Exosomes: A Common Pathway for a Specialized Function

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Exosomes are membrane vesicles that are released by cells upon fusion of multivesicular bodies with the plasma membrane. Their molecular composition reflects their origin in endosomes as intraluminal vesicles. In addition to a common set of membrane and cytosolic molecules, exosomes harbor unique subsets of proteins linked to cell type–associated functions. Exosome secretion participates in the eradication of obsolete proteins but several findings, essentially in the immune system, indicate that exosomes constitute a potential mode of intercellular communication. Release of exosomes by tumor cells and their implication in the propagation of unconventional pathogens such as prions suggests their participation in pathological situations. These findings open up new therapeutic and diagnostic strategies.

Key words: ESCRT machinery, exosomes, multivesicular bodies (MVBs), intercellular communication, ransmissible pathogens.

Abbreviations: MVBs, multi-vesicular bodies; ILVs, intra-luminal vesicles; ESCRT, endosomal sorting complex required for transport; MHC, major histocompatibility complex; TfR, transferrin receptor; IEC, intestinal epithelial cells; GluR 2/3, glutamate receptor subunits 2/3; Hsc, heat shock cognate; Hsp, heat shock protein; PrP, prion protein.

Multivesicular bodies (MVBs), and their intraluminal vesicles (ILVs), are involved in the sequestration of proteins destined for degradation in lysosomes (*1*). An alternative fate of MVBs is their exocytic fusion with the plasma membrane leading to the release of the 50–90 nm ILVs into the extracellular milieu (Fig. 1). The secreted ILVs are then called exosomes (reviewed in Refs. *2* and *3*). After their initial description as vesicles of endosomal origin secreted by reticulocytes during differentiation (*4*), vesicles with the hallmarks of exosomes appeared to be released by other cells. Exosomes are present in the culture supernatant of several cell types of hematopoietic origin [B cells (*5*), dendritic cells (*6*), mast cells (*7*), T cells (*8*) and platelets (*9*)] and of non hematopoietic origin [intestinal epithelial cells (*10*), tumor cells (*11*), Schwann cells (*12*) and neuronal cells (*13*)]. In addition there is increasing evidence for the presence of exosomes in physiological fluids such as plasma (*14*), malignant and pleural effusions (*15*, *16*) and urine (*17*).

As a consequence of proteins and lipids sorting at the limiting membrane of endosomes during the formation of the ILVs in MVBs, exosomes harbour a specific set of molecules. The sorting process and the generation of ILVs require the recognition of cargo proteins by a series of multiprotein complexes that form the ESCRT machinery [for review (*18*)] (Fig. 2). There is increasing evidence, however, that cargo sorting and MVB generation is not solely dependent on ESCRT components. The mechanisms leading to the fusion of MVBs with the plasma membrane and the consequent release of exosomes are unknown,

but could be reminiscent of those involved in the secretory process of lysosome related organelles (*19*).

Selected cell types appear to use exosome secretion to further their own function. Exosome release by reticulocytes clears unwanted proteins and allows net loss of the cell surface membrane, thus contributing to red blood cell differentiation (*20*). Beyond this clearing function of exosomes, the presence of proteins with adhesion properties (*21*) may confer the exosomes other roles. Phenotypical and functional analyses of exosomes from immune cells support the idea that these endosomal-derived vesicles establish a cellular-independent mode of communication. After capture, exosomes attribute new functions to the recipient cell (reviewed in Refs. *2* and *3*). Epithelial cell–derived exosomes, through their ability to be secreted apically and basolaterally, may be used for elimination of unwanted molecules in the lumen and for the transfer of luminal antigenic information towards the mucosal immune tissue (*10*).

The presence of exosomes in biological fluids such as urine (*17*) and blood plasma (*14*) could be exploited as biomarkers for diagnosis purposes. The ability of exosomes from antigen presenting cells (APCs) to stimulate T cells and to induce the eradication of tumors in mice has led to their use as therapeutic agents for the stimulation of anti-tumoral immune responses. Their release from tumor cells and their presence, *in vivo*, in malignant effusions (*11*, *15*) remains puzzling as they could reveal the immunogenic properties of the tumor (*11*, *15*) despite being found to contribute towards tumor progression (*22–25*). Another role for exosomes in disease has emerged with their possible implication in the replication and propagation of transmissible pathogens. Recent studies indicate that the prion protein is released from cells in association with exosomes (*26*). A direct implication of exosomes in

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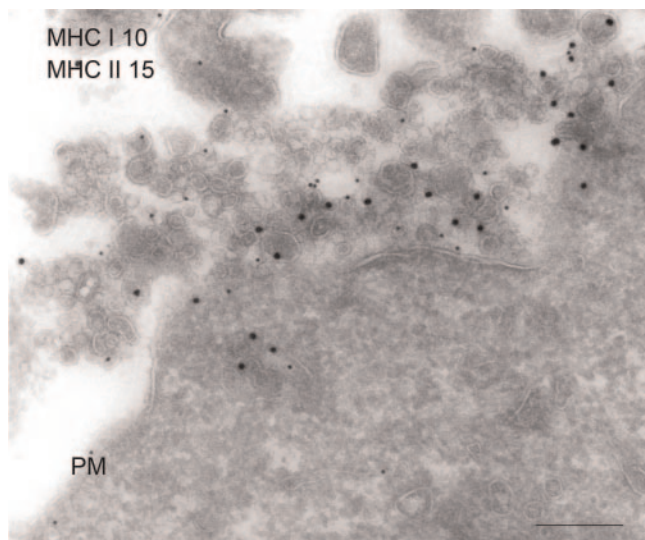
Fig. 1. **MVBs fusion profile.** Fusion of MVBs with the cell surface and exosome release. Detail of an ultrathin cryosection of a B lymphocyte double immunogold labeled for MHC class II (protein A gold 15 nm) and MHC class I (protein A gold 10 nm). MHC class I and class II positive exosomes are observed in the extracellular space closely apposed to the plasma membrane (PM), Bar: 200 nm.

retroviral transmission remains a plausible hypothesis (*27*), however exosomes could also promote HIV trans-infection by providing co-factors such as adhesion molecules (*28*). The aim of this review is to describe the nature of exosomes and demonstrate their relevance in health and disease through highlighting several important findings.

Generation of exosomes

Exosomes can be defined as the ILVs of MVBs once secreted into the extracellular milieu. The ILVs which progressively accumulate during endosome maturation, are formed by inward budding and scission of vesicles from the limiting membrane into the endosomal lumen. During this process, transmembrane and peripheral membrane proteins are incorporated into the invaginating membrane, maintaining the same topological orientation as at the plasma membrane, while cytosolic components are engulfed and enclosed into the small 50–90 nm vesicles (Fig. 2). Exosomes from different cellular origins sequester a common set of molecules but also display cell-type specific components (*2*).

