FORUM *hypothesis*

Jamming the endosomal system: lipid rafts and lysosomal storage diseases

Kai Simons and Jean Gruenberg

Some lysosomal storage diseases result from the accumulation of lipids in degradative compartments of the endocytic pathway. Particularly striking is the example of the Niemann–Pick (NP) syndrome. NP syndromes types A and B are characterized by the accumulation of sphingomyelin, whereas cholesterol typically accumulates in NP type C. These two different lipids, sphingomyelin and cholesterol, are normal constituents of specific lipid microdomains called rafts. Because accumulation of raft lipids is observed not only in NP diseases but also in many other lipidoses, we forward the hypothesis that lysosomal storage diseases can be caused by the accumulation of lipid rafts in late endosomes/lysosomes.

Lipid rafts are assemblies of sphingolipids (sphingomyelin and glycosphingolipids) and cholesterol, forming a separate liquid-ordered phase in the liquid-disordered matrix of the lipid bilayer[1,2]. The preferential packing of sphingolipids with cholesterol is facilitated by the long saturated hydrocarbon chains of the sphingolipids, and perhaps also by intermolecular hydrogen bonds, especially those involving the carbohydrate head groups of the glycosphingolipids – although the role of the latter mechanism is controversial[3]. The size of the individual rafts is small and below the resolution of light microscopes: most recent estimates are about 50 nm for the diameter[4]. Rafts function as platforms to which distinct classes of proteins are associated, such as glycosylphosphatidylinositol (GPI)-anchored proteins, transmembrane proteins (often palmitoylated) and di-acylated proteins.

How lipid rafts are handled and transported in the endocytic pathways is not well understood. Rafts can be internalized individually through clathrin-coated vesicles, but there might also be, at least in some cell types, raft pathways for endocytosis. For instance, caveolae comprise lipid rafts that form invaginations on the cell surface and contain the cholesterol-binding protein caveolin[5]. These structures seem to have the capacity to be internalized, especially in endothelial cells, where they have been implicated in transcytosis. In most cells, endocytosed molecules, including raft constituents, are delivered to endosomes. From there, raft components can be returned to the cell surface to be reutilized or transported towards late endosomes/lysosomes[6]. Several lines of evidence suggest that lipid rafts are restricted from entering the degradative compartments, at least in some cell types[7]. Studies using fluorescent sphingolipid derivatives showed that, once internalized, they are rapidly returned back to the cell surface via recycling endosomes, much like recycling receptors. Internalized GPI-anchored proteins follow the same pathway, albeit with slower kinetics, and their retention in endosomes depends on membrane cholesterol[8]. In addition, recent data demonstrate that recycling endosomes in Madin–Darby canine kidney (MDCK) cells are enriched in both sphingolipids and cholesterol[9], whereas these lipids are depleted from late endosomes and lysosomes in some mammalian cells[7]. Also, in the yeast *Saccharomyces cerevisiae*, the vacuole contains little ergosterol and sphingolipid[10]. This suggests that endocytosed raft lipids are not distributed stochastically within endosomal membranes and thus raises the question of whether they are sorted away from the pathway leading to degradation. By contrast, Mukherjee *et al.* found that the lipid analogue DiC16, which had previously been shown to partition into lipid rafts on the cell surface[11], is delivered to late endosomes in Chinese hamster ovary (CHO) cells. One possibility is that cell-type-specific differences in trafficking might account for this apparent discrepancy because endosomal organization varies quite significantly in different cell types[12,13]. Alternatively, we can speculate that DiC16 is not a natural raft lipid and might be sorted on the basis of its structure. While rafts might be excluded from the degradation pathway, individual lipids might be selectively incorporated into (or excluded from) the highly curved membranes that invaginate within the lumen of endosomes along the degradation pathway[14].

