Circulation of Cholesterol between Lysosomes and the Plasma Membrane*

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The cholesterol in the lysosomes of cultured human fibroblasts was determined to constitute ~6% of the cell total. This pool was enlarged by as much as 10-fold in Niemann-Pick type C cells. Certain amphiphiles (e.g. U18666A, progesterone, and imipramine) caused lysosomal cholesterol to increase to similarly high levels at a rate of ~0.8% of cell cholesterol/h. Lysosomal cholesterol accumulated even in the absence of exogenous lipoproteins. Furthermore, nearly all of the lysosomal cholesterol in both of the two perturbed systems was shown to be derived from the plasma membrane. Oxysterols known to alter cholesterol movement and homeostasis blocked lysosomal cholesterol accretion in amphiphile-treated cells, suggesting that this process is regulated physiologically.

Treating cells with amphiphiles slightly reduced the efflux of cholesterol from lysosomes and slightly increased the influx from the plasma membrane, causing the lysosomal cholesterol compartment to double in size in ~15 h. After more prolonged amphiphile treatments, a population of buoyant lysosomes appeared that exchanged cholesterol with the plasma membrane completely but slowly. Niemann-Pick type C lysosomes were similarly buoyant and sluggish.

We conclude that cholesterol circulates bidirectionally between the plasma membrane and lysosomes. The massive accumulation of lysosomal cholesterol in the perturbed cells does not appear to reflect disabled lysosomal transport but rather the formation of lysosomes modified for lipid storage, i.e., lamellar bodies.

Cellular cholesterol resides primarily in plasma membranes and in the endocytic compartments derived therefrom (1–3). Only a few percent of cell cholesterol is found in the ER1 (4) and lysosomes (5, 6), although these are the sites of its biosynthesis and assimilation from ingested LDL. The mechanisms controlling the distinctive and nonuniform distribution of cholesterol in animal cells are not well understood (3).

There is strong evidence for the transport of sterols both from the ER to the plasma membrane (7, 8) and in the opposite direction (9–11). This bidirectional flow of cholesterol could serve to inform the homeostatic elements in the ER as to the needs of the plasma membrane by setting the size of the ER cholesterol pool accordingly (4, 12). A similar bidirectional cholesterol flux may occur between the cell surface and mitochondria (13, 14).

Cholesterol is also transported rapidly from the lysosomes to the cell surface (6, 15–18). That cholesterol accumulates to excess in the lysosomes of cells treated with certain amphiphiles as well as in NPC disease has led to the suggestion that lysosomal cholesterol export is mediated by a specific, inhibited process (17–22). NPC1, a putative integral membrane protein capable of binding cholesterol, has been implicated in NPC disease (23, 24) and also could be the target of these amphiphiles.

It is reasonable to suppose that cell surface cholesterol moves to the lysosomes, given the massive internalization of the plasma membrane bilayer through the endocytic pathway (25, 26). A circuit of cholesterol between the cell surface and lysosomal compartments could provide a regulated link that apportion cholesterol between these two pools. However, such a system has not been documented. We now present evidence for a brisk circulation of cholesterol between lysosomes and plasma membranes. In addition, our results suggest that the standing pool of lysosomal cholesterol in normal and cholesterol-enriched fibroblasts is derived primarily from the plasma membrane and may be regulated.

EXPERIMENTAL PROCEDURES

Cell Culture—Human foreskin fibroblasts were obtained as described (27). NPC fibroblasts were the generous gift of Dr. Peter Pentchev (National Institutes of Health). We used NPC line 93.59 here; where tested, the findings were confirmed with line 31.23. Cells were cultured in Dulbecco’s minimal essential medium supplemented with 10% bovine fetal serum plus 100 units/ml penicillin and 100 µg/ml streptomycin sulfate. To deplete their cholesterol, cells were incubated in medium supplemented with 5% LPDS for 24 h (28). Solvent alone was added to the controls for drug treatments.