Protein composition of exosomes. The protein composition of exosomes purified from different sources, and in particular from cell culture supernatants of B lymphocytes, DCs, intestinal epithelial cells, Schwann cells and neurons, has been analyzed by proteomics, Facs, Western-blotting and immunocytochemistry at the electron microscopical level (*10*, *12*, *13*, *29*, *31*). This composition reflects the cell type from which they are secreted and their endosomal origin; as well as their possible physiological role and targeting properties (reviewed in Refs. *2* and *3*). Numerous examples of cell-type specific proteins have been described: MHC class II and class I molecules (antigen presenting cells), transferrin receptor (TfR) (reticulocytes), A33 antigen (IEC), CD3 (T cells), and the GluR2/3 subunits of

glutamate receptors (neurons). Similarly different sets of adhesion molecules appear to reflect the cell type (integrins, CAMs, tetraspanins) and end up exposed at the exosomal surface. Exosomes also contain common components such as chaperones (Hsc70 and Hsp90); subunits of trimeric G proteins; cytoskeletal proteins (*e.g.*, actin, tubulin, moesin); ESCRT proteins (Tsg 101, Alix); clathrin; proteins involved in transport and fusion (Rab 7, Rab 2, Annexins); and several enzymes and elongation factors (reviewed in Ref. *2*). Some of these common components (Tsg 101, Alix) are certainly involved in the generation of exosomes (see below) but the functional significance of other proteins (annexins, Rabs) is not fully understood.

Exosomes biogenesis. Due to the diversity of exosomal proteins: transmembrane, peripherally associated membrane proteins, cytosolic proteins and chaperones, their sorting at the endosomal level into ILVs is thought to involve several participants such as ESCRT components, lipids and/or tetraspanins-enriched microdomains (Fig. 2A). Based on studies of protein sorting to ILVs of MVBs, the sequestration of transmembrane proteins in exosomes could require (mono)-ubiquitination and the ESCRT machinery. This model would predict the sequential involvement of three proteins complexes (ESCRT I, II, III) which would sort cargo proteins at the limiting membrane of endosomes. This sorting is preceded by the recognition of the mono-ubiquitinated cargo proteins by Hrs, which associates in a complex with STAM, Eps15 and Clathrin. Hrs recruits Tsg101 of the ESCRT I complex, that is also able to recognize ubiquitin moieties. ESCRT I is then thought to recruit ESCRT III via ESCRT II. Finally, the dissociation and recycling of the ESCRT machinery requires interaction with the AAA-ATPase Vps4. These sequential interactions permit the sequestration of cargo proteins into the inward budding vesicles of maturing MVBs (for review Ref. *18*). Recent studies indicate that ESCRT II may not be directly involved in the biogenesis of MVBs or may be redundant in the process of cargo sorting, or its sorting function just limited to particular cargo (*32*). The presence in exosomes of components of the ESCRT machinery such as Alix and Tsg101 (*3*) and clathrin (*31*) suggest that this machinery is implicated in the sorting of exosomal proteins. To date, the only example of an exosomal-associated protein that appears to require the ESCRT components is the transferrin receptor in reticulocytes. This receptor, however, is not ubiquitinated but associates with Alix to recruit the ESCRT machinery (*33*). These findings, together with observations that other proteins not modified by ubiquitination utilize some (Hrs, VPS4) but not all (tsg101) of the components of the ESCRT machinery (*34*) imply that different protein interactions can provide access to the final steps of ESCRT processing.

ESCRT-independent mechanisms are also likely to operate for sorting of transmembrane cargo proteins in ILVs of MVBs. One example is the premelanosomal protein Pmel17 that has been shown to be present in exosomes (*11*) and whose sorting to ILVs of MVBs is independent of ubiquitination, Hrs and ESCRT I components (*35*). It is also unlikely (although it cannot be excluded) that the sorting of cytosolic and peripherally associated proteins requires the ESCRT machinery. Sorting of cytosolic proteins can be explained by a "random" engulfment of small
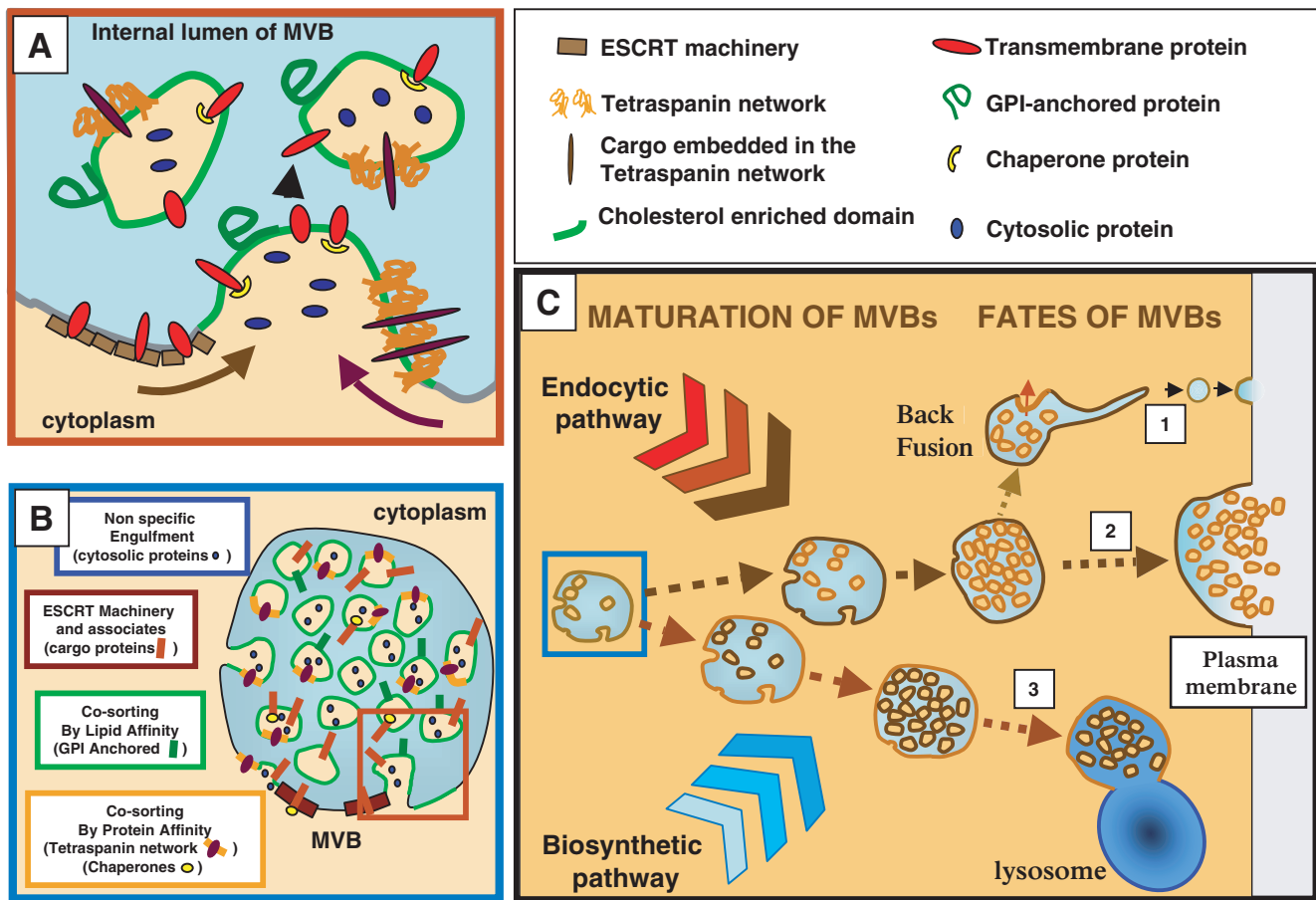
Fig. 2. **Biogenesis of exosomes.** A: At the limiting membrane of MVBs, several mechanism act jointly to allow specific sorting of transmembrane, chaperones, membrane associated and cytosolic proteins on the forming ILVs. B: Presence of several sorting mechanisms may induce heterogeneity in the population of ILVs in single MVBs by acting separately on different domains of the limiting membrane. C: Receiving lipids and proteins from the endocytic and the biosynthetic pathway, different subpopulations of MVBs may be generated whose composition confers them different fate: (1) back fusion of the ILVs with the limiting membrane. During this process molecules previously sequestered on the ILVs are recycled to the limiting membrane and to the cytosol. Change in the composition of the limiting membrane may be responsible for the tubulation allowing plasma membrane expression of endosomal proteins. (2) Unknown mechanism may lead MVBs toward the plasma membrane where proteins such as SNAREs and synaptogamins would allow their fusion and the consequent release of the ILVs in the extracellular medium as exosomes. (3) Similarly, the composition of the limiting membrane would preferentially induce fusion of MVBs with lysosomes leading to the degradation of the molecules sorted on ILVs.