Lysobisphosphatidic acid-rich membranes of late endosomes

One lipid that is enriched in late endosomes is lysobisphosphatidic acid (LBPA), which is a characteristic molecule of this degradative organelle in the endocytic pathway[15]. LBPA is localized to the complex system of membranes present in the lumen of late endosomes and is an abundant constituent of these membranes (4–17 mole percent)[15]. A unique property of LBPA is that the lipid is a poor substrate for phospholipases and hence is resistant to lysosomal enzyme degradation[16]. The lipid is presumably synthesized *in situ* within the acidic organelles of the endocytic pathway[17] and has an inverted cone shape. This structure might facilitate the formation of the invaginations that form the multivesicular elements of late endosomes and multivesicular bodies[7]. Sandhoff and collaborators have demonstrated

Kai Simons is at the Max Planck Institute for Molecular Cell Biology and Genetics, Pfotenhauerstrasse, 01307 Dresden, Germany, and EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany; and Jean Gruenberg is in the Dept of Biochemistry, Sciences II, University of Geneva, 30 quai E. Ansermet, 1211, Geneva 4, Switzerland. E-mail: jean.gruenberg@biochem.unige.ch

 0962-8924/00/$ – see front matter
PII: S0962-8924(00)01847-X

TABLE 1 – LIPID AND PROTEIN DISTRIBUTION IN SPHINGOLIPIDOSES

Disease cells	BODIPY-LacCer in lysosomes	Protein redistribution	Cholesterol accumulation
Fabry's disease	+		+
GM1 gangliosidosis	+	+ (NPC1)	+
GM2 gangliosidosis (Tay–Sachs)	+		–
GM2 gangliosidosis (Sandhoff)	+		+
Mucolipidosis type IV	+		+
Metachromatic leucodystrophy	+		+
Niemann–Pick type A	+	+ (NPC1)	+
Niemann–Pick type B	+		+
Niemann–Pick type C	+	+ (MPR)	+
Prosaposin deficiency	+		+

This table summarizes data published mostly by Pagano and his collaborators. They showed that the fluorescent analogue of lactosylceramide, BODIPY-LacCer accumulated in the lysosomes of fibroblasts from patients with the indicated sphingolipidosis, whereas it is routed to the Golgi complex in control cells[31]. Cholesterol accumulation occurred in all sphingolipidoses tested, except Tay–Sachs[30], in agreement with Ref. 20. The distribution of the NPC1 protein was changed in GM1 gangliosidosis and NP type A[30]. The mannose-6-phosphate receptor (MPR) was redistributed to late endosomes in NP type C fibroblasts, whereas it was found in the *trans*-Golgi network of control fibroblasts[15].

that incorporation of negatively charged phospholipids, in particular LBPA itself, into liposomes containing glucosylceramide greatly facilitates the degradation of the glycosphingolipid by glucosylcerebrosidase and sphingolipid activator protein C (Ref. 18). LBPA also facilitates the degradation of GM2 by hexosaminidase A and GM2-activator[19], as well as ceramide by acid ceramidase/SAP-B and sphingomyelin by acid sphingomyelinase (K. Sandhoff, pers. commun.). LBPA seems not only to play a role in facilitating sphingolipid degradation but functions also in cholesterol efflux from late endosomes/lysosomes[20]. If antibodies against LBPA are internalized by fluid-phase endocytosis, they bind to LBPA and accumulate in late endosomes. Under these conditions, cholesterol released from low-density lipoprotein (LDL) remains trapped in the late endosomes and cannot be transported out from this organelle as would normally occur if the antibody were absent. The network of membrane tubules and vesicles within the lumen of late endosomes might thus have an important function in sphingolipid degradation and cholesterol distribution in the cell. Accumulation of endocytosed antibodies against LBPA also results in the defective sorting/trafficking of proteins that transit via late endosomes, presumably because membrane properties are altered[15]. The function and maintenance of the highly curved membrane structures are still poorly understood, but obviously LBPA membrane domains do contribute to the selectivity in handling of lipid rafts in the endocytic pathway. Alterations in these processes appear to cause not only accumulation of lipid rafts but also result in disturbances in protein traffic.

Lipid storage diseases

A relatively large subgroup of lysosomal storage diseases are caused by the accumulation of lipids in late endosomes or lysosomes[21,22]. The Niemann–Pick (NP) syndrome, which is now known to have more than one cause[23,24], is of particular interest; NP types A and B are characterized by sphingomyelin accumulation, whereas cholesterol typically accumulates in the third form, NPC. In NPC, accumulation of cholesterol in degradative compartments of the endocytic pathway is apparently due to a failure in the mechanism responsible for redistribution of cholesterol taken up by endocytosis of LDL, in contrast to other storage diseases caused by defective metabolic enzymes. Recent studies showed that the disease can be caused by mutations in the gene encoding NPC-1 (Refs 25 and 26). Although the precise function of the NPC-1 protein is unclear, it shares a cholesterol-sensing domain with proteins involved in cholesterol homeostasis [HMG-CoA reductase and SCAP, the sterol-regulatory-element-binding protein (SREBP) cleavage-activating protein] and with the morphogen receptor Patched. One interesting feature of NPC is that, in addition to the major defect in cholesterol transport, sphingolipids also accumulate in the multivesicular compartments of the endocytic pathway[27–29]. This varies from tissue to tissue. In neurons, the gangliosides GM3 and GM2 have been reported to accumulate, whereas, in spleen, sphingomyelin is increased. Conversely, in NPA disease, which results from a defect in sphingomyelinase, sphingomyelin accumulation is accompanied by cholesterol accumulation in the spleen and in the liver[27,30]. Thus, it seems as though increased storage of one raft lipid can lead to the concomitant increase of other raft lipids.

