

Exosomes: Specific Intercellular Nano-Shuttles?

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Abstract: The term “exosome” was coined in 1981 (Trams et al, *Biochim Biophys Acta*, 1981, 645:63) when the presence of “exfoliated membrane vesicles with 5’-nucleotidase activity” was first reported. Since then, in the biomedical literature the term exosome has evolved to designate both the above mentioned small vesicles of endocytic origin and the 3’->5’ exonuclease complex involved in RNA processing and degradation. In the present review we will focus on the original definition of exosomes and particularly on their emerging role as intercellular signaling devices.

Exosomes are secreted by a variety of cells, particularly antigen-presenting cells such as DCs, B cells and macrophages. Enriched in MHC class I and II antigens and costimulatory molecules, they are considered to be an alternative pathway of antigen delivery and presentation. The use of exosomes engineered to prime the immune system against tumor antigens is a promising new arm of cancer immunotherapy. On the other hand, exosomes released by the tumor itself may provoke a tolerogenic response. Participation of exosomes in other immune mechanisms, such as platelet activation, mast cell degranulation, germinal center reaction and engulfment of apoptotic cells has also been postulated.

An evolutionary link between retroviruses and exosomes has recently been proposed. In general, viruses can use the host’s intracellular machinery for their budding, and exosomes may constitute a vehicle for transmission of pathogens and interaction with the immune system. A deeper knowledge of the cells targeted by exosomes and the mechanisms governing these interactions will give a clear picture of the role of exosomes as intercellular messengers.

Keywords: Exosomes, antigenic presentation, multivesicular bodies, endosomes, LCMV.

INTRODUCTION

Many scientists are still skeptical about exosomes. Indeed, these elusive nano-structures seem to provoke the same reaction as their “cousins”, lipid rafts or caveolae did some years ago. Although described for the first time in the early 80s, exosomes have been relegated to the obscure group of “bubbles” that shuttle molecules between intracellular compartments, of interest only to zealous cell biologists passionate about “trafficking” issues. Neither exosomes’ well-studied role in transferrin receptor disposal during reticulocyte differentiation [1] nor anecdotic reports of exosome-like particle release in semen [2] and mast cells [3] gave them enough recognition.

They emerged though to the spotlight after studies appeared describing B and dendritic cell (DC)-derived exosomes as tiny antigen-presenting machineries, capable of stimulate T-cells [4, 5]. Moreover, exosomes loaded with tumor peptides can elicit potent anti-tumor responses, a fact being presently tested in clinical trials.

Suddenly, everybody was looking for exosomes: and they seem to be everywhere. Not only immune cells such as DCs, B cells and mast cells, but also platelets, tumor cells, renal and prostatic epithelial cells produce exosomes (for excellent reviews, see [6-9]). Exosomes were detected in biological fluids such as serum [10, 11], urine [12],

malignant effusions [13, 14] and bronchoalveolar lavage fluid [10, 15]. Advanced proteomic analysis would reveal not only a set of molecules common to all exosomes, but also cell type-specific differences. The latter, of course, is related to the different functions of exosomes- functions that seem straightforward on occasions. Here we can include the previously mentioned transferrin receptor disposal by differentiating reticulocytes [1, 16-20] or the provision of MHC class II antigens to follicular DCs by B-cell produced exosomes [21]. In most of the cases, however, many questions remain. What is the *in vivo* relevance of exosomes in antigen presentation and T-cell priming? Is the concentration of antigenic peptides, MHC antigens and costimulatory molecules high enough *in vivo* to achieve a significant response? Is it a true “long-distance” mechanism capable of transferring the antigenic stimulation from an antigen-presenting cell (APC) to a distant T-cell? Or are exosomes only metabolic by-products of signaling events occurring on the cell surface (particularly lipid rafts), which must be shed and disposed of by other cells [22]?

