Exosome Function: From Tumor Immunology to Pathogen Biology

Jeffrey S. Schorey¹,* and Sanchita Bhatnagar¹,²

¹Center for Global Health and Infectious Diseases, Department of Biological Sciences, University of Notre Dame, 130 Galvin Life Science Center, Notre Dame, IN 46556, USA
²Current address: Departments of Immunobiology and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06519, USA
*Corresponding author: Jeffrey S. Schorey, schorey.1@nd.edu

Exosomes are the newest family member of ‘bioactive vesicles’ that function to promote intercellular communication. Exosomes are derived from the fusion of multivesicular bodies with the plasma membrane and extracellular release of the intraluminal vesicles. Recent studies have focused on the biogenesis and composition of exosomes as well as regulation of exosome release. Exosomes have been shown to be released by cells of hematopoietic and non-hematopoietic origin, yet their function remains enigmatic. Much of the prior work has focused on exosomes as a source of tumor antigens and in presentation of tumor antigens to T cells. However, new studies have shown that exosomes might also promote cell-to-cell spread of infectious agents. Moreover, exosomes isolated from cells infected with various intracellular pathogens, including Mycobacterium tuberculosis (M. tb) and Toxoplasma gondii, have been shown to contain microbial components and that these exosomes have immune modulatory activity. In this review, we give a general summary of MVBs and exosomes but focus primarily on their diverse functions as well as their potential usefulness as vaccines and disease biomarkers.

MVB: Biogenesis and Functions

Eukaryotic cells secrete proteins from the biosynthetic pathway by constitutive exocytosis of secretory vesicles or by regulated release of secretory/storage granules upon appropriate stimulation. However, recently, the endocytic network has also been demonstrated to contain an alternative secretory pathway (6). Pan and Johnstone were the first to describe this pathway in reticulocytes (7,8). Subsequent to its discovery in reticulocytes, it has been shown to be present in many cell types including B lymphocytes (9), dendritic cells (DCs) (10), platelets (11), epithelial cells (12) and neurons (13). The studies in reticulocytes demonstrated that this alternative secretory pathway involves the fusion of MVBs with the plasma membrane and extracellular release of the material present within the ILVs. The MVB is an intermediate but a well-defined compartment that is formed from endosomes by invagination of the limiting endosomal membrane.

Although it has been almost 30 years since electron microscopy (EM) studies suggested the presence of...
MVBs in cells, there are still significant gaps in our understanding of MVB biogenesis (14). The process has to be well co-ordinated as it dictates the composition and the fate of ILVs. Only recently have certain mechanisms for protein sorting to MVBs been elucidated and include (i) ubiquitination of the target and (ii) preferential aggregation. Using a *Saccharomyces cerevisiae* experimental system, it has been shown that monoubiquitination of endosomal proteins serves as a signal for sorting to MVBs (15). Some studies have also suggested that oligoubiquitination may also be a sorting signal for trafficking to MVBs, which may increase MVB sorting efficiency (16). A key player in MVB biogenesis is the hetero-oligomeric protein complex, endosomal sorting complex required for transport (ESCRT). ESCRT-I, -II and -III recognize monoubiquitinated cargoes and promote their inclusion in MVBs (14). Once completed, the ESCRT complex dissociates from the MVB membrane aided by the adenosine triphosphatase vacuolar protein sorting 4 (Vps4) and is recycled for subsequent cargo (17). As summarized in Figure 1, hepatocyte growth factor regulated tyrosine kinase substrate (HRS) binds ubiquitinated cargo while simultaneously recruiting the ESCRT family of proteins and mobilizes the cargo for inclusion into MVBs.

However, some proteins such as the transferrin receptor are present in ILVs but are not ubiquitinated. These proteins, which lack the sorting signal for ubiquitination, are partitioned into the ILVs based on their intrinsic physical properties and preference to segregate into raft-like microdomains (18). Molecular aggregation of transferrin receptor in reticulocytes reroutes the receptor from the recycling compartment to the MVB (19). Studies by Geminard et al. indicate that the transferrin receptor can interact with the ESCRT machinery despite the lack of ubiquitination (20). Additional studies have demonstrated that protein aggregation induced by antibodies led to the defective sorting of antigens to MVBs (19). Thus, protein clustering appears to be a major determinant in protein trafficking to the MVB. Recent studies by Theos et al. demonstrated a trafficking of melanosomal protein Pmel17 to MVBs in an ubiquitin- and ESCRT-independent manner.

**Figure 1:** MVB biogenesis and exosome release. Monoubiquitination or aggregation provides the signal for trafficking of proteins and lipids to MVBs. The machinery for sorting ubiquitinated proteins involves the multi-domain Vps27/HRS protein that acts as a bridge between monoubiquitinated transmembrane proteins and clathrin on endosomes (94). ESCRT is also a key player in MVB biogenesis. ESCRT-I, -II and -III recognize monoubiquitinated cargoes and promote their inclusion in MVBs (95). Once completed, the ESCRT complex dissociates from the MVB membrane. Interestingly, the proteins within the ILVs are enriched for ubiquitin, indicating that not all of the ubiquitin is removed from proteins upon targeting to the MVB. Fusion of the MVB and release of the ILVs as exosomes are regulated and are known to require PLD, calcium and Rab11. PLD, phospholipase D.
In summary, not all cargoes are recruited to MVBs by the same mechanism, and there are still significant gaps in our understanding of how different cargoes are targeted to ILVs and how the ILV formation occurs.

In contrast to our limited knowledge of MVB biogenesis, their function has been well defined and plays a central role in endocytic trafficking. In most cell types, the MVBs fuse with the lysosomal compartment and thus shuttle MVB cargo for degradation. However, MVBs can also fuse with the plasma membrane and release their ILVs as ‘exosomes’ (22).

