# Differential rate of cholesterol efflux from the apical and basolateral membranes of MDCK cells

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**Abstract** **Epithelial cells contain two distinct membrane surfaces, the apical and basolateral plasma membranes, which have different lipid and protein compositions. In order to assess the effect of the compositional differences of the apical and basolateral membranes on their ability to undergo cholesterol efflux, MDCK cells were radiolabeled with [$^3$H]cholesterol and grown as a polarized monolayer on filter inserts, that separate the upper apical compartment from the lower basolateral compartment. The rate of cholesterol efflux from the basolateral membrane into media containing HDL in the basolateral compartment was 6.3%/h ± 0.7, whereas HDL-mediated efflux from the apical membrane was approximately 3-fold slower (1.9%/h ± 0.3). In contrast, Fu5AH cells, which do not form distinct polarized membrane domains, had a similar rate of HDL-mediated cholesterol efflux into the apical and basolateral compartments. Similar to HDL, other cholesterol acceptors, namely LDL, bovine serum albumin, and a lipid emulsion, also showed a decreased rate of cholesterol efflux from the apical membrane surface versus the basolateral membrane. Compared to the basolateral membrane, the apical membrane was also found to be more resistant to cholesterol oxidase treatment, to bind less HDL, and to take up less cholesterol from the medium. In conclusion, cholesterol efflux occurred less readily from the apical membrane than from the basolateral membrane for all types of acceptors tested. These results suggest that differences in the composition of the apical and basolateral membrane lead to a relative decrease in cholesterol desorption from the apical membrane and hence a reduced rate of cholesterol efflux.—**Remaley, A. T., B. D. Farsi, A. C. Shirali, J. M. Hoeg, and H. B. Brewer, Jr. **Differential rate of cholesterol efflux from the apical and basolateral membranes of MDCK cells. *J. Lipid Res.* 1998.** 39: **1231–1238.**

**Supplementary key words** cholesterol efflux • high density lipoprotein • cholesterol • atherosclerosis

Epithelial cells contain two distinct plasma membrane domains, the apical and basolateral membranes, which face an external lumen and the underlying cell layer, respectively (1). The two membrane domains are separated by tight junctions that prevent the translocation of lipids and proteins between the two membranes (2). Each plasma membrane domain has a specialized function and contains a different composition of lipids and proteins. The basolateral membrane of epithelial cells is similar to the plasma membrane of nonepithelial cells, in terms of lipid and protein composition, as well as function (3, 4). In contrast, the apical membrane of epithelial cells consists of a unique composition of lipids and proteins, which is consistent with their function of providing a protective barrier (3, 4). The exofacial side of the lipid bilayer of apical membranes is enriched in glycosphingolipids and cholesterol and is relatively depleted of phospholipids (3–8). The rigidity and impermeability of the apical membrane of epithelial cells has been proposed to be due to the intermolecular H-bonding of glycosphingolipids (3).

Reverse cholesterol transport is the process whereby cholesterol is removed from peripheral tissues and is delivered to the liver for subsequent excretion into bile (9–11). High density lipoprotein (HDL) has been proposed to play a protective role in preventing atherosclerosis by being the principal acceptor of cholesterol that effluxes from peripheral cells. HDL mediates cholesterol removal by a process referred to as aqueous diffusion (11), which involves the desorption of cholesterol from the plasma membrane and its diffusion and binding onto extracellular acceptors, such as HDL. Different cell types differ in their rate of cholesterol efflux to HDL (10). The mechanism for the difference in the rate of cholesterol efflux between cells is not fully understood, but has been proposed to be due to differences in the lipid composition of the membrane (12–14). Sphingomyelin enrichment of membranes, for example, has been shown to reduce the rate of cholesterol efflux because of its ability to bind and retain cholesterol in the plasma membrane (12, 13). An increase in unsaturated fatty acids on phospholipids and an increase in the cholesterol to phospholipid ratio has also been shown to affect the rate of cholesterol efflux (11).

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; MDCK cells, Madin-Darby canine kidney cells; PC, phosphatidylcholine; BSA, bovine serum albumin.

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MDCK cells are an interesting model to investigate the process of cholesterol efflux, because they are epithelial cells that grow in a polarized manner and form both an apical and basolateral plasma membrane domain in vitro (15). When grown as a cell monolayer on a membrane-permeable support, which separates the upper apical compartment from the lower basolateral compartment, MDCK cells become morphologically and functionally polarized. MDCK cells, therefore, provide a way to examine the mechanism of cholesterol efflux from different plasma membrane domains of a single cell type. Furthermore, because some polarized cells, such as endothelial cells, participate in reverse cholesterol transport, information on the process of cholesterol efflux from the apical and basolateral membranes should be relevant to a full understanding of how reverse cholesterol transport occurs in vivo.

In this study, we examined the cholesterol efflux from the apical and basolateral membranes of MDCK cells and show that cholesterol efflux occurs less readily from the apical membrane. The difference in the rate of cholesterol efflux was not specific for the type of cholesterol acceptor and is, therefore, most likely due to a differential rate of desorption of cholesterol from the two plasma membrane domains.

