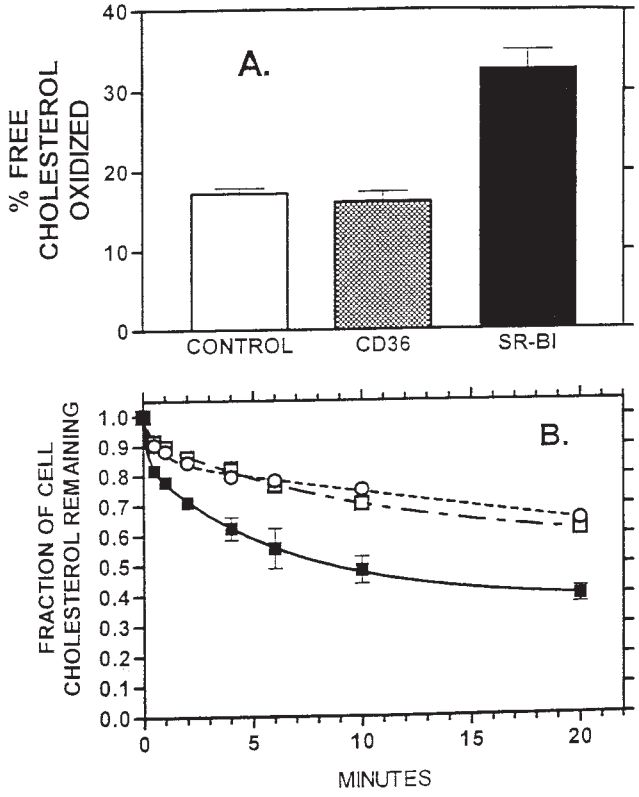

Fig. 13. Effect of expression of SR-BI on the organization of cholesterol in the plasma membrane. Transfected COS-7 cells expressing SR-BI, CD36, or vector DNA were prepared and labeled with [^{3}H]cholesterol. Panel A: Cholesterol oxidation assay. Labeled cell monolayers were fixed with 1% glutaraldehyde for 10 min and incubated with cholesterol oxidase for 30 min at 37°C as described by Slotte and Bierman (93). After separation by thin-layer chromatography, radioactive cholesterol and cholestanone were quantitated. Data taken from reference 78. Panel B: Efflux of cell cholesterol to 50 mm 2-hydroxypropyl-β-cyclodextrin. Labeled cells were placed in suspension and efflux to the cyclodextrin was measured at the indicated times; ○, control cells; □, CD36 transfected cells; ■, SR-BI transfected cells. Details for the measurement of efflux and the calculation of efflux values are as described in reference 13. (G. Weibel and G. H. Rothblat, unpublished data).

trated in panels 3 and 4 are dependent on receptors only expressed in some cells.

Mechanism 1: Desorption of cholesterol molecules out of the plasma membrane and diffusion through the aqueous phase to phospholipid-containing lipoproteins (Fig. 14, panel 1)

The simplest and most basic mechanism responsible for cellular cholesterol efflux is the process in which individual cholesterol molecules desorb from the plasma membrane, diffuse through the aqueous phase, and are subsequently incorporated into PL-containing acceptor particles; this process involving diffusion of cholesterol down a concentration gradient has been extensively reviewed (6, 7, 36). The rate of cholesterol release by aqueous diffusion is influenced by both cell and acceptor properties. The desorption step has a high activation energy (7, 13). The rate of cholesterol desorption can be modulated by the distribution of lipids between membrane domains. Cholesterol efflux kinetics show fast and slow pools, most convincingly demonstrated using fluorescent sterol analogs (82) or cyclodextrin acceptors (13) that reflect the distribution of cholesterol in such membrane domains. It has been established that cholesterol within the slow pool can replenish the fast pool with half times ranging between 20–35 min (13). As this rate is faster than the $t_{1/2}$ for cholesterol efflux to any phospholipid-containing acceptor, it can be concluded that the movement of cholesterol from slow to fast pool is not a rate-limiting step. It has, however, been established that the relative distribution of cholesterol among fast and slow pools varies among different cell types, and that there is a general correlation between the relative size of the fast pool and the rate of cholesterol efflux from different cell types (13, 51). The organization of cholesterol in plasma membrane domains can be expected to change depending on the amount of cholesterol in the membrane (83, 84), and membrane cholesterol content can therefore influence the rate of release. (Fig. 14, panel 1).

Rates of cholesterol release mediated by a pure aqueous diffusion process are significantly influenced by acceptor

