Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies

Caroline Subra a, Karine Laulagnier b, Bertrand Perret a, Michel Record a, *

a INSERM U563, Département “Lipoprotéines et Médiateurs Lipidiques”, CPTP, CHU Purpan, Place Baylac, BP 3028, 31024 Toulouse Cedex3, France
b Département de Biochimie, Université Sciences II, 30 quai Ernest Ansermet, 1211 Genève 4, Switzerland

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Abstract

Exosomes are part of the family of “bioactive vesicles” and appear to be involved in distal communications between cells. They vehiculate bioactive lipids and lipolytic enzymes and their biogenesis require specific lipids and a membrane reorganisation. Their biogenesis pathway could be a way to secrete enzymes involved in lipid signalling and to generate “particulate agonists”. However, this pathway seems also to be used by pathogens such as HIV. This review will consider several aspects of lipidomics studies which might help to understand the fate and role of these fascinating vesicles.

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1. Introduction

Bioactive vesicles are receiving increasing interest since they are an important process to enhance life diversity. Exosomes are the only type of bioactive vesicles originating from an intracellular compartment, namely MultiVesicular Bodies (MVB, or Late Endosomes) and released out of the cell. Several reviews describing “the exosome pathway” are now available [1,2]. First observed in 1987 during reticulocyte maturation [3] and extensively investigated in that field [4], the interest towards these vesicles was triggered again 10 years later, when it was observed they were enriched in MHC II and released out of B lymphocytes following cell activation [5]. The strong potency of exosomes loaded with a specific antigen to eradicate tumors in mice was demonstrated two years later [6]. Phase I clinical trials were completed a year ago in humans, and exosomes appear as a promising tool for autologous treatments in cancer [7].

In that context exosome lipidomics appears as a requirement for the elaboration of “reconstituted exosomes”, with the purpose of elaborating a tool in cancer treatment independent of any biological source. Vehiculating therapeutic drugs by encapsulation inside a liposome has been the dream of lipidologists for decades and many attempts are currently undergoing [8]. In all cases, lipid composition of liposomes has been empirical, based on the knowledge acquired for years on lipid organisation in membranes and subsequent physicochemical properties. Instead, exosomes offer the first opportunity of a “therapeutic vesicle” whose lipid composition and organisation could be a starting point to devise efficient liposomes, vehiculating tumor antigens to boost the immune response towards tumors.

During these last years, lipidomics has appeared as an emerging field, since genomics and proteomics could not bring
all the responses scientists were expecting in the perspective to cure a panel of pathologies [9]. The lack of powerful tools to analyse the complexity of lipids has been overcome by novel analytical approaches, including liquid chromatography coupled to powerful mass spectrometry equipments [10] easier to use than in the past, allowing for example the elaboration of “lipid arrays” [11]. This review will summarize the present knowledge on exosome lipidomics, in order to better understand how to regulate their biogenesis which undoubtedly requires appropriated lipid mediators.

2. Analytical lipidomics

This part will consider the lipid composition and membrane organisation of exosomes.

2.1. Phospholipid composition

Three major works in this field have been performed so far, on exosomes derived either from guinea-pig reticulocytes (1989) [12], or from a B lymphocyte cell line (2003) [13], a rat mast cell line or from human dendritic cells (2004) [14].