## METHODS

### Cell culture

MDCK II cells (15) and Fu5AH cells (American Type Culture Collection, Rockville, MD) were grown in 6-well plates with Eagle's minimum essential medium (GIBCO BRL, Gaithersburg, MD), supplemented with 10% fetal calf serum, 2 mm glutamine, 100 IU/ml of penicillin, and 100 μg/ml of streptomycin (EMEM10), as previously described (16). Polarized cells were grown as a monolayer on Anopore (Nunc, Napersville, IL) membrane tissue culture inserts (0.02 μm pore), which contain a membrane that supports the cell monolayer and separates the upper apical compartment from the lower basolateral compartment. The integrity and confluence of the cell monolayer were assessed morphologically by light microscopy.

### Cholesterol efflux assay

The cholesterol efflux assay was performed as previously described (10, 17), except for the following modifications. Confluent MDCK cells grown on tissue culture inserts were incubated for the indicated time with EMEM10 containing 1 μCi/ml of [1,2-$^3$H]cholesterol (50 Ci/mmol)(DuPont; Wilmington, DE) for 48 h. Typically, this labeling protocol resulted in approximately 100,000 CPM/well or 1000 CPM/μg protein. Cells were washed three times with serum-free EMEM containing 1 mg/ml bovine serum albumin (EMEM/BSA), and efflux was assessed with the indicated cholesterol acceptors prepared in EMEM/BSA. After the efflux period, media were collected, centrifuged (10,000 *g* for 5 min) and an aliquot of the media was counted for radioactivity by liquid scintillation counting. The residual radioactivity in the cell fraction was determined after an overnight extraction with isopropanol. The percent efflux was calculated by dividing the radioactive counts in the efflux media by the sum of the radioactive counts in the media plus the cell fraction.

### Lipoprotein and lipid emulsion preparation

LDL (d 1.019–1.063 g/ml) and total HDL (d 1.063–1.21 g/ml) were isolated from human plasma by density gradient ultracentrifugation, as previously described (18). Lipid emulsion was prepared by a 10-fold dilution of 20% Liposyn (Abbott, Abbot Park, IL) with EMEM/BSA. Unless otherwise indicated, all lipoprotein concentrations were determined as μg of total protein per ml. Phosphatidylcholine (PC) was determined enzymatically (Wako Bioproducts, Richmond, VA).

### HDL binding assay

HDL was iodinated with $^{125}$I by the iodide monochloride method (19) to a specific activity of $2 \times 10^6$ cpm/μg. Confluent MDCK cells grown on tissue culture inserts were incubated with the indicated concentration of iodinated HDL for 3 h at 37°C in the presence and absence of a 50-fold excess of unlabeled HDL. Cells were rapidly washed three times with EMEM/BSA at 4°C. After dissolving the cell fraction with 0.1 m NaOH and 0.1% SDS, radioactive counts bound to the cells were determined by gamma counting.

### Cholesterol oxidase treatment

MDCK cells were treated with cholesterol oxidase as previously described (20), except for the following modifications. After a 2-h pulse labeling with [$^3$H]cholesterol in EMEM10, the cells were washed and incubated with cholesterol oxidase in EMEM/BSA for 15 min at 37°C. The cells were then washed and extracted with isopropanol, and cholesterol and cholestenone were separated by thin-layer chromatography (20) and quantitated by liquid scintillation counting.

## RESULTS

In order to investigate cholesterol efflux from the apical and basolateral plasma membranes, MDCK cells were grown as a cell monolayer on tissue culture inserts. The bottom of the insert contains a semipermeable membrane that supports the cell monolayer and the sides of the insert form a container that separates the upper apical compartment from the lower basolateral compartment. MDCK cells were grown for 3 days after reaching confluence on tissue culture inserts to allow for the formation of tight junctions. After labeling both compartments for 48 h with [$^3$H]cholesterol followed by a wash, HDL was added to either the apical (**Fig. 1**, panel A) or to the basolateral compartment (Fig. 1, panel B) or to both (Fig. 1, panel C). The side without HDL was incubated with EMEM/BSA, which served as a control. As can be seen by comparing panel A with B, less cholesterol efflux occurred when the HDL was in the apical compartment compared to the basolateral compartment. As expected, the side containing only EMEM/BSA media showed much reduced cholesterol efflux compared to the opposite side containing HDL (Fig. 1, panels A and B). By calculating the initial rate of efflux within the first 4 h, the apical membrane effluxed cholesterol approximately 3 times slower than the basolateral membrane. The initial rate of cholesterol efflux from the apical membrane was 1.9 ± 0.3%/h, whereas the rate from the basolateral membranes was 6.3 ± 0.7/h. When HDL was added to both compartments simultaneously (Fig. 1, panel C), the rate of cholesterol ef-