Labeling Lysosomes with [3H]Cholesterol via LDL—Plasma obtained from healthy human volunteers was labeled with [3H]cholesterol linoleate (40 Ci/mmol) from Amersham Pharmacia Biotech, as described (29). For the uptake of labeled lipoproteins, the serum in the medium was replaced with 5% LPDS. [3H]LDL was added to 40–100 µg of protein/ml, and the cells were incubated, as indicated, either at 37 °C (allowing lysosomal digestion to proceed) or at room temperature, causing ingested LDL to accumulate in a predigestive compartment (15).

Labeling of Cell Surfaces with [3H]Cholesterol—Cells were rinsed in the flask and covered with phosphate-buffered saline containing ~1 µCi of [3H]cholesterol in the benign vehicle, Triton WR-1339 (final concentration, 0.025%). The flasks were incubated for 10–15 min at 37 °C, the labeled medium was removed, the monolayers were rinsed twice with phosphate-buffered saline containing 1 mg/ml bovine serum albumin, and fresh medium was added for further incubation (11).

Sucrose Gradients (11)—Homogenates of cell suspensions were mixed throughout linear gradients of 8–48% sucrose (w/v) containing 5 mM NaP, (pH 7.5) plus 1 mM MgCl2. The gradients were centrifuged in...
Somes in fibroblasts. Cells were homogenized, spun to equilibrium on sucrose gradients, and assayed for the distribution of 5′-nucleotidase, cholesterol mass, and acid β-galactosidase.

A Beckman SW 50.1 rotor for >10^6 g_w × min at 4 °C and fractionated from the bottom.

Percoll Gradients (6)—Cell homogenates were mixed with 30% Percoll in 0.25 m sucrose, 5 mM NaPi (pH 7.5) plus 1 mM EDTA and layered on a cushion of 56% sucrose in 5 mM NaPi (pH 7.5). The tubes were centrifuged for 30 min at 35,000 rpm at 3 °C in a Beckman SW 50.1 rotor. Fraction 1 was the dense end of the gradient.

Other Assays—Lysosomal β-galactosidase (6), 5′-nucleotidase (30), galactosyltransferase (31), cholesterol mass (27), and protein (32) were determined as described.

RESULTS

Strategy

To analyze the relationship between the lysosomal and plasma membrane compartments, we followed the intracellular distribution and movement of cholesterol in normal human fibroblasts, which contain little lysosomal cholesterol, and in two perturbed fibroblast systems with increased lysosomal cholesterol. One of the perturbed systems was fibroblasts cultured from patients with NPC disease (21, 22). The other was created by treating cultured fibroblasts with class 2 amphiphiles, a diverse group that includes steroids and hydrophobic amines (14, 17–20, 33, 34). We also tested two oxysterols, potent effectors of cholesterol homeostasis and movement (4, 35), which oppose the effects of class 2 amphiphiles on cholesterol homeostasis (14).

The lysosomal cholesterol pool was labeled selectively by feeding cells LDL labeled with [3H]cholesterol linoleate (6); the plasma membrane pool was labeled with a pulse of exogenous [3H]cholesterol (11). Although plasma membrane radiocholesterol has been probed with cholesterol oxidase (Ref. 2; see also Ref. 18) and cycloextrin extraction (cf. Ref. 36), we used density gradient centrifugation to obtain a more definitive analysis of the subcellular localization of cholesterol pools. We found that the buoyant density distribution of the plasma membranes and normal (dense) lysosomes overlapped on sucrose density gradients (e.g. Fig. 1), while the buoyant density distribution of the plasma membranes and perturbed (buoyant) lysosomes overlapped on Percoll gradients (e.g. Figs. 4 and 5). However, a judicious choice of gradients allowed us to resolve these membranes in the experiments described below.

Effect of Class 2 Amphiphiles on the Distribution of Intracellular Cholesterol

Virtually all of the cholesterol in the control fibroblasts equilibrated on density gradients as a unimodal peak at ~35% sucrose, congruent with the plasma membrane marker, 5′-nucleotidase (Fig. 1; see also Ref. 30). Lysosomes, represented by acid (β-galactosidase), were typically found in a broader and somewhat denser peak, which overlapped the plasma membrane (Fig. 1).

