Secretary Vesicular Transport from the Golgi Is Altered during ATP-binding Cassette Protein A1 (ABCA1)-mediated Cholesterol Efflux

Received for publication, January 17, 2003
Published, JBC Papers in Press, January 27, 2003, DOI 10.1074/jbc.C300024200

Xiaohui Zha§§, Andre Gauthier, Jacques Genest‡, and Ruth McPherson‡

From The Lipoprotein & Atherosclerosis Group, University of Ottawa Heart Institute, Ottawa, Ontario K1Y 4W7, Canada and the ¶Division of Cardiology, McGill University Health Center, Montreal, Quebec H3A 1A1, Canada

Apolipoprotein A1 (apoA1)-mediated cholesterol efflux is a process by which cells export excess cellular cholesterol to apoA1 to form high density lipoprotein. ATP-binding cassette protein A1 (ABCA1) has recently been identified as the key regulator of this process. The pathways of intracellular cholesterol transport during efflux are largely unknown nor is the molecular mechanism by which ABCA1 governs cholesterol efflux well understood. Here, we report that, in both macrophages and fibroblasts, the secretory vesicular transport changes in response to apoA1-mediated cholesterol efflux. Vesicular transport from the Golgi to the plasma membrane increased 2-fold during efflux. This increase in vesicular transport during efflux was observed in both raft-poor and raft-rich vesicle populations originated from the Golgi. Importantly, enhanced vesicular transport in response to apoA1 is absent in Tangier fibroblasts, a cell type with deficient cholesterol efflux due to functional ABCA1 mutations. These findings are consistent with an efflux model whereby cholesterol is transported from the storage site to the plasma membrane via the Golgi. ABCA1 may influence cholesterol efflux in part by enhancing vesicular trafficking from the Golgi to the plasma membrane.

ApoA1-mediated cholesterol efflux is one of the major events in “reverse cholesterol transport,” a process that generates HDL and transports excess cholesterol from the peripheral tissues, including the arterial wall, to the liver for biliary secretion. At the cellular level, apoA1 is capable of triggering a cascade of intracellular events, leading to transport of stored cholesterol to the cell surface. ApoA1 then acquires lipids including cholesterol to form mature HDL. ApoA1-mediated cholesterol efflux is thus the key step in the maintenance of healthy levels of HDL in human. This process is absent in a genetic disorder, Tangier disease, due to functional mutations in ABCA1 (1). Tangier patients develop extensive lipid accumulations in various tissues, extremely low HDL-C levels, and elevated risk for coronary artery disease. ABCA1 is found predominantly in the Golgi and the plasma membrane including endosomes in various cell types. The molecular mechanism by which ABCA1 governs cholesterol efflux is not well understood. Nor is the pathway by which cholesterol is transported to the cell surface for efflux.

The net effect of apoA1-mediated efflux is to eliminate excess cholesterol from the cell. Intracellular cholesterol transport during the process is thought to be initiated from lipid droplets to the plasma membrane (2). Cellular cholesterol plays a critical role in several important cell functions, including protein trafficking, membrane vesiculation, and signal transduction (3). Mammalian cells have developed highly sophisticated mechanisms to ensure adequate cholesterol levels. Plasma membrane cholesterol content, for example, is regulated through a feedback mechanism controlled by sterol regulatory element binding protein-2 (SREBP-2) (4). SREBP-2 senses membrane cholesterol levels and regulates the transcription of genes encoding proteins required for endogenous cholesterol synthesis and uptake of plasma lipoproteins. Under most circumstances SREBP-2 can effectively control cellular cholesterol levels. There are, however, situations in which large amounts of cholesterol are taken into cells by other pathways involving receptors not regulated by SREBP-2. This leads to accumulation of intracellular cholesterol. To maintain normal cell function, most mammalian cells are capable of sequestering excess cholesterol into the lipid droplets as a temporary storage system. Cholesterol may be used as additional energy source in the future. It is, however, far more common in modern societies that the this storage system is overloaded, leading to macrophage foam cell formation with potentially lethal effects due to the build-up of unstable coronary artery plaque. It is thus critical that the storage capacity be efficiently renewed by the removal of excess cholesterol from cells. ApoA1-mediated efflux is by far the most significant process to remove excess lipid, including cholesterol, under physiological conditions. How stored cholesterol in the lipid droplets reaches apoA1 on the cell surface during this process is not clear at present. As cholesterol is known to actively shuttle between the lipid droplets and the endoplasmic reticulum (ER) (2), it is likely that stored cholesterol is first released from lipid droplets into the ER and from there to the cell surface to interact with apoA1. The secretory vesicular pathway is thus likely involved in transporting lipids including cholesterol to the plasma membrane.

