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Exosomes biological significance: A concise review☆

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Abstract

Exosomes were initially thought to be a mechanism for removing unneeded membrane proteins from reticulocytes. Current studies have shown that the process of exosome formation extends to many mammalian cells. This concise review highlights the findings reported at a Workshop on Exosomes. Full knowledge of the contribution of exosomes to intercellular information transmission and the potential medical application of this knowledge will depend on the ingenuity of future investigators and their insight into biological processes.

Keywords: Exosomes; Vesicle secretion; Cancer therapy

Exosomes—current state

The intracellular production of small vesicles containing a highly selective content of plasma membrane proteins was described over 25 years ago in maturing reticulocytes of sheep [1–3]. Many plasma membrane proteins are known to disappear during the maturation of the reticulocyte to the final, nucleus-free, mitochondria free, concave-disc-shaped mammalian erythrocyte. While the lysosomal compartment has been the generally recognized site for the degradation of unwanted, denatured or otherwise obsolete or obsolescent membrane proteins, developing red cells face an additional challenge. As the cells mature, and presumably the "aging" or unwanted proteins have to be eliminated, the lysosomal structures are also being lost or destroyed.

The red cell is known to have a well-documented and explored pathway for degrading cytosolic proteins using the ubiquitin-dependent proteosome route [4,5]. However, other mechanisms clearly exist to destroy or diminish selectively the protein "menu" of the plasma membrane. In the transition between the recognizable reticulocyte and mature erythrocyte, approximately one third of the surface area is lost. The protein composition of the remaining membrane in the erythrocyte is sharply different from that in the reticulocyte [6]. At the same time, some membrane proteins seem to be fully retained between erythrocyte and reticulocyte [7] so that, by comparison, the retained proteins form a higher percentage of the total plasma membrane proteins.

The major mechanism of loss of plasma membrane proteins appears to be through the selective intracellular budding of lipidoprotein vesicles into a late endosomal compartment [3,8]. Following fusion of the latter compartment with the plasma membrane, the contained vesicular bodies (named exosomes [6]) are released into the circulation. The circulating exosomes can be retrieved by centrifugation (or immobilized by binding to a protein-selective column), and their protein and lipid contents identified and characterized. Whether the exosomes released from reticulocytes play a further role in the physiology of the organism, such as transferring some of their components to other cells or tissues, remains unknown. Transferrin receptor-containing exosomes bind Fe-transferrin with the same avidity as the native reticulocyte [9]. When engulfed by phagocytic cells, exosomally bound iron would be delivered to those cells. Such a mechanism could provide a hitherto unexplored route for iron uptake by a small population of cells.

☆ This review is based on the reports presented at a Workshop on the Biological Significance of Exosomes in Montreal, Canada, May 20–21, 2005. The references in this short report are neither complete nor extensive. The definitive references to each of the topics addressed may be found in the articles cited from Workshop on "Exosomes" published in vol. 34 and 35 in BCMD in 2005.

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A synopsis of views and outstanding issues from the first workshop on exosomes

Mammalian red cells lose the majority of their intracellular organelles during maturation into reticulocytes [10]. Whether exosome formation is linked to the extrusion and destruction of organelles like the nucleus and mitochondria is still under investigation [11]. Studies by Johnstone and colleagues [12] have concluded that the processes are independent based on the observation that native avian red cells, which retain both their nuclei and mitochondria, still form exosomes upon maturation. Moreover, a transformed erythroblast avian cell line when induced to differentiate into a definitive red cell starts to secrete transferrin-receptor containing exosomes [13] but without loss of either the nucleus or the mitochondria. Such data suggest the absence of an essential interaction between exosome formation and organelle loss.

On the obverse side of the coin, Vidal and his associates [11] have found that proteins released during mitochondrial destruction and enucleation are likely to have a role in enhancing exosome release. Proteins such as Alix, derived from the destruction of mitochondria, enhance exosome formation [12]. Clearly, the resolution of the participation of proteins derived from organelle destruction, either as modifiers or essential factors for exosome formation during red cell maturation, awaits further study. Two factors identified as essential in exosome formation in K562 cells (a human erythroleukemic cell line) are Rab11 (a GTP-dependent protein involved in fusion events) and Ca^{++} [14].