The phospholipid composition of reticulocyte-derived exosomes appears quite similar to that of plasma membrane (see Table 1), which is identical to the phospholipid composition of erythrocytes. No increase in cholesterol/phospholipid ratio was observed in exosomes as compared with the parent cells, at the opposite of what was reported for B cell-derived exosomes whose ratio was increased by three times (Table 1). In the latter case, however, only MHCII-enriched exosomes were considered (Table 1) since a complete phospholipid composition of the other subpopulations was not reported [13]. We have shown that mast cell-derived exosomes contain at least three subpopulations according to the type of protein and lipid content [15]. Considering the bulk of mast cells (RBL-2H3)-derived exosomes, we have observed no increase in the cholesterol/phospholipid ratio compared with parent cells. However, this ratio is fairly low in mast cells when comparing to other analysis reported in reticulocytes and B lymphocyte cell line, but this observation does not exclude the clustering of cholesterol molecules that was detected by immunoelectron microscopy in B cell-derived exosomes [13]. In that respect exosomes released by reticulocytes contain typical rafts [16]. Exosomes are distinct from rafts, as we reported from the lipidic composition of mast cell-derived exosomes which feature a sphingomyelin/cholesterol/glycerophospholipids molar ratio of 1/1/4, respectively, whereas the corresponding molar ratio in rafts would be 1/2.2/1.3 [14]. In addition rafts exhibit

Table 1
Comparative phospholipid composition of exosomes and their parent cells

<table>
<thead>
<tr>
<th></th>
<th>SM</th>
<th>PC</th>
<th>PE</th>
<th>PS + PI</th>
<th>LPC</th>
<th>CHOL/Plip</th>
<th>Plip/Prot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exosomes</td>
<td>15.9</td>
<td>44.4</td>
<td>23.9</td>
<td>15.8</td>
<td></td>
<td>0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25</td>
</tr>
<tr>
<td>Parent cells</td>
<td>12.1</td>
<td>43.2</td>
<td>28.3</td>
<td>16.4</td>
<td></td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>1.31</td>
<td>1.03</td>
<td>0.84</td>
<td>0.96</td>
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<tr>
<td>Mast Cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exosomes</td>
<td>14</td>
<td>33</td>
<td>27</td>
<td>18</td>
<td>7</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td>Parent cells</td>
<td>5</td>
<td>50</td>
<td>25</td>
<td>15</td>
<td>5</td>
<td>0.18</td>
<td>0.35</td>
</tr>
<tr>
<td>Ratio</td>
<td>2.8</td>
<td>0.66</td>
<td>1.08</td>
<td>1.2</td>
<td>1.4</td>
<td>1</td>
<td>0.48</td>
</tr>
<tr>
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<td>Exosomes</td>
<td>20</td>
<td>26</td>
<td>26</td>
<td>19</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parent cells</td>
<td>9</td>
<td>43</td>
<td>23</td>
<td>12</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>2.2</td>
<td>0.6</td>
<td>1.13</td>
<td>1.66</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MHC II exosomes</td>
<td>39.7</td>
<td>35.1</td>
<td>25.2</td>
<td>0.73&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Ratio</td>
<td>2.3</td>
<td>0.76</td>
<td>0.7</td>
<td>3.04</td>
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</table>

Results are expressed in moles percent of total phospholipids. Cholesterol/phospholipid values are expressed as molar ratio “b” (it is not known whether values “a” and “c” represent mass or molar ratios). Phospholipid/protein are mass ratios (w/w). Values in italics represent ratios between exosomes over parent cell phospholipid contents. Note the decrease in PLIP/protein ratio in RBL-2H3 mast cells exosomes (last column). EthLip, ether lipids; CHOL, cholesterol; PLIP, phospholipids; Prot, proteins; SM, sphingomyelins; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PS, phosphatidylserines; PI, phosphatidylinositol; LPC, lysophosphatidylcholines.
a low protein-to-lipid ratio and float on the top of a density gradient (average density of 1.07 [17]), whereas it is quite the opposite for exosomes which feature a high protein-to-lipid ratio (Table 1) and a buoyant density of 1.12–1.15 [18] from cells or 1.21–1.28 from blood [19].

The exosome biogenesis pathway appears to be “hijacked” by pathogens such as HIV, viruses budding inside the MVB similarly as exosomes [20]. The comparison between HIV lipid composition and the parent T cell line [21] exhibits similarities with exosome lipid composition reported in Table 1, specifically with B cell-derived exosomes which are enriched in cholesterol.