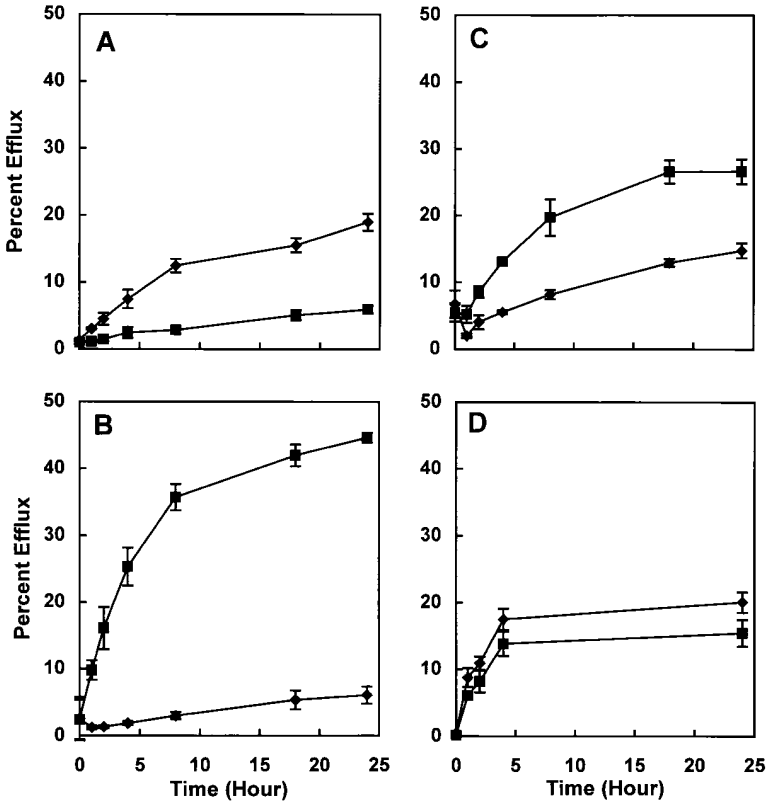


**Fig. 1.** Cholesterol efflux from apical and basolateral membranes. MDCK cells (panels A, B, and C) or Fu5AH cells (panel D) were labeled with [$^3$H]cholesterol for 24 h, followed by cholesterol efflux with 50 μg/ml of HDL or EMEM/BSA for up to 48 h. The following acceptors were added to either the apical (◆) or basolateral (■) compartment: (panel A) apical-HDL, basolateral-EMEM/BSA, (panel B) apical-EMEM/BSA, basolateral-HDL, (panel C) apical-HDL, basolateral-HDL, (panel D) apical-HDL, basolateral-HDL. The results represent the mean of triplicates ± 1 SD.

flux from the apical membrane was again observed to be decreased relative to the basolateral membrane. As a control, Fu5AH cells, a nonpolarized cell line that does not form distinct membrane domains in vitro, were also grown on tissue culture inserts. In contrast to MDCK cells, there was no significant difference in cholesterol efflux between the apical and basolateral compartment for Fu5AH cells (Fig. 1, panel D).

The impermeability of the MDCK and Fu5AH cell monolayer to HDL was evaluated in **Fig. 2**. Radiolabeled HDL was added to either the apical (Fig. 2, panels A and C) or to the basolateral compartment (panels B and D) and the transfer of HDL to the other compartment was assessed over time. MDCK cells (panels A and B) almost completely prevented the transfer of HDL to the opposite compartment over a 24-h time period, which confirms the integrity of the cell monolayer and its formation of tight junctions separating the apical and basolateral compartments. This finding validates the experimental approach of selectively measuring cholesterol efflux from just one compartment at a time by adding HDL to only that compartment. In contrast to MDCK cells, Fu5AH cells (panels C and D) transferred appreciable amounts of HDL to the other compartment over time, which is consistent with the fact that Fu5AH cells do not form tight junctions between cells, thereby allowing for the diffusion of HDL across the cell monolayer. The transfer of HDL in Fu5AH cells, however, was negligible within the first 4 h. During this time frame any difference in cholesterol efflux from the apical and basolateral compartments should have been apparent. As can be seen in Fig. 1 (panel D), there was no significant difference in cholesterol efflux during the first 4 h for Fu5AH cells, which excludes the possibility that the similar initial rate of cholesterol efflux from the apical and basolateral compartments in Fu5AH cells was the result of diffusion of HDL across the cell monolayer.

A difference in the dose–response relationship between the HDL concentration and cholesterol efflux was examined (**Fig. 3**) to determine whether it accounted for the difference in the rate of cholesterol efflux from the apical and basolateral membranes. Both the apical and basolateral membranes showed similar shaped curves that began to saturate at about 200 μg/ml. At all HDL concentrations tested, however, there was approximately a 3.0-fold increase in cholesterol efflux from the basolateral membrane compared to the apical membrane, which suggests that a difference in the dose–response curves does not account for the differential rate of cholesterol efflux from the two plasma membrane domains.

In Figs. 1 and 3, the MDCK cells were labeled simultaneously in both compartments for 48 h in order to uniformly label both plasma membrane domains. Because the percent efflux is calculated by dividing the radioactive counts that appear in the media by the total radioactive counts, which includes the radioactive counts in the media plus the residual radioactive counts in the cells (see methods), a difference in the incorporation of the cholesterol into the apical versus the basolateral membranes could potentially account for an apparent difference in the percent efflux. Cholesterol efflux from MDCK cells was reassessed in **Fig. 4**. by selectively labeling only one membrane surface at a time, to account for any possible