As with avian red cells, destruction of basic cellular organelles is not obligatory for the process of exosome formation in cells of the immune system. In this collective class of cells, a similar process of exosome formation and release also occurs, but no organelles are destroyed. (However, a cautionary note is necessary. Because the machinery required for exosome formation has not been identified, it is conceivable that inherent differences may exist between the cells of the immune system and developing mammalian red cells with respect to the availability of existing cofactors for exosome formation, thereby bypassing the need for organelle destruction.) In the cells of the immune system, there is striking similarity to red cells with respect to exosome formation. The exosomes are accumulated in multivesicular sacs followed by fusion with the plasma membrane and the release of vesicles of a size comparable to that of the red cell exosomes [15–17]. However, the protein composition of the exosomes from the two sources shows substantial differences. For example, the major protein of red cell exosomes is the transferrin receptor, a protein which represents about 2% of the plasma membrane proteins in the newly released circulating reticulocyte. In contrast, MHC Class I and II complex molecules form a major fraction of the proteins in exosomes derived from lymphocytes and dendritic cells but show little or no transferrin receptor [15,16,18].

Nonetheless, some proteins appear to be common in exosomes from a variety of sources, including the chaperone, HSP 70, and tetraspan proteins. The particulars of the exosomal protein composition no doubt reflect the cell of origin. It has been suggested that only proteins which are not attached to the cytoskeleton are targeted for externalization [11], an observation which is worthy of further study to obtain insight into this method of shedding specific membrane proteins.

Understanding the signals that lead to exosome formation remains a major challenge. No less a challenge is recognizing whether a special class of membrane proteins is subject to elimination by exosome formation in the various cell types where the phenomenon occurs. Two proposals have been put forth with respect to targeting the designated proteins, that of Vidal et al. [11] mentioned above and a second proposal by Stoorvogel [19]. The latter proposes and provides evidence that the proteins targeted are first monoubiquitinated. These two considerations are not necessarily exclusive, but whether they act together has not been examined.

What targets protein elimination via exosomes?

As previously noted, the proteins targeted for elimination by exosomes depend on the cell type. Even in a single type of cell, such as the mammalian red cell, the same protein may be targeted for elimination to a very different extent in different species. For example, while the transferrin receptor is uniformly lost in all known species of adult red cells, sheep cells retain most of their glucose transporters and pig cells retain their nucleoside transporters, but not the reverse, into maturity [6,20–22]. Not only transmembrane proteins are eliminated via exosomes but also GPI-anchored proteins. Thus, GPI-anchored acetylcholine esterase [23] and GPI-anchored prions, both infectious PrPsc and native PrP, are secreted via exosomes [24,25].

In searching for markers on exosomes, which could play a role in recognition, a prominent peptide of dendritic cell exosomes, lactadherin, was proposed as a promising candidate for targeting released exosomes to distal dendritic cells with the transfer relevant antigens [26]. However, direct examination of this proposal showed that the absence of lactadherin had little effect on the recognition process [26].

At present, neither the recognition signals which target some proteins for release in exosomes nor the proteins in exosomes which participate in the transfer of exosomal proteins to distal cells have been identified.

Sorting signals to escape degradation in lysosomes

MVB (multivesicular body) formation has been described in yeast as well as a number of mammalian cells as a means for selective sorting and targeting of proteins to the recycling and the degradative pathways respectively. The internal vesicles of the MVB destined for degradation are presumed to undergo monoubiquitination and release into the lysosomal lumen upon fusion of the MVB with the lysosomal membrane. The residual membrane of the MVB, on the other hand, is believed to retain those proteins which will escape degradation and continue to recycle to the plasma membrane [27]. The studies presented at the Exosome Workshop dealt exclusively with a population of vesicles that are not targeted for degradation before they leave

the cell of origin. The discussion focused on MVBs which fuse with the plasma membrane and release their contents ("exosomes") into the ambient medium, or in vivo, into the circulation.

There are reasons to question whether the factors governing fusion of MVBs with the lysosomal membrane are identical to those for fusion at the plasma membrane. Although the enzymatic requirements for fusion to both these membranes may be similar and vesicle secretion into the lysosome or into the external milieu may be considered release into an "extracellular milieu", it is also self evident that the recognition processes must have substantial differences. For one, the outcome of the two fusion events is different. In one case, the cargo of the fusion event is subject to degradation, while the other escapes degradation and continues to have potential for biological activity. Furthermore, it appears highly unlikely that the proteins of the plasma membrane facing the cytoplasm are completely identical or analogous to the proteins lining the cytoplasmic face of the lysosomes. The differences at the two membrane faces would entail the need for differences in the recognition markers for fusion, at least in a quantitative sense. Furthermore, the fact that some vesicles in MVBs evade degradation indicates preexisting differences.