Prolonged treatment of fibroblasts with several class 2 amphiphiles caused their cell cholesterol to increase up to 2-fold in growth medium containing the indicated amphiphiles and then extracted for analysis (see “Experimental Procedures”). Data represent duplicate assays on duplicate flasks that agreed to ±2%.

| Agent         | Concentration (µM) | Cholesterol (%) |
|---------------|--------------------|----------------|
| None          | 0                  | 42.6           |
| U18666A       | 5                  | 90.8           |
| Progesterone  | 10                 | 50.3           |
| Trifluoperazine| 15                 | 55.4           |
| Imipramine    | 25                 | 79.7           |

FIG. 1. Buoyant density profile of plasma membranes and lysosomes in fibroblasts. Cells were homogenized, spun to equilibrium on sucrose gradients, and assayed for the distribution of 5′-nucleotidase (○), cholesterol mass (●), and acid β-galactosidase (●).

FIG. 2. Buoyant density profile of organelles after prolonged treatment with U18666A. Fibroblasts were incubated for 44 h in growth medium containing 5 µM U18666A. The medium was replaced with one containing 5% LPDS plus 5 µM U18666A and LDL labeled with [3H]cholesterol linoleate (100 µg of protein/ml). After a 2-h incubation at 37 °C, the labeled medium was removed, and medium containing 10% serum plus 5 µM U18666A was added for a 28-h chase. Homogenates were then spun to equilibrium on sucrose gradients, and marker distributions were determined. A, cholesterol mass (●); galactosyltransferase (△); and [3H]cholesterol (dotted line). B, acid β-galactosidase (●) and 5′-nucleotidase (○).
The accumulation of cholesterol in dense lysosomes, resolved from plasma membranes on Percoll gradients (see below), commenced immediately upon treatment of cells with class 2 amphiphiles and proceeded at a constant uptake rate of 0.8% of total cell cholesterol per h (S.D. = 0.3% per h; n = 13; range = 0.4–1.3). The buoyant lysosome population did not appear until after 1 day of treatment. The β-galactosidase activity was never found at an intermediate buoyant density, suggesting that the two populations of lysosomes might be discrete and different.

These results corroborate earlier reports that excess cholesterol accumulates in the lumen of lysosomes in cells treated with certain amphiphiles (20), conferring unusual lysosomal buoyancy. While many of the class 2 amphiphiles are weak bases, their effect on cholesterol accumulation was probably not related to the discharge of lysosomal pH. First, progesterone and other agents that induce lipidosis are not known to be acidotropic (33). Second, none of the agents we tested reduced the punctate pattern of lysosome staining by the acidotropic fluorescent dye, acridine orange (37). Finally, it has been observed that perturbing lysosomal acidification in fibroblasts with the lysosomotropic class 2 amphiphile, chloroquine, stimulated the redistribution of lysosomal membrane markers to the plasma membrane (38), an effect opposite to that seen here for cholesterol.

**Does the Accumulation of Cholesterol in Buoyant Lysosomes Depend on Ingested LDL?**

When fibroblasts were incubated with [U-18666A] in medium containing 5% LPDS rather than 10% whole serum, their lysosomes still shifted to a more buoyant density (Fig. 3A) and accumulated extra cholesterol (Fig. 3B). While these effects were less striking than those seen in the presence of 10% whole serum (Fig. 2), they nevertheless suggest that lysosomes might receive cholesterol from a source other than ingested LDL.

**Oxysterols Counteract the Effect of Amphiphiles**

Most of the lysosomes from normal cells were dense in Percoll gradients (Fig. 4A). Treatment of cells with [U-18666A] for more than 1 day shifted most of the lysosomes from the dense to the buoyant region (Fig. 4B). This effect was reversible. For example, two-thirds of the large buoyant peak of acid β-galactosidase that formed in fibroblasts incubated with imipramine for 40 h disappeared during a subsequent overnight incubation without the agent (not shown).

Two oxysterols, 25-hydroxycholesterol and 7-ketocholesterol, abolished the buoyant peak of lysosomes both in normal cells (Fig. 4, compare A and C) and in cells treated with [U-18666A] (Fig. 4, compare B and D). [U-18666A] by itself caused the cell cholesterol to double (see the Fig. 4 legend and Table I); the oxysterols reversed this accumulation and also caused a slight diminution of the cholesterol content of the control cells. We infer that class 2 amphiphiles and oxysterols have opposing effects on cholesterol accumulation and, consequently, on lysosomal buoyancy.