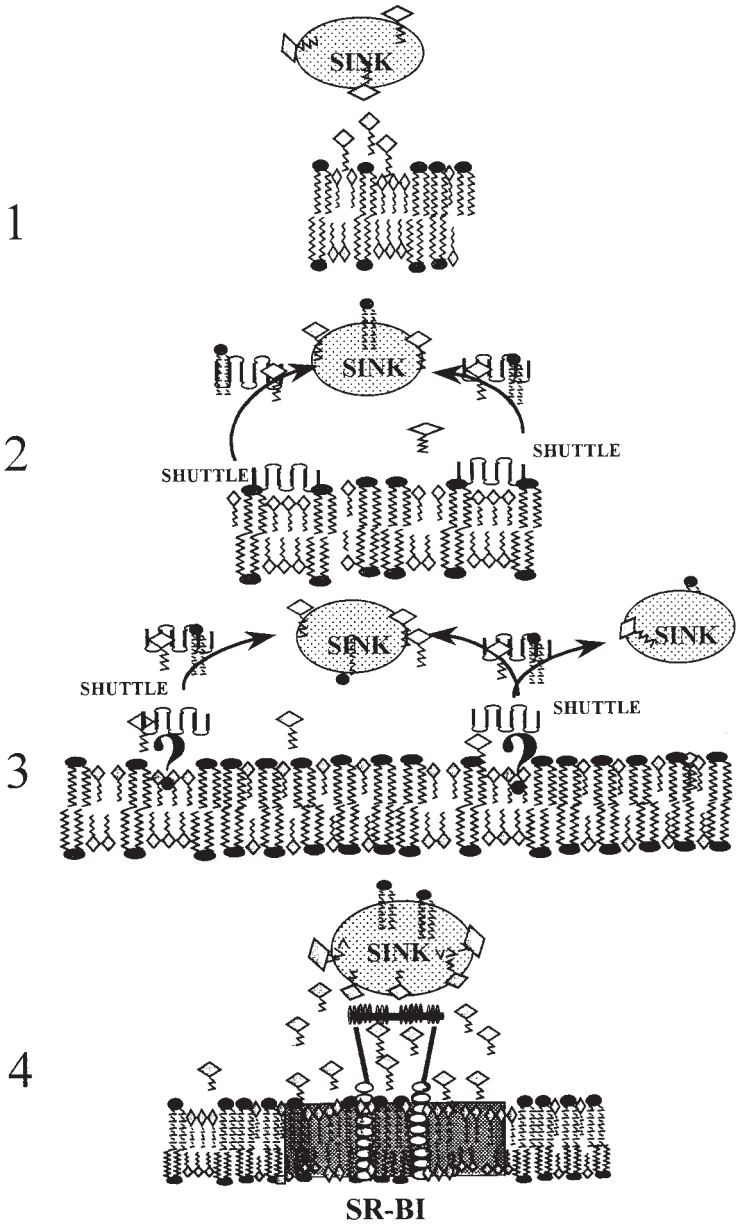

Fig. 14. Mechanisms participating in the efflux of cellular plasma membrane cholesterol. Mechanisms 1 and 2 operate in all cells types, whereas mechanisms 3 and 4 promote cell cholesterol efflux under some conditions in some cells. Panel 1: Unmediated aqueous diffusion. Cholesterol molecules desorb from the plasma membrane into the aqueous phase and are subsequently incorporated into PL-containing acceptors. Diffusion and mixing of cholesterol molecules in the aqueous phase is limited by the unstirred water layer, and access to the cell surface is reduced as acceptor size is increased. The rate of desorption is influenced by the cholesterol/PL domain structure in the membrane and enrichment of the plasma membrane with cholesterol changes these domains and increases efflux rates. Efflux by simple diffusion is a relatively inefficient process. Panel 2: Lipid-free and lipid-poor apolipoprotein interactions with plasma membrane lipid domains. Lipid-free or unassociated apolipoproteins, indicated here as shuttles, interact through their amphipathic domains with lipid domains within the plasma membrane and acquire both PL and cholesterol in a membrane microsolubilization process. These newly lipidated particles participate in the transfer of membrane cholesterol to larger particles that serve as cholesterol sinks. This transfer of cholesterol can occur through the modification of the initial acceptor, or by transfer of FC or CE, in part mediated by LCAT and CETP. Apolipoprotein/membrane interactions occur with all cells, and removal of cholesterol and phospholipid is increased upon enrichment of the membrane with cholesterol. Panel 3: Membrane microsolubilization mediated by apolipoprotein interactions with plasma membrane receptors. As in panel 2, lipid-free apolipoproteins interact with cell plasma membrane to acquire both PL and FC; however, in this case there is a protein/protein interaction between the apolipoprotein and specific receptors (?, putative receptor) in the membrane. In addition to binding the apolipoprotein, the expression of the receptor may organize lipid domain structure within the membrane to enhance PL and FC transfer to the apolipoprotein. The presence of the receptor increases the rate of transfer of membrane lipids to the apolipoprotein shuttle. Panel 4: SR-BI-mediated efflux. SR-BI expressed on the plasma membrane both binds acceptors and changes the lipid domain organization within the membrane. The binding of the lipoprotein results in an increase in the efflux rate of membrane cholesterol; however, the additional effects of SR-BI are likely to result from the membrane reorganization that increases the rate of exchange of FC between cells and PL-containing acceptors. This latter effect will be particularly significant at acceptor concentrations where SR-BI is saturated. Because this is an efficient process in which FC molecules move directly between larger acceptors and plasma membrane, there is a reduced need for efflux mediated by lipid-free or lipid-poor apolipoproteins serving as shuttles.

properties (6, 7, 39). Acceptor size is an important factor as diffusional considerations and the presence of extracellular matrix limit the access to the cell surface. Thus, small phospholipid-containing particles can more readily approach the cell surface, reducing the thickness of the aqueous layer that desorbed cholesterol molecules have to traverse. However, it has been estimated that even small phospholipid-containing particles cannot directly contact the plasma membrane because of phospholipid to phospholipid repulsion factors (85), and therefore an aqueous layer would always restrict cholesterol movement mediated by this process. Thus, desorption and aqueous diffusion can be viewed as a relatively inefficient mechanism that operates in all cell types. Its relative contribution to cellular cholesterol efflux varies among cell types, depending on the availability of additional mechanisms that can enhance cholesterol efflux efficiency.