In this review we intend to focus on exosomes as part of a dynamic intra and intercellular transport system. At the intracellular level exosomes are still parts of multivesicular bodies (MVBs) and constitute an integral part of the endosomal system, involved in the transport, recycling or degradation of molecules. If released into the extracellular milieu, however, exosomes seem to act as messengers delivering specific molecules to target cells. At this level they can be used for clinical purposes, either purified from the organism or artificially synthesized. Particular emphasis will be placed on the crossroad of viral propagation and

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exosome release, and how it could affect the host's immune response.

EXOSOMES INSIDE AND OUTSIDE THE CELL

Exosomes are classically defined as vesicles with a diameter of 40-100 nm that originate in MVBs of the endosomal system. Endosomal compartments are a set of heterogeneous, membrane-enclosed tubes extending from the periphery of the cell to the perinuclear region, where it is often close to the Golgi apparatus. A tracer molecule added to the extracellular medium appears within a minute or so in early endosomes, just beneath the plasma membrane. After 5–15 minutes, it moves to late endosomes, close to the Golgi apparatus and near the nucleus. Early and late endosomes differ in their protein compositions; for example, they are associated with different Rab proteins. In general, later endosomes are more acidic than early endosomes. This acidic environment has a crucial role in the function of these organelles. The early endosomes form a compartment that acts as the main sorting station in the endocytic pathway, just as the *cis* and *trans* Golgi networks serve this function in the biosynthetic-secretory pathway. In the acidic

environment of the early endosome, many internalized receptor proteins change their conformation and release their ligand. Those endocytosed ligands that dissociate from their receptors in the early endosome are usually doomed to destruction in lysosomes, along with the other soluble contents of the endosome. Some other endocytosed ligands, however, remain bound to their receptors, and thereby share the fate of the receptors.

The fate of the receptor proteins—and of any ligands remaining bound to them—vary according to the specific type of receptor. For a recent review of signaling events in the endocytic pathway, see [23].

Late endosomes are characterized by the presence of internally pinched-off vesicles and are often referred to as MVBs. Maturing MVBs accumulate internal vesicles that have 3 potentially distinct fates.

1. Target proteins and lipids to lysosomal degradation: It is still uncertain how endocytosed molecules move from the early to the late endosomal compartment so as to end up in lysosomes. A current view is that portions of the early endosomes migrate slowly along microtubuli toward the cell interior, shedding vesicles