These findings bear on three other issues. First, they support the premise that the action of the class 2 amphiphiles on the lysosomes is unrelated to their luminal pH, upon which oxysterols are not known to act. Second, they support the hypothesis (14) that oxysterols stimulate the movement of cholesterol between membranes, in opposition to class 2 agents, rather than acting exclusively upon homeostatic proteins in the ER (39). Finally, they suggest that oxysterols might also be found to reverse the accumulation of cholesterol in the trans-Golgi membranes and the lysosomes of NPC cells (19) and perhaps in naturally occurring lamellar bodies (see below).

**Movement of Lysosomal Cholesterol to the Plasma Membrane in Cells Treated with Amphiphiles**

Fibroblasts were preincubated for 15 h with [U-18666A] to build their pool of lysosomal cholesterol; they were then allowed to accumulate LDL labeled with [3H]cholesterol in endocytic compartments for 2 h at 22 °C (6, 15); finally, they were chased for 1 h at 37 °C. During the chase, free [3H]cholesterol was liberated from LDL in the lysosomes from whence it moved to the plasma membrane. In control experiments, we found that limiting the duration of the amphiphile pretreatment to less than 20 h avoided the formation of buoyant lysosomes (compare Fig. 5, A and C, with Fig. 4B), so that Percoll gradient centrifugation cleanly separated plasma membranes from lysosomes. It was also established that, in the absence of a chase, only a small fraction of the free [3H]cholesterol was present in the region of Percoll gradients containing the plasma membrane.

In three experiments such as that shown in Fig. 5B, 60% (S.D. = 2%; range = 59–62%) of the free [3H]cholesterol liberated from the labeled LDL ingested by untreated cells reached the plasma membrane after a 1-h chase. Similar transport rates were reported previously (15–18). In two experiments in which cells were preincubated with [U-18666A] for 14–15 h, 40 and 38% of the lysosomal [3H]cholesterol moved to the plasma membrane in the 1-h chase, as illustrated in Fig. 5D. Two earlier studies also showed modest inhibition of lysosomal export by treatment with [U-18666A] (17, 18), while another indi-
with U18666A and oxysterols. In two separate experiments, repli-
cated no reduction of the egress of lysosomal \(^{3}H\)cholesterol after a short preincubation with class 2 agents (6).

Values obtained in this way are likely to underestimate the rate of cholesterol transport, because the lysosomal probe is replenished by ongoing hydrolysis of the \(^{3}H\)cholesterol ester precursor during the 1-h chase period. In addition, the true value for cholesterol efflux should take into account the unla-
gmented radioactivity and pool sizes, we determined that, in three
experiments like that in Fig. 5, 11 % ± 3% and 10 ± 2% of total
cell cholesterol left the lysosomes of control and U18666A-
treated cells, respectively, in a 1-h incubation. We conclude
therefore that the lysosomes in cells treated for —15 h with
U18666A exhibited no significant reduction of their absolute
capacity to export cholesterol despite a moderate decrease in their fractional rate of transport.

It follows from these observations that lysosomal cholesterol export can appear to be inhibited by amphiphiles simply be-
cause of the isotope dilution of the ingested probe in the ex-
panded lysosomal cholesterol pool.

Movement of Plasma Membrane Cholesterol to Lysosomes

Exogenous pulses of \(^{3}H\)cholesterol were introduced into the surfaces of fibroblasts (11). As determined by Percoll gradient
centrifugation of normal control cells, 4.7% of the probe moved
from plasma membranes to lysosomes during a 1-h chase (S.D. = 2.3%; n = 6; range = 2.9–9.1%). Similar kinetics were
observed in cells pretreated with U18666A for 5–16 h; 5.8% of the plasma membrane \(^{3}H\)cholesterol was transported to lys-
somes in the 1-h chase (S.D. = 2.7%; n = 3; range = 3.3–8.7%). From these values, we calculated rate constants for the corre-
sponding first order transport processes of 0.048 h