Cholesterol enrichment has been reported to occur in MVB intralumenal vesicles, which are precursors of exosomes, in the RN B cell line. They appear to concentrate 63% of the cholesterol located within the endocytic track [22]. LBPA, which has been claimed to associate with cholesterol [23], starts to accumulate in MVBs (14% of total phospholipids in late endosomes [MVB] versus 1.3% in early endosomes of BHK cells), and could be prerequisite for intralumenal MVB vesicle formation. No enrichment in this phospholipid was noticed in biochemical analysis of mast cell (RBL-2H3)-derived exosomes as well as in B cell exosomes [13,14]. It seems conceivable that LBPA remains on the MVB limiting membrane once the intralumenal vesicle has been pinched off.

Detailed composition of RBL and DC-derived exosomes is presented in Table 2. We observed a noticeable amount of disaturated molecular species in phosphatidylethanolamine and phosphatidylcholine classes. Disaturated PC and PE increase by 2.6 and 3.7 times, respectively, as compared to parent cells, similarly to the increase in disaturated PC reported for HIV (Fig. 1) [21]. This observation strengthens the “Trojan horse hypothesis” [24] suggesting that HIV would be an “exosome-like” particle.

Noteworthy, we observed that the amount of diglycerides was two times less in mast cell (RBL-2H3)-derived exosomes than in parent cells [14]. Diglycerides have been shown to modulate phospholipid membrane packing, and their limited amount in exosome membrane together with a high content in disaturated species suggested an elevated membrane rigidity.

<table>
<thead>
<tr>
<th>Table 2</th>
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</thead>
<tbody>
<tr>
<td>Typical lipid composition of mast cell (RBL-2H3)-derived exosomes</td>
</tr>
<tr>
<td>Lyso-phosphatidylcholine</td>
</tr>
<tr>
<td>Sphingomyelin</td>
</tr>
<tr>
<td>Disaturated phosphatidylcholine</td>
</tr>
<tr>
<td>Phosphatidylcholine (other species)</td>
</tr>
<tr>
<td>Disaturated phosphatidylethanolamine</td>
</tr>
<tr>
<td>Phosphatidylethanolamine (other species)</td>
</tr>
<tr>
<td>Phosphatidylserine + phosphatidylinositol</td>
</tr>
<tr>
<td>Lyso-bis phosphatidic acid (BMP)</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>Diglyceride</td>
</tr>
</tbody>
</table>

Values are in mole percent of total. Note the presence of saturated phospholipids (8.7% of total), almost 1/1 ratio between sphingomyelin and cholesterol, and the low amount of LBPA (<1%).

2.2. Membrane organisation

Remarkably membrane rigidity of RBL-derived exosomes increases between acidic to neutral pH [14], suggesting that some membrane reorganisation occurs when exosomes are released out of the MVB in the extracellular medium. Exosomes in a neutral pH environment thus feature a tight lipid packing on their surface but an elevated transmembrane movement of lipids [14]. Such a rapid flip-flop is not in favour of an asymmetrical distribution of phospholipids between the two membrane leaflets. Effectively, we have observed an equal distribution of PE between the two leaflets of exosome membrane [14], whereas it is well-known that they are enriched in the plasma membrane inner leaflet in cells [25].

Interestingly, proteomics analysis of RBL-derived exosomes unravelled the presence of a phospholipid scramblase (C.S, M.R., unpublished data), which is involved in the mixing of phospholipids between membrane leaflets [25]. No translocase, involved in the active maintain of PE and PS [25] in the inner leaflet was detected. These results indicate that not only PE, as we reported, but also all the other phospholipids, are probably randomly distributed between the two membrane leaflets of exosomes.

Noteworthy, the absence of translocase was also reported in reticulocyte-derived exosomes, even though PE displayed an asymmetrical distribution in these exosomes [12], similarly to what occurs in reticulocyte plasma membrane. It was...
concluded that PE were maintained in the inner leaflet because of the high curvature of exosome membrane, or via interaction with proteins present in the lumen of the vesicles. Because of their phospholipid composition which resembles that of plasma membrane (Table 1) and the presence of a phospholipid asymmetry, reticulocyte-derived exosomes appear quite different from those released from immunocompetent cells, and more specifically those derived from the mast cell line RBL-2H3.