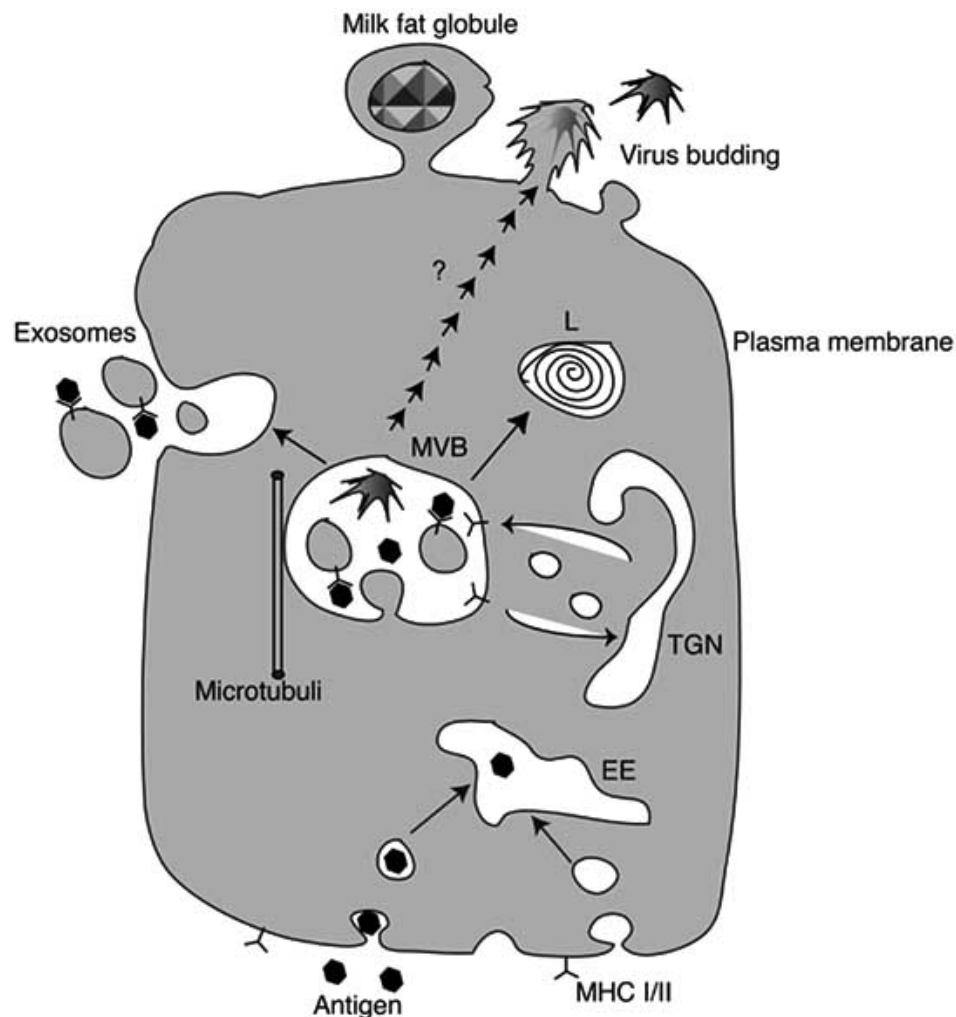


Fig. (1). Schematic representation of the endosomal system and the biogenesis of exosomes. Exosome generation occurs through inward budding, similar to milk-fat globule secretion and viral budding. It has been recently hypothesized that retroviruses use the exosome pathway to their propagation. EE: early endosomes; MVB: multivesicular bodies; L: lysosomes, TGN: *trans*-Golgi-network.

of material to be recycled to the plasma membrane. It is unknown whether multivesicular bodies eventually fuse with a late endosomal compartment or if they fuse instead with each other to become late endosomes. At the end of this pathway, the late endosomes are converted to lysosomes as a result of their fusion with hydrolase-bearing transport vesicles from the *trans* Golgi network and their increased acidification.

- In DCs, MVBs can also serve as temporal storage compartments of MHC class II molecules and their associated invariant chain [24]. Thus the MHCII-Ii complexes can avoid processing, which is triggered upon stimulation of the DC. Then, the intraluminal vesicles fuse back with the limiting membrane, allowing peptide loading onto the MHCII molecule after removal of the Ii [7, 23]. Moreover, MHC class I antigens can also be found in MVBs, acquiring predominantly peptides from exogenous endocytosed proteins [5]. This alternative pathway is important in the process of cross-priming of antigens derived mainly from virus-infected or tumor cells (reviewed by [25]). Mast cell granules are reservoirs of exosomes containing MHC antigens and costimulatory molecules, which are released upon treatment of mast cells with IL4 [26].
- Secretion as exosomes: If MVBs are released by the fusion of their limiting membranes with the plasma membrane, they are termed exosomes. The process of exosome release is, similarly to virus budding and milk-fat globule secretion, a reverse budding event,

where exosomes will contain cytosol inside, exposing the extracellular (or luminal when coming from the endosomal pathway) domain of receptors. A schematic representation of exosome biogenesis is shown in (Fig. 1).

EXOSOME COMPOSITION: GENERAL AND CELL-DEPENDENT

Exosomes can be identified both by morphological and biochemical criteria. Electron microscopic images show the exocytic fusion of MVBs and the presence of characteristic “saucer-like” particles, a flattened sphere limited by a lipid bilayer [6].

Purification of exosomes can be achieved both from cells and, most commonly, from cellular supernatants. Purification methods usually involve a series of filtration/ultracentrifugation steps. To ensure highly purified exosome preparations, usually a gradient centrifugation step is included. Exosomes float on sucrose gradients, at a density between 1.15-1.18 g/ml, depending on the originating cell type.