Mechanism 2: Efflux of membrane cholesterol and phospholipid to lipid-free or lipid-poor apolipoproteins (Fig. 14, panels 2 and 3)

The second mechanism for cellular cholesterol efflux involves lipid-depleted apolipoproteins as cholesterol acceptors. The extent to which the membrane microsolubilization process (64) contributes to overall efflux depends on cell type. In this mechanism the apolipoproteins directly interact with the plasma membrane, during which time they remove membrane lipid and thus serve as transporters to move cholesterol away from the cell surface. In this manner unassociated apolipoproteins form small HDL particles and can function as cholesterol transporters, or shuttles, by acting as the initial acceptors of plasma membrane cholesterol. These initial acceptors subsequently transport this cholesterol to larger lipoproteins that serve as cholesterol sinks (23, 56, 59, 86). However, unlike cyclodextrin shuttles that act catalytically as they acquire membrane cholesterol, unassociated apolipoproteins probably undergo metabolic transformations mediated by CETP, PLTP, and LCAT as the cholesterol moves from initial acceptors to sinks (15, 59, 64).

The release of cholesterol to lipid-free apolipoproteins is linked to the release of cell membrane phospholipid (Fig. 14, panel 2). The magnitude of the response varies among cells (63, 71), and is also a function of the metabolic status of the cells. The two metabolic manipulations of cells that have been shown to enhance apolipoprotein-mediated lipid efflux are cholesterol enrichment (Fig. 14, panel 2) and cAMP treatment (Fig. 14, panel 3) (66). These two treatments appear to operate by different mechanisms as the stimulation of efflux appears to be additive when FC-enriched cells are exposed to cAMP (67). It has been proposed that the cholesterol enrichment modifies the membrane lipid domain structure, resulting in a greater interaction between the lipid-free apolipoproteins and membrane lipid domains. The nature of these membrane domains remains to be resolved, but it is probable that they are associated with the caveolae and detergent-resistant membranes (DRMs) that have been implicated as structures that play an important role in cell cholesterol flux (84, 87–89). The mechanism underlying the action of cAMP involves the induction of apolipoprotein binding sites on the cell surface (66, 67) (Fig. 14, panel 3).The observation that apoA-I specific binding is increased in parallel to the increased efflux upon treatment of cells with cAMP (Table 1) indicates that the release of cholesterol to lipid-free apolipoproteins is linked to the binding of the apolipoprotein to receptors on the cell membrane. However, the fact that the EC_{50} for efflux to apoA-I is considerably lower than the K_d for apoA-I binding suggests that only a subset of the binding sites is involved in efflux, perhaps because they are in a particular membrane domain.

Mechanism 3: Exchange of free cholesterol mediated by SR-BI (Fig. 14, panel 4)

The third mechanism that participates in the movement of cholesterol between cell plasma membranes and lipoproteins occurs in those cells expressing the SR-BI receptor (50, 79). As discussed earlier, the efficiency of efflux of cellular cholesterol to PL-containing acceptors is enhanced by the expression level of SR-BI protein on the surface of donor cells. In addition, the ability of PL supplementation of serum or HDL to enhance the rate of cholesterol efflux is also improved by SR-BI expression. This increase in cholesterol flux is not entirely accomplished by the tethering of the acceptor particle to the membrane, but rather it is largely due to changes in the organization of the lipids in the plasma membrane (80). Because at least some of the SR-BI tends to locate in caveolae (78, 90) it is likely that the SR-BI-induced changes in plasma membrane organization involve caveolae and/or lipid rafts (84, 91).

The presence of SR-BI on the plasma membrane creates an environment whereby the rate of exchange of FC molecules is increased (i.e., the exchange is facilitated). In such a situation the net movement of FC between cells and lipoproteins is not influenced by SR-BI. Instead, the net movement of FC is a reflection of the cholesterol gradient that exists between the lipoproteins (acceptors) and the cells. Just as in the case of simple aqueous diffusion (Fig. 14, panel 1) (7, 41), these gradients result from the complex interactions between lipoprotein composition, as influenced by such factors as LCAT, CETP, and PLTP, differences in plasma membrane and acceptor PL/cholesterol ratios, and the activity of ACAT within the cells. When there is increased flux between SR-BI positive cells and PL-rich HDL, the need for lipid-free/poor cholesterol shuttles is diminished. Thus, the stimulation of cholesterol flux by lipid-free apolipoproteins or small, HDL is probably of greatest importance in those cells that do not express SR-BI. In addition to increasing the rate of exchange of FC between HDL and cell membranes, the presence of SR-BI also promotes the uptake of HDL-CE (74, 76). It is interesting that in Fu5AH cells, which express very high levels of the receptor, the influx of HDL-FC greatly exceeds the uptake of HDL-CE (92).

FUTURE DIRECTIONS

Understanding the biochemical and morphological nature of kinetically distinct cellular cholesterol pools

Studies on the kinetics of cholesterol flux have shown the occurrence of multiple membrane cholesterol pools. Although these are typically referred to as slow and fast pools and may represent lipid rafts or domains in the membrane, at present there is no information about their biochemical nature. There is increasing evidence that structures such as caveolae play an important role in cholesterol flux, but direct evidence linking specific membrane domains or structures to the kinetic pools remains to be obtained. Thus, an important challenge for future studies is to identify these pools biochemically and to learn how alterations in membrane domains influence cholesterol efflux rates. An initial step will require efflux studies with artificial membranes in which domain organization can be systematically altered to elucidate how alterations in membrane domains may influence cholesterol flux in living cells. It will be more difficult, but important, to test and extend hypotheses generated with artificial systems to cholesterol flux in intact cells.