However, when making a ratio between the proportion of the same phospholipid present in exosomes and their parent cells, sphingomyelin is the only one to increase in reticulocyte as well as in immunocompetent exosomes (Table 1). Exosomes might be a new type of sphingomyelin domain. It has been shown that sphingomyelin only-rich domains are present in Jurkat T cells plasma membrane and are distinct from SM/Cholesterol/GM1 enriched domains called “rafts” [26].

3. Cellular lipidomics

This part of the review will consider the cellular origin of lipids recovered in exosome composition.

3.1. Sphingomyelin

Cross-linking of plasma membrane sphingomyelin (SM) by an appropriated probe (lysenin) triggers calcium influx and ERK phosphorylation, but not tyrosine phosphorylation, indicating that these domains could be specific signalling platforms [26]. Since plasma membrane is the cellular compartment with the highest SM content, exosome SM enrichment could originate from plasma membrane, because plasma membrane lipid rafts (SM/Cholesterol/GM1 domains) can be endocytosed and reach the late endosomes [27], corresponding to MVBS.

We have approached the analysis of lipid flux from parent cell compartments towards exosomes by means of fluorescent lipids [15]. In our conditions NBD-PC labelled only the plasma membrane of RBL cells and was weakly recovered in exosomes upon ionomycin-induced degranulation [15]. Instead lipid fluxes from the Golgi and from the MVB limiting membrane appear as the main pathways to supply lipids in exosome biogenesis. This is in favour of a direct flux of SM from Golgi to exosomes via the MVB compartment.

In addition, the increase of SM in exosomes appears to occur at the expense of PC (Table 1). In that respect, sphingomyelin synthase (SMS) can potentially regulate in opposite direction the levels of ceramides and diacylglycerols [28] which can be used by the CDP-choline phosphotransferase to give sphingomyelins or phosphatidyleholines, respectively. By driving the phosphocholine pool towards catalytic reaction with ceramide instead of diglycerides, the SMS could account for the high level of SM in exosomes, at the expense of PC. Precisely, the SMS has been located in the Golgi [29].

3.2. Lyso-bis phosphatidic acid (bis monoacylglycerol phosphate)

The biogenesis of exosomes occurring at the level of a cell compartment enriched in LBPA [30], namely the MVBs, attention has been focused on this fascinating lysolipid which seems to arrive from nowhere between early and late endosomes. In addition this compound tends to accumulate in organelles derived from MVBS, i.e. Multilamellar Bodies [22]. LBPA, also called BMP for bis monoacylglycerol phosphate, rises from 1 to 15% of total phospholipids between early and late endosomes of BHK cells [30]. What metabolically occurs between these two compartments? Comparison of their phospholipid composition shows a decrease in SM content (−5.8%), PE (−4.7%) and PS (−4.6%) in late endosomes, thus giving no specific clue on how this lysolipid could be formed. Phosphatidylglycerol (PG), which has been shown to be the BMP (LBPA) precursor [31], has not been measured in endosomes from BHK cells. Fatty acids (mainly oleic acid and docosahexaenoic acid) [32] which esterify the glycerol backbone appears to be brought by poorly characterized transacylases, directly at the sn-2 position. Degradation pathways in cells are also unknown since this compound appears to be refractory to in vitro lipolytic enzyme attack [33]. However, conformation of the fatty acids might hide the polarity of the phosphate head group since LBPA migrates close to neutral lipids on thin layer chromatography [30]. Such a conformation could impair some lipolytic enzyme attacks. In addition, LBPA has been claimed as the only phospholipid in cells with an “unnatural” configuration [34], being assigned to be a sn-1–sn′1 configuration when “natural” phospholipids exhibit a sn-3 configuration. Analysis of the stereoconfiguration of this lipid has to be revisited since the rationale on investigating that point by several authors a few decades ago is not clear [34]. In addition, an isomerase activity is required to obtain a 1–1′ LBPA compound from natural PG, and such isomerase has never been characterized. In any case a 1–1′ compound and a 3–3′ one should display a similar three-dimensional shape since both molecules exhibit the same symmetrical axis. In that respect, although the most efficient LBPA molecule able to trigger intraliposomal vesicle formation at acidic pH (pH 5.5, similar to pH of MVBS) has been reported as being a 1–1′ LBPA esterified at the sn-2 position [35], a 3–3′ LBPA esterified also at the sn-2 position would certainly have been as efficient.