portions of cytosol during the inward budding process and/or by their transient association with transmembrane proteins. For example, the presence of chaperones such as Hsp70 and Hsc70 in exosomes from most cells could be driven by their co-sorting with other proteins. In agreement with this, both Hsp and Hsc70 interact with the TfR and the interaction of TfR with Hsc 70 regulates its release *via* exosomes (*33*).

In relation to GPI-anchored proteins (for example the Prion protein (PrP)) and other "raft"-associated proteins such as flotillin, stomatin or lyn; their sequestration in exosomes, reflects the presence, in exosomal membranes, of lipid raft-like domains. The lipid microdomains and their components could be themselves involved in the generation of the ILVs (*36*), or in concert with other proteins with affinity for "raft-like domains" such as tetraspanins. Tetraspanins interact together to form a network in which integrins, signaling molecules or MHC II could be incorporated. Due to their affinity for cholesterol and sphingolipids, they form membranous microdomains involved in adhesion, motility or signaling (*37*). The presence of high amounts of tetraspanins in exosomes (*2,3*) suggests that recruitment of other membrane proteins from the limiting membrane of endosomes into the ILVs of MVBs could involve their early incorporation into tetraspanin-containing detergent-resistant membrane domains (*31*).

Several mechanisms are thus likely to be involved in cargo protein sorting and ILV formation. This raises the question of whether they all act jointly to generate a unique MVB population or reveal different MVBs subpopulations. The analysis of potentially mixed populations of exosomes recovered from cell culture supernatants or biological fluids cannot answer this question. Electron microscopical observations of B lymphocytes and DCs indicate that the content of a single MVB is heterogeneous with respect to the morphological appearance, the size and the composition of the ILVs, suggesting that multiple mechanisms

operate in a single compartment (our unpublished observations) (Fig. 2B). On the other hand, distinct MVB populations are also likely to be generated in cells (Fig. 2C). Independently of exosome secretion, studies on the EGF receptor have recently shown that the receptor traffics through a subpopulation of MVBs that are distinct from morphologically identical vacuoles that label for lysobisphosphatidic acid (LBPA) (*38*). Moreover, previous findings in B lymphocytes indicate that morphologically similar MVBs contain different amounts of cholesterol. Interestingly those MVBs enriched for cholesterol preferentially fuse with the cell surface (*39*). These data emphasize that the biogenesis of exosomes certainly involves multiple mechanisms and the existence of MVBs "specialized" for secretory purposes cannot be excluded.

Release of exosomes. By analogy with the process of exocytic fusion of secretory lysosomes (*19*), the release of ILVs in the extracellular space requires the transport of the formed MVB towards the cell periphery and its docking and fusion with the plasma membrane. These processes may be dependent on Rab 11, where Rab11 acts on the behaviour of MVBs in living cells and possibly in the process of their secretion (*40*). It is not clear, whether the effects on exosome secretion observed with Rab11 mutants are due to the involvement of this Rab protein in fusion events or a consequence of the alterations of the biogenesis of MVBs. Alternatively, the association of Rab proteins with lipids present in MVBs, could regulate the motility of these compartments. The accumulation of cholesterol in endosome membranes increases the amount of membrane-associated Rab7 and inhibits the motility of late endocytic structures (*41*). Therefore, sequestering specific lipids away from the limiting membrane by sorting them in ILVs, could affect the motility of the late endosytic structures and thus the docking of MVBs with the plasma membrane. As discussed previously, exosomes from different sources contain proteins involved in membrane transport and in fusion events (Rab 5, Rab 7, ARF, rap1B, annexins). At the MVB level these proteins are enclosed in ILVs, but they could be recruited to the cytosolic side of the limiting membrane by a process of back-fusion, which was initially proposed to occur in DCs (*42*). After docking, MVBs fuse with the plasma membrane to release their contents (Fig. 2C). This docking/fusion process is likely to be mediated by SNAREs proteins and synaptotagmin family members. VAMP7, Syntaxin 7 and synaptotagmin 7 are known to be implicated in the fusion of "conventional" lysosomes with the plasma membrane (*43*). It is not known whether the exocytic fusion of MVBs is similarly modulated and/or controlled by the same fusion machinery. Exosome release is sensitive to changes in intracellular calcium in mast cells (*7*) and in a human erythroleukemia cell line (*40*). Depolarisation induced by K^+ appears to increase the secretion of neuronal exosomes (*13*). Crosslinking of CD3 in T cells stimulates exosome release by T cells but the mechanisms underlying such stimulation are not known (*8*). Future work is clearly needed to highlight the molecular mechanisms leading to the release of exosomes, an essential step to further understand their functions and significance.