This is true not only for the NP diseases: other sphingolipid storage diseases accumulate different sphingolipid classes in late endosomes/lysosomes. It turns out that cells from patients with sphingolipidoses also exhibit increased cholesterol levels in endocytic organelles (revealed by staining cholesterol with filipin)[30]. One notable exception is Tay–Sachs disease[20,30] (see Table 1). Pagano and collaborators have made the interesting observation that the trafficking of a fluorescent derivative of lactosylceramide, a glycosphingolipid carrying the fluorescent BODIPY moiety in the fatty acid amide bonded to sphingosine, is disturbed in both cholesterol and sphingolipid storage disorders[31] (Table 1). They have demonstrated that BODIPY lactosylceramide, after insertion into the plasma membrane of normal fibroblasts, is internalized and accumulates in the Golgi complex. In the lipid storage diseases, however, it enters into late endosomes/lysosomes instead. This simple assay can be used in clinical diagnosis. Although the mechanism involved is not

known, this different internalization behaviour might reflect disturbed functions of the degradative endocytic pathway.

A unified working hypothesis

These findings led us to formulate a model for the normal handling of lipid rafts in the endocytic pathway, which might serve to clarify some of the problems associated with lipid storage diseases. The basis of our postulate is that the amounts of raft lipids tolerated by late endosomes are limited. In some cell types, with a high hydrolytic capacity, sphingolipids might be transported to late endosomes and then be rapidly degraded after incorporation into LBPA-internal membranes, where the lysosomal hydrolysis mechanisms are probably mostly operating. It is also possible that sphingolipids can be partially recycled after reaching late endosomes. Alternatively, endocytosed sphingolipids might be excluded from entering the pathway to late endosomes and lysosomes, the small amounts still transported to late endosomes accounting for normal turnover. In any case, the idea that we propose is that degradation of sphingolipids is normal when cholesterol is exiting normally from late endosomes. Similarly, cholesterol removal from late endosomes would operate normally only when amounts of sphingolipid in late endosomes are low. In lipid storage diseases involving raft lipids, the accumulation of one raft lipid class – for example cholesterol, sphingomyelin or glucosylceramide – would slowly lead to trapping of other raft lipids in late endosomes. Deregulated accumulation of either raft lipid would then jam both sphingolipid degradation and cholesterol transport processes.

Raft lipid accumulation in late endosomes/lysosomes is also likely to lead to a traffic jam[32] affecting the distribution of other lipids and proteins. Three, not mutually exclusive, processes might contribute to protein mislocalization.

- First, mistargeting of raft components in lipid storage disorders might cause some proteins, which would normally be associated with rafts in the peripheral plasma membrane or early endosomal circuit[2,7], to be redistributed to late endosomes and lysosomes. Recent studies indicate that annexin II is redistributed from its normal localization in early endosomes and the plasma membrane, to late endosomes in NPC cells (N. Mayran, R. Parton and J. Gruenberg, unpublished).
- Second, raft lipid accumulation is expected to alter the properties of late endosomal/lysosomal membranes, including LBPA-rich membranes, so that they start to form lysosomes containing lipid lamellae characteristic of lipid storage diseases[21]. This trapping could be caused by the preferential association of sphingolipids with cholesterol[30], which is the driving force for raft assembly. Raft accumulation might flatten the highly curved internal membranes within late endosomes and transform the internal membranes into lamellae. This accumulation and the resulting transformation would interfere with the normal sorting/trafficking capacity of this organelle, as appears to be the case in NPC fibroblasts[20]. Indeed, the mannose 6-phosphate receptor is found in late endosomes in NPC fibroblasts but is in the Golgi complex in normal fibroblasts. Also, the distribution of the NPC-1 protein is altered in GM1 gangliosidosis and NP type A[30].
- Finally, one can predict that such a protein/lipid traffic jam in lipid storage disorders results in more general perturbations of late endocytic functions, including lysosome biogenesis and autophagy.