Biochemical studies of exosomes, either by immunogold staining or using proteomic analysis when highly purified, have confirmed their MVB origin. The presence of lysosomal proteins in exosomes is partial and dependent on their cellular origin [6]. Thus, exosomes in general do not contain lysosomal proteases or other soluble residents. For instance B-cell exosomes do not contain the invariant chain CD74 and LAMP-2, although the latter is present in DC-

Table 1. Protein Families Present in Exosomes (Adapted from [9])

Protein family	Representative molecules	Reference
Antigen-presentation	MHC class I and II (B-cell and DC) costimulatory molecules: CD86, CD80	[31]
Adhesion molecules	Tetraspanins: CD9, CD63, CD81, CD82 Integrins: 3, 4, M, L, 1, 2 MFG-E8 (only murine cells) CD11b, CD18	[6, 9] [6, 9] [50] [28]
Immunoglobulin family members	ICAM/CD54 P-selectin A33 antigen	[6, 26, 31] [29] [48]
Membrane transport and fusion	RAP1B/RABGDI, Rab7, Rab2 Annexins I, II, IV, V, VI, VII	[6, 28] [26, 28]
Heat-shock proteins	HSC70, HSP84/90	[28, 37, 48]
Cytoskeletal proteins	Actin, cofilin, tubulin, moesin.	[28, 35]
Raft-associated proteins and glycolipids	Flotillin, CD55, CD59, GM1, GM3, Gi2 Lyn	[27]
Enzymes	Pyruvate kinase, -enolase Phospholipase 2	[32]
Others	Elongation factor 1, 14-3-3, clathrin, ferritin ESCRT proteins: Alix, Tsg101 Thioredoxin peroxidase CBL/LCK NADPH-oxidase GAPDH	[28] [28] [33] [34] [35]

derived exosomes. Lipid composition of exosomes is similar to that of lipid rafts in that it is enriched in cholesterol and sphingomyelin [7]. Other raft markers have been detected in exosomes, such as GM1, GM3, flotillin and the src protein kinase Lyn [27]. Of particular interest is the identification in exosomes of molecules involved in MVB biogenesis, such as Tsg101 and Alix [28]. The latter has been confirmed in exosomes released by renal epithelial cells and isolated from human urine. Interestingly, proteins associated to renal or systemic disease such as hypertension could also be detected, indicating a possible use of urine exosomes as biomarkers [12]. Exosomes from platelets and cytotoxic cells contain specific proteins such as von Willebrand factor [29] and perforin and granzymes [30], respectively. In Table 1. a summary of the main protein families encountered in exosomes is shown.

EXOSOMES: FUNCTION

One of the most exciting features of immune cell-derived exosomes is their ability to stimulate immune responses. This is based on their molecular composition: a condensed array of MHC antigens, costimulatory molecules as well as adhesion molecules, capable of targeting exosomes to specific cellular targets. Antigenic peptides can be found in certain exosomal preparations (for instance from tumoral cells, [36, 37]), or can be added artificially.

It is not clear yet which cells are the *in vivo* targets for exosomes. Direct effect of exosomes derived from B-cells and DCs on T-cells has been observed [4, 38], but the general consensus based on *in vitro* experiments is that the stimulatory response is greatly enhanced if APCs such as DCs are present [39]. *In vivo* tracking experiments have shown that fluorescent-labeled exosomes are taken up in the spleen by immature DCs and phagocytes, as well as by hepatic Kupffer cells. This interaction is mediated by an array of adhesion molecules: MFG-E8, CD11a, CD154, phosphatidylserine and tetraspanins [40]. Mast cell-derived exosomes presented mitogenic activity on T and B-cells, both *in vitro* and *in vivo* [26] and can induce maturation of DCs [41]. Interestingly, the presence of TCTP, a histamine-releasing factor participating in inflammatory responses, has also been detected in exosomes [42].