Exosomes are 30- to 100-nm lipid bilayer vesicles with a density of 1.13 g/mL (for B cell derived) to 1.19 g/mL (for intestinal cell derived). Biophysically, exosomes are equivalent to cytoplasm enclosed in a lipid bilayer with the external domains of transmembrane proteins exposed to the extracellular environment. EM studies have demonstrated the fusion of the limiting membrane of MVB with the plasma membrane as well as release of ILVs in different cell types of hematopoietic origin, such as Epstein-Barr virus (EBV)-transformed B cells (9), mastocytes (23), DCs (10,24), platelets (11), macrophages (25) and cells of non-hematopoietic origin like neurons and epithelial cells (13).

Exosome Composition

The lipid and protein content of exosomes has been extensively analyzed by various techniques including Western blotting, fluorescence-activated cell sorting, immuno-EM and mass spectrometry. Exosome composition varies depending on the cell type of origin. Nevertheless, exosomes contain a number of common protein components (26). As shown in Figure 2, the cytosolic proteins present on exosomes include Rabs, which promote exosome docking and the membrane fusion events (27). The annexins, including annexin I, II, V and VI, may regulate membrane cytoskeleton dynamics and membrane fusion events (28). Several adhesion molecules such as intercellular adhesion molecule-1, CD146, CD9, milk-fat-globule EGF-factor VIII (MFG-E8), CD18, CD11a, CD11b, CD11c, CD166 and LFA-3/CD58 have also been identified in exosomal preparations.

Figure 2: Protein composition of exosomes indicating their name, their location (i.e. membrane bound or soluble) and in some cases their function. GDI, GTP dissociation inhibitor; ICAM1, intercellular adhesion molecule-1; CAP-1, adenylyl cyclase-associated protein; LAMP, lysosomal associated membrane protein-1; PGRL, PG regulatory-like protein.
(26,27). In addition, several proteins involved in apoptosis are present on exosomes including thioredoxin peroxidase II, Alix, 14-3-3 and galectin 3. Exosomes also contain heat-shock proteins Hsp70 and Hsp90, which can facilitate peptide loading onto major histocompatibility complex (MHC)I and MHCII (29). One of the characteristic features of exosomes is the tetraspanins, which include CD9, CD63, CD81 and CD82. Exosomes also carry some cell-specific proteins like MHCII and CD86 present only on exosomes isolated from antigen-presenting cells (APCs) (30) and MFG-E8/lactadherin present on exosomes from immature DCs (31). Exosomes are also enriched in proteins that participate in vesicle formation and trafficking like the lysobisphosphatidic acid (LBPA)-binding protein Alix (28). Other proteins detected on exosomes are the metabolic enzymes such as peroxidases, pyruvate and lipid kinases and enolase-1 (32). Consistent with their endosomal origin, exosomes typically do not contain endoplasmic reticulum, mitochondria or nuclear proteins.

Similar to proteins, lipids present on exosomes are characteristic of the cell origin, with most of the lipid analytical work being performed on exosomes derived from reticulocytes (33), mast cells (34), B lymphocyte cell lines (35) and human DCs (34). The typical lipid composition of mast cell-derived exosomes includes lysophosphatidylcholine, sphingomyelin, phosphatidylyceroline, phosphatidylethanolamine, cholesterol and diglyceride (34). Although most of these lipids are also present on exosomes isolated from other cell types, and the ratios of these lipids vary. For instance, the cholesterol/phospholipid ratio is higher in B-cell-derived exosomes compared with exosomes from mast cells and reticulocytes (36). Phospholipids like LBPA accumulate in MVBs and appear to play a key role in ILV formation (37). Recent studies by de Gassart et al. have demonstrated the presence of lipid rafts on the exosomes (18). These low-density, Triton-insoluble fractions are enriched in cholesterol and glycosphingolipids and contain different acylated proteins such as glycosyl-phosphatidylinositol-anchored proteins and tyrosine kinases of the Src family (38).

**Exosome Function**

The release of exosomes provides another mechanism of intercellular communication. Exosome function will depend on the cell type from which they were derived and the composition of exosomes in terms of lipids, carbohydrates and proteins. Because exosomes contain a spectrum of surface molecules, they provide a mechanism to engage different cell receptors simultaneously and for exchange of material between cells.

**Alternative secretion of proteins by exosomes**

Exosome release was initially characterized as a mechanism to eliminate obsolete proteins during reticulocyte maturation and differentiation. Johnstone et al. showed that sheep red blood cells lose their transferrin receptor during *in vivo* and *in vitro* maturation by exosome release (39). The transferrin receptor is tightly associated with Hsp70 on exosomes, and the heat-shock protein may play a role in exosome release from reticulocytes. How soluble proteins that lack a signal sequence are released from cells has been the focus of considerable research, and some mechanisms have recently been defined. One method is through association with exosomes, as observed for the translationally controlled tumor protein (TCTP), a cytoplasmic protein that facilitates an inflammatory response by stimulating histamine release. Tumor suppressor activated pathway 6 (TSAP6), a p53-inducible 5–6 transmembrane protein, has been reported to associate with exosomes as well as with cytoplasmic TCTP, thus facilitating the release of TCTP through exosomes (40). A more recent study by Yu et al. demonstrated that exosomes function in the release of some p53-regulated extracellular proteins and that this effect stems from the upregulation of TSAP6 expression by p53 (41). Another function attributed to exosomes includes the constitutive extracellular release of tumor necrosis factor (TNF) receptor 1 (42). This established a new mechanism for release of soluble cytokine receptors, which could compete for ligand binding. The cytokine interleukin (IL)-1β and possibly the chemokine regulated activation normal T cell expressed and secreted (RANTES) may also be secreted by exosomes (43). Other studies have defined a role for exosomes in ectodomain shedding and consequently a vehicle for the cellular export of soluble molecules like L1 (CD171) and CD44 in ovarian carcinoma cells (44). A study by Azevedo et al. showed that in sepsis, nitric oxide and bacterial elements are responsible for type-specific platelet-derived exosome generation (45). These exosomes play an active role in vascular signaling as redox active particles, which induced endothelial cell caspase-3 activation and apoptosis of vascular cells (46).