Functions of exosomes

The molecular composition of exosomes reflects the specialized function(s) of their original cells. Through their ability to bind target cells, they are likely to modulate selected cellular activities, such as vascular homeostasis, and antigen presentation. The presence of exosomes in blood and tissues *in vivo*, suggests their participation in physiological and/or pathological processes. Their particular lipid composition (*31*) and the presence of protective proteins against complement such as CD55 and CD59 may contribute to their stability in the extracellular environment (*44*).

Exosomes from reticulocytes. Exosome release has been demonstrated *in vivo* and *in vitro*, to be a route by which the red cell, during maturation of reticulocytes to erythrocytes, decreases in size and sheds specific plasma membrane activities (*20*). If the cell benefits from exosome release to undergo differentiation, the fate and role of reticulocyte exosomes once released is not clear. The association of Milk Fat globule EGF factor 8 with exosomal membranes may facilitate clearance of reticulocyte exosomes by phagocytes (*45*). The presence of decay-accelerating factor (CD55) and membrane inhibitor of reactive lysis (CD59) known to protect against complement attack (*44*) or the capacity of exosomes to bind endothelial cells through alpha4beta1 integrins/VCAM-1 interactions (*21*) might confer additional functions to exosomes that await further investigation.

Exosomes from immune cells. The presence of molecules involved in antigen presentation give immune cell–derived exosomes a status of potential modulators of the immune response. Initial studies on B-EBV lymphocytic cell lines highlighted the capacity of APCs to secrete exosomes (*5*). Due to the presence of MHC I and II, co-stimulatory and adhesion molecules, B-cell exosomes have the ability to induce antigen-specific MHC class II–restricted T cell responses *in vitro*. One possible fate of B-cell exosomes *in vivo* is their targeting to FDCs in germinal centers, as illustrated by the finding that FDCs in tonsils are likely to acquire MHC II molecules and certainly other molecules *via* exosomes adsorbed at their surface (*46*). In addition, B-cell exosomes express functional integrins, which are capable of mediating anchorage to extracellular matrix components and to cytokine-activated fibroblasts. Notably, exosome adhesion to TNF-alpha–activated fibroblasts triggers integrin-dependent changes in cytosolic calcium suggesting that exosomes may deliver adhesion signals at distances (*47*). As with B cell exosomes, DC-derived exosomes display functional MHC class I and class II, and accessory molecules. These exosomes are able to prime specific cytotoxic T lymphocytes *in vivo* when pulsed with tumor antigen and after injection into mice bearing tumours although they do not have the ability to stimulate directly T cells (*6*). DC-derived exosomes appear to mediate transfer of MHC class II-peptide complexes to an "intermediate" recipient cell, such as a neighbouring DC, then able to activate CD4+T cells in an antigen specific manner (*48*). Such transfer results in an amplification of the immune response due to an increase in the number of cells bearing the significant MHC–peptide complexes. These interactions are possibly primed by the particular sets of adhesion molecules present on exosomes and target

cells. Recent studies indicate that the capacity of DC-derived exosomes to directly stimulate T cells is dependent on their composition, which changes during DC maturation. The presence of I-CAM1 in exosomes from mature DCs appears to be essential for an indirect stimulation of T cells (*49*). In addition to antigen presentation, DC-derived exosomes could also support naive CD4+ T cell survival *via* NF-(kappa) B activation (*50*). In these studies exosomes from APCs may act directly once bound to the cell surface or after capture by a target cell. Internalization of exosomes by target cells is a possible mechanism underlying exosome capture but it is still unclear what endocytic pathway is operating (*51*). It is still also unknown by which pathway mast cell derived exosomes display mitogenic activity on B and T cells *in vitro* and *in vivo* (*52*) or induce up-regulation of PAI-1 secretion from endothelial cells (*53*). T cells have also been shown to release exosomes that could be involved in down regulation of CD3 (*8*). Other studies indicate that the pro-apoptotic membrane associated form of Fas L, can be released on T cell exosomes. FasL on exosomes retained its activity and was able to trigger Fas-dependent apoptosis (*54*), suggesting that exosomes could, by this way, silence an inopportune cell response.

Exosomes in epithelia. The capacity of epithelial cells to secrete exosomes was first described in IECs (*10*). Interestingly, biochemical and morphological analysis showed that exosomes were secreted from the apical and the basolateral sides of the polarized cell monolayers. Both populations shared common proteins such as MHC I, tetraspanins and Hsp, but also contain specific sets of molecules. Apical exosomes contain syntaxin 3, microsomal dipeptidase CD26 and are typically addressed to the apical membrane. Release of apical exosomes in the intestinal lumen suggests their involvement in a clearance process. The presence, however, of High-mobility group box 1 (HMGB1), a cytokine-like pro-inflammatory protein, on immunostimulated Caco-2 cell derived apical exosomes can influence epithelial permeability (*55*). Basolateral exosomes are constitutively enriched in A33 antigen and in MHC II molecules after activation by γIFN (*56*). The presence of the porous basal membrane between epithelium and mucosal immune system suggests a role for basolateral exosomes in antigen presentation independently of direct cellular contact with effector cells. Interestingly murine epithelial cell exosomes display immunostimulatory properties against food antigens when injected intraperitoneally (*56*) whereas exosome-like structures named tolerosomes, isolated from rat IECs and serum, were shown to induce tolerance (*57*). This discrepancy can be explained by a different site of injection of exosomes in the animal, or the activation state of the recipient cells. Tolerizing properties were further emphasized for exosomes extracted from the serum containing weak amounts of A33 antigens. However the same study also showed that "tolerosomes" induce *in vivo* and *in vitro* activation of specific T cells (*57*). The presence of exosomes in serum (*57*) and potentially in cells entering mesenteric lymph nodes (*56*) may confer the ability of static epithelial cells to act at a distance. Exosomes of intestinal epithelium seem therefore to constitute antigen-carrying structures establishing a link between luminal antigens and the local immune system. They could act as sensors of the antigenic information present in the intestinal lumen, their capacity to induce immune response would be then influenced by the inflammation.