Understanding the mechanism of cholesterol efflux to lipid-poor acceptors

Another important issue is the efflux of cell cholesterol to lipid-free/lipid-poor acceptors. Efflux to such acceptors occurs with a number of cell types, but is prominent in macrophages. These cells play a major role in cholesterol accumulation, and understanding this mechanism will provide new ways to enhance cholesterol removal from arterial lesions. At present we have circumstantial evidence that a membrane receptor(s) is necessary for this process in macrophages and other cells. However, this receptor(s) has not been identified. An important focus of research is to identify and clone the macrophage receptor(s) for lipid-free apolipoproteins and to determine its tissue distribution. This accomplishment would open the way for molecular studies on this process, both in cell culture and in vivo.

Understanding the structure and function of SR-BI and its role in lipid flux

SR-BI is the first membrane protein that has been shown to influence cholesterol flux across the membrane and to alter the organization of cholesterol within the membrane. It is expressed most highly in steroidogenic cells where it mediates the uptake of cholesteryl ester as well as the flux of free cholesterol. SR-BI is also substantially expressed in the liver and in macrophages where it may impact on reverse cholesterol transport and atherosclerosis. Studies seeking to understand how SR-BI works at the molecular level can be expected to provide key information, not just about this receptor, but also about fundamental aspects of lipid flux and membrane domain organization in intact cells. It will be important to learn how SR-BI works at the molecular level, how it is organized in membrane domains, and how its expression is regulated. Such studies will provide fundamental information as well as new targets for the development of therapies to influence reverse cholesterol transport and atherosclerosis.

Understanding how cellular and lipoprotein phospholipids modulate net cholesterol flux

A new area is the study of the physiological role of phospholipid in the cholesterol efflux process. Although both the phospholipid composition and organization of the plasma membrane modulate cellular cholesterol flux, clearly the concentration and nature of the phospholipid associated with extracellular acceptors play a fundemental role in mediating their interaction with cells. Phospholipids establish the cholesterol concentration gradients that regulate the rate, magnitude, and direction of net cholesterol movement. Studies in which the amount and type of phospholipid in reconstituted and native lipoproteins is manipulated will further elucidate the role of these lipids in the acceptors. Moreover, elucidation of the origin and regulation of lipoprotein phospholipids, and specifically HDL phospholipids, will be important. Manipulating cell membrane phospholipid composition will be much more difficult; however, the study of cells expressing mutations in phospholipid metabolism may provide useful models.

Understanding the physiological components leading to net cholesterol flux

Finally, a fundamental question that remains is the relative contribution of the various efflux mechanisms to cholesterol flux. Clearly this will depend on cell type, metabolic status, and tissue location. Assessment of the contributions of each mechanism may be achieved by exposing various cell types to medium containing different mixtures of acceptors. In addition, sera from transgenic or KO animals can be used to probe the contribution of serum components, such as a specific apolipoprotein. However, a clear understanding of the mechanism of efflux in vivo will not be possible until new technologies are developed that lead to a more precise understanding of the lipoprotein and apolipoprotein composition of interstitial fluids.