DC-derived exosomes loaded with acid-eluted tumoral peptides elicited impressive anti-tumor effect in mice [5]. Tumor cell-derived exosomes could not induce direct cytotoxic response *in vitro*, however, they were able to induce a potent T cell response when loaded onto human DCs [37]. Exosome-based immunotherapy has become an attractive new anti-tumor modality with promising results (for review, see [43]).

Exosomes can also mediate tolerogenic responses. Exosomes derived from a donor given before transplantation may induce tolerance rather than immunity, prolonging allograft survival [44]. Also, exosomes derived from melanoma cells present HLA-G, suggesting involvement in a tolerogenic response against the tumor [45] and Fas-L, provoking apoptosis of T-cells [46]. "Tolerosomes" corresponding to exosome-like structures are produced by intestinal epithelial cells and could induce tolerance to oral antigens [47, 48]. However, in another report, when injected

into mice these exosomes induced an immunogenic rather than tolerogenic response [49].

Interestingly, immature DCs lacking MFG-E8 were not capable of engulfing apoptotic cells [50]. MFG-E8 can potentially bind integrins expressed by DCs and macrophages and thus is responsible for cell-cell contacts. Targeting MFG-E8 via exosomes to DCs and phagocytes in general could be a way of amplification of a "scavenger" response to take up apoptotic cells.

Platelet degranulation. Activated platelets secrete exosomes by fusion of alpha-granules and MVBs with the plasma membrane. Platelet exosomes are probably not involved in coagulation as they do not interact with annexin V and do not bind prothrombin or factor X [29].

A new and intriguing function of exosomes could be that of alternative cytokine delivery. Exosomes from serum and lung epithelial lining fluid-derived contain uncleaved TNFR1 [10], suggesting a new mechanism of generation of soluble cytokine receptors, which could compete for ligand binding. The chemokine receptor CCR5 (principal co-receptor for HIV-1) [51] and possibly the chemokine RANTES too [52] are also secreted in exosomes.

EXOSOMES AND VIRUS: SIMILAR MECHANISM OR EXPLOITATION?

It is impossible not to find similarities in the way both exosomes and viruses leave the cells, taking with themselves a representation of the host's proteins. In fact, the presence of host proteins in budding HIV particles prompted Gould and collaborators to enunciate the "Trojan exosome" hypothesis [53], which, albeit controversial, still elegantly argues for the exploitation by retroviruses of the exosome secretion pathway. It could explain the easy propagation of retroviruses and their poor capacity to stimulate immune responses, due to their expression of host proteins. It would be also advantageous for the retrovirus to accumulate in MVBs and thus "hide". One of the aspects that requires further investigation is the localization of virus budding, which can be different even for the same virus in different cell types. For instance, HIV-1 budding has been associated to plasma membrane rafts in T-cells, but in macrophages it appears to happen through MVBs and the exosome pathway. HIV assembly is driven by Gag, a viral protein that associates with Tsg101, a protein demonstrated to reside in MVBs and exosomes [54-56].

Other groups have also detected the presence of viral particles such as hepatitis C (HCV) in exosomes. Thus, the complex formed between HCV and CD81 would leave the infected cells in the form of exosomes and use the fusogenic capacity of these particles to propagate even in the presence of neutralizing antibodies. [11].

Our group has preliminarily explored the role of exosomes in T-cell priming during LCMV infection. LCMV, the archetypal Arenavirus, buds from the plasma membrane of infected cells [57-59]. When exosome preparations were obtained from *in vitro* LCMV-infected DCs or DCs from LCMV-infected mice, they did contain viral particles as shown by plaque assays and the presence of viral proteins by Western blot. Even after UV-irradiation

(which kills the virus), exosomes were still able to prime both memory and naïve CD8⁺ T-cells to proliferate and produce IFN. In concordance to results from other labs, this stimulation was suboptimal when exosomes were directly added to responder T-cells, but higher in the presence of DCs. Moreover, the stimulatory effect was greatly enhanced when IL2 or CD4⁺ cells were added to the system.