As many lipids are known to be transported from the Golgi to the plasma membrane exclusively through vesicular mechanisms, we hypothesized that the Golgi may play a role in transporting newly released cholesterol to the plasma mem-
brane during apoAI-mediated efflux and ABCA1 may influence this pathway.

We therefore took advantage of recent development in green fluorescent proteins (GFP) techniques and designed a series of experiments to quantitatively measure whether vesicular transport in secretory pathway is affected when cells are actively pumping cholesterol to apoAI on the cell surface. We also investigated whether vesicular transport is affected in response to apoAI in Tangier cells with functional mutations in ABCA1. We report here that there is enhanced vesicular transport from the Golgi to the plasma membrane in cells in which cholesterol efflux is elicited by exposure to apoAI. This enhanced vesicular transport was not observed in Tangier cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—Brefeldin A (BFA) and phosphatidylinositol-specific phospholipase C (PI-PLC) were purchased from Molecular Probes. Yellow fluorescent protein-vesicular stomatitis virus glycoprotein (YFP-VSVG) and yellow fluorescent protein-glycosyl-phosphatidylinositol-anchored protein (YFP-GPI) in adenoviral vectors were gifts from Drs. P. Keller and K. Simons, EBI. Recombinant apoAI was obtained from Drs. R. Kiss and Y. Marcel, Ottawa University Heart Institute.

**Cholesterol Efflux**—Cholesterol efflux was performed using J774 macrophages and human primary fibroblasts (from a normal individual and a Tangier patient). The Tangier patient is a compound heterozygote in which one allele gives the mutation C1477R and the other one is a Gly → Cys mutation at an exon/intron boundary, causing a splice site mutation and thus a truncated mRNA (5). Cells were cholesterol-loaded with acetylated low-density lipoprotein (75 μg/ml) for 1–2 days along with [3H]cholesterol. Cells were then changed into medium containing 8-Bromo-cAMP (50 μM) and BSA (2 mg/ml) overnight. Cholesterol efflux was induced by incubating cells with 10 μg/ml apoAI for various time. The medium was then collected and counted for [3H]cholesterol. In BFA experiments, cells were preincubated 15 min with BFA (10 μM) before apoAI. BFA was present throughout the efflux period. Cells were then lysed and counted for total cellular [3H]cholesterol. Efflux is presented as percentage [3H]cholesterol in the medium of total cellular [3H]cholesterol.

**YFP-GPI and YFP-VSVG Transport Assay**—Macrophages and fibroblasts were loaded with cholesterol and treated with cAMP/BSA over night. Cells were then transfected with YFP proteins using adenoviral vectors. For the transport from the Golgi to the plasma membrane, transfected cells were incubated at 19.5 °C for 2–4 h to accumulate YFP protein in the Golgi. Cycloheximide was added for the last 30 min of the incubation to stop further protein synthesis. Cells were then moved to 32 °C to allow the transport from the Golgi to the plasma membrane in the presence or absence of apoAI. For quantitative transport measurement, cells were fixed at the end of the 32 °C incubation with 4% paraformaldehyde and analyzed by quantitative fluorescent microscopy.

**Fluorescent Microscopy and Imaging Analysis**—To measure the transport of YFP proteins, fluorescent images of fixed cells were acquired on an Olympus IX70 microscope using a 40× (NA 0.75) objective and a 12-bit CCD digital camera IMAGO. For Golgi to plasma membrane transport, an average fluorescent intensity in the Golgi area was measured using TILLvision software. After background correction, Golgi fluorescence intensity was ratioed to the average fluorescent intensity from whole cell to give an indication of the rate of transport in that cell. For each treatment, 50–200 individual cells were imaged and quantified.

**RESULTS**

To test whether the Golgi is indeed involved in apoAI-mediated cholesterol efflux, we first examined the effect of BFA in the process. BFA disassembles the Golgi and redistributes it into the ER. This leads to a complete inhibition in secretory vesicular transport (6). In J774 macrophages, we found that cholesterol efflux is sensitive to BFA. When treated with BFA the Golgi was disassembled within 10 min (not shown), and there was about 50% inhibition of apoAI-mediated cholesterol efflux during 2 h following BFA treatment (Fig. 1a). Cholesterol efflux from human primary fibroblasts was similarly affected and, as expected, Tangier fibroblasts failed to produce any cholesterol efflux treated with or without BFA (Fig. 1b). Prolonged BFA treatment (>4 h) produces much more severe inhibition in both macrophages and fibroblasts (not shown), in agreement with previous observations (7). BFA effects within 2 h are likely due to specific blockage of vesicular transport. The inhibition by BFA thus suggests that apoAI-mediated efflux involves a vesicular process in the secretory pathway.