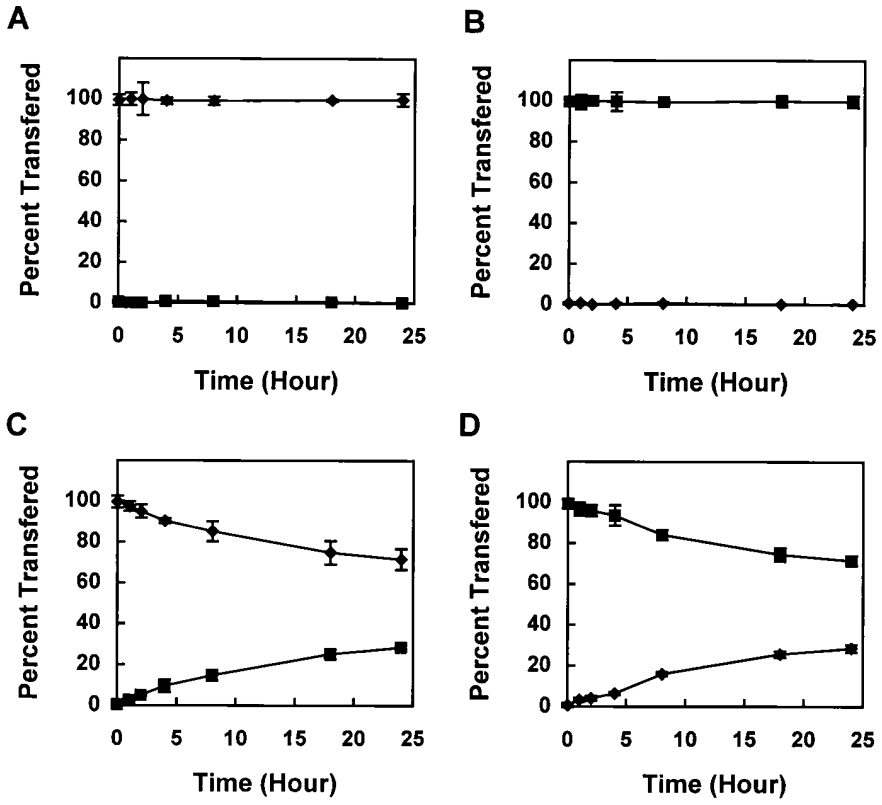


**Fig. 2.** Percent transfer of HDL between apical and basolateral compartment. Radioiodinated HDL was added to either the apical (panels A and C) or the basolateral compartment (panels B and D) of MDCK (panels A and B) or Fu5AH cells (panels C and D). TCA-precipitable radioactive counts in the apical (◆) and basolateral (■) compartment were determined at the indicated time points. The results represent the mean of triplicates ± 1 SD.

differences in the incorporation of radiolabeled cholesterol. This was performed by pulse labeling the cells for a short time, which has been successfully used before to selectively label only the plasma membrane of cells (20). In panel A, the apical surface was pulse labeled for 2 h and then HDL was added to both compartments. In panel B, the basolateral surface was labeled and HDL was added to both compartments. In panel C, both compartments were labeled simultaneously and HDL was added to both sides. Although we overall observed greater cholesterol efflux from the short term labeling protocol (Fig. 4) compared to uniformly labeling the cells (Fig. 1), there was a similar pattern of reduced cholesterol efflux from the apical membrane compared to the basolateral membrane. The selective labeling of the apical and basolateral surface was confirmed by cholesterol oxidase treatment (**Fig. 5**). After labeling either the apical or basolateral compartment with radiolabeled cholesterol, cholesterol oxidase was added to either the same compartment that was labeled or the opposite compartment. When the basolateral compartment was labeled and cholesterol oxidase was added to that compartment, over 60% of the cholesterol was converted to cholestenone by cholesterol oxidase (Fig. 5, BB). In contrast, when the basolateral compartment was labeled and cholesterol oxidase was added to the apical compartment, only trace amounts of the cholesterol were modified by cholesterol oxidase (Fig. 5, BA). This result indicates that the pulse incubation of the basolateral compartment successfully labeled only the basolateral membrane and not the apical membrane. The converse experiment of labeling the apical compartment was also performed with similar results, except for two differences. First, there was approximately a 50% reduction in the incorporation of the radiolabeled cholesterol into the apical membrane compared to the basolateral membrane. Second, although the results of the cholesterol oxidase treatment are consistent with the selective labeling of the apical surface, cholesterol oxidase less efficiently modified cholesterol that was incorporated into the apical surface compared to the basolateral membrane (Fig. 5, AA).

Several cells have been shown to have high affinity binding sites for HDL, and the direct binding of HDL to cells has been proposed to play a role in facilitating reverse cholesterol transport (17). In **Fig. 6**, the binding of HDL to the apical and basolateral membranes of MDCK cells was examined. Compared to apical membranes, the basolateral membrane bound approximately 3 times the amount of HDL. Excess unlabeled HDL competed for the binding of radiolabeled HDL from both the apical and basolateral membranes, but greater competition was observed from basolateral membranes, which also suggests a