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REFERENCES

1. Bailey, J. M. 1965. Lipid metabolism in cultured cells IV: serum alpha globulins and cellular cholesterol exchange. *Exp. Cell Res.* **37:** 175–182.
2. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
3. Oram, J. F., and S. Yokoyama. 1996. Apolipoprotein-mediated removal of cellular cholesterol and phospholipids. *J. Lipid Res.* **37:** 2473–2491.
4. Liscum, L., and N. K. Dahl. 1992. Intracellular cholesterol transport. *J. Lipid Res.* **33:** 1239–1254.
5. Johnson, W. J., F. H. Mahlberg, G. K. Chacko, M. C. Phillips, and G. H. Rothblat. 1988. The influence of cellular and lipoprotein cholesterol contents on the flux of cholesterol between fibroblasts and high density lipoprotein. *J. Biol. Chem.* **263:** 14099–14106.
6. Johnson, W. J., F. H. Mahlberg, G. H. Rothblat, and M. C. Phillips. 1991. Cholesterol transport between cells and high density lipoproteins. *Biochim. Biophys. Acta.* **1085:** 273–298.
7. Phillips, M. C., W. J. Johnson, and G. H. Rothblat. 1987. Mechanisms and consequences of cellular cholesterol exchange and transfer. *Biochim. Biophys. Acta.* **906:** 223–276.
8. Pitha, J., T. Irie, P. B. Sklar, and J. S. Nye. 1988. Drug solubilizers to aid pharmacologists: amorphous cyclodextrin derivatives. *Life Sci.* **43:** 493–502.
9. Duchene, D. 1990. Minutes of the 5th International Symposium on Cyclodextrins, Paris, France: Editions de Sante, 1990.
10. Christian, A. E., M. P. Haynes, M. C. Phillips, and G. H. Rothblat. 1997. Use of cyclodextrins for manipulating cellular cholesterol content. *J. Lipid Res.* **38:** 2264–2272.
11. Kilsdonk, E. P. C., P. Yancey, G. Stoudt, F. W. Bangerter, W. J. Johnson, M. C. Phillips, and G. H. Rothblat. 1995. Cellular cholesterol efflux mediated by cyclodextrins. *J. Biol. Chem.* **270:** 17250–17256.
12. Kritharides, L., M. Kus, A. J. Brown, W. Jessup, and R. T. Dean. 1996. Hydroxypropyl-β-cyclodextrin-mediated efflux of 7-ketocholesterol from macrophage foam cells. *J. Biol. Chem.* **271:** 27450–27455.
13. Yancey, P. G., W. V. Rodrigueza, E. P. C. Kilsdonk, G. W. Stoudt, W. J. Johnson, M. C. Phillips, and G. H. Rothblat. 1996. Cellular cholesterol efflux mediated by cyclodextrins: demonstration of kinetic pools and mechanism of efflux. *J. Biol. Chem.* **271:** 16026–16034.
14. Atger, V. M., M. de la Llera-Moya, G. W. Stoudt, W. V. Rodrigueza, M. C. Phillips, and G. H. Rothblat. 1997. Cyclodextrins as catalysts for the removal of cholesterol from macrophage foam cells. *J. Clin. Invest.* **99:** 773–780.
15. Forte, T. M., J. K. Bielicki, R. Goth-Goldstein, J. Selmek, and M. R. McCall. 1995. Recruitment of cell phospholipids and cholesterol by apolipoproteins A-II and A-I: Formation of nascent apolipoprotein-specific HDL that differ in size, phospholipid composition, and reactivity with LCAT. *J. Lipid Res.* **36:** 148–157.
16. Li, Q., and S. Yokoyama. 1995. Independent regulation of cholesterol incorporation into free apolipoprotein-mediated cellular lipid efflux in rat vascular smooth muscle cells. *J. Biol. Chem.* **270:** 26216–26223.
17. Liang, H-Q., K-A. Rye, and P. J. Barter. 1995. Cycling of apolipoprotein A-I between lipid-associated and lipid- free pools. *Biochim. Biophys. Acta.* **1257:** 31–37.
18. Asztalos, B. F., C. H. Sloop, L. Wong, and P. S. Roheim. 1993. Comparison of apoA-I-containing subpopulations of dog plasma and prenodal peripheral lymph: evidence for alteration in subpopulations in the interstitial space. *Biochim. Biophys. Acta.* **1169:** 301–304.
19. Lefevre, M., C. H. Sloop, and P. S. Roheim. 1988. Characterization of dog prenodal peripheral lymph lipoproteins. Evidence for the peripheral formation of lipoprotein-unassociated apoA-I with slow pre-β electrophoretic mobility. *J. Lipid Res.* **29:** 1139–1148.
20. Asztalos, B. F., and P. S. Roheim. 1995. Presence and formation of 'free apolipoprotein A-I-like' particles in human plasma. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1419–1423.
21. Roheim, P. S., L. Dory, M. Lefevre, and C. H. Sloop. 1990. Lipoproteins in interstitial fluid of dogs: implications for a role in reverse cholesterol transport. *Eur. Heart J.* **11:** 225–229.
22. Sloop, C. H., L. Dory, and P. S. Roheim. 1987. Interstitial fluid lipoproteins. *J. Lipid Res.* **28:** 225–237.
23. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
24. Davidson, W. S., D. L. Sparks, S. Lund-Katz, and M. C. Phillips. 1994. The molecular basis for the difference in charge between pre-β- and α-migrating high density lipoproteins. *J. Biol. Chem.* **269:** 8959–8965.