Perspectives

Beyond the striking similarities in raft lipid redistribution in lipid storage diseases, great variations are observed in the types of affected tissues and in the clinical pictures. These variations might be caused by cell-type-specific expression of sphingolipids, as well as by differences in the residual activity of an affected enzyme (threshold theory), which might lead to an adult (high residual activity) or infantile (low residual activity) onset of the disease (discussed in Ref. 22). Also, we know little about the functional importance of different glycolipid head groups. Different glycosphingolipids might interact specifically with different proteins and interfere with their function individually. Lipid rafts play a key role not only in intracellular transport and protein sorting but also in many signal-transduction processes[33]. Thus the disturbed distribution of raft lipids and proteins that could result from the jamming of the endocytic pathways might lead to functional impairment of cellular signalling. Also, the regulation of the immune responses is dependent on normal raft function. The variable effects on these and other possible targets might explain some of the different clinical outcomes of raft lipid storage diseases.

In the secretory and endocytic pathways, the late endosomes/lysosomes are not the only compartments with a low raft lipid content – this characteristic is also shared by the endoplasmic reticulum (ER). Although the mechanisms responsible for raft exclusion are still not known, it seems that, in both cases, the raft lipid content in the membranes is used as a sensing device in biosynthesis or in degradation. The late endosomes/lysosomes constitute the degradative compartments for sphingolipids. Degradation depends on many factors, such as the overall load of raft lipids being endocytosed and recycled, and the balance between cholesterol and sphingolipids. Raft lipids not associated with rafts in the sorting endosomes would be allowed to enter into the degradative pathway, and, in this way, the correct balance for each lipid class that forms rafts might be maintained.

The ER, by contrast, constitutes the sensing station for cholesterol levels. A decrease in cholesterol levels beyond a given threshold causes cleavage of the transmembrane precursor of the SREBP transcription factor, which is then free to activate genes involved in cholesterol biosynthesis[34]. It has been demonstrated that hydrolysis of sphingomyelin at the plasma membrane with exogenous

sphingomyelinase causes the cell to react as if cholesterol levels had been increased[35]. In addition, recent studies show that ER cholesterol content is tightly regulated by plasma membrane cholesterol[36]. A simple interpretation is that, when the cholesterol-binding capacity of rafts is decreased, cholesterol is released and transported to the ER, where increased levels are registered by the sensing system. Obviously, more work will be necessary to define how cholesterol and sphingolipids are distributed in cells and how the regulation of their location affects cellular function. Clearly, one might also expect cholesterol-sensing mechanisms to be somehow affected in lysosomal storage disorders.

We speculate that endosomal traffic jams caused by raft accumulation in the degradative compartments might contribute to the clinical features associated with each lysosomal storage disorder. Altered trafficking and mistargeting of proteins might be modulated in more or less subtle ways in various disorders, accounting for the complex spectrum of pathologies.