Exosomes from other cell types. Despite the lack of evidence for their physiological function *in vivo*, exosomes appear to constitute a new way of communication shared by an increasing number of cell types. In addition to distinct vesicles called microvesicles, platelets secrete exosomes; but the direct implication of these exosomes in blood clotting awaits further studies (*9*). Primary cortical neurons release vesicles with the hallmarks of exosomes harbouring glutamate receptors (GlR2/3). Their secretion is stimulated by depolarization, but their role is still poorly investigated (*13*). Exosomes appear also to be secreted by microglial cells. These exosomes bear the aminopeptidase CD13, which remains active in its ability to cleave neuropeptides (*58*).

With the caution that should be given to the characterization of membrane vesicles as exosomes, many other cells have been proposed to secrete exosomes or exosome-like vesicles. Inflamed intraocular epithelia appeared to release the target antigen hr44 and CD63 *via* exosomes, which may influence the maintenance of an ocular immune privilege (*59*). Kidney epithelial cells also appear to produce exosomes from their apical side, which can be recovered from urine (*17*). Finally, salivary gland epithelial cells (*60*) and the cauda epididymal region (*61*) have been also described to release exosomes. So-called "Epididymosomes" could play a role in sperm epididymal maturation by transferring P25b, which belongs to a family of sperm surface proteins required for the binding to the surface of the egg.

Exosomes in disease

The advantages of an exosomal-acellular mode of communication should aid the development of diagnostic and therapeutic strategies: they are non living, they contain sorted sets of molecules involved in many different cellular processes, they have the capacity to transmit antigenic information and can be easily recovered from fluids. Based on these properties, diagnostic protocols and clinical assays for anti-tumoral immunotherapy are under development. The advantages however are in detriment to the potential consequences that exosomes pose to human health, where exosomes may be used by tumoral cells to invade normal tissue, and by pathogens such as prions and HIV to maximise their spreading in between cells.

Exosomes in diagnosis and therapies. The presence in accessible fluids (blood plasma, urine or broncho-alveaolar lavage fluid) of exosomes containing biomarkers, is already exploited in clinical studies and may be useful for early detection of a disease status. Hundreds of proteins, including multiple protein products of genes already known to be responsible for renal and systemic diseases such as aquaporin-2 have been found in exosomes isolated from urine (*17*).

A potential approach to cancer immunotherapy based on exosomes has arisen from initial studies showing that DC-derived exosomes loaded with tumor peptides are capable of priming cytotoxic T cells and can mediate the

rejection of tumours expressing the relevant antigens in mice (*6*). These "Dexosomes" also promote NK cell activation in immunocompetent mice and NK cell–dependent anti-tumor effects. Based on these results, clinical trials are in progress (*62*). Vaccination strategies could also be envisioned using exosomes from tumor cells that carry tumor antigens (*11*, *63*). By using different sources of tumour exosomes such as Plasmacytoma cells–derived exosomes (*64*), several groups have shown that exosomes can induce tumor-specific immunity, prevent tumor development and are a potential strategy for future therapeutic tumour vaccination.

Some novel properties of exosomes were recently described which included the suppression of inflammatory and autoimmune responses induced by IL10-treated immature DCs derived exosomes (*65*) and protection against infection with T. gondii-pulsed DC-derived exosomes (*66*). Therefore innovative strategies based on exosomes could emerge in the forthcoming years for developing a broad range of novel diagnostics and therapeutics.

Exosomes in cell transformation. The biological significance of exosome secretion by tumor cell lines, and the presence of exosomes in malignant effusions is not clear (*11*, *15*). Despite their immunogenic properties when captured by DCs (*11*), tumoral exosomes may also favour tumor invasion (*25*, *67*). Tumor exosomes bearing NKG2D ligands decrease the capacity of CD8+ T cells to kill target cells (*22*). This discrepancy can be attributed to the presence of DCs in the vicinity of exosomes or to the presence of Hsp with activating properties on exosomes (*68*). Similarly, LMP1, an EBV protein, was found on exosomes from EBV-positive tumor cells. This protein mediates immunosuppressive effects on tumor-infiltrating lymphocytes and LMP1-containing exosomes were shown to inhibit the proliferation of peripheral blood mononuclear cells (*23*). Exosomes could also support tumor cell survival by promoting their invasive properties. The presence on mesothelioma cell–derived exosomes of a strong angiogenic factor (developmental endothelial locus-1 or DEL-1) could participate in the vascular development in the neighbourhood of the tumor (*24*). Other mechanisms contributing to cancer immune evasion have been described. Tumor-derived exosomes could block IL2-mediated activation of NK cells (*69*) or induce Fas Ligand-mediated T-cell apoptosis (*67*). These studies should warrant further investigations considering that the composition of exosomes may determine the capacity of tumor exosomes to induce anti tumor-immune response (*68*).

Exosomes and prions. The finding that the cellular PrP (PrPc) and its scrappie isoform (PrPsc) are associated with exosomes together with the proposal that exosomal membranes constitute a mean of intercellular communication, opens the possibility that exosomes may potentially participate in the propagation of this unusual infectious agent (*26*). Based on Western blot, mass spectrometry and morphological analysis, PrPc and PrPsc in the cell culture supernatants of PrP-expressing rabbit kidney epithelial cells and Schwann cells appeared to be associated with exosomes. Furthermore, PrPsc-containing exosomes inoculated in transgenic mice induced a typical neurological disorder (*12*). Recent studies on platelets are also suggestive of such a role for exosomes in prion diseases (*70*). Exosome delivery from infected to recipient non-infected cells could be relevant to understanding the transconformation process. First the raft-like nature of exosomal membranes (*31*) could be a favourable environment for transconformation and amyloid fiber formation. Second, it has been reported that conversion of PrPc into PrPsc is greatly facilitated when the cellular and the transconformed PrP proteins are in contiguous rather than in facing membranes. Exchange of membranes can result in insertion of incoming PrPsc into the raft domains of recipient cells and exosomes bearing PrPsc released from infected cells could undergo fusion with the plasma membrane of non-infected cells (reviewed in Ref. *26*). Perhaps a more likely scenario would involve internalization of exosomes and fusion with endosomal limiting membranes, similar to the process of back fusion of the internal vesicles of MVBs reported to occur in DCs (*42*).