**Antigen presentation**

Pioneering studies by Raposo et al. showed that exosomes secreted by EBV-transformed B cells can stimulate human CD4+ T-cell clones in an antigen-specific manner (9). Their studies were the first to document the release of intact MHCII on the exosomes secreted by both human and murine B-cell lines. Subsequent studies have shown that exosomes produced by mouse DCs pulsed with tumor peptides induced the rejection of established tumors in a T-cell-dependent fashion (10). Exosomes can also transfer antigens from tumor cells to DCs (47) and therefore functions in antigen cross-presentation. Like DC-derived exosomes, exosomes from tumor cells carry MHC molecules along with tumor antigens like melan-A/MART1 (melanoma tumor), which can be recognized by T cells (27). Together, the data implicate that exosomes can function in presenting tumor antigens to sensitized T cells and can promote tumor rejection *in vivo* (10,43). A number of excellent reviews are available on exosomes role in tumor immunity (48–50). Interestingly, recent studies by Muntasell et al. showed that in a 24-h period, ~12% of the surface-bound peptide–MHCII complex is endocytosed,
trafficked to MVBs and released on exosomes, suggesting that exosome discharge may be a common mechanism to seed secondary lymphoid organs with membrane-bound antigen (51).

In addition to their role in antigen presentation, exosomes have also been implicated in immune suppression. This was nicely demonstrated by Peche et al. who showed that injecting donor-haplotype exosomes from bone marrow DCs before transplantation leads to a significantly prolonged heart allograft survival in congenic and fully MHC-mismatched Lewis rats (52). Moreover, in vivo studies showed a significant decrease in CD4⁺ T cells in the exosome-treated recipient, suggesting an immunotolerance effect (52). Exosomes isolated from immature DCs treated with cytokines, such as IL-4 and IL-10, when injected into mice reduced the severity of established collagen-induced arthritis (53,54). DCs that were virally transduced to produce Fas ligand (FasL) also produced exosomes with anti-inflammatory activity (65). Therefore, the use of exosomes may be a better therapeutic approach compared with DCs for the treatment of autoimmune diseases such as rheumatoid arthritis. ‘Tolerosomes’ corresponding to exosome-like structures are produced by intestinal epithelial cells and could induce tolerance to oral antigens (56–58). Moreover, exosomes from T cells, melanoma cells and ovarian tumor cells have been shown to carry FasL, which could induce T-cell apoptosis (59,60). Tumor-derived exosomes may also impair DC development and induce myeloid-suppressive cells (61).

**Shuttle for RNA**

Elegant studies by Valadi et al. showed that exosomes are enriched in messenger RNA and micro RNA. The exosomes derived from a human (HMC-1) and mouse (MC/9) mast cell lines were found to transport RNA to neighboring mast cells, which was then translated indicating that the transferred RNA was biologically active. The RNA transferred through exosomes (exosomal shuttle RNA) can confer new functions to the cells (62).

**Shuttle for infectious agents**

The cellular process associated with MVB biogenesis and release has been commandeered during evolution by various pathogens, including viruses, to provide an escape mechanism from the host immune response. Indeed, an evolutionary link between retroviruses and exosome biogenesis has been proposed (63). Studies with retroviruses have revealed the ability of viruses to hijack the intracellular machinery of MVBs for their budding at the cell surface (64). HIV utilizes MVBs as the major site for accumulation in human macrophages (25,63), and the viruses released have markers commonly found on exosomes. However, although the HIV particles and exosome contain similar components, they may have different origins (5). Interestingly, recent studies in HIV-1-infected macrophages have suggested that HIV-1 is present within internally sequenced CD63-positive plasma membrane domains but not in endosomes (65).

New studies have revealed an unexpected role for exosomes in the spread of prions. Prion diseases are fatal neurodegenerative disorders that affect both humans and animals. Raposo and colleagues have demonstrated that prion protein (PrP) in both its normal (PrP⁰) and its scrappie (PRPsc) conformation are trafficked to MVBs and released on exosomes (66).

**Pathogen immune surveillance – a novel function of exosomes**

Recent work has yielded substantial insight into the immune responses required for controlling an infection and how pathogens circumvent these mechanisms. It is now apparent that innate effector mechanisms are initiated through specific detection of microbial patterns, which facilitate an immune response. These microbial signatures are referred to as pathogen-associated molecular patterns (PAMPs) and are specifically recognized by the host’s pattern recognition receptors (67). Therefore, PAMPs expressed on the surface or released by the pathogen play an essential role in stimulating immunity. By their nature, intracellular pathogens show a more limited exposure to the immune system compared with extracellular pathogens, and this includes limited exposure of PAMPs. We and others hypothesized that release of exosomes from infected cells may be one mechanism by which this sequestration of PAMPs can be overcome.

The first evidence to support this hypothesis came from a series of insightful experiments by Russell and colleagues where they identified a number of the mycobacteria cell wall components that were trafficked inside the infected cell (68). EM studies by Beatty et al. identified mycobacterial PAMPs including lipoarabinomannan (LAM) and phosphatidylymyo-inositol mannosides (PIM) in the endocytic compartment of Mycobacterium bovis BCG-infected macrophages (68). Density gradient electrophoresis analysis of the infected cells also suggested that released mycobacterial lipids coalesce in the late endosomal/lysosomal compartments, including MVBs (68). Studies directed toward the trafficking of Mycobacterium avium glycopeptidolipids (GPL) also indicated a similar release and trafficking of the GPL inside the macrophages (69). Moreover, these studies indicated that the mycobacterial PAMPs were not confined to the infected cells but were also trafficked to the neighboring bystander cells (68–71). These results raised an important question about the mechanism of mycobacterial component secretion.