That in red cells the contents of the MVBs evade degradation by lysosomes is perhaps not surprising since lysosomes are also undergoing degradation during the course of maturation. But the same does not apply to other cells with active lysosomes that are known to release intact exosomes. It is therefore self evident that the signaling and/or recognition required for fusion of MVBs with the plasma membrane, and not the lysosomes, requires further elucidation. It also seems self-evident that, if monubiquitination is required for lysosomal targeting of designated proteins, it cannot be the sole signal for recognition of a protein to be released intact in an exosome, as for example, the transferrin receptor. The relative frequency of protein removal from the cell by exosome secretion rather than by lysosomal degradation needs further investigation to establish the extent and potential impact of exosome release on protein homeostasis.

Exosomes beyond the blood cell systems

While exosome formation is now well established in red cells and cells of the immune system, an example of an unexpected cell type contributing to exosome release is the intestinal epithelial cell. While engaged in its classical role in nutrient absorption, the intestinal cell has a number of characteristics of a cell of the immune system. Exosomes released from the basolateral surface of enterocytes may carry antigens and act as a link between the local immune system and the digestive tract, thus providing balance and tolerance between food antigens and the local immune system [28].

In man, the cells of the small intestinal epithelium have MHC II-rich MVBs which are found at both apical and basolateral compartments. In biopsies of human intestinal sections and in human intestinal cell lines, similar peptides, including MHC I and II, are found in the vesicular compartments at both luminal and basolateral surfaces. Only the compartments at the apical side show the presence of HLA-DM. Exosome release occurs only at the basolateral surface [28,29]. Whether the presence of HLA-DM is a key component in restricting exosome formation at the luminal side has not been addressed.

The release of intact exosomes in intestinal cells, which contain lysosomes [29], supports a sorting mechanism which targets certain types of "exosomes" in MVBs to lysosomes for degradation, while other types, perhaps those bearing as yet unrecognized markers, are "secreted" upon fusion of the MVBs with the plasma membrane. The released intestinal exosomes may play a role in some aspects of signal or information transmission since epithelial exosomes can transfer antigens from the intestine to local dendritic cells [28]. Furthermore, in the immuno-suppressive microenvironment of the intestine, the recognition of exosomal MHC class II peptide complexes by mucosal lymphocytes might drive the tolerogenic response [28,29].

The epididymis, like the intestinal epithelium, also shows vesicular secretions, named epididysomes, that are exosome-like secretions rich in sphingomyelin. Proteins in these exosome-like secretions are apparently essential for the maturation of the male gamete. In a manner analogous to the transfer of proteins via exosomes between the cells of the immune system, there is transfer of proteins of the epididymis via epididysomes to the spermatozoa along their lengthy passage through the epididymis. The transfer of these epididymal proteins, including a protein essential for binding of the egg, underlines the significance of the externalized vesicles for optimal reproduction [30].

Recently, studies have appeared which show that kidney tubules secrete exosomes containing Aquaporin [31] into the urine. This is the first example of an exosome being excreted from the body with a recognizable, characteristic protein of kidney tubules. Whether additional proteins in these exosomes will also be identified and provide a means to characterize changes in kidney function or incipient malfunction provides a new challenge for kidney research and medical applications for exosomes. Although the work on kidney exosomes was not addressed at the Exosome Workshop, the study indicates that the phenomenon of exosome release may be more widespread than currently appreciated and with yet unrecognized potential.

Exosomes and the activation of the immune system

Exosomes derived from the multivesicular bodies of dendritic cells (DC) have taken on important immunological functions. Mature dendritic cell exosomes are two orders of magnitude more effective than those from immature dendritic cells in inducing antigen-specific T-cell activation [32].

While immature DCs derived from spleen produce greater numbers of MVBs and exosomes than mature cells, the former are weaker in their ability to stimulate T cells, presumably indicative of differences in their protein composition. Exosomes from tumor-antigen-pulsed DC cells have the capacity to elicit antitumor responses in vivo [33]. The protective effect is, however, non-specific for tumor type, raising the question

whether other molecules, different from the newly acquired tumor antigen, are the responsible agents [33]. This behavior may be an example of the well-known need for co-stimulatory signals in eliciting an immune reaction.