25. Zhao, Y., D. L. Sparks, and Y. L. Marcel. 1996. Specific phospholipid association with apolipoprotein A-I stimulates cholesterol efflux from human fibroblasts. *J. Biol. Chem.* **271:** 25145–25151.
26. Bielicki, J. K., W. J. Johnson, J. M. Glick, and G. H. Rothblat. 1991. Efflux of phospholipids from fibroblasts with normal and elevated levels of cholesterol. *Biochim. Biophys. Acta.* **1084:** 7–14.
27. Hara, H., A. Komaba, and S. Yokoyama. 1992. α-Helical requirements for free apolipoproteins to generate HDL and to induce cellular lipid efflux. *Lipids.* **27:** 302–304.
28. Mendez, A. J., G. M. Anantharamaiah, J. P. Segrest, and J. F. Oram. 1994. Synthetic amphipathic helical peptides that mimic apolipoprotein A-I in clearing cellular cholesterol. *J. Clin. Invest.* **94:** 1698–1705.
29. Yancey, P. G., J. K. Bielicki, W. J. Johnson, S. Lund-Katz, M. N. Palgunachari, G. M. Anantharamaiah, J. P. Segrest, M. C. Phillips, and G. H. Rothblat. 1995. The efflux of cellular cholesterol and phospholipid to lipid- free apolipoproteins and class A amphipathic peptides. *Biochemistry.* **34:** 7955–7965.
30. Barkia, A., P. Puchois, N. Ghalim, G. Torpier, R. Barbaras, G. Ailhaud, and J-C. Fruchart. 1991. Differential role of apolipoprotein AI-containing particles in cholesterol efflux from adipose cells. *Atherosclerosis.* **87:** 135–146.
31. Hodenberg, E. v., S. Heinen, K. E. Howell, C. Luley, W. Kubler, and H. M. Bond. 1991. Cholesterol efflux from macrophages mediated by high-density lipoprotein subfractions, which differ principally in apolipoprotein A-I and apolipoprotein A-II ratios. *Biochem. Biophys. Res. Commun.* **1086:** 173–184.
32. Ohta, T., R. Nakamura, Y. Ikeda, M. Shinohara, A. Miyazaki, S. Horiuchi, and I. Matsuda. 1992. Differential effect of subspecies of lipoprotein containing apolipoprotein A-I on cholesterol efflux from cholesterol-loaded macrophages: functional correlation with lecithin: cholesterol acyltransferase. *Biochim. Biophys. Acta.* **1165:** 119–128.
33. Okawa, S., A. J. Mendez, J. F. Oram, E. L. Bierman, and M. C. Cheung. 1993. Effects of high-density lipoprotein particles containing apo A-I, with or without A-II, on intracellular cholesterol efflux. *Biochim. Biophys. Acta.* **1165:** 327–334.
34. Lagrost, L., C. Dengremont, A. Athias, C. De Geitere, J-C. Fruchart, C. Lallemant, P. Gambert, and G. Castro. 1995. Modulation of cholesterol efflux from Fu5AH hepatoma cells by the apolipoprotein content of high density lipoprotein particles. Particles containing various proportions of apolipoproteins A-I and A-II. *J. Biol. Chem.* **270:** 13004–13009.
35. Johnson, W. J., E. P. C. Kilsdonk, A. Van Tol, M. C. Phillips, and G. H. Rothblat. 1991. Cholesterol efflux from cells to immunopurified subfractions of human high density lipoproteins: LP-AI and LP-AI / AII. *J. Lipid Res.* **32:** 1993–2000.
36. Johnson, W. J., M. C. Phillips, and G. H. Rothblat. 1997. Lipoproteins and cellular cholesterol homeostasis. *In* Cholesterol: Its Metabolism and Functions in Biology and Medicine. R. Bittman, editor. Plenum Press, New York. 235–276.
37. Davidson, W. S., K. L. Gillotte, S. Lund-Katz, W. J. Johnson, G. H. Rothblat, and M. C. Phillips. 1995. The effect of high density lipoprotein phospholipid acyl chain composition on the efflux of cellular free cholesterol. *J. Biol. Chem.* **270:** 5882–5890.
38. Davidson, W. S., S. Lund-Katz, W. J. Johnson, G. M. Anantharamaiah, N. Palgunachari, J. P. Sergrest, G. H. Rothblat, and M. C. Phillips. 1994. The influence of apolipoprotein structure on the efflux of cellular free cholesterol to high density lipoprotein. *J. Biol. Chem.* **269:** 22975–22982.
39. Davidson, W. S., W. V. Rodrigueza, S. Lund-Katz, W. J. Johnson, G. H. Rothblat, and M. C. Phillips. 1995. Effects of acceptor particle size on the efflux of cellular free cholesterol. *J. Biol. Chem.* **270:** 17106–17113.
40. Gillotte, K. L., W. S. Davidson, S. Lund-Katz, G. H. Rothblat, and M. C. Phillips. 1996. Apolipoprotein A-I structural modification and functionality of reconstituted high density lipoprotein particles in cellular cholesterol efflux. *J. Biol. Chem.* **271:** 23792–23798.
41. Johnson, W. J., M. J. Bamberger, R. A. Latta, P. E. Rapp, M. C. Phillips, and G. H. Rothblat. 1986. The bidirectional flux of cholesterol between cells and lipoproteins. *J. Biol. Chem.* **261:** 5766–5776.
42. Rothblat, G. H., M. Bamberger, and M. C. Phillips. 1986. Reverse cholesterol transport. *Methods Enzymol.* **129:** 628–644.
43. Jian, B., M. de la Llera-Moya, L. Royer, G. H. Rothblat, O. Fran-