Beatty et al. originally determined that vesicles the size of exosomes contain mycobacterial components (68). Additional studies indicated that the mycobacterial components, including GPL, LAM, PIM, trehalose dimycolate and phenolic glycolipids, were released from mycobacterial-infected...
macrophages through exosomes (Figure 3A) (69,71) and that these exosomes were captured by the bystander uninfected cells. Interestingly, some of these lipids have been shown to induce a proinflammatory response when introduced to uninfected macrophages. For instance, PIM2 coated on microspheres could induce TNF-α and mycobacteria-containing phagosome-1 in interferon-γ-primed bone marrow macrophages or thioglycollate-elicited peritoneal macrophages (71). Moreover, as indicated above, exosomes can function to modulate immune responses, including immune stimulation and immune suppression. Our recent studies demonstrated that exosomes derived from Mycobacterium-infected cells were capable of inducing a proinflammatory response as indicated by the TNF-α and RANTES secretion and inducible nitric oxide synthase (iNOS) induction in naïve cells (Figure 3B) (69,72). This response was completely dependent on MyD88, an adaptor molecule required for most Toll-like receptor signaling (69). The stimulatory activity of exosomes were also replicated in vivo where mice injected with exosomes derived from M. bovis bacille Calmette–Guérin (BCG) or M. tb-infected cells induced a TNF-α and IL-12p40 response as well as neutrophil and alveolar macrophage migration into the bronchoalveolar lavage fluid (BALF) (69). Exosomes have been shown to be released in the biological fluids like urine (73), amniotic fluid (74), BALF (75) and plasma (76). Exosomes isolated from the BALF of the mice infected with M. bovis BCG also contained PAMPs and could induce a proinflammatory response in treated macrophages (72). These results, for

Figure 3: Composition and trafficking of exosomes from infected macrophages with a focus on mycobacterial-infected cells. A) A list of microbial or infectious components that have been shown to be on exosomes. These include ‘infectious’ proteins such as prions as well as HIV. Also shown are microbial molecules, which have been released from the pathogen and trafficked to MVBs and present on exosomes. B) General diagram of how mycobacterial PAMPs are released from the mycobacteria within a phagosome and transported to the MVB for release on exosomes. The exosomes containing the PAMPs bind to pattern recognition receptors (PRRs) on surrounding macrophages leading to macrophage activation. LPS, lipopolysaccharide; MCP, mycobacteria-containing phagosome; FAP, fibronectin attachment protein; iNOS, inducible nitric oxide synthase; MAPK, mitogen activated protein kinase.
the first time, reveal exosomes ability to induce a proinflammatory response both in vitro and in vivo.

These experiments supported a role for exosomes in the intercellular transport of mycobacterial components; however, which component(s) induced the proinflammatory response in naıve macrophages was not defined. Insight into this question was provided by our recent studies using the M. tb H37Rv lspaA knockout strain, which lacks the 19-kDa lipoprotein (77). Previous studies have indicated that the 19-kDa lipoprotein of M. tb interacts with TLR2 and can induce IL-12p40 production in macrophages (78). Exosomes isolated from macrophages infected with the LspA-deficient M. tb failed to induce TNF-α secretion or iNOS production in uninfected macrophages, whereas cells treated with exosomes from wild-type M. tb or the LspaA-complemented M. tb strain induced both TNF-α secretion and iNOS production (Figure 4). Together, these data indicate that the 19-KDa lipoprotein is present on exosomes from M. tb-infected macrophages and is responsible, at least in part, for the exosomes proinflammatory activity.

Interestingly, this phenomenon of immune surveillance in the host by exosomes was not unique to the Mycobacterium genus as exosomes isolated from Salmonella typhi-}

**Exosomes as vaccine candidates**

The use of exosomes has garnered considerable interest as vaccine candidates for tumor immunotherapy (79). Much of this interest stems from the difficulty associated with DC-based immunotherapy and how an exosome-based approach can overcome some of these difficulties. Tumor cell-derived exosomes containing tumor antigens plus MHC class I molecules can transfer tumor antigens to DCs to induce a CD8+ T-cell dependent anti-tumor immune response (80). Exosomes released from DCs pulsed with tumor antigens were also shown to elicit strong anti-tumor responses. Data obtained in mice have shown that exosomes obtained from DCs pulsed with tumor peptides could prime specific cytotoxic T lymphocytes (CTLs) in vivo and limit or suppress growth of established murine tumors in a T-cell-dependent manner (10,81). Interestingly, tumor-derived exosomes may have broader activity than previously believed as one study showed that exosomes isolated from different tumors inhibited not only syngeneic but also allogenic tumor growth, indicating that tumor-derived exosomes may harbor some common tumor antigens (47). Together, these studies indicate that exosomes can be isolated from tumor cells or from DCs pulsed with tumor antigens to deliver a target immunogen capable of inducing an effective immune response and that they may represent a new cell-free vaccine. Some phase I clinical trials have been completed, and although problems with their use remain, the data suggest that exosome-based therapy is a viable approach (82).