Cholesterol is relatively enriched in the plasma membrane and in the Golgi (8). It is believed that cholesterol/sphingomyelin-rich membrane domains (rafts) are first formed in the Golgi and, from there, transported to the plasma membrane. Golgi to plasma membrane vesicular transport could be used to transport lipid including cholesterol to the cell surface during apoAI-mediated efflux. We therefore measured the rate of vesicular transport from the Golgi to the plasma membrane in cells undergoing apoAI-mediated cholesterol efflux and compared it with cells treated identically but without exposure to apoAI. In non-polarized cells, membrane vesicles budding from the Golgi are known to have at least two distinct populations, namely raft-rich and raft-poor vesicles in terms of their membrane lipid compositions. Each population of vesicles is known to carry a specific set of cargo proteins. To accurately estimate overall membrane vesicular movement from the Golgi to the plasma membrane, we chose two well characterized markers, a raft-associated YFP-GPI and a non-raft-associated YFP-VSVG. This allowed us to target both vesicle populations, respectively (9). These proteins were expressed using adenovirus vectors and accumulated in the Golgi by a 19.5 °C temperature block (Fig. 2a, top row). Upon switching to 32 °C, YFP-GPI or YFP-VSVG entered budding vesicles, exited the Golgi, and moved to the plasma membrane. The rate of transport was measured in individual cells by quantifying YFP fluorescence intensities in the Golgi as a function of time. In cholesterol-loaded J774
mature macrophages, we found that, after switching temperature to 32 °C for 10 min, YFP fluorescent intensity in the Golgi decreased more rapidly in cells undergoing efflux (+apoAI, apoAI), when compared with control cells (−apoAI). Correspondingly, there was more YFP-GPI on the plasma membrane in cell exposed to apoAI (Fig. 2a, bottom row, right panel). Quantitative analysis from a large number of individual cells further confirmed that the transport of YFP-GPI from the Golgi to the plasma membrane was significantly increased in cells incubated with apoAI (Fig. 2b, gray line) relative to control cells (Fig. 2b, black line).

To further verify this, we performed similar experiments using YFP-GPI in cholesterol-loaded normal primary fibroblasts. These cells are also capable of mobilizing cholesterol when exposed to apoAI (Fig. 1b). Under these conditions (+apoAI), we indeed observed an enhanced Golgi to plasma membrane transport in comparison with cells under control conditions (−apoAI) (Fig. 3a), similar to what was observed in macrophages. To confirm that increased vesicular transport in the presence of apoAI is related to cholesterol efflux, we then measured Golgi to plasma membrane transport in Tangier fibroblasts. These cells are incapable of apoAI-mediated cholesterol efflux due to functional ABCA1 mutations (Fig. 1b). Indeed, we could not detect any change in vesicular transport in Tangier cells when these cells were incubated with apoAI (Fig. 3b). This observation was further supported by biochemical analysis. YFP-GPI on the cell surface can be cleaved by a membrane-impermeable PI-PLC to release YFP to the medium (10). Fibroblasts were transfected with YFP-GPI and underwent a 19.5 °C temperature block to accumulate YFP-GPI in the Golgi. Cells were then switched to 32 °C for 20 min. PI-PLC was applied to cleave the GPI protein that had reached cell surface during 32 °C incubation, and media were collected to quantify YFP using a fluorometer. Consistent with our imaging analysis with individual cells, we found twice as much YFP in the medium from normal fibroblasts exposed to apoAI relative to control (−apoAI) (Fig. 3c). Tangier cells again showed no response to apoAI (Fig. 3b). The increased YFP-GPI...
transport from the Golgi to the plasma membrane in the presence of apoAI is therefore associated with functional ABCA1 and cholesterol efflux.

We also analyzed the rate of transport from the Golgi to the plasma membrane using YFP-VSVG, a non-raft protein. YFP-VSVG was again expressed in fibroblasts and incubated at 19.5 °C to accumulate proteins in the Golgi. We then analyzed the exit rate of YFP-VSVG in these cells after switching to 32 °C. We found that the transport of YFP-VSVG was similarly increased in normal fibroblasts when cholesterol efflux was induced by apoAI (Fig. 3c). Tangier fibroblasts did not show any changes in vesicular transport rate upon apoAI treatment. It is thus apparent that overall vesicular transport from the Golgi to the plasma membrane is up-regulated during apoAI-mediated cholesterol efflux. Both raft-rich and raft-poor populations of secretory vesicles from the Golgi are likely involved in this process. We therefore conclude that vesicular transport from the Golgi to the plasma membrane may participate in transporting lipids, including cholesterol, to apoAI on the cell surface. Functional ABCA1 is required for this up-regulation of vesicular transport in response to apoAI.