cone, and J. B. Swaney. 1997. Modification of the cholesterol efflux properties of human serum by enrichment with phospholipid. *J. Lipid Res.* **38:** 152–162.
44. de la Llera-Moya, M., V. Atger, J. L. Paul, N. Fournier, N. Moatti, P. Giral, K. E. Friday, and G. H. Rothblat. 1994. A cell culture system for screening human serum for ability to promote cellular cholesterol efflux: relationships between serum components and efflux, esterification and transfer. *Arterioscler. Thromb.* **14:** 1056–1065.
45. Friedman, M., and S. O. Byers. 1956. Role of hyperphospholipidemia and neutral fat increase in plasma in the pathogenesis of hypercholesterolemia. *Am. J. Physiol.* **186:** 13–18.
46. Williams, K. J., V. P. Werth, and J. A. Wolff. 1984. Intravenously administered lecithin liposomes: a synthetic antiatherogenic lipid particle. *Perspect. Biol. Med.* **27:** 417–431.
47. Fournier, N., M. de la Llera-Moya, B. Burkey, J. Swaney, J. Jr., Paterniti, N. Moatti, V. Atger, and G. H. Rothblat. 1996. The role of HDL phospholipids in efflux of cell cholesterol to whole serum: studies with human apoA-I transgenic rats. *J. Lipid Res.* **37:** 1704–1711.
48. Fournier, N., J. L. Paul, V. Atger, M. de la Llera Moya, G. Rothblat, and N. Moatti. 1997. HDL phospholipid content and composition as a major determinant of cholesterol efflux to whole serum. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2685–2691.
49. Chiesa, G., C. Parolini, M. Canavesi, N. Colombo, C. R. Sirtori, R. Fumagalli, G. Franceschini, and F. Bernini. 1998. Human apolipoproteins A-I and A-II in cell cholesterol efflux: studies with transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1417–1423.
50. Jian, B., M. de la Llera-Moya, Y. Ji, N. Wang, M. C. Phillips, J. B. Swaney, A. R. Tall, and G. H. Rothblat. 1998. Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors. *J. Biol. Chem.* **273:** 5599–5606.
51. Mahlberg, F. H., and G. H. Rothblat. 1992. Cellular cholesterol efflux: role of cell membrane kinetic pools and interaction with apolipoproteins AI, AII, and Cs. *J. Biol. Chem.* **267:** 4541–4550.
52. Nemecz, G., and F. Schroeder. 1988. Time-resolved fluorescence investigation of membrane cholesterol heterogeneity and exchange. *Biochemistry.* **27:** 7740–7749.
53. Rothblat, G. H., F. H. Mahlberg, W. J. Johnson, and M. C. Phillips. 1992. Apolipoprotein, membrane cholesterol domains, and the regulation of cholesterol efflux. *J. Lipid Res.* **33:** 1091–1098.
54. Fielding, C. J. 1991. Reverse cholesterol transport. *Curr. Opin. Lipidol.* **2:** 376–378.
55. Rodrigueza, W. V., K. J. Williams, G. H. Rothblat, and M. C. Phillips. 1997. Remodeling and shuttling: mechanisms for the synergistic effects between different acceptor particles in the mobilization of cellular cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **17:** 383–393.
56. Francone, O. L., and C. J. Fielding. 1990. Initial steps in reverse cholesterol transport: the role of short-lived cholesterol acceptors. *Eur. Heart J.* **11:** 218–224.
57. Asztalos, B., W. Zhang, P. S. Roheim, and L. Wong. 1997. Role of free apolipoprotein A-I in cholesterol efflux: formation of pre-α-migrating high density lipoprotein particles. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1630–1636.
58. Zhang, W., B. Asztalos, P. S. Roheim, and L. Wong. 1998. Characterization of phospholipids in pre-α HDL: selective phospholipid efflux with apolipoprotein A-I. *J. Lipid Res.* **39:** 1601–1607.
59. Chung, B. H., F. Franklin, B. H. S. Cho, J. P. Segrest, K. Hart, and B. E. Darnell. 1998. Potencies of lipoproteins in fasting and postprandial plasma to accept additional cholesterol molecules released from cell membranes. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1217–1230.
60. Liang, H-Q., K-A. Rye, and P. J. Barter. 1994. Dissociation of lipid-free apolipoprotein A-I from high density lipoproteins. *J. Lipid Res.* **35:** 1187–1199.
61. Savion, N., A. Gamliel, J-P. Tauber, and D. Gospodarowicz. 1987. Free apolipoproteins A-I and A-IV present in human plasma displace high-density lipoprotein on cultured bovine aortic endothelial cells. *Eur. J. Biochem.* **164:** 435–443.
62. Hara, H., and S. Yokoyama. 1991. Interaction of free apolipoproteins with macrophages. *J. Biol. Chem.* **266:** 3080–3086.
63. Komaba, A., Q. Li, H. Hara, and S. Yokoyama. 1992. Resistance of smooth muscle cells to assembly of high density lipoproteins with extracellular free apolipoproteins and to reduction of intracellularly accumulated cholesterol. *J. Biol. Chem.* **267:** 17560–17566.
64. Gillotte, K. L., W. S. Davidson, S. Lund-Katz, G. H. Rothblat, and M. C. Phillips. 1998. Removal of cellular cholesterol by pre-β -HDL involves plasma membrane microsolubilization. *J. Lipid Res.* **39:** 1918–1928.
65. Bielicki, J. K., W. J. Johnson, R. B. Weinberg, J. M. Glick, and G. H. Rothblat. 1992. Efflux of lipid from fibroblasts to apolipoproteins: dependence on elevated levels of cellular unesterified cholesterol. *J. Lipid Res.* **33:** 1699–1710.
66. Smith, J. D., M. Miyata, M. Ginsberg, C. Grigaux, E. Shmookler, and A. S. Plump. 1996. Cyclic AMP induces apolipoprotein E binding activity and promotes cholesterol efflux from a macrophage cell line to apolipoprotein acceptors. *J. Biol. Chem.* **271:** 30647–30655.
67. Sakr, S. W., D. L. Williams, G. W. Stoudt, M. C. Phillips, and G. H. Rothblat. 1999. Cholesterol efflux from macrophages. *Biochim. Biophys. Acta.* In press.
68. Barbaras, R., P. Puchois, A. Pradines-Figuereso, A. Steinmetz, V. Clavey, N. Ghalim, J-C. Fruchart, and G. Ailhaud. 1990. Binding of apolipoproteins A to adipose cells: role of receptor sites in cholesterol efflux and purification of binding protein(s). *In* Hypercholesterolemia, hypocholesterolemia, and hypertriglyceridemia. C. L. Malmendier, editor. Plenum Press, New York. 85–92.
69. Li, Q., H. Czamecka, and S. Yokoyama. 1995. Involvement of a cellular surface factor(s) in lipid-free apolipoprotein-mediated cellular cholesterol efflux. *Biochim. Biophys. Acta.* **1259:** 227–234.
70. Mendez, A. J., and J. F. Oram. 1997. Limited proteolysis of high density lipoprotein abolishes its interaction with cell-surface binding sites that promote efflux. *Biochim. Biophys. Acta.* **1346:** 285–299.
71. Li, Q., A. Komaba, and S. Yokoyama. 1993. Cholesterol is poorly available for free apolipoprotein-mediated cellular lipid efflux from smooth muscle cells. *Biochemistry.* **32:** 4597–4603.
72. Forte, T. M., R. Goth-Goldstein, R. W. Nordhausen, and M. R. McCall. 1993. Apolipoprotein A-I-cell membrane interaction: extracellular assembly of heterogeneous nascent HDL particles. *J. Lipid Res.* **34:** 317–324.
73. Acton, S., A. Rigotti, K. T. Landschulz, S. Xu, H. H. Hobbs, and M. Krieger. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* **271:** 518–520.
74. Rigotti, A., B. Trigatti, J. Babitt, M. Penman, S. Xu, and M. Krieger. 1997. Scavenger receptor BI—a cell surface receptor for high density lipoprotein. *Curr. Opin. Lipidol.* **8:** 181–188.
75. Rigotti, A., S. Acton, and M. Krieger. 1995. The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. *J. Biol. Chem.* **270:** 16221–16224.
76. Xu, S., M. Laccotripe, X. Huang, A. Rigotti, V. I. Zannis, and M. Krieger. 1997. Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-B1, an HDL receptor that mediates selective lipid uptake. *J. Lipid Res.* **38:** 1289–1298.
77. Krieger, M., and J. Herz. 1994. Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Annu. Rev. Biochem.* **63:** 601–637.
78. Babitt, J., B. Trigatti, A. Rigotti, E. J. Smart, R. G. W. Anderson, S. Xu, and M. Krieger. 1997. Murine SR-B1, a high density lipoprotein receptor that mediates selective lipid uptake, is *N*-glycosylated and fatty acylated and colocalizes with plasma membrane caveolae. *J. Biol. Chem.* **272:** 13242–13249.
79. Ji, Y., B. Jian, N. Wang, Y. Sun, M. de la Llera-Moya, M. C. Phillips, G. H. Rothblat, J. B. Swaney, and A. R. Tall. 1997. Scavenger receptor B1 promotes high density lipoprotein-mediated cellular cholesterol efflux. *J. Biol. Chem.* **272:** 20982–20985.
80. de la Llera-Moya, M., G. H. Rothblat, M. A. Connelly, G. Kellner-Weibel, S. W. Sakr, M. C. Phillips, and D. L. Williams. 1999. Scavenger receptor BI (SR-BI) mediates free cholesterol flux independently of HDL tethering to the cell surface. *J. Lipid Res.* **40:** 575–580.
81. Calvo, D., D. Gomez-Coronado, Y. Suarez, M. A. Lasuncion, and M. A. Vega. 1998. Human CD36 is a high affinity receptor for the native lipoproteins HDL, LDL, and VLDL. *J. Lipid Res.* **39:** 777–788.
82. Schroeder, J. K. Woodford, J. Kavecansky, W. G. Wood, and C. Joiner. 1995. Cholesterol domains in biological membranes. *Mol. Membrane Biol.* **12:** 113–119.
83. Schroeder, F., J. R. Jefferson, A. B. Kier, J. Knittel, T. J. Scallen, W. G. Wood, and I. Hapala. 1991. Membrane cholesterol dynamics: Cholesterol domains and kinetic pools. *Proc. Soc. Exp. Biol. Med.* **196:** 235–252.
84. Brown, D., and E. London. 1998. Structure and origin of ordered lipid domains in biological membranes. *J. Membr. Biol.* **164:** 103–114.
85. McIntosh, T. J., S. Advani, R. E. Burton, D. V. Zhelev, D. Needham, and S. A. Simon. 1995. Experimental tests for protrusion and undulation pressures in phospholipid bilayers. *Biochemistry.* **34:** 8520–8532.