While T-cell activation generally depends on the recognition of peptidic fragments bound to the major histocompatibility complex on antigen-presenting cells, a co-stimulatory signal, present elsewhere on the antigen-presenting cells, is usually an additional requirement. From such studies, it is perhaps expected that T cells would not undergo activation directly by exosomes derived from antigen-presenting cells which did not contain the required co-stimulatory peptide. However, under specified conditions, the need for additional signals may be bypassed. Sprent [34] has shown that exosomes may be directly immunogenic for $CD8^+$ T cells in absence of APCs provided that the exosomes express high densities of MHC I peptide. Such a result suggests that the lack of activation of T-cells by exosomes alone may be concentration-dependent and capable of being compensated by other factors when the critical MHC peptide is present at inadequate concentrations. Whether differences in concentration of specific peptides are also responsible for the relative effects of exosomes from mature and immature dendritic cells on T-cell activation (mentioned above) remains to be clarified.

The ability of exosomes to transfer peptide "information" to another cell has important biological implications. Exosomes from immature DCs are known to transfer functional MHC–peptide complexes to other DCs [32]. Similarly, it has been proposed that the prion protein released in exosomes may fuse with the cell surface of uninfected cells, thereby spreading the passage of prion protein [25]. A route for releasing prions via exosomes allows transmission without cell to cell contact. This could have both disease-preventing as well as deleterious consequences to the organism. On the positive side, it could lead to novel methods for preventing the spread of prions. On the negative side, it may allow the spread of prions to escape detection when attention is focused on cell to cell contact for transmission. A number of cell surface antigens released in exosomes could become vehicles for surface protein transfer, without involving direct cell to cell contact. The transfer of antigenic markers, bypassing cell to cell contact, provides an escape route from the scanning mechanism for foreign markers of the body's immune system.

Transfer of infectious agents via exosomes

The insidious nature of the propagation of agents of disease by exosomal transfer is self-evident. Such pathways have already been proposed for spread of retroviruses, including HIV [35,36]. Both Gould [36] and Marsh [35] addressed the retroviral issue at the Exosome Workshop. In primary macrophages of man, HIV may assemble in internal compartments with the characteristics of late endosomal multivesicular compartments [35], which accumulate exosome-like bodies. This type of compartment appears related to that in which budding of non-viral proteins occurs since it contains a number of tetraspannins characteristic of the MVBs in dendritic cells. Upon fusion of the MVB with the plasma membrane of the macrophages, infectious particles can be released into the circulation. After release, the viral particles are again free to infect other cells and are not subject to immune surveillance as "foreign cells", thus avoiding the normal scanning methods and contributing to persistence of the infection [35]. While HIV transmission through exosomes formed in macrophages is a minor route for transmission of HIV particles, given the severity of the disease, the consequent problems become exacerbated. Moreover, since this route of viral transmission is insensitive to the antiviral agents used to control the spread of the disease, a minor mode of transmission takes on additional importance. It is noteworthy that, in dendritic cells [35] which capture and internalize HIV into an endosomal compartment, the viral antigens colocalize with the tetraspannins that are the characteristic markers of released exosomes from MVBs. The subcellular endosomal compartment appears to have been exploited and adapted by the viral machinery to enhance its propagation [35].

Gould [36] focused on the release of exosome-like particles, which envelop the HIV complex, in a specialized type of blebbing or budding directly from the cell surface rather than into an intracellular multivesicular compartment. This proposal depends on the presence of discrete domains on the T-cell plasma membrane, which are enriched in endosomal markers from which budding can take place. The data obtained [36] were consistent with the proposal, showing the presence of such patches on the T-cell membrane as well as surface budding. HIV Gag was sorted to these domains to form the surface buds which, when released, became "exosomes".

To date, it remains uncertain whether the processes of HIV budding (or other vesicle budding) from the cell surface directly and that into the internal MVBs are identical processes. In cells which form and release exosome-like structures at the plasma membrane, do the plasma membranes simply contain an enrichment of the proteins and lipids which characterize the internal endosomal membrane, thus permitting exosome formation? Or, are there more fundamental differences between two related processes which occur to varying degrees in different cell types? Furthermore, is it correct to assume that there are no fundamental differences underlying viral budding and non-viral particle budding? These questions await resolution.