Because the small size of the normal lysosomal pool under-
mind its rigorous analysis, we preincubated cells with amphi-
philes for prolonged intervals and then followed the redistribu-
tion of \(^{3}H\)cholesterol from their dense plasma membranes to
the buoyant lysosome fraction on sucrose gradients. In un-
treated cells, the probe remained congruent with the plasma
membrane (Fig. 6A; see also Ref. 30), presumably because the
small lysosomal cholesterol pool was obscured by the plasma
membrane peak. In contrast, the surface label moved to the
buoyant lysosomes in cells pretreated for 32 h with U18666A
(Fig. 6B). The time course of such redistribution is shown in
Fig. 7. The specific activity of the \(^{3}H\)cholesterol in the buoyant
lysosomes approached that of the plasma membranes with first
order kinetics and an apparent half-time of 26 h. That the specific activity ratio was initially close to 0 shows that the appearance of label in the buoyant fraction was not a gradient artifact but rather was dependent on a slow cellular process. That the ratio of specific activities approached unity in the plateau strongly implies complete exchange between the plasma membrane and lysosome pools; i.e. it appears that all of the cholesterol in the membranes and lumens of the buoyant lysosomes was derived from the plasma membrane. Similar results were obtained with imipramine (data not shown).

NPC Cells

Movement of Lysosomal Cholesterol to the Plasma Membrane—As noted previously (19), the behavior of cholesterol in NPC cells was strikingly similar to that in amphiphile-treated cells. In particular, the cholesterol content of NPC cells was 3 times higher than that of normal fibroblasts (113 ± 14 μg of cholesterol/mg of protein (n = 9) versus 37 ± 3 μg of cholesterol/mg of protein (n = 13)). Furthermore, the bulk of the lysosomal marker in NPC cells was buoyant in sucrose (Figs. 8 and 9) as well as on Percoll gradients (not shown, but see Ref. 40). Most of the cell cholesterol coincided with the buoyant lysosomal marker, while the remainder was distributed with the plasma membrane.

The transfer of LDL [3H]cholesterol from buoyant lysosomes to plasma membranes in NPC cells was much slower than normal (not shown, but see Ref. 41), and meaningful kinetic measurements were not obtained. Nevertheless, after a 7-h incubation with [3H]LDL and a 23-h chase, the [3H]cholesterol had equilibrated between the lysosomal and plasma membrane peaks (Fig. 8B), suggesting that the entire pool is dynamic.

Movement of Plasma Membrane Cholesterol to Lysosomes—[3H]cholesterol introduced into the plasma membranes of NPC fibroblasts appeared in their buoyant lysosomal fraction after a 1-h chase (Fig. 9A); eventually, the specific activity of the
the plasma membranes, which contain cholesterol/phospholipid ratio must be much lower than that in
resides in the lysosomes of normal fibroblasts, the lysosomal total membrane bilayer (26) but only 6% of the cholesterol provided further evidence that the lysosomes derived most of their
in medium lacking serum lipoproteins. This observation pro-
ilar values were obtained when the cells were grown overnight
Matched confluent NPC cell cultures were
surface-labeled with a pulse of [3H]cholesterol and chased in fresh
[3H]cholesterol (44).
lysosomes in NPC cells.
lysosomal pool approached that of the plasma membranes (Fig.
B). The kinetics of isotopic equilibration were first order, with
an apparent half-time of 35 h in several experiments (Fig. 10).

Size of the Normal Lysosomal Cholesterol Pool

The dense peak of lysosomes on Percoll gradients from fibro-
blasts grown in normal medium (10% serum) contained
roughly 10% of cellular cholesterol. However, it also contained traces of two plasma membrane constituents not normally present in lysosomes: 5'-nucleotidase (42) and [14C]cholesterol briefly delivered to intact cells on ice to block its redistribution to the cell interior (11). Correcting for plasma membrane contamination by means of these markers, we estimated that the lysosomes in normal human fibroblasts contained 6% of total cell cholesterol (S.D. = 2.3%; range = 4.1–10.2%; n = 7). Similar values were obtained when the cells were grown overnight in medium lacking serum lipoproteins. This observation provided further evidence that the lysosomes derived most of their cholesterol from sources other than ingested LDL. If 30% of the total membrane bilayer (26) but only 6% of the cholesterol resides in the lysosomes of normal fibroblasts, the lysosomal cholesterol/phospholipid ratio must be much lower than that in the plasma membranes, which contain ~90% of cell cholesterol and half its phospholipid (44).