86. Castro, G. R., and C. J. Fielding. 1988. Early incorporation of cell-derived cholesterol into pre-B-migrating high-density lipoprotein. *Biochemistry.* **27:** 25–29.
87. Chang, W-J., K. G. Rothberg, B. A. Kamen, and R. G. W. Anderson. 1992. Lowering the cholesterol content of MA104 cells inhibits receptor-mediated transport of folate. *J. Cell Biol.* **118:** 63–69.
88. Fielding, P. E., and C. J. Fielding. 1995. Plasma membrane caveolae mediate the efflux of cellular free cholesterol. *Biochemistry.* **34:** 14288–14292.
89. Hailstones, D., L. S. Sleer, R. G. Parton, and K. K. Stanley. 1998. Regulation of caveolin and caveolae by cholesterol in MDCK cells. *J. Lipid Res.* **39:** 369–379.
90. Webb, N. R., P. M. Connell, G. A. Graf, E. J. Smart, W. J. S. De Villiers, F. C. de Beer, and D. R. van der Westhuyzehn. 1998. SR-BII, an isoform of the scavenger receptor BI containing an alternate cytoplasmic tail, mediates lipid transfer between high density lipoprotein and cells. *J. Biol. Chem.* **273:** 15241–15248.
91. Ahmed, S. N., D. A. Brown, and E. London. 1997. On the origin of sphingolipid/cholesterol-rich detergent-insoluble cell membranes: physiological concentrations of cholesterol an sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes. *Biochemistry.* **36:** 10944–10953.
92. Bamberger, M., S. Lund-Katz, M. C. Phillips, and G. H. Rothblat. 1985. Mechanism of the hepatic lipase induced accumulation of high density lipoprotein cholesterol by cells in culture. *Biochemistry.* **24:** 3693–3701.
93. Slotte, J. P., and E. L. Bierman. 1988. Depletion of plasma-membrane sphingomyelin rapidly alters the distribution of cholesterol between plasma membranes and intracellular cholesterol pools in cultured fibroblasts. *Biochem. J.* **250:** 653–658.