CONCLUSION

Exosomes should be studied in their appropriate context: either as part of the intracellular endosomal system, as intercellular messengers delivering cell-specific signals, or as possible immunotherapeutic tools. In the third context they have already been validated as having a clinical effect. It remains to be elucidated what the exact biological role of this nanoparticles is, either inside or outside the cell. As it seems that exosomes have a particular cell-specific composition, and thus also a cell-specific role, this task won't be simple. Particularly interesting would be to know if pathogens (specially viruses) use this pathway, and how. This could open new avenues to prevent, control or treat many human clinical disorders.

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REFERENCES

- [1] Johnstone RM. The Jeanne Manery-Fisher Memorial Lecture 1991 Maturation of reticulocytes: formation of exosomes as a mechanism for shedding membrane proteins. *Biochem Cell Biol* 1992; 70: 179-190.
- [2] Ronquist G, Brody I. The prostasome: its secretion and function in man. *Biochim Biophys Acta* 1985; 822: 203-218.
- [3] Raposo G, Tenza D, Mecheri S, Peronet R, Bonnerot C, Desaynard C. Accumulation of major histocompatibility complex class II molecules in mast cell secretory granules and their release upon degranulation. *Mol Biol Cell* 1997; 8: 2631-2645.
- [4] Raposo G, Nijman HW, Stoorvogel W, *et al.* B lymphocytes secrete antigen-presenting vesicles. *J Exp Med* 1996; 183: 1161-1172.
- [5] Zitvogel L, Regnault A, Lozier A, *et al.* Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat Med* 1998; 4: 594-600.
- [6] Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002; 2: 569-579.
- [7] Stoorvogel W, Kleijmeer MJ, Geuze HJ, Raposo G. The biogenesis and functions of exosomes. *Traffic* 2002; 3: 321-330.
- [8] Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ. Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* 2000; 113: 3365-3374.
- [9] Fevrier B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr Opin Cell Biol* 2004; 16: 415-421.
- [10] Hawari FI, Rouhani FN, Cui X, *et al.* Release of full-length 55-kDa TNF receptor 1 in exosome-like vesicles: a mechanism for generation of soluble cytokine receptors. *Proc Natl Acad Sci USA* 2004; 101: 1297-1302. Epub 2004 Jan 1226.
- [11] Masciopinto F, Giovani C, Campagnoli S, *et al.* Association of hepatitis C virus envelope proteins with exosomes. *Eur J Immunol* 2004; 34: 2834-42.
- [12] Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci USA* 2004; 101: 13368-13373.
- [13] Andre F, Scharz NE, Movassagh M, *et al.* Malignant effusions and immunogenic tumour-derived exosomes. *Lancet* 2002; 360: 295-305.
- [14] Bard MP, Hegmans JP, Hemmes A, *et al.* Proteomic analysis of exosomes isolated from human malignant pleural effusions. *Am J Respir Cell Mol Biol* 2004; 31: 114-121. Epub 2004 Feb 2019.
- [15] Admyre C, Grunewald J, Thyberg J, *et al.* Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. *Eur Respir J* 2003; 22: 578-583.
- [16] Geminard C, de Gassart A, Vidal M. Reticulocyte maturation: mitoptosis and exosome release. *Biocell* 2002; 26: 205-215.
- [17] Grdisa M, Mathew A, Johnstone RM. Expression and loss of the transferrin receptor in growing and differentiating HD3 cells. *J Cell Physiol* 1993; 155: 349-357.