The successful use of exosomes in cancer immunotherapy has lead to the hypothesis that they could function as vaccine candidates in the context of infectious diseases. Aline et al. demonstrated that exosomes derived from DCs pulsed with T. gondii tachyzoite sonicates could induce a protective immune response against T. gondii infection. These exosomes primed an antigen-specific cellular and humoral immune response, which provided a good protection against both acute and chronic toxoplasmosis (83). Moreover, CBA/J mice vaccinated with exosomes isolated from T. gondii antigen-pulsed DCs exhibited significantly fewer brain cysts (84). Another application of exosomes in immunotherapy has been implicated in the treatment of pneumococcal infection in mice (85). Colino and Snapper

**Figure 4:** Exosomes isolated from macrophages infected with an LspaA-deficient H37Rv fail to stimulate macrophage activation. Exosomes were isolated from infected J774 cells as described (81) and used to treat bone marrow-derived murine macrophages. After 24 h, supernatants were harvested and analyzed for iNOS expression by Western blot as described (81). Total MAPK p38 was used as a loading control. COMP, the LspaA mutant complemented with the wild-type (WT) LspaA gene; MUT, H37Rv mutant that lacks LspaA and therefore fails to make the 19-KDa lipoprotein; RC, untreated cells; UI, exosomes from uninfected cells.

Traffic 2008; 9: 871–881
showed that murine bone marrow-derived DCs (BMDCs)
pulsed in vitro with intact diphtheria toxin (DT)-released
exosomes, which upon injection into mice induce immuno-
globulin G (IgG)2b and IgG2a responses specific for DT
(86). Exosomes have also been evaluated in the context of
Streptococcus infections. Invasive strains of Streptococ-
cus pneumoniae are leading causes of meningitis and major
causes of otitis media and bacteremia in children and
pneumonia in the elderly (87). Vaccine-mediated protection against S. pneumoniae infection is based on
humoral immunity specific for S. pneumoniae capsular
polysaccharides (Cps) (88). Similar to the DT exosomes,
BMDCs treated with Cps14 released exosomes enriched
in Cps14. These purified exosomes could induce a S.
pneumoniae-protective Cps14-specific immunoglobulin
M and IgG3 response in naive recipients (85). Exosomes
isolated from M. bovis BCG-infected macrophages could
stimulate splenic T cells isolated from BCG-infected mice
(U. S., unpublished data), but whether these exosomes
can function as vaccine candidates awaits further study.
Exosomes as a vaccine has also been explored for atypical
severe acute respiratory syndrome (SARS) caused by the
positive-stranded RNA virus, SARS-associated coronavirus
(SARS-CoV). Studies by Kuate et al. showed that exo-
somes containing spike S protein of SARS-CoV induced
neutralizing antibody titres (89). This immune response
was further accentuated by priming with the SARS-S
exosomal vaccine and then boosting with the currently
used adenoviral vector vaccine (89).

In addition to the potential use of exosomes as vaccines
against infectious diseases, exosomes have also proved
useful in treatment of autoimmune diseases in animal
models. This is illustrated in studies by Kim et al. who
showed that administration of exosomes derived from
DCs-expressing recombinant IL-4 was able to modulate
the activity of APC and T cells in vivo, partly through a Fasl/
Fas-dependent mechanism, resulting in effective treat-
ment against collagen-induced arthritis through suppres-
sion of the delayed-type hypersensitivity inflammatory
response (90).

Exosomes as biomarkers
The proteins associated with renal diseases could be
detected on exosomes isolated from urine, indicating
a possible use for urine exosomes as biomarkers (91).
For instance, Pisitkun et al. demonstrated the excretion
of exosomes containing aquaporin-2 protein in autosomal
dominant and autosomal recessive nephrogenic diabetes
insipidus patients (73). Similar proteomic studies per-
formed on urinary exosomes generated a long list of
molecular signatures, illustrating valuable potential for
diagnostic, prognostic and pathophysiological discovery
(92). Similar to reno pathologies, exosomes are also an
attractive biomarker candidate for cancer diagnosis with
most of the focus centered on bladder cancer. The differ-
entially expressed proteins include psoriasin, kertain-14,
galectin-7, epidermal fatty acid binding protein (E-FABP),
migration inhibitor factor-related protein (MRP8) and 14
and stratfin, which may be useful markers for the diagno-
sis of bladder cancer (91). Exosomes may also be valuable
as biomarkers for infectious diseases, mainly in the
context of defining treatment success. Unfortunately, to
date, this has not been tested. Exosomes may be parti-
cularly useful in the context of tuberculosis (TB) as the
time required to test a new TB drug treatment protocol is
extensive, leading to high drug development cost as well
as delays in the introduction of new medication. A major
limitation in developing an efficient drug treatment for TB
is the lack of available methodology to identify an early
infection as well as determine drug treatment efficacy.
Currently, a major goal is to identify disease biomarkers in
biological fluids that can be measured relatively inexpen-
sively for early diagnosis of disease and treatment suc-
cess. We hypothesize that exosomes, whose composition
may change during the course of an M. tb infection and
treatment, may be such a biomarker.

Exosome display technology
Exosome display technology is a novel technique of
manipulating the molecular composition of the exosomes
and tailoring exosomes with new desirable properties.
Recently, exosome display was applied for the induction
of epitope-specific antibody response against tumor bio-
markers (93). This technology opens up new possibilities in
designing novel therapies and generating new diagnostic
tools. Exosome display has been used to prepare recombi-
ant vesicles carrying cytokines as well as tumor antigens
that may or may not have been previously found on
exosomes (79). The targeted co-delivery of antigens with
the activators of DCs, B-, T- or natural killer cells may also
accentuate the exosomes efficacy as a vaccine.

Concluding Remarks and Future Direction
It has become increasingly clear, as new exosome studies
are published, that these small bioactive vesicles are
important in a number of biological functions. From their
original discovery in the removal of unwanted proteins
from maturing reticulocytes to their role in immune sur-
veillance, the inventory of functions continues to grow. As
cancer phase I clinical trails have shown, our knowledge
of exosomes can be applied therapeutically and the use
of exosomes in treatment and diagnostics is also likely
to grow. Nevertheless, there are still many unanswered
questions including (i) how is fusion of a MVB with the
plasma membrane and release of exosomes regulated, (ii)
under what circumstances do exosomes function in vivo,
and what are the consequences of their expression and (iii)
how can we modify exosome composition to maximize
their efficacy as vaccines or therapeutic agents. As our
understanding of exosome formation and function contin-
ues to expand, answers to these and many other ques-
tions should be forthcoming.
References

1. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987; 262:9412–9420.