Clinical trials and applications of exosomes

While these basic questions are being pursued, attempts to find practical applications for the released exosomes continue to expand. Harvested vesicles, bearing specific protein markers derived from dendritic cells, have already been used to treat metastatic melanoma patients [37,38]. These exosomes contain functional MHC class II peptide complexes known to be capable of promoting immune responses and tumor rejection. The preliminary results in both man and experimental animals have been encouraging since attrition of the malignancy has been seen. Clinical investigations with the tumor-derived "exosomes" (as well as exosomes from dendritic cells) have reported some success in tumor growth reversal [37–39].

Given the success of some treatments with exosomes on retarding the growth of malignant cells in culture as well as in the clinic, attempts have been made to improve their efficacy by introducing proteins of known potency into cells which produce exosomes naturally. The technology to produce these "enriched exosomes" (named Exosome Display Technology) can then be used to target malignant cells with antibodies to specific antigens and to develop diagnostic and therapeutic agents. The particular example discussed, as well as its efficacy, was that of the generation of antibodies against the tumor biomarkers of the HLA/peptide complex [40].

Conflicting results have, however, been obtained with vesicles derived from tumor cells. The extent to which the inconsistencies originate as a result of inadequate identification of the vesicles released by tumor cells is a central issue (see below). Since the tumor-derived vesicles bear tumor antigens, investigators have used them to induce $CD8^+$ T cell responses by allowing these vesicles to adsorb to mature dendritic cells. Despite positive outcomes noted above, other studies [41,42] report contrary results, namely, that exposure to tumor-derived exosomes resulted in a decreased antitumor activity on the part of T cells. The latter is in line with the well-known immune suppression by tumors.

The key problem with these diverse effects may be that neither the origin nor properties of the "exosomes" derived from tumor cells have been closely examined. Few comparative studies have been carried out between tumor exosomes and those from well-established sources. Many malignant cells release small, membrane-bound vesicles into the circulating medium [43–46]. Some investigators have called the vesicles exosomes [43,46,47]. Taylor and colleagues [46,47] used the name because the vesicles have the size and density of exosomes produced in MVBs [16].

However, the origin of the tumor exosomes has yet to be addressed, and the possibility exists that the vesicles [46,47] released by tumor cells may be budded from the cell surface and show distinctive properties. It is known that some cells may shed surface vesicles as well as those harbored in MVBs [48]. The lack of information and definition of the term "exosome" may lie at the core of the conflicting results between the antitumorogenic responses to dendritic-cell-derived exosomes and tumor-cell-derived exosomes. The same type of vesicle may not be derived from all the reported cases.

Furthermore, a single cell may produce different populations of vesicles. With few exceptions, most investigators have harvested the exosomes as if they represented a single population of identical vesicles. Using phospholipids tagged with three different fluorescent probes (Bodipy-Cer, Bodipy-PC, NBD-PC), Record and his collaborators [49] showed that, with RBL-2H3 cells, this single cell line produced different populations of vesicles, enriched in different proteins and phospholipids. MHC-II-containing exosomes were enriched in one of the phospholipids, whereas exosomes containing the two tetraspan proteins (CD 63 or CD 81) were labeled with two of the three tagged phospholipids. Interestingly, the phospholipid species targeted to the plasma membrane, NBD-PC, labeled few exosomes. Using a single fluorescent label, it was possible to quantify the respective amounts of exosomes released containing CD 63 (47%) MHC II (32%) and CD 81 (21%) showing that the released exosome population is not uniform.

Given the conflicting reports on to the efficacy of harvested exosomes in the treatment of specific malignancies, perhaps greater attention is required to address the uniformity of the exosomes used in the controversial studies and to use selected populations of exosomes.