- [18] Johnstone RM, Bianchini A, Teng K. Reticulocyte maturation and exosome release: transferrin receptor containing exosomes shows multiple plasma membrane functions. *Blood* 1989; 74: 1844-1851.
- [19] Johnstone RM, Mathew A, Mason AB, Teng K. Exosome formation during maturation of mammalian and avian reticulocytes: evidence that exosome release is a major route for externalization of obsolete membrane proteins. *J Cell Physiol* 1991; 147: 27-36.
- [20] Vidal M, Mangeat P, Hoekstra D. Aggregation reroutes molecules from a recycling to a vesicle-mediated secretion pathway during reticulocyte maturation. *J Cell Sci* 1997; 110: 1867-1877.
- [21] Denzer K, van Eijk M, Kleijmeer MJ, Jakobson E, de Groot C, Geuze HJ. Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. *J Immunol* 2000; 165: 1259-1265.
- [22] Joly E. Hypothesis: could the signalling function of membrane microdomains involve a localized transition of lipids from liquid to solid state? *BMC Cell Biol* 2004; 5: 3.
- [23] Gonzalez-Gaitan M, Stenmark H. Endocytosis and signaling: a relationship under development. *Cell* 2003; 115: 513-521.
- [24] Kleijmeer M, Ramm G, Schuurhuis D, *et al.* Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. *J Cell Biol* 2001; 155: 53-63.
- [25] Jondal M, Schirmbeck R, Reimann J. MHC class I-restricted CTL responses to exogenous antigens. *Immunity* 1996; 5: 295-302.
- [26] Skokos D, Le Panse S, Villa I, *et al.* Mast cell-dependent B and T lymphocyte activation is mediated by the secretion of immunologically active exosomes. *J Immunol* 2001; 166: 868-876.
- [27] de Gassart A, Geminard C, Fevrier B, Raposo G, Vidal M. Lipid raft-associated protein sorting in exosomes. *Blood* 2003; 102: 4336-4344. Epub 2003 Jul 4324.
- [28] Thery C, Boussac M, Veron P, *et al.* Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. *J Immunol* 2001; 166: 7309-7318.
- [29] Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood* 1999; 94: 3791-3799.
- [30] Peters PJ, Borst J, Oorschot V, *et al.* Cytotoxic T lymphocyte granules are secretory lysosomes containing both perforin and granzymes. *J Exp Med* 1991; 173: 1099-1109.
- [31] Clayton A, Court J, Navabi H, *et al.* Analysis of antigen presenting cell derived exosomes based on immuno-magnetic isolation and flow cytometry. *J Immunol Methods* 2001; 247: 163-174.
- [32] Laulagnier K, Grand D, Dujardin A, *et al.* PLD2 is enriched on exosomes and its activity is correlated to the release of exosomes. *FEBS Lett* 2004; 572: 11-14.
- [33] Blanchard N, Lankar D, Faure F, *et al.* TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J Immunol* 2002; 168: 3235-3241.
- [34] Janiszewski M, Do Carmo AO, Pedro MA, Silva E, Knobel E, Laurindo FR. Platelet-derived exosomes of septic individuals possess proapoptotic NAD(P)H oxidase activity: A novel vascular redox pathway. *Crit Care Med* 2004; 32: 818-825.
- [35] Wubbolts R, Leckie RS, Veenhuizen PT, *et al.* Proteomic and biochemical analyses of human B cell-derived exosomes Potential implications for their function and multivesicular body formation. *J Biol Chem* 2003; 278: 10963-10972. Epub 12003 Jan 10967.
- [36] Andre F, Scharz NE, Chaput N, *et al.* Tumor-derived exosomes: a new source of tumor rejection antigens. *Vaccine* 2002; 20: A28-31.