DISCUSSION

Unlike most kinds of lysosomal cargo, which can be rapidly hydrolyzed to their water-soluble constituents, cholesterol is not broken down and exits the lysosomes intact. Most and perhaps all of this cholesterol is transported directly to the plasma membrane (6, 15–18). We have now characterized the circuit that arises from the transport of cholesterol between plasma membranes and lysosomes.

The mediator of cholesterol transport from the plasma membrane to the lysosomes could be a soluble cytoplasmic carrier protein; however, such a mechanism has not been documented convincingly (3). Perhaps more likely would be the flow of endocytic plasma membrane bilayer (45). In fact, because the observed rate of movement of plasma membrane cholesterol to lysosomes (~5%/h) matches that determined for other fibroblast plasma membrane constituents (43), no other mechanism need be postulated. Since the entire plasma membrane is internalized on the time scale of an hour in cultured fibroblasts (25), only a small portion of cell surface cholesterol appears to reach the lysosomes, the bulk presumably being intercepted by endosomal processing (cf. Refs. 25, 38, and 43). Supporting this hypothesis is evidence that other cell surface lipids are normally internalized, sorted, and delivered to lysosomes (46). Furthermore, the types of lipids that accumulate in the lysosomes of NPC cells generally correspond to those found in plasma membranes (21). There is also evidence for activities that could serve to return ingested plasma membrane constituents from the lysosomes to the cell surface (e.g. 38, 47–50). However, the degree to which lysosomal cholesterol follows such a path is unknown.

Normal cells ingested LDL at a rate of 2.0–3.7% of total cell cholesterol/h; this value was only modestly altered in the perturbed systems (not shown; see Refs. 17 and 19). Since the accretion of cholesterol in the lysosomes of cells treated with U18666A proceeded at ~0.8% of total cell cholesterol/h, all of the excess lysosomal cholesterol could have been provided by ingested LDL. On the other hand, the [3H]cholesterol derived from ingested LDL promptly left the lysosomes (Fig. 5 and Refs. 6, 15, and 16) and was replaced by a larger stream of cholesterol entering from the plasma membrane (Figs. 7 and 10). This raises the possibility that the plasma membrane is the predominant source of lysosomal cholesterol in both control and perturbed fibroblasts. Supporting the latter hypothesis is the observation that the accumulation of cholesterol in the lysosomes of cells treated with class 2 amphiphiles does not require the ingestion of LDL (Fig. 3). In addition, the size of the lysosomal pool does not fall when LDL is withheld. Furthermore, Figs. 7 and 10 show that the entire pool of cholesterol in the lysosomes is in exchange equilibrium with the plasma

![Fig. 9. Transfer of [3H]cholesterol from plasma membrane to lysosomes in NPC cells.](Image)

![Fig. 10. Kinetics of transfer of plasma membrane [3H]cholesterol to lysosomes in NPC cells.](Image)
membrane, even in cells fed saturating levels of LDL. Therefore, the size of the lysosomal pool could be set independently of cholesterol ingestion.

In the simplest case, the lysosomal pool could be set by the first-order rate constants for its equilibration with the plasma membrane. Values for these parameters were estimated from the 1-h incubation values described under “Results,” using the expression \( k = \ln(N/N_0) / t \), where \( k \) is a first-order rate constant; \( N_0 \) and \( N \) are the \(^{3}H\)cholesterol levels in a given compartment at times 0 and \( t \), respectively, and \( t \) is the chase time. For control fibroblasts, the calculated \( k \) values are 0.94 h\(^{-1}\) for lysosomal cholesterol transport to plasma membranes and 0.048 h\(^{-1}\) for plasma membrane cholesterol transport to lysosomes. The ratio of the sizes of the equilibrating compartments is given by the quotient of these rate constants (here ~20). Making the realistic assumption that almost all of the cell cholesterol was confined to these two compartments, this calculation assigns 5% of total cell cholesterol to the lysosomes. The good agreement of this value with that determined above by direct measurement, ~6%, supports the simple model of first order kinetics.