Exosomes and virus. HIV-1 and other retroviruses accumulate in compartments with features of late endosomes/MVBs because of their ability to exploit the machinery of MVBs generation. Retroviruses and ILVs/Exosomes share several characteristics with relation to their composition and both are released into the extracellular milieu upon fusion of "hijacked" MVBs with the cell surface (reviewed in Ref. *27*). A direct implication of exosomal membranes in HIV infection is not clear but recent observations indicate that HIV particles captured by DCs are internalized into MVB-like compartments (*71*) and are able to be transmitted to T cells by exocytosis without de novo infection. Productive infections of CD4(+) target cells could be facilitated by an association of HIV particles with exosome-like vesicles (*28*).

Concluding remarks

In recent years a wide range of regulatory functions have been attributed to membrane vesicles with the hallmarks of exosomes. The identification of vesicles as exosomes is based on both morphological and biochemical criteria although particular attention should be given to the purification and characterization methods. Exosomes are the ILVs of MVBs and are thus endosome-derived membrane vesicles. An "exosomal-type" of communication is certainly not limited to the immune system and may be exploited by cells to interact with each other, locally or at a distance. Highlighting the cellular and molecular basis of exosome targeting to recipient cells will be invaluable to understand how different populations of exosomes maintain their own specificity. Exosome biogenesis is still far from being well understood and the mechanisms involved in the fusion of "secretory" MVBs with the plasma membrane are unknown. In the forthcoming years, light should be shed on the mechanisms regulating these processes so that methods can be designed to interfere with exosome secretion. With this advance, much more could be discovered on how exosomes function in physiological and pathological situations.

REFERENCES

1. Gruenberg, J. and Stenmark, H. (2004) The biogenesis of multivesicular endosomes. *Nat. Rev. Mol. Cell. Biol.* **5**, 317–323

2. Fevrier, B. and Raposo, G. (2004) Exosomes: endosomal-derived vesicles shipping extracellular messages. *Curr. Opin. Cell Biol.* **16**, 415–421

3. Thery, C., Zitvogel, L., and Amigorena, S. (2002) Exosomes: Composition, biogenesis and function. *Nature Rev. Immunol.* **2**, 569–579

4. Pan, B.T., Teng, K., Wu, C., Adam, M., and Johnstone, R.M. (1985) Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J. Cell Biol.* **101**, 942–948

5. Raposo, G., Nijman, H.W., Stoorvogel, W., Liejendekker, R., Harding, C.V., Melief, C.J., and Geuze, H.J. (1996) B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.* **183**, 1161–1172

6. Zitvogel, L., Regnault, A., Lozier, A., Wolfers, J., Flament, C., Tenza, D., Ricciardi-Castagnoli, P., Raposo, G., and Amigorena, S. (1998) Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nat. Med.* **4**, 594–600

7. Raposo, G., Tenza, D., Mecheri, S., Peronet, R., Bonnerot, C., and Desaymard, C. (1997) Accumulation of Major Histocompatibility Complex Class II Molecules in Mast Cell Secretory Granules and Their Release upon Degranulation. *Mol. Biol. Cell* **8**, 2631–2645

8. Blanchard, N., Lankar, D., Faure, F., Regnault, A., Dumont, C., Raposo, G., and Hivroz, C. (2002) TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J. Immunol.* **168**, 3235–3241

9. Heijnen, H.F., Schiel, A.E., Fijnheer, R., Geuze, H.J., and Sixma, J.J. (1999) Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood* **94**, 3791–3799

10. Van Niel, G., Raposo, G., Candalh, C., Boussac, M., Hershberg, R., Cerf-Bensussan, N., and Heyman, M. (2001) Intestinal epithelial cells secrete exosome-like vesicles. *Gastroenterology* **121**, 337–349

11. Wolfers, J., Lozier, A., Raposo, G., Regnault, A., Thery, C., Masurier, C., Flament, C., Pouzieux, S., Faure, F., Tursz, T., Angevin, E., Amigorena, S., and Zitvogel, L. (2001) Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nat. Med.* **7**, 297–303

12. Fevrier, B., Vilette, D., Archer, F., Loew, D., Faigle, W., Vidal, M., Laude, H., and Raposo, G. (2004) Cells release prions in association with exosomes. *Proc. Natl. Acad. Sci. USA* **101**, 9683–9688

13. Faure, J., Lachenal, G., Court, M., Hirrlinger, J., Chatellard-Causse, C., Blot, B., Grange, J., Schoehn, G., Goldberg, Y., Boyer, V., Kirchhoff, F., Raposo, G., Garin, J., and Sadoul, R. (2006) Exosomes are released by cultured cortical neurones. *Mol. Cell. Neurosci.* **31**, 642–648

14. Caby, M.P., Lankar, D., Vincendeau-Scherrer, C., Raposo, G., and Bonnerot, C. (2005) Exosomal-like vesicles are present in human blood plasma. *Int. Immunol.* **17**, 879–887

15. Andre, F., Schartz, N.E., Movassagh, M., Flament, C., Pautier, P., Morice, P., Pomel, C., Lhomme, C., Escudier, B., Le Chevalier, T., Tursz, T., Amigorena, S., Raposo, G., Angevin, E., and Zitvogel, L. (2002) Malignant effusions and immunogenic tumour-derived exosomes. *Lancet* **360**, 295–305

16. Bard, M.P., Hegmans, J.P., Hemmes, A., Luider, T.M., Willemsen, R., Severijnen, L.A., van Meerbeeck, J.P., Burgers, S.A., Hoogsteden, H.C., and Lambrecht, B.N. (2004) Proteomic analysis of exosomes isolated from human malignant pleural effusions. *Am. J. Respir. Cell. Mol. Biol.* **31**, 114–121

17. Pisitkun, T., Shen, R.F., and Knepper, M.A. (2004) Identification and proteomic profiling of exosomes in human urine. *Proc. Natl. Acad. Sci. USA* **101**, 13368–13373

18. Babst, M. (2005) A protein's final ESCRT. *Traffic* **6**, 2–9

19. Stinchcombe, J., Bossi, G., and Griffiths, G.M. (2004) Linking albinism and immunity: the secrets of secretory lysosomes. *Science* **305**, 55–59

20. Johnstone, R.M. (2006) Exosomes biological significance: A concise review. *Blood Cells Mol. Dis.* **36**, 315–321

21. Rieu, S., Geminard, C., Rabesandratana, H., Sainte-Marie, J., and Vidal, M. (2000) Exosomes released during reticulocyte maturation bind to fibronectin via integrin alpha4beta1. *Eur. J. Biochem.* **267**, 583–590

22. Clayton, A. and Tabi, Z. (2005) Exosomes and the MICA-NKG2D system in cancer. *Blood Cells Mol. Dis.* **34**, 206–213

23. Flanagan, J., Middeldorp, J., and Sculley, T. (2003) Localization of the Epstein-Barr virus protein LMP 1 to exosomes. *J. Gen. Virol.* **84**(Pt 7), 1871–1879