**DISCUSSION**

In the present study, we demonstrated that vesicular transport is responsive to apoAI-mediated cholesterol efflux. In particular, Golgi to plasma membrane transport is increased during this cholesterol export process. A functional ABCA1 or active cholesterol efflux is required for this enhancement as Tangier cells fail to show any alterations in vesicular transport. This conclusion was not only supported by two independent vesicular markers, YFP-GPI and YFP-VSVG, but also by both quantitative fluorescent imaging analysis and biochemical assays. Interestingly, both raft-rich (YFP-GPI) and raft-poor (YFP-VSVG) vesicle populations budding from the Golgi are similarly accelerated by apoAI-mediated cholesterol efflux, indicating a general accelerated membrane trafficking to the plasma membrane.

As lipids are the most abundant component of these transporting vesicles, increased vesicular transport means that more lipids including cholesterol are delivered to the plasma membrane during apoAI-mediated cholesterol efflux. Our results thus suggest that vesicular transport from the Golgi to the plasma membrane is one of the means for cells to move stored cholesterol to the cell surface where it can be transferred to the cholesterol acceptor, apoAI. It is unlikely, however, that this vesicular transport is the sole pathway responsible for cholesterol export, since BFA only partially inhibited cholesterol efflux. This suggests that other pathways must be working in parallel to supply lipid including cholesterol to the plasma membrane during efflux. Cholesterol, for instance, can traffic through the cytoplasm to different cellular locations via carrier proteins such as sterol-carrier protein 2 (SCP2) (11) or caveolin (12). This would provide a non-vesicular transport pathway to translocate intracellular stored cholesterol to the cell surface. This scenario is consistent with what is known for newly synthesized cholesterol transport (13). The relative contribution from different transport components, such as non-vesicular versus vesicular, may vary to facilitate the cholesterol flow during apoAI-mediated cholesterol efflux at a time when cells are transporting more cholesterol to the cell surface.

It is interesting that ABCA1 has a direct impact on the up-regulation of Golgi to plasma membrane trafficking during apoAI-mediated cholesterol efflux. Non-functional mutations in ABCA1 as in Tangier fibroblasts not only abolish cholesterol efflux, but also diminish apoAI-induced enhancement in vesicular transport from the Golgi to the plasma membrane. It is not clear how ABCA1 up-regulates vesicular transport from the Golgi. It is likely, however, that apoAI acquires lipids, including cholesterol, from the cell surface and this action depends on a functional ABCA1. Lipid depletion on the cell surface may initiate intracellular signaling processes that signal cells to increase the delivery machinery required to supply more lipids including cholesterol to the plasma membrane. Vesicular transport from the Golgi to the plasma membrane is one of components of this process. Without effective cholesterol depletion on the cell surface as in Tangier cells, there may be no need to up-regulate the transport system. Alternatively, ABCA1 may directly influence membrane vesiculation process at the Golgi, especially when cells are under stimulated conditions, such as apoAI-mediated efflux. This would imply that apoAI might be able to initiate a signal transduction process from the cell surface to regulate ABCA1 function in the Golgi. ABCA1 has been identified as a phosphatidylinerse flipase (14) and shown to influence vesiculation processes on the plasma membrane (15). Golgi membranes, especially the membrane in trans-Golgi network, is thought to share many characteristics, such as lipid content, with the plasma membrane. Thus, it would not be entirely surprising if ABCA1 actually functions in the Golgi to directly influence vesicular transport to the plasma membrane, even though the mechanism may not be identical as on the plasma membrane. This notion is further supported by the fact that a morphologically abnormal Golgi has been frequently observed in Tangier cells (15).

In summary, we have identified the involvement of the Golgi in ABCA1-mediated cholesterol efflux. We demonstrate for the first time that membrane trafficking from the Golgi is regulated under physiological conditions, such as apoAI-mediated efflux. As enhanced Golgi to plasma membrane trafficking likely represents only part of a complex intracellular process, it will be now important to investigate other cellular transport events that may interplay with the Golgi to facilitate cholesterol transport to the cell surface during efflux.