2. Couzin J. Cell biology: the ins and outs of exosomes. Science 2005; 308:1862–1863.

3. Gould SJ, Booth AM, Hildreth JE. The Trojan exosome hypothesis. Proc Natl Acad Sci U S A 2003;100:10592–10597.

4. Fang Y, Wu N, Gan X, Yan W, Morrell JC, Gould SJ. Higher-order oligomerization targets plasma membrane proteins and HIV gag to exosomes. PLoS Biol 2007;5:e158.

5. Chen BJ, Lamb RA. Mechanisms for enveloped virus budding: can some viruses do without an ESCRT? Virology 2007;372:221–232.

6. van Niel G, Porta-Carreiro I, Simoes S, Raposo G. Exosomes: a common pathway for a specialized function. J Biochem 2006;140:13–21.

7. Pan BT, Teng K, Wu C, Adam M, Johnstone RM. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol 1985;101:942–948.

8. Pan BT, Bloxstein R, Johnstone RM. Loss of the transferrin receptor during the maturation of sheep reticulocytes in vitro. An immunological approach. Biochem J 1983;210:37–47.

9. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996;183:1161–1172.

10. Zitvogel L, Regnault A, Lozier A, Wolters J, Flament C, Tenza D, Riccardi-Castagnoli P, Raposo G, Amigorena S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med 1998;4:594–600.

11. 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.

12. Wilson JM, Whitney JA, Neutra MR. Biogenesis of the apical endosomal-lysosome complex during differentiation of absorptive epithelial cells in rat ileum. J Cell Sci 1991;100:133–143.

13. Marcesco AM, Janich P, Wilsch-Brauninger M, Dubreuil V, Langenfeld K, Corbeil D, Huttner WB. Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells. J Cell Sci 2005;118:2849–2858.

14. Pier RC, Katzmann DJ. Biogenesis and function of multivesicular bodies. Annu Rev Cell Dev Biol 2007;23:519–547.

15. Katzmann DJ, Sarkar S, Chu T, Audhya A, Erm SD. Multivesicular body sorting: ubiquitin ligase Rsp5 is required for the modification and sorting of carboxypeptidase S. Mol Biol Cell 2004;15:488–480.

16. van Niel G, Wubbolts R, Ten Broeke T, Buschow SI, Ossendorp FA, van Niel G, Wubbolts R, Ten Broeke T, Buschow SI, Ossendorp FA. Multivesicular body sorting of carboxypeptidase S. Mol Biol Cell 2004;15:468–480.

17. de Gassart A, Geminard C, Fevrier B, Raposo G, Vidal M. Lipid raft-associated protein sorting in exosomes. Blood 2003;102:4336–4344.

18. 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.

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

20. Teohs AC, Truschel ST, Tenza D, Hubrain I, Harper DC, Berson JF, Thomas PC, Raposo G, Marks MS. A lumenal domain-dependent pathway for sorting to intraluminal vesicles of multivesicular endosomes involved in organelle morphogenesis. Dev Cell 2006;10:343–354.

21. 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.

22. Raposo G, Tenza D, Mecheri S, Peronnet R, Bonnerot C, Desaymard 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.

23. Thery C, Regnault A, Garin J, Wolters J, Zitvogel L, Riccardi-Castagnoli P, Raposo G, Amigorena S. Molecular characterization of dendritic cell-derived exosomes. Selective accumulation of the heat shock protein hsc73. J Cell Biol 1999;147:599–610.

24. Nguyen DG, Booth A, Gould SJ, Hildreth JE. Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. J Biochem 2003;278:52347–52354.

25. Thery C, Boussac M, Peron P, Riccardi-Castagnoli P, Raposo G, Garin J, Amigorena S. Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. J Immunol 2001;168:7309–7318.

26. Mears R, Craven RA, Hanrahan S, Totty N, Upton C, Young SL, Patel P, Selby PJ, Banks RE. Proteomic analysis of melanoma-derived exosomes by two-dimensional polyacrylamide gel electrophoresis and mass spectrometry. Proteomics 2004;4:4019–4031.

27. Futter CE, White JI. Annexins and endocytosis. Traffic 2007;8:951–958.

28. Gastpar R, Gehrmann M, Bausero MA, Asea A, Gross C, Schroader JA, Multhoff G. Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells. Cancer Res 2005;65:5238–5247.

29. Segura E, Amigorena S, Thery C. Mature dendritic cells secrete exosomes with strong ability to induce antigen-specific effector immune responses. Blood Cells Mol Dis 2005;35:89–93.

30. Veron P, Segura E, Sugano G, Amigorena S, Thery C. Accumulation of MFG-E8/actadherin on exosomes from immature dendritic cells. Blood Cells Mol Dis 2005;35:81–88.

31. Hegmans JP, Barden MP, Hemmes A, Luider TM, Kleijmeer MJ, Prins JB, Zitvogel L, Burgers SA, Hoogsteden HC, Lambrecht BN. Proteomic analysis of exosomes secreted by human mesothelioma cells. Am J Pathol 2004;164:1807–1815.

32. Vidal M, Sainte-Marie J, Philippot JR, Bienvenue A. Asymmetric distribution of phospholipids in the membrane of vesicles released during in vitro maturation of guinea pig reticulocytes: evidence precluding a role for “aminophospholipid translocase”. J Cell Physiol 1989; 140:455–462.