24. Hegmans, J.P.J.J., Bard, M.P.L., Hemmes, A., Luider, T.M., Kleijmeer, M.J., Prins, J.-B., Zitvogel, L., Burgers, S.A., Hoogsteden, H.C., and Lambrecht, B.N. (2004) Proteomic Analysis of Exosomes Secreted by Human Mesothelioma Cells. *Am. J. Pathol.* **164**, 1807–1815

25. Koga, K., Matsumoto, K., Akiyoshi, T., Kubo, M., Yamanaka, N., Tasaki, A., Nakashima, H., Nakamura, M., Kuroki, S., Tanaka, M., and Katano, M. (2005) Purification, characterization and biological significance of tumor-derived exosomes. *Anticancer Res.* **25**, 3703–3707

26. Fevrier, B., Vilette, D., Laude, H., and Raposo, G. (2005) Exosomes: a bubble ride for prions? *Traffic* **6**, 10–17

27. Pelchen-Matthews, A., Raposo, G., and Marsh, M. (2004) Endosomes, exosomes and Trojan viruses. *Trends Microbiol.* **12**, 310–316

28. Wiley, R.D. and Gummuluru, S. (2006) Immature dendritic cell-derived exosomes can mediate HIV-1 trans infection. *Proc. Natl. Acad. Sci. USA* **103**, 738–743

29. Clayton, A., Court, J., Navabi, H., Adams, M., Mason, M.D., Hobot, J.A., Newman, G.R., and Jasani, B. (2001) Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry. *J. Immunol. Methods* **247**, 163–174

30. Thery, C., Regnault, A., Garin, J., Wolfers, J., Zitvogel, L., Ricciardi-Castagnoli, P., Raposo, G., and Amigorena, S. (1999) Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. *J. Cell Biol.* **147**, 599–610

31. Wubbolts, R., Leckie, R.S., Veenhuizen, P.T., Schwarzmann, G., Mobius, W., Hoernschemeyer, J., Slot, J.W., Geuze, H.J., and Stoorvogel, W. (2003) Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. *J. Biol. Chem.* **278**, 10963–10972

32. Bowers, K., Piper, S.C., Edeling, M.A., Gray, S.R., Owen, D.J., Lehner, P.J., and Luzio, J.P. (2006) Degradation of endocytosed epidermal growth factor and virally ubiquitinated major histocompatibility complex class I is independent of mammalian ESCRTII. *J. Biol. Chem.* **281**, 5094–5105

33. Geminard, C., De Gassart, A., Blanc, L., and Vidal, M. (2004) Degradation of AP2 during reticulocyte maturation enhances binding of hsc70 and Alix to a common site on TFR for sorting into exosomes. *Traffic* **5**, 181–193

34. Hislop, J.N., Marley, A., and Von Zastrow, M. (2004) Role of mammalian vacuolar protein-sorting proteins in endocytic trafficking of a non-ubiquitinated G protein-coupled receptor to lysosomes. *J. Biol. Chem.* **279**, 22522–22531

35. Theos, A.C., Truschel, S.T., Tenza, D., Hurbain, I., Harper, D.C., Berson, J.F., Thomas, P.C., Raposo, G., and Marks, M.S. (2006) A lumenal domain-dependent pathway for sorting to intralumenal vesicles of multivesicular endosomes involved in organelle morphogenesis. *Dev Cell* **10**, 343–354

36. de Gassart, A., Geminard, C., Fevrier, B., Raposo, G., and Vidal, M. (2003) Lipid raft-associated protein sorting in exosomes. *Blood* **102**, 4336–4344

37. Hemler, M.E. (2003) Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu. Rev. Cell Dev. Biol.* **19**, 397–422

38. White, I.J., Bailey, L.M., Aghakhani, M.R., Moss, S.E., and Futter, C.E. (2006) EGF stimulates annexin 1-dependent inward vesiculation in a multivesicular endosome subpopulation. *EMBO J.* **25**, 1–12

39. Mobius, W., van Donselaar, E., Ohno-Iwashita, Y., Shimada, Y., Heijnen, H.F., Slot, J.W., and Geuze, H.J. (2003) Recycling compartments and the internal vesicles of multivesicular bodies harbor most of the cholesterol found in the endocytic pathway. *Traffic* **4**, 222–231

40. Savina, A., Fader, C.M., Damiani, M.T., and Colombo, M.I. (2005) Rab11 promotes docking and fusion of multivesicular bodies in a calcium-dependent manner. *Traffic* **6**, 131–143

41. Lebrand, C., Corti, M., Goodson, H., Cosson, P., Cavalli, V., Mayran, N., Faure, J., and Gruenberg, J. (2002) Late endosome motility depends on lipids via the small GTPase Rab7. *EMBO J.* **21**, 1289–1300

42. Kleijmeer, M., Ramm, G., Schuurhuis, D., Griffith, J., Rescigno, M., Ricciardi-Castagnoli, P., Rudensky, A.Y., Ossendorp, F., Melief, C.J., Stoorvogel, W., and Geuze, H.J. (2001) Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. *J. Cell Biol.* **155**, 53–63

43. Rao, S.K., Huynh, C., Proux-Gillardeaux, V., Galli, T., and Andrews, N.W. (2004) Identification of SNAREs involved in synaptotagmin VII-regulated lysosomal exocytosis. *J. Biol. Chem.* **279**, 20471–20479

44. Rabesandratana, H., Toutant, J.P., Reggio, H., and Vidal, M. (1998) Decay-accelerating factor (CD55) and membrane inhibitor of reactive lysis (CD59) are released within exosomes during In vitro maturation of reticulocytes. *Blood* **91**, 2573–2580

45. Blanc, L., De Gassart, A., Geminard, C., Bette-Bobillo, P., and Vidal, M. (2005) Exosome release by reticulocytes–an integral part of the red blood cell differentiation system. *Blood Cells Mol. Dis.* **35**, 21–26

46. Denzer, K., van Eijk, M., Kleijmeer, M.J., Jakobson, E., de Groot, C., and Geuze, H.J. (2000) Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. *J. Immunol.* **165**, 1259–1265

47. Clayton, A., Turkes, A., Dewitt, S., Steadman, R., Mason, M.D., and Hallett, M.B. (2004) Adhesion and signaling by B cell-derived exosomes: the role of integrins. *FASEB J.* **18**, 977–979

48. Thery, C., Duban, L., Segura, E., Veron, P., Lantz, O., and Amigorena, S. (2002) Indirect activation of naive CD4+ T cells by dendritic cell-derived exosomes. *Nat. Immunol.* **3**, 1156–1162