In cells treated with U18666A for 16 h or less, the first order rate constant for lysosomal cholesterol transport to the plasma membrane was 0.49 h\(^{-1}\), and that for plasma membrane cholesterol transport to lysosomes was 0.060 h\(^{-1}\). These values suggest that the amphiphile caused a slight reduction of lysosomal cholesterol efflux and a slight increase in its influx. The quotient of these rate constants, 8.2, predicts that the lysosomal cholesterol efflux and a slight increase in its influx. The good agreement of this value with that determined above by direct measurement, ~6%, supports the simple model of first order kinetics.

On the other hand, the first order model, using the kinetic constants observed after less than 16 h of treatment with U18666A, does not account for the continued accumulation of lysosomal cholesterol to massive levels. It is also not apparent why the buoyant form of lysosomes did not appear in the amphiphile-treated cells until after a lag of about a day and why lysosomes of intermediate density were not observed. It may be that the delayed appearance of buoyant lysosomes bearing the bulk of the excess cholesterol represents their transformation to a new form. Note that we cannot now ascribe the sluggish dynamics of the lysosomal \(^{3}H\)cholesterol in NPC cells and cells treated for many hours with amphiphiles (Figs. 2, 7, and 10; Refs. 21 and 36) to grossly altered transport kinetics, since they might reflect instead the isotope dilution of the probe in the large lysosomal cholesterol pool.

The new form of lysosomes appears to be lamellar bodies, long known to act as a store of membrane-derived lipids in many physiological and pathological settings (for a review, see Ref. 34). For example, lamellar bodies in various epithelial cells accumulate and secrete lipids as lung surfactant, skin coating, and joint lubricant. Other cells (e.g., macrophages in the arterial intima) gather lipid debris in their lamellar bodies, possibly for later secretion. The lamellar bodies in NPC and amphiphile-treated fibroblasts may similarly represent the cell’s attempt to reduce an abnormal endogenous cholesterol load through storage and secretion (34).

Our data therefore suggest the following sequence of events. Class 2 amphiphiles (and perhaps the genetic lesion in NPC cells) affect a central homeostatic mechanism that misdirects cells to accumulate extra cholesterol. At least in the case of the amphiphiles, an immediate and approximately constant accrual of cholesterol ensues. Part of the excess is sequestered in preexisting lysosomes by means of minor physiological adjust-ments in the kinetic constants for its transport. These lysosomes remain dense even as they accumulate cholesterol (e.g. Fig. 5). Lamellar bodies appear as buoyant lysosomes after about 1 day of treatment and eventually acquire the bulk of the excess cholesterol.

Our findings also suggest a parallel in the mechanisms of cholesterol management by the ER, mitochondria, and lysosomes. First, there seems to be a circulation of cholesterol between the plasma membrane and each of these three cytoplasmic organelles (see “Results” and Refs. 12 and 14). Second, class 2 amphiphiles appear to perturb the cholesterol flux between the plasma membrane and each of these organelles. Third, in each case, oxysterols counter the action of the amphiphiles. Finally, the defect in NPC disease appears to affect not just lysosomal cholesterol but also that in the ER (19, 40).

NPC1, the protein defective in NPC disease (23, 24), could participate in the transfer of plasma membrane cholesterol to the ER and mitochondria as well as the export of lysosomal cholesterol to the plasma membrane. It could act directly in intermembrane cholesterol transport; alternatively, it could serve a regulatory sensor function. In either case, NPC1 activity might be altered, directly or indirectly, by interactions with class 2 amphiphiles and oxysterols. For example, if the transport of lysosomal cholesterol to the cell surface were to utilize the return pathway for endocytosed plasma membrane bilayer, NPC1 might manage the entry of cholesterol into that stream. Alternatively, the pool of lysosomal cholesterol or the formation of lamellar bodies were physiologically regulated, lysosomal cholesterol accumulation in NPC disease and in the lipidoses could represent a secondary response to a more central defect.

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