Since this peculiar phospholipid appears at the step of MVB formation along the endocytic pathway, it certainly plays a key role in the dynamic of that compartment [36]. However, the efficiency of such compound to trigger exosome biogenesis in cells remains to be established.

For stereoconfiguration, we refer to the nomenclature based on the position of the phosphate moiety linked to the position 3 or 1 of the glycerol backbone. The works reported in Refs. [33,35] refer to the stereoconfiguration of the carbon 2 of the glycerol.
3.3. Cholesterol

LBPA has been proposed to interact with cholesterol [23] and the effect of this neutral lipid in exosome biogenesis should also require attention. In the “RN” B cell line, MVB displays a high content in cholesterol and a low content in LBPA, the respective proportions of the two lipids becoming opposite in Multilamellar Bodies [22]. Except that cell line, the cholesterol/phospholipid ratio is similar between parent cells and exosomes derived from either mast cells, dendritic cells or reticulocytes [12,14]. In that case also, cholesterol might remain on the MVB limiting membrane once the intraluminal vesicle has been pinched off.

3.4. Lysophosphatidylcholine

On the reverse, Lysophosphatidylcholine (LPC) present in cells is also recovered on exosomes from dendritic cells or RBL mast cells, and accounts for about 10% of phospholipids [14]. As depicted in Fig. 2, the generation of LPC on MVB outer layer accounts for the membrane curvature required to make the neck of the vesicle, since the planar surface occupied by the LPC polar head group is higher than the surface of the fatty acid. Exosome thus appears as a new vector for LPC. Concentrations between 10 and 40 μM of LPC bound to serum albumin have been reported to trigger cell response [37]. A recent work has established the presence of circulating exosomes in blood, with an average amount of 60 μg proteins for 300 ml of blood [19]. In our hands this amount of exosome represents 20 nmoles phospholipids, which would carry around 2 nmoles of LPC (see Table 1). Such an amount of LPC for 300 ml of blood is equivalent to a concentration of about 7 nM. Therefore, exosomes would represent a minor source of circulating LPC.

4. Functional lipidomics

This part will consider the dynamics of lipids involved in exosome biogenesis and the role of some lipolytic enzymes on their biological functions.

However, exosome-bound LPC might act efficiently in target cells such as dendritic cells (DC). Exosomes appear to be endocytosed by immature dendritic cells and to enhance both cell maturation and antigen presentation [38]. Mature DC cells will then release new exosomes more efficient than those originating from immature cells [39]. It has been shown that LPC can trigger DC maturation [40], and it is conceivable that exosome-bound LPC could participate in that process. Also exosome-bound LPC could trigger lymphocyte chemotaxis via the G protein coupled receptor named “G2A” [37], and this could also account for exosome-mediated activity of T cells in lymph nodes. The membrane-bound LPC on exosomes, although representing a limited amount, could display a “bioactivity” more efficient than the “soluble” LPC: exosomes could thus be considered as “vehicles of particulate agonists”.