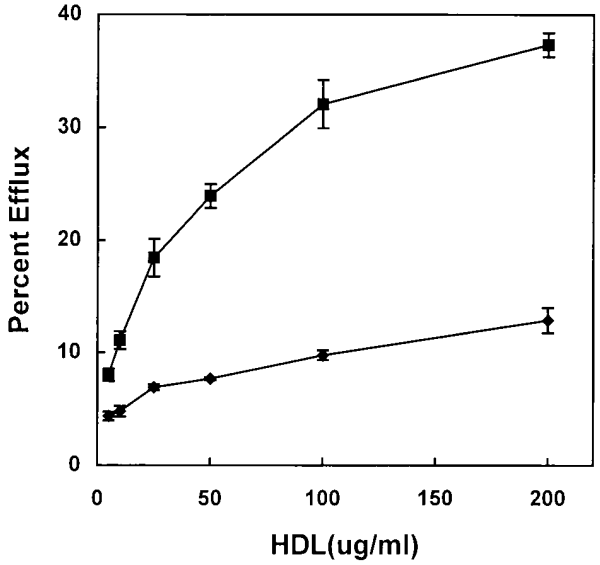


**Fig. 3.** Dose–response curve for efflux of cholesterol from apical and basolateral membranes. MDCK were labeled for 48 h with [$^3$H]cholesterol, followed by cholesterol efflux for 24 h with the indicated concentration of HDL in both the apical (◆) and basolateral (■) compartment. The results represent the mean of triplicates ± 1 SD.

greater number of high affinity binding sites for HDL on basolateral membranes.

In order to determine whether the increased binding of HDL to the basolateral membrane accounted for the increased efflux from the basolateral surface, several nonspecific acceptors, namely LDL, lipid emulsion, and BSA, were also tested for their ability to efflux cholesterol from the apical and basolateral membrane. These acceptors remove cholesterol by an aqueous diffusion mechanism and do not require a direct interaction with cells (11, 21). As shown in **Fig. 7**, although there were some differences in the overall ability of the various acceptors to efflux cholesterol, all of the acceptors tested removed a greater amount of cholesterol from the basolateral membrane than the apical membrane, which suggests that the increased efflux from the basolateral membrane ocurred by an aqueous diffusion mechanism.

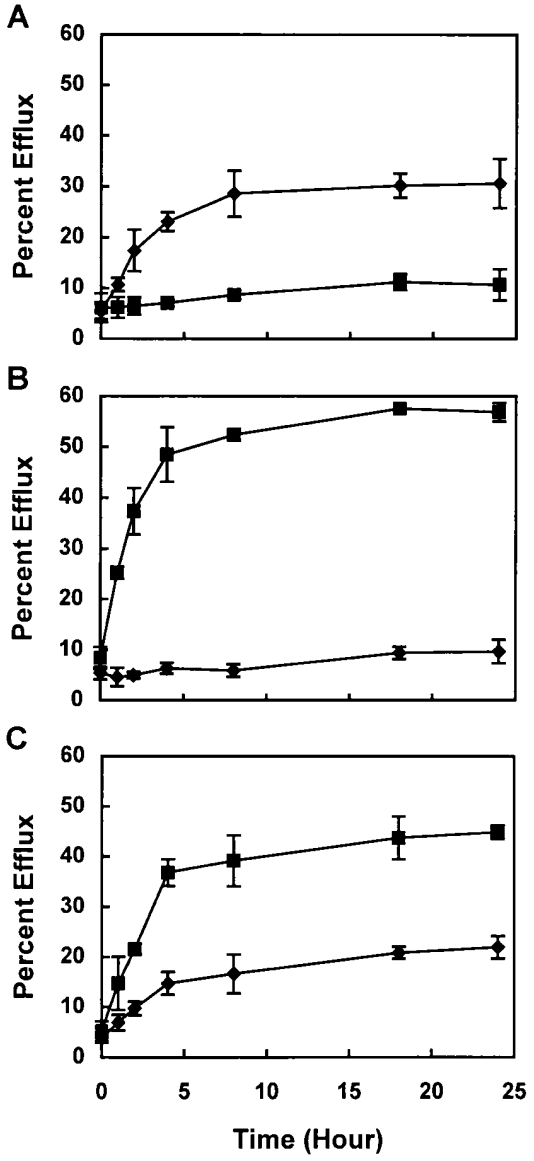


**Fig. 4.** Cholesterol efflux from apical and basolateral membranes. MDCK cells were pulse labeled with [$^3$H]cholesterol for 2 h in either the apical (panel A) or basolateral (panel B) compartment or both (panel C), followed by cholesterol efflux with 50 mg/ml of HDL in both the apical (◆) and basolateral (■) compartment for up to 24 h. The results represent the mean of triplicates ± 1 SD.

## DISCUSSION

The ability of cells to participate in reverse cholesterol transport by donating cholesterol to HDL has been proposed to be critical in establishing the proper distribution of cholesterol in peripheral cells. Patients with reduced levels of HDL have been postulated to have an increased risk for developing atherosclerosis because of a decrease in HDL-mediated cholesterol efflux from peripheral tissues. Besides the level of HDL, cellular factors may also be important in modulating the effectiveness of the reverse cholesterol transport pathway. Different cell types are known to differ in their ability to efflux cholesterol, but the mechanism for the difference is not known (10, 14). In this study, two different plasma membrane domains from a single cell are shown to differ in their ability to efflux cholesterol.