References

1 Brown, D.A. and London, E. (1998) Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* 14, 111–136
2 Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. *Nature* 387, 569–572
3 Ostermeyer, A.G. *et al.* (1999) Glycosphingolipids are not essential for formation of detergent-resistant membrane rafts in melanoma cells. Methyl-beta-cyclodextrin does not affect cell surface transport of a GPI-anchored protein. *J. Biol. Chem.* 274, 34459–34466
4 Pralle, A. *et al.* (2000) Sphingolipid-cholesterol rafts diffuse as small entities in the plasma membrane of mammalian cells. *J. Cell Biol.* 148, 997–1008
5 Parton, R.G. Caveolae and caveolins. (1996) *Curr. Opin. Cell Biol.* 8, 542–548
6 Gruenberg, J. and Maxfield, F. (1995) Membrane transport in the endocytic pathway. *Curr. Opin. Cell Biol.* 7, 552–563
7 Kobayashi, T. *et al.* (1998) Lipids and lipid domains in endocytic membrane traffic. *Semin. Cell Dev. Biol.* 9, 517–526
8 Mayor, S. *et al.* (1998) Cholesterol-dependent retention of GPI-anchored proteins in endosomes. *EMBO J.* 17, 4626–4638
9 Gagescu, R. *et al.* (2000) The recycling endosome of MDCK cells is a mildly acidic compartment rich in raft components. *Mol. Biol. Cell* 11, 2775–2791
10 Zinser, E. and Daum, G. (1995) Isolation and biochemical characterization of organelles from the yeast, *Saccharomyces cerevisiae*. *Yeast* 11, 493–536
11 Thomas, J.L. *et al.* (1994) Large-scale co-aggregation of fluorescent lipid probes with cell surface proteins. *J. Cell Biol.* 125, 795–802
12 Geuze, H.J. (1998) The role of endosomes and lysosomes in MHC class II functioning. *Immunol. Today* 19, 282–287
13 Tooze, J. and Hollinshead, M. (1991) Tubular early endosomal network in AtT20 and other cells. *J. Cell Biol.* 115, 635–654
14 Mukherjee, S. and Maxfield, F.R. (2000) Role of membrane organization and membrane domains in endocytic lipid trafficking. *Traffic* 1, 203–211
15 Kobayashi, T. *et al.* (1998) A lipid associated with the antiphospholipid syndrome regulates endosome structure/function. *Nature* 392, 193–197
16 Brotherus, J. *et al.* (1974) Novel stereochemical configuration in lysobisphosphatidic acid of cultured BHK cells. *Chem. Phys. Lipids* 13, 178–182
17 Amidon, B. *et al.* (1996) Transacylase and phospholipases in the synthesis of bis(monoacylglycero)phosphate. *Biochemistry* 35, 13995–14002
18 Wilkening, G. *et al.* (1998) Lysosomal degradation on vesicular membrane surfaces. Enhanced glucosylceramide degradation by lysosomal anionic lipids and activators. *J. Biol. Chem.* 273, 30271–30278
19 Kolter, T. *et al.* (1999) Recent advances in the biochemistry of glycosphingolipid metabolism. *Biochem. Soc. Trans.* 409–415
20 Kobayashi, T. *et al.* (1999) Late endosomal membranes rich in lysobisphosphatidic acid regulate cholesterol transport. *Nat. Cell Biol.* 1, 113–118
21 Neufeld, E.F. Lysosomal storage diseases. (1991) *Annu. Rev. Biochem.* 60, 257–280
22 Kolter, T. and Sandhoff, K. (1998) Recent advances in the biochemistry of sphingolipidoses. *Brain Pathol.* 8, 79–100
23 Liscum, L. and Klansek, J.J. (1998) Niemann-Pick disease type C. *Curr. Opin. Lipidol.* 9, 131–135
24 Kolodny, E.H. (2000) Niemann-Pick disease. *Curr. Opin. Hematol.* 7, 48–52
25 Carstea, E.D. *et al.* (1997) Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. *Science* 277, 228–231
26 Loftus, S.K. *et al.* (1997) Murine model of Niemann-Pick C disease: mutation in a cholesterol homeostasis gene. *Science* 277, 232–235
27 Stanbury, J.B. *et al.* (1983) *The Metabolic Basis of Inherited Disease.* (Fifth edn) pp. 820–969, McGraw-Hill
28 Watanabe, Y. *et al.* (1998) Increased levels of GM2 ganglioside in fibroblasts from a patient with juvenile Niemann-Pick disease type C. *Brain Dev.* 20, 95–97
29 Vanier, M.T. (1999) Lipid changes in Niemann-Pick disease type C brain: personal experience and review of the literature. *Neurochem. Res.* 24, 481–489
30 Puri, V. *et al.* (1999) Cholesterol modulates membrane traffic along the endocytic pathway in sphingolipid-storage diseases. *Nat. Cell Biol.* 1, 386–388
31 Chen, C-S. *et al.* (1999) Broad screening test for sphingolipid-storage diseases. *Lancet* 354, 901–905
32 Liscum, L. (2000) Niemann-Pick type C mutations cause lipid traffic jam. *Traffic* 1, 218–225
33 Simons, K. and Toomre, D. Lipid rafts and their role in signal transduction. *Nat. Rev. Mol. Cell. Biol.* (in press)
34 Brown, M.S. and Goldstein, J.L. (1997) The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 89, 331–340
35 Slotte, J.P. and Bierman, E.L. (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
36 Lange, Y. *et al.* (1999) Regulation of endoplasmic reticulum cholesterol by plasma membrane cholesterol. *J. Lipid Res.* 40, 2264–2270