Fig. 2. Exosome biogenesis in cell and at the molecular level inside the Multivesicular Body (MVB) compartment. (A) Intralumenal vesicles, precursors of exosomes, are generated inside the MVB compartment (late endosomes), corresponding to secretory granules in hematopoietic cells. Details of the biogenesis of the intralumenal vesicles are depicted in B and C. The budding vesicle membrane is based on the exosome lipid composition and the absence of phospholipid transmembrane asymmetry reported for exosomes derived from rat mast cells (RBL-2H3) and human dendritic cells (DC) [14]. (B) The donor membrane for the budding vesicle is the MVB membrane, whose composition is based on data reported in BHK cells [30]. We postulated that no phospholipid asymmetry was present in this membrane. The initial event triggering vesicle budding would be a higher molecular area of LBPA molecules which organize in clusters at acidic pH (see arrows), creating an excess of lipids on the MVB inner leaflet. Lipid sorting during that process would select more SM, less PC and about the same amount of PE and PS, whereas negatively charged lipids (LPC, PA) with the help of positively charged BAR domains from appropriate proteins, trigger MVB outer leaflet bending at the neck of the vesicle. (C) Fission of the vesicle occurs by the mixing of each leaflet induced by fusogenic lipids, PA and LPC on one leaflet, and by LBPA accumulated on the other leaflet at the neck of the vesicle. LBPA would remain on the MVB membrane after separation of the vesicle since no enrichment of this lipid has been found on immunocompetent-derived exosomes [14].
These “vehicles” appear very efficient when injected in a whole organism and it has been suspected that MFG-E8/lactadherin which binds strongly phosphatidylserine and which is highly enriched in exosomes [41] could drive them towards target cells. However, invalidation of lactadherin gene has not evidenced a key role of PS/lactadherin in attracting exosomes to appropriated sites [41].

Since the recent work of Caby et al. [19] it is clear there is a circulating network of exosomes in the organism, organizing distal communication between cells. Positive aspects of this network are for example the enhancement of immune response or the maturation of reticulocytes [42], one negative aspect being that the “exosome pathway” is “hijacked” by pathogens, infectious (HIV [24], mycobacteria [43]) or not (prion [44]). Therefore, regulating the exosome biogenesis appears critical to either enhance their production in order to boost the immune response towards tumors for example, or to block their production in order to stop the spreading of infectious or prion diseases. The knowledge in the protein machinery involved in exosome biogenesis [45] is by far much more advanced than the lipids participating to that process. However, all protein interactions lead to lipid modifications of the bilayer, in order to promote both the bending of the membrane and the biogenesis of fusogenic lipids. Subsequent fusion membrane of the opposite bilayers will occur at the level of the neck of the vesicle to trigger its removal from the parent membrane. A possible process of pre-exosome vesicle biogenesis, i.e. the intraluminal vesicles generated inside the MVB, is represented in Fig. 2.

The initiation event for the vesicle formation relies on the observation of Matsuo et al. [35], who showed that in a giant liposome with a similar size (600 nm) and composition as the MVB (i.e. containing 15 mol% of LBPA), adjusting the intraliposomal pH down to 5.5 (pH of the MVB) induces spontaneously the formation of small vesicles inside the giant liposome. With a neutral intraliposomal pH, nothing occurs. A hypothesis would be that LBPA molecular area increases at acidic pH and the lipid becomes organized in clusters, inducing an excess of lipids on the inner leaflet of the liposome, with subsequent budding of this inner leaflet. This budding will then drive the liposome outer leaflet to form a vesicle. This process will eliminate the excess of lipids on the inner leaflet of the donor membrane and reequilibrate the lipid content of the two leaflets. Indeed, in a vesicle of 60 nm diameter (the average size of an intralumenal vesicle or an exosome), two-third of the lipids are present on the outer layer [14]. It should be borne in mind that the inner leaflet of the MVB membrane becomes the outer leaflet of the exosomes membrane (see Fig. 2.). In MVB like in giant liposomes, internal vesicle budding will then remove two times more lipids from the inner leaflet than from the outer one. When considering the three-dimensional aspect of this process, inner leaflet LBPA has to be self-organized in such a way it triggers a tubule formation of 60 nm wide, which will be further “cut” by constriction to form a vesicle released by fission from the parent membrane. Tubule generation on liposomes has been shown to be triggered by proteins such as kinesin [46] or endophilin [47]; thus the effect of LBPA alone would be quite unique. Since this lipid spontaneously triggers vesicle formation in a lipid phase, this process has to be controlled in cells. Indeed various LBPA interacting proteins have been described. The protein Alix [35], present in cytosol of BHK cells inhibits the formation of vesicles when added to the outside of LBPA-containing liposomes. Similarly, another LBPA interacting protein, Saposin C [48], inhibits MVB formation in human fibroblasts. Therefore, Alix and Saposin C interaction with LBPA appears to regulate the intraluminal vesicle formation in MVBs.