Whether MDCK cells were labeled to equilibrium (Fig. 1) or selectively labeled one membrane domain at a time (Fig. 2), it was observed that the apical membrane effluxed cholesterol significantly slower than the basolateral membrane. In contrast, Fu5AH cells, which do not form separate polarized membrane domains, did not show a difference in cholesterol efflux from the apical versus the basolateral compartment. The difference in cholesterol efflux could not be explained by a change in the dose–response relationship between the HDL concentration and efflux for the apical and basolateral membranes (Fig.

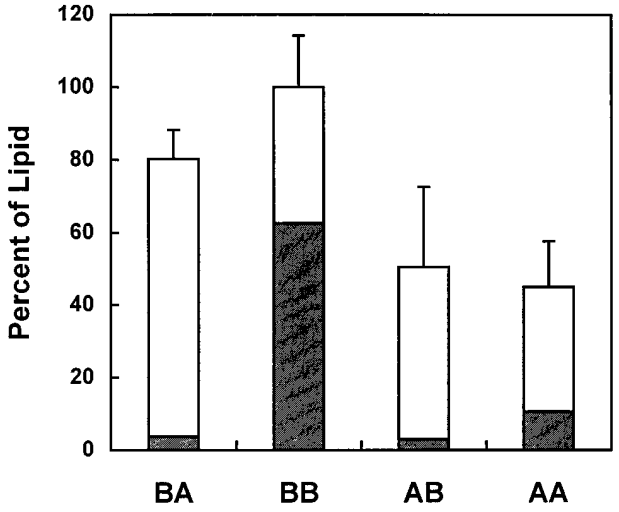


**Fig. 5.** Cholesterol oxidase treatment of apical and basolateral membranes. Either the apical or the basolateral membranes of MDCK cells were pulse labeled with [$^3$H]cholesterol for 2 h and then treated with cholesterol oxidase for 15 min at 37°C in EMEM/BSA in either the apical or basolateral compartment, according to the following protocol: basolateral–pulse labeled and apical–cholesterol oxidase-treated (BA); basolateral–pulse labeled and basolateral–cholesterol oxidase-treated (BB); apical–pulse labeled and basolateral–cholesterol oxidase-treated (AB); apical–pulse labeled and apical–cholesterol oxidase-treated (AA). Solid rectangles indicate the percent of cholesterol converted to cholestenone and open rectangles indicate the percent of radioactive counts as cholesterol. Results are normalized as the percent of the total radioactive counts obtained for the BB protocol. The results represent the mean of triplicates ± 1 SD.

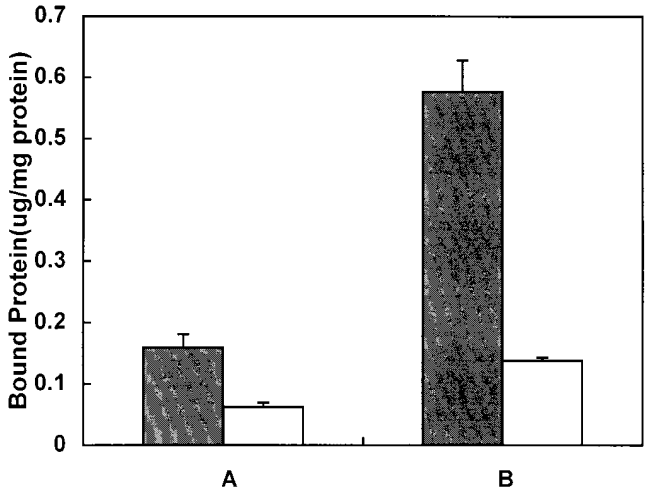


**Fig. 6.** Binding of HDL to the apical and basolateral membranes of MDCK cells. Radioiodinated HDL (5 μg/ml) was added to either the apical (A) or the basolateral compartment (B) and cell bound radioactive counts were determined after 3 h at 37°C. Bound radioactive counts were determined in the presence (open rectangles) and in the absence (solid rectangles) of 50-fold excess unlabeled HDL. The results represent the mean of triplicates ± 1 SD.

3). Interestingly, cholesterol oxidase treatment (Fig. 5), which was used to show the selective labeling of one membrane domain at a time, also showed a difference in the susceptibility of the apical and basolateral membranes to the cholesterol oxidase. Cholesterol on the basolateral membranes was more readily oxidized by cholesterol oxidase than was cholesterol on the apical side. In addition, it was observed that greater incorporation of cholesterol occurred into the basolateral side than the apical side. Both of these results, as well as the difference in cholesterol efflux, are likely a consequence of differences in the structure and composition of the two membrane domains (1, 3, 4).