49. Segura, E., Nicco, C., Lombard, B., Veron, P., Raposo, G., Batteux, F., Amigorena, S., and Thery, C. (2005) ICAM-1 on exosomes from mature dendritic cells is critical for efficient naive T-cell priming. *Blood* **106**, 216–223

50. Matsumoto, K., Morisaki, T., Kuroki, H., Kubo, M., Onishi, H., Nakamura, K., Nakahara, C., Kuga, H., Baba, E., Nakamura, M., Hirata, K., Tanaka, M., and Katano, M. (2004) Exosomes secreted from monocyte-derived dendritic cells support in vitro naive CD4+ T cell survival through NF-(kappa)B activation. *Cell Immunol.* **231**, 20–29

51. Morelli, A.E., Larregina, A.T., Shufesky, W.J., Sullivan, M.L., Stolz, D.B., Papworth, G.D., Zahorchak, A.F., Logar, A.J., Wang, Z., Watkins, S.C., Falo, L.D., Jr., and Thomson, A.W. (2004) Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood* **104**, 3257–3266

52. Skokos, D., Le Panse, S., Villa, I., Rousselle, J.C., Peronet, R., David, B., Namane, A., and Mecheri, S. (2001) Mast cell-dependent B and T lymphocyte activation is mediated by

the secretion of immunologically active exosomes. *J. Immunol.* **166**, 868–876

53. Al-Nedawi, K., Szemraj, J., and Cierniewski, C.S. (2005) Mast cell-derived exosomes activate endothelial cells to secrete plasminogen activator inhibitor type 1. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1744–1749

54. Alonso, R., Rodriguez, M.C., Pindado, J., Merino, E., Merida, I., and Izquierdo, M. (2005) Diacylglycerol kinase alpha regulates the secretion of lethal exosomes bearing Fas ligand during activation-induced cell death of T lymphocytes. *J. Biol. Chem.* **280**, 28439–28450

55. Liu, S., Stolz, D.B., Sappington, P.L., Macias, C.A., Killeen, M.E., Tenhunen, J.J., Delude, R.L., and Fink, M.P. (2005) HMGB1 is secreted by immunostimulated enterocytes and contributes to cytomix-induced hyperpermeability of Caco-2 monolayers. *Am. J. Physiol. Cell Physiol.* **290**, C990–C999

56. Van Niel, G., Mallegol, J., Bevilacqua, C., Candalh, C., Brugiere, S., Tomaskovic-Crook, E., Heath, J.K., Cerf-Bensussan, N., and Heyman, M. (2003) Intestinal epithelial exosomes carry MHC class II/peptides able to inform the immune system in mice. *Gut* **52**, 1690–1697

57. Ostman, S., Taube, M., and Telemo, E. (2005) Tolerosome-induced oral tolerance is MHC dependent. *Immunology* **116**, 464–476

58. Potolicchio, I., Carven, G.J., Xu, X., Stipp, C., Riese, R.J., Stern, L.J., and Santambrogio, L. (2005) Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13 in neuropeptide catabolism. *J. Immunol.* **175**, 2237–2243

59. McKechnie, N.M., Copland, D., and Braun, G. (2003) Hr44 secreted with exosomes: Loss from ciliary epithelium in response to inflammation. *Invest. Ophthalmol. Vis. Sci.* **44**, 2650–2656

60. Kapsogeorgou, E.K., Abu-Helu, R.F., Moutsopoulos, H.M., and Manoussakis, M.N. (2005) Salivary gland epithelial cell exosomes: A source of autoantigenic ribonucleoproteins. *Arthritis Rheum.* **52**, 1517–1521

61. Gatti, J.-L., Metayer, S., Belghazi, M., Dacheux, F., and Dacheux, J.-L. (2005) Identification, proteomic profiling, and origin of ram epididymal fluid exosome-like vesicles. *Biol. Reprod.* **72**, 1452–1465

62. Morse, M.A., Garst, J., Osada, T., Khan, S., Hobeika, A., Clay, T.M., Valente, N., Shreeniwas, R., Sutton, M.A., Delcayre, A., Hsu, D.H., Le Pecq, J.B., and Lyerly, H.K. (2005) A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *J. Trans. Med.* **3**, 9

63. Andre, F., Schartz, N.E., Chaput, N., Flament, C., Raposo, G., Amigorena, S., Angevin, E., and Zitvogel, L. (2002) Tumor-derived exosomes: a new source of tumor rejection antigens. *Vaccine* **20 Suppl 4**, A28–A31

64. Altieri, S.L., Khan, A.N., and Tomasi, T.B. (2004) Exosomes from plasmacytoma cells as a tumor vaccine. *J. Immunother.* **27**, 282–288

65. Kim, S.H., Lechman, E.R., Bianco, N., Menon, R., Keravala, A., Nash, J., Mi, Z., Watkins, S.C., Gambotto, A., and Robbins, P.D. (2005) Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. *J. Immunol.* **174**, 6440–6448

66. Aline, F., Bout, D., Amigorena, S., Roingeard, P., and Dimier-Poisson, I. (2004) Toxoplasma gondii antigen-pulsed-dendritic cell-derived exosomes induce a protective immune response against T. gondii infection. *Infect. Immun.* **72**, 4127–4137

67. Taylor, D.D. and Gercel-Taylor, C. (2005) Tumour-derived exosomes and their role in cancer-associated T-cell signalling defects. *Br. J. Cancer* **92**, 305–311

68. Gastpar, R., Gehrmann, M., Bausero, M.A., Asea, A., Gross, C., Schroeder, J.A., and Multhoff, G. (2005) Heat shock protein 70

surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. *Cancer Res.* **65**, 5238–5247

69. Liu, C., Yu, S., Zinn, K., Wang, J., Zhang, L., Jia, Y., Kappes, J.C., Barnes, S., Kimberly, R.P., Grizzle, W.E., and Zhang, H.G. (2006) Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. *J. Immunol.* **176**, 1375–1385

70. Robertson, C., Booth, S.A., Beniac, D.R., Coulthart, M.B., Booth, T.F., and McNicol, A. (2006) Cellular prion protein is released on exosomes from activated platelets. *Blood* **107**, 3907–3911

71. Garcia, E., Pion, M., Pelchen-Matthews, A., Collinson, L., Arrighi, J.F., Blot, G., Leuba, F., Escola, J.M., Demaurex, N., Marsh, M., and Piguet, V. (2005) HIV-1 trafficking to the dendritic cell-T-cell infectious synapse uses a pathway of tetraspanin sorting to the immunological synapse. *Traffic* **6**, 488–501