- [37] Wolfers J, Lozier A, Raposo G, *et al.* Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nat Med* 2001; 7: 297-303.
- [38] Hwang I, Shen X, Sprent, J. Direct stimulation of naive T cells by membrane vesicles from antigen-presenting cells: distinct roles for CD54 and B7 molecules. *Proc Natl Acad Sci USA* 2003; 100: 6670-6675. Epub 2003 May 6612.
- [39] Thery C, Duban L, Segura E, Veron P, Lantz O, Amigorena S. Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. *Nat Immunol* 2002; 3: 1156-1162.
- [40] Morelli AE, Larregina AT, Shufesky WJ, *et al.* Endocytosis Intracellular Sorting and Processing of Exosomes by Dendritic Cells. *Blood* 2004; 104:3257-66. Epub 2004 Jul 29.
- [41] Skokos D, Botros HG, Demeure C, *et al.* Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J Immunol* 2003; 170: 3037-3045.
- [42] Amzallag N, Passer BJ, Allanic D, *et al.* TSAP6 facilitates the secretion of TCTP/HRF via a non-classical pathway. *J Biol Chem* 2004; 279:46104-12. Epub 2004 Aug 19.
- [43] Chaput N, Taieb J, Scharzt NE, Andre F, Angevin E, Zitvogel L. Exosome-based immunotherapy. *Cancer Immunol Immunother* 2004; 53: 234-239. Epub 2004 Jan 2016.
- [44] Peche H, Heslan M, Usal C, Amigorena S, Cuturi MC. Presentation of donor major histocompatibility complex antigens by bone marrow dendritic cell-derived exosomes modulates allograft rejection. *Transplantation* 2003; 76: 1503-1510.
- [45] Riteau B, Faure F, Menier C, *et al.* Exosomes bearing HLA-G are released by melanoma cells. *Hum Immunol* 2003; 64: 1064-1072.
- [46] Andreola G, Rivoltini L, Castelli C, *et al.* Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. *J Exp Med* 2002; 195: 1303-1316.
- [47] Karlsson M, Lundin S, Dahlgren U, Kahu H, Pettersson I, Telemo E. "Tolerosomes" are produced by intestinal epithelial cells. *Eur J Immunol* 2001; 31: 2892-2900.
- [48] van Niel G, Raposo G, Candalh C, *et al.* Intestinal epithelial cells secrete exosome-like vesicles. *Gastroenterology* 2001; 121: 337-349.
- [49] van Niel G, Mallegol J, Bevilacqua C, *et al.* Intestinal epithelial exosomes carry MHC class II/peptides able to inform the immune system in mice. *Gut* 2003; 52: 1690-1697.
- [50] Miyasaka K, Hanayama R, Tanaka M, Nagata S. Expression of milk fat globule epidermal growth factor 8 in immature dendritic cells for engulfment of apoptotic cells. *Eur J Immunol* 2004; 34: 1414-1422.
- [51] Mack M, Kleinschmidt A, Bruhl H, *et al.* Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. *Nat Med* 2000; 6: 769-775.
- [52] Catalfamo M, Karpova T, McNally J, *et al.* Human CD8+ T cells store RANTES in a unique secretory compartment and release it rapidly after TcR stimulation. *Immunity* 2004; 20: 219-230.
- [53] Gould SJ, Booth A M, Hildreth JE. The Trojan exosome hypothesis. *Proc Natl Acad Sci USA* 2003; 100: 10592-10597. Epub 12003 Aug 10528.
- [54] Pelchen-Matthews A, Kramer B, Marsh M. Infectious HIV-1 assembles in late endosomes in primary macrophages. *J Cell Biol* 2003; 162: 443-455. Epub 2003 Jul 2028.
- [55] Ono A, Freed EO. Cell-type-dependent targeting of human immunodeficiency virus type 1 assembly to the plasma membrane and the multivesicular body. *J Virol* 2004; 78: 1552-1563.
- [56] Nguyen DG, Booth A, Gould SJ, Hildreth JE. Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. *J Biol Chem* 2003; 278: 52347-52354. Epub 52003 Oct 52314.
- [57] Zeller W, Bruns M, Lehmann-Grube F. Viral nucleoprotein can be demonstrated on the surface of lymphocytic choriomeningitis virus-infected cells. *Med Microbiol Immunol (Berl)* 1986; 175: 89-92.
- [58] Zeller W, Bruns M, Lehmann-Grube F. Lymphocytic choriomeningitis virus X Demonstration of nucleoprotein on the surface of infected cells. *Virology* 1988; 162: 90-97.
- [59] Bruns M, Kratzberg T, Zeller W, Lehmann-Grube F. Mode of replication of lymphocytic choriomeningitis virus in persistently infected cultivated mouse L cells. *Virology* 1990; 177: 615-624.