The basolateral membranes also showed increased binding of HDL compared to the apical membrane (Fig. 6). Two different mechanisms have been proposed for HDL-mediated cholesterol efflux from cells (9). Cholesterol can desorb from cells into the aqueous compartment and then bind to HDL or to other acceptors in a process referred to as aqueous diffusion (11). Numerous experimental studies with model membranes, as well as cells, have shown that this process does occur and accounts for the majority of cholesterol efflux from cells. In addition, however, it has been shown that cells contain high affinity binding sites for HDL (17). The binding of HDL to cells has been shown to stimulate various second messengers, which have been proposed to facilitate cholesterol efflux by mobilization of intracellular pools of cholesterol (20). In Fig. 7, the mechanism for cholesterol efflux was evaluated by assessing the ability of different cholesterol acceptors, besides HDL, to efflux cholesterol from the apical and basolateral membranes. These non-HDL acceptors are believed to remove cholesterol by the aqueous diffusion process and do not depend on a direct interaction with cells for cholesterol efflux (11, 21). Although there were some differences in the overall efflux of the various

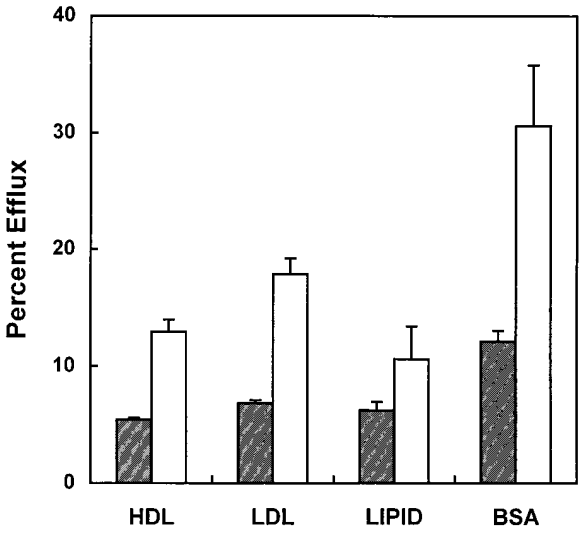


**Fig. 7.** Efflux of cholesterol from MDCK cells with various acceptors. MDCK cells were labeled with [$^3$H]cholesterol for 48 h, followed by cholesterol efflux from the apical (■) and the basolateral (□) compartment for 24 h with the following acceptors: HDL (50 μg total protein/ml; 100 μg PC/ml), LDL (50 μg total protein/ml; 133 μg PC/ml), lipid emulsion (10% (V/V), 1.2 mg PC/ml), BSA(50 mg/ml). The results represent the mean of triplicates ± 1 SD.

acceptors at the doses tested, all of the acceptors showed reduced cholesterol efflux from the apical membrane compared to the basolateral membrane. This result is consistent with a differential rate of cholesterol desorption from the apical and basolateral membranes as being the mechanism for the difference in cholesterol efflux. Because the rate-limiting step in aqueous diffusion is the desorption of cholesterol from the membrane (11), a reduced rate of desorption of cholesterol from the apical membrane would account for the relative decrease in cholesterol efflux from the apical membrane.

The apical and basolateral membranes of epithelial cells are composed of different lipids, which is believed to contribute to the different physical and functional properties of the two membrane surfaces (3, 4). The basolateral membrane of epithelial cells is similar in its lipid composition to the plasma membrane of nonpolarized cells but the apical membrane has a relatively unique lipid composition. There is an enrichment of glycolipids and a relative depletion of phospholipids in the apical membrane of epithelial cells (3), including MDCK cells (3–8). This results in an increase in the cholesterol to phospholipid ratio and an increase in the sphingomyelin to phospholipid ratio for the apical membrane, which has been previously shown from model membrane systems, as well as from tissue culture studies, to increase and decrease, respectively, the rate of cholesterol efflux from membranes (11). In addition to the differences in the overall lipid composition of the apical and the basolateral membrane, the two sides of the apical membrane bilayer differ in their lipid composition. Glycosphingolipids are almost exclusively located on the exofacial side of the bilayer (3, 4). Based on the lipid composition of the apical membrane, the exofacial side has been proposed to be relatively depleted in phospholipids, except for sphingomyelin, and to consist primarily of glycosphingolipids and cholesterol (3, 4). The molar ratio of glycosphingolipids, cholesterol and phospholipids has been proposed to be 1:1:1 in the exofacial side of the apical membrane (3, 4). Glycosphingolipids readily form intermolecular H-bonds and increase the lateral packing density of a lipid bilayer, which results in decreased fluidity and permeability of the apical membrane (3, 4) and may also result in decreased cholesterol efflux (11). Reducing membrane fluidity by increasing the content of unsaturated fatty acids in phospholipids has been shown to decrease cholesterol efflux from membranes (11). The relative enrichment of sphingomyelin on the exofacial side of the apical membrane would also be predicted to reduce cholesterol efflux because of its interaction with cholesterol and its ability to decrease the desorption of cholesterol from membranes (12, 13). Similarly, a potential interaction of cholesterol with glycosphingolipids could also contribute to a reduced rate of cholesterol efflux.

In addition to the lipid composition, the protein components on cell membranes may also play a role in modulating the rate of cholesterol efflux. Although changes in the lipid composition of cell membranes can affect the rate of cholesterol efflux, the change in efflux is not as large as what is observed with model membranes, which do not contain proteins (12, 13). In addition, lipid vesicles produced from lipid extracts from cells with different rates of cholesterol efflux do not show significant differences in the rate of cholesterol efflux (14). These studies suggest that the protein composition and/or the effect of membrane proteins on the lipid organization of the cell membrane can also affect the rate of cholesterol efflux. Apical and basolateral membranes have numerous differences in their protein composition, most notably the enrichment of GPI-linked proteins on the apical surface (1), and differences in protein composition may also be a factor for the difference in cholesterol efflux. Finally, glycosaminoglycans on the surface of the apical side could also potentially limit the rate of cholesterol efflux by increasing the unstirred water layer and therefore increase the diffusion barrier for cholesterol desorption (11).