The second step is the constriction of the neck of the vesicle and its release. Separate events occur on each leaflet. In cells, mixing of the MVB outer leaflet at the level of the neck of the vesicle (Fig. 2B) might involve lipid mediators such as phosphatidic acid (PA), and lyso-PC (LPC). These negatively charged lipids will allow BAR domain-containing proteins to bind and to bend the membrane in order to accelerate the process of vesicle invagination [47]. Because PA is a fusogenic lipid in presence of calcium [49] it can help the mixing of opposite bilayers. As a consequence, the LBPA clusters in the MVB inner leaflet would accumulate at the neck of the vesicle (Fig. 2C) thus reaching the critical concentration allowing its fusogenic properties. LBPA has been shown to be fusogenic at pH 5.5 in membranes containing 20–30% of the lipid [33]. Consequently, the vesicle will then be released out of the parent membrane, and the transmembrane distribution of lipids will be re-organized to equilibrate with two-third of them on the outer layer of the vesicle.

Intraluminal vesicle formation thus requires enzymatic modification of lipids on the MVB membrane. Interplay between PLA2 and PLD has been observed in cells [49]. The presence of a calcium-independent PLA2 has been reported in reticulocyte-derived exosomes [50], and we also observed a PLA2 activity [51] in mast cell (RBL-2H3)-derived exosomes. The role of PA appears to depend upon the type of enzyme generating this mediator. Inhibition of Diacylglycerol (DG) kinase is required to enhance Jurkat T cell-derived exosomes [52], whereas we have reported that overexpression of an active PLD2 multiply by two the amount of exosomes produced by the mast cell line [51]. This differential effect of PA producing-enzymes might depend upon the molecular species of PA generated. Interestingly, some of the lipolytic enzymes involved in intraluminal vesicle formation will be trapped inside exosomes, and then released out of the cell and later on in the blood stream. Exosomes thus appear as vehicles for lipolytic enzymes.

Beside lipolytic enzymes, exosomes contain proteins participating in vesicle formation and trafficking. As an example they contain small G proteins and the LBPA-binding protein Alix [18]. GTPase might activate lipolytic enzymes, and LBPA concentration might control the sorting function of Alix. The lipid flux during exosomes biogenesis regulates the type and the amount of proteins which can be inserted in the nascent exosome bilayer. Therefore, regulating lipid exosomes biosynthesis appears as a key to either stimulate exosome production in order to boost the immune response, or on the opposite block their production in the case of HIV infection.
5. Conclusion

Exosomes are a new type of bioactive vesicles which are suspected to play a key role in many biological responses in the body [53]. On model membranes, LBPA (BMP) seems to be essential for their biogenesis, but this requires confirmation in cells. In any cases, a better understanding on the metabolism of this lipid is necessary. Differences in exosomes lipid composition and membrane organisation have appeared between reticulocyte and immunocompetent cell-derived exosomes. Lipid composition of exosomes might in fact condition their fate and function in the body. An exhaustive lipidomics study of exosomes from various sources appears necessary for a better understanding on how these vesicles operate in the organism and how their biogenesis pathway can be “hijacked” by pathogens.

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