All vesicles may not be exosomes: establishing guidelines for a definition

With red cells and cells of the immune system, exosomes were identified intracellularly in MVBs prior to being released into the extracellular milieu [3,8,48,50]. In addition to size and shape, a number of specific proteins such as tetraspannins, membrane-bound proteins and chaperones have been shown as characteristic residents of this particle from a variety of sources [6,15–18,51,52]. In many studies with other cells, including most malignant cells, the major characteristic upon which the conclusion was based that shed vesicles were "exosomes", and identical to those derived from the aforementioned cells was that of size and density (for example the work reported by Taylor [46]). Yet, the literature records a much wider variation in size in tumor-derived "exosomes" than those described from the cells of the hematopoietic system. In an early description of the microvesicles called exosomes originating mainly from tumor or transformed cell lines, Trams et al. [43] reported sizes varying from 500 to 1000 nm as well as 40 nm.

At present, there are several considerations which argue in favor of keeping separate the nomenclature of vesicles budded directly from the cell surface from those budded into an internal compartment which subsequently fuses with the plasma membrane. In the literature, there are a wide variety of reports of vesicles formed under many conditions from many types of cells (see below). Left ill-defined, this amorphous pool of vesicles, all called "exosomes", will become increasingly difficult to redefine and sort out in the future. The ability to identify a specific tag by which to recognize a protein destined to be packaged into an exosome in a multivesicular sac (perhaps monoubiquitination [19]) could be a means of providing for a particular definition.

The major issue on which consensus eluded the Workshop on "Exosomes: Biological Significance" was precisely the definition of what constitutes an exosome. As alluded to above, the word has been used in different contexts. Excluding a current usage of the word in relation to nucleic acid metabolism, the term was introduced independently to describe at least three separate events. It was coined by Johnstone et al. [6] to describe the phenomenon of specific protein elimination in maturing reticulocytes [3,8].

An earlier use of the word " exosomes" by Trams et al. [43] described exfoliation of membrane bound ecto-enzymes (e.g. ecto 5′nucleotidase) from normal and malignant cells. These exfoliated structures were capable of dephosphorylating cell surface enzymes.

Nickel [53] used the term exosome for membrane vesicles budding directly from the cell surface that appear to be involved in galectin secretion in epithelial cells and macrophages (Mehul and Hughes [54]). These vesicles arise without prepackaging in ER-derived membrane components. A variety of such budding events at the cell surface have been described in recent years which involve secretion of vesicles by routes that are independent of the ER/Golgi apparatus. Proteins lacking a leader sequence (hence soluble proteins) are entrapped in a vesicle of endosomal origin. The vesicle releases its contents into the environment upon fusion with the plasma membrane [53].

Yet, another type of vesicle has been reported for human polymorphonuclear neutrophils. It is released by a process called ectocytosis (Hess et al. [55]) and hence named ectosome. The latter contains a subset of cell surface proteins such as integrins, selectins and active enzymes such as elastase and matrix metalloproteinase-9. Reports by Stein and Luzio [56] also demonstrated selected protein and lipid loss in vesicles, apparently directly from the surface of neutrophils. Finally, mechanical stress in fibroblasts (Chamson, Lee and Grinnell [57]) also leads to a release of a type of membrane vesicle.

Clearly, release of vesicles directly from the cell surface has been reported by several investigators. On the whole, their components have been less rigorously examined than those from the internal MVBs. At issue is whether the same cellular machinery is used in packaging the buds which are formed at the cell surface and released at that site and those buds which form in a late endosomal structure (MVB) and release occurs when the MVB fuses with the plasma membrane. Given the variety of forms of surface budding described above, the likelihood that more than a single process may be involved in surface budding alone is significant. The lack of a differentiating nomenclature between budding into an intracellular compartment and surface budding reduces the ability to differentiate between the many varieties of secreted vesicles.

To prevent confusion, it would seem useful to apply a more rigorous definition of the term “exosomes”, a term which includes their origin in MVBs and at least one or two proteins, such as HSP 70, which have been commonly characterized in exosomes from different cells.

It may eventually become apparent that one type of surface budding is closely related to the budding into an internal MVB. At present, a differentiating nomenclature would reduce confusion for both incoming and established investigators in the field. It would therefore appear practical to retain names that offer the least likelihood of confusion, i.e. ‘exosome’ for secreted vesicles originating from the multivesicular sacs and ‘ectosome’ for vesicles released directly from the surface membrane. This has the advantage of making a clear differentiation based on existing experimental findings. Since HIV budding can occur from an infected cell at the surface and also into MVBs (at least in macrophages), HIV release would take place by both ‘exosome’ and ‘ectosome’ formation. Final assessment of the nomenclature awaits definitive studies on unraveling the budding and protein recognition processes.

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