In summary, the apical membrane showed a relative decrease in cholesterol efflux compared to basolateral membrane for all acceptors tested and is most likely mediated by a decreased rate of cholesterol desorption due to differences in the composition and structure of the two membrane surfaces. Future studies aimed at further examining the mechanism for the difference in cholesterol efflux between the two plasma membrane domains of epithelial cells should potentially lead to interesting new insights into the cellular factors that can affect reverse cholesterol transport.

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## REFERENCES


1. Hubbard, A. L. 1991. Targeting of membrane and secretory proteins to the apical domain in epithelial cells. *Semin. Cell Biol.* **2:** 365–374.
2. Van Meer, G. and K. Simons. 1986. The function of tight junctions in maintaining differences in lipid composition between the apical and the basolateral cell surface domains of MDCK cells. *EMBO J.* **5:** 1455–1464.
3. Simons, K. and G. Van Meer. 1988. Lipid sorting in epithelial cells. *Biochemistry.* **27:** 6197–6202.
4. Van't Hof, W. and G. Van Meer. 1994. Lipid polarity and sorting in epithelial cells. *Curr. Top. Membranes.* **40:** 539–563.
5. Van Meer, G. and K. Simons. 1982. Viruses budding from either the apical or the basolateral plasma membrane domain of MDCK cells have unique phospholipid composition. *EMBO J.* **11:** 847–852.
6. Van Genderen, I. L., G. Van Meer, J. W. Slot, H. J. Gueze, and W. F. Voorhout. 1991. Subcellular localization of Forssman glycolipid in epithelial MDCK cells by immuno-electronmicroscopy after freeze substitution. *J. Cell Biol.* **115:** 1009–1019.
7. Van Meer, G., E. H. K. Stelzer, R. W. Wijnaendts-van-Resandt, and K. Simons. 1987. Sorting of sphingolipids in epithelial cells. *J. Cell Biol.* **105:** 1623–1635.
8. Nichols, G. E., T. Shiraishi. and W. W. Young, Jr. 1988. Polarity of neutral glycolipids, gangliosides, and sulfated lipids in MDCK cells. *J. Lipid Res.* **29:** 1205–1213.
9. Hoeg, J. M. and A. T. Remaley. 1994. Reverse cholesterol transport. *In* Genetic Factors in Heart Disease. U. Goldbourt, U. deFaire, and K. Berg, editors. Kluwer Academic Publishers, Norwell, MA. 351–372.
10. Rothblat, G. H., M. Banberger, and M. C. Phillips. 1986. Reverse cholesterol transport. *Methods Enzymol.* **129:** 628–644.
11. Phillips, M. C., W. J. Johnson. and G. H. Rothblat. 1987. Mechanism and consequences of cellular cholesterol exchange and transfer. *Biochim.Biophys. Acta.* **906:** 223–276.

12. Gold, J. C. and M. C. Phillips. 1990. Effects of membrane lipid composition on the kinetics of cholesterol exchange between lipoproteins and different species of red blood cells. *Biochim. Biophys. Acta*. **1027:** 85–92.
13. Gold, J. C., and M. C. Phillips. 1992. Effect of membrane lipids and proteins and cytoskeletal proteins on the kinetics of cholesterol exchange between high density lipoprotein and human red blood cell ghosts andmicrovesicles. *Biochim. Biophys. Acta*. **1111:** 103–110.
14. Bellini, F., M. C. Phillips, C. Pickell, and G. H. Rothblat. 1984. Role of the plasma membrane in the mechanisms of cholesterol efflux from cells.*Biochim. Biophys. Acta*. **777:** 209–215.
15. Richardson, J. C. W., V. Scalera, and N. L. Simmons. 1981. Identification of two strains of MDCK cells which resemble separate nephron tubule segments. *Biochim. Biophys. Acta*. **673:** 26–36.
16. Remaley, A. T., and J. M. Hoeg. 1995. Polarized secretion of apoA-I and apoA-II by transfected MDCK cells. *J. Lipid Res*. **36:** 407–413.
17. Oram J. F. 1986. Receptor-mediated transport of cholesterol between cultured cells and high density lipoproteins. *Methods Enzymol*. **129:** 629–659.
18. Schumaker, V. N., and D. L. Puppione. 1986. Sequential flotation-ultracentrifugation. *Methods Enzymol*. **128:** 155–169.
19. Schaefer, E. J. and J. M. Ordovas. 1986. Metabolism of apolipoproteins A-I, A-II, and A-IV. *Methods Enzymol*. **129:** 420–413.
20. Mendez, A. J., J. F. Oram, and E. L. Bierman. 1991. Protein kinase C as a mediator of high density lipoprotein receptor-dependent efflux of intracellular cholesterol. *J. Biol. Chem*. **266:** 10104–10111.
21. Chau, I. Y., and R. P. Geyer. 1978. The effects of serum albumin and phospholipid on sterol excretion in tissue culture cells. *Biochim. Biophys. Acta*. **542:** 214–221.