33. Laulagnier K, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux JF, Mom JL, Thuillier R, Casanove B, Delage A, Vidal M. Heat shock protein 70 surface-positive tumor exosomes: proteomic analyses 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.
36. Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. Biochimie 2007;89:205–212.
37. Chu Z, Witte DP, Qi X. Saposin C-LBPA interaction in late-endosomes/lysosomes. Exp Cell Res 2008;303:300–307.
38. Echarri A, Muriel O, Del Pozo MA. Intracellular trafficking of raft/caveola domains: insights from integrin signaling. Semin Cell Dev Biol 2007;18:627–637.
39. Johnstone RM, Adam M, Pan BT. The fate of the transferrin receptor during maturation of sheep reticulocytes in vitro. Can J Biochem Cell Biol 1984;62:1246–1254.
40. Amzallag N, Passer BJ, Allanic D, Segura E, Thery C, Goud B, Amson R, Telemann A. T6ASP facilitates the secretion of translationally controlled tumour protein/histamine-releasing factor via a nonclassical pathway. J Biol Chem 2004;279:46104–46112.
41. Yu X, Harris SL, Levine AJ. The regulation of exosome secretion: a novel function of the p53 protein. Cancer Res 2006;66:4795–4801.
42. Zhang HG, Liu C, Su K, Yu S, Zhang L, Zhang S, Wang J, Cao X, Grizzle W, Kimberly RP. A membrane form of TNF-alpha is presented by exosomes delays T cell activation-induced cell death. J Immunol 2006;176:7385–7393.
43. Chen W, Wang J, Shao C, Liu S, Yu Y, Wang Q, Cao X. Efficient induction of antimurin T cell immunity by exosomes derived from heat-shocked lymphoma cells. Eur J Immunol 2006;36:1598–1607.
44. Stocek A, Keller S, Riedle S, Sanderson MP, Runz S, Le Naour F, Gutwein P, Ludwig A, Rubinstein E, Altevogt P. A role for exosomes in the constitutive and stimulus-induced ectodomain cleavage of L1 and CD44. Biochem J 2006;393:609–618.
45. Azevedo LC, Janiszewski M, Pontieri V, Pedro MA, Bassi E, Tucci PJ, Laurindo FR. Platelet-derived exosomes from septic shock patients induce myocardial dysfunction. Crit Care 2007;11:R120.
46. Gambim MH, Caram AO, Marti L, Verissimo-Filho S, Lopes LR, Janiszewski M. Platelet-derived exosomes induce endothelial cell apoptosis through peroxynitrite generation: experimental evidence for a novel mechanism of septic vascular dysfunction. Crit Care 2007;11:R107.
47. Wolfers J, Lozier A, Raposo G, Regnaut A, Thery C, Masurier C, Flamant C, Pouzieux S, Faure F, Tursz T, Angève C, Amigorena S, Zitvogel L. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nat Med 2001;7:297–303.
48. Andre F, Schartz NE, Chaput N, Flamant C, Raposo G, Amigorena S, Angève C, Zitvogel L. Tumor-derived exosomes: a new source of tumor rejection antigens. Vaccine 2002;20 (Suppl. 4):A28–A31.
49. Chaput N, Flamant C, Viaud S, Taieb J, Roux S, Spatz A, Andre F, Lepeceq JB, Boussac M, Garin J, Amigorena S, Cervel T, Zitvogel L. Dendritic cell-derived exosomes: biology and clinical implications. J Leukoc Biol 2008;80:471–478.
50. Andre F, Escudier B, Angévin E, Tursz T, Zitvogel L. Exosomes for cancer immunotherapy. Ann Oncol 2004;15 (Suppl. 4):v141–v144.
51. Muntassil A, Berger AC, Roche PA. T cell-induced secretion of MHC class II peptide complexes on B cell exosomes. EMBO J 2007;26:4263–4272.
52. 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.
53. Kim SH, Lechman ER, Bianco NR, Menon R, Keravala A, Nash J, Mi Z, Watkins SC, Gabbotto A, Robbins PD. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. J Immunol 2006;174:6440–6448.
54. Kim SH, Bianco NR, Shufesky WJ, Morelli AE, Robbins PD. Effective treatment of inflammatory disease models with exosomes derived from dendritic cells genetically modified to express IL-4. J Immunol 2007;179:2242–2249.
55. Kim SH, Bianco N, Menon R, Lechman ER, Shufesky WJ, Morelli AE, Robbins PD. Exosomes derived from genetically modified DC expressing Fasl are anti-inflammatory and immunosuppressive. Mol Ther 2006;13:289–300.
56. Karlsson M, Lundin S, Dahlgren U, Kahu H, Pettersson I, Tellemo E. “Tolerosomes” are produced by intestinal epithelial cells. Eur J Immunol 2001;31:2892–2900.
57. van Niel G, Mallegol J, Bevilaqua C, Candalh C, Brugiere S, Tomaskovic-Crook E, Heath JK, Cerf-Bensussan N, Heyman M. Intestinal epithelial exosomes carry MHC class II peptides able to inform the immune system in mice. Gut 2003;52:1690–1697.
58. Mallegol J, van Niel G, Lebreton C, Lepelletier Y, Candalh C, Dugave C, Heath JK, Raposo G, Cerf-Bensussan N, Heyman M. T84-intestinal epithelial exosomes bear MHC class I/peptide complexes potentiating antigen presentation by dendritic cells. Gastroenterology 2007;132:1866–1876.
59. Andreola G, Rivoltini L, Castelli C, Huber V, Pereg P, Deho P, Squarcina P, Accornero P, Lozupone F, Rugini L, Stringaro A, Molinari A, Arancia G, Gentile M, Parmiani G et al. Induction of lymphocyte apoptosis by tumour cell secretion of Fas-L-bearing microvesicles. J Exp Med 2002;195:1303–1316.
60. Martinez-Lorenzo MJ, Anel A, Gamen S, Monle NI, Larrad L, Pineda A, Alava MA, Naval J. Activated human T cells release bioactive Fas ligand and APO2 ligand in microvesicles. J Immunol 1999;163:1274–1281.
61. Iero M, Valenti R, Huber V, Filipazzi P, Parmiani G, Fais S, Rivoltini L. Tumour-released exosomes and their implications in cancer immunity. Cell Death Differ 2008;15:80–88.
62. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654–659.
63. Pelchen-Matthews A, Raposo G, Marsh M. Endosomes, exosomes and Trojan viruses. Trends Microbio 2004;12:310–316.
64. Jouve M, Soul-Foulon N, Watson S, Schwartz O, Benaroch P. HIV-1 buds and accumulates in “nonacidic” endosomes of macrophages. Cell Host Microbe 2007;2:85–95.
65. Deneka M, Pelchen-Matthews A, Byland R, Ruiz-Mateos E, Marsh M. In macrophages, HIV-1 assembles into an intracellular plasma membrane domain containing the tetraspanins CD81, CD9, and CD53. J Cell Biol 2007;177:329–341.
66. Porto-Carreiro I, Fevrier B, Paquet S, Vilette D, Raposo G. Prions and macrophages: “Tolerosomes” are produced by intestinal epithelial cells. Eur J Immunol 2001;31:2892–2900.
67. Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol 2001;1:135–145.
68. Beatty WL, Rhoades ER, Ullrich HJ, Chatterjee D, Heuser JE, Russell DG. Trafficking and release of mycobacterial lipids from infected macrophages. Traffic 2000;1:135–145.
69. Beatty WL, Ullrich HJ, Russell DG. Mycobacterial surface moieties are released from infected macrophages by a constitutive exocytic event. Eur J Cell Biol 2001;80:31–40.
70. Rhoades E, Hsu F, Torrelles JB, Turk J, Chatterjee D, Russell DG. Identification and macrophage-activating activity of glycolipids released from intracellular Mycobacterium bovis BCG. Mol Microbiol 2003;48:875–888.
Bhatnagar S, Shinagawa K, Castellino FJ, Schorey JS. Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response in vitro and in vivo. Blood 2007;110:3234–3244.

73. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A 2004;101:13368–13373.

74. Keller S, Rupp C, Stoeck A, Runz S, Fogel M, Lugert S, Hager HD, Abdel-Bakky MS, Gutwein P, Altevogt P. CD24 is a marker of exosomes secreted into urine and amniotic fluid. Kidney Int 2007;72:1095–1102.

75. Admyre C, Grunewald J, Thyberg J, Gripenback S, Tornling G, Eklund A, Scheunius A, Gabrielsson S. Exosomes with major histocompatibility complex class II and co-stimulatory molecules are present in human BAL fluid. Eur Respir J 2003;22:578–583.

76. Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C. Exosomal-like vesicles are present in human blood plasma. Int Immunol 2005;17:879–887.

77. Banaiee N, Kincaid EZ, Buchwald U, Jacobs WR Jr, Ernst JD. Potent inhibition of macrophage responses to IFN-gamma by live virulent Mycobacterium tuberculosis is independent of mature mycobacterial lipoproteins but dependent on TLR2. J Immunol 2006;176:3019–3027.

78. Stewart GR, Wilkinson KA, Newton SM, Sullivan SM, Neyrolles O, Wain JR, Pool KL, Young DB, Wilkinson RJ. Effect of deletion or overexpression of the 19-kilodalton lipoprotein Rv3763 on the innate response to Mycobacterium tuberculosis. Infect Immun 2005;73:6831–6837.

79. Delcayre A, Le Pecq JB. Exosomes as novel therapeutic nanodevices. Curr Opin Mol Ther 2006;8:31–38.

80. Hao S, Bai O, Yuan J, Guresi M, Xiang J. Dendritic cell-derived exosomes stimulate stronger CD8+ CTL responses and antitumor immunity than tumor cell-derived exosomes. Cell Mol Immunol 2006;3:205–211.

81. Hao S, Bai O, Li F, Yuan J, Laferte S, Xiang J. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity. Immunology 2007;120:90–102.

82. Hao S, Moyana T, Xiang J. Review: cancer immunotherapy by exosome-based vaccines. Cancer Biother Radiopharm 2007;22:692–703.

83. Aline F, Bout D, Amigorena S, Roingeard P, Dimier-Poission I. Toxoplasma gondii antigen-pulsed-dendritic cell-derived exosomes induce a protective immune response against T. gondii infection. Infect Immun 2004;72:4127–4137.

84. Beauvillain C, Ruiz S, Guiton R, Bout D, Dimier-Poission I. A vaccine based on exosomes secreted by a dendritic cell line confers protection against T. gondii infection in syngeneic and allogeneic mice. Microbes Infect 2007;9:1614–1622.

85. Colino J, Snapper CM. Dendritic cell-derived exosomes express a Streptococcus pneumoniae capsular polysaccharide type 14 cross-reactive antigen that induces protective immunoglobulin responses against pneumococcal infection in mice. Infect Immun 2007;75:220–230.

86. Colino J, Snapper CM. Exosomes from bone marrow dendritic cells pulsed with diphtheria toxoid preferentially induce type 1 antigen-specific IgG responses in naive recipients in the absence of free antigen. J Immunol 2006;177:3757–3762.

87. Lopez R. Pneumococcus: the sugar-coated bacteria. Int Microbiol 2006;9:179–190.

88. Makwana N, Riordan FA. Bacterial meningitis: the impact of vaccination. CNS Drugs 2007;21:355–366.

89. Kuate S, Cinati J, Doerr HW, Uberla K. Exosomal vaccines containing the S protein of the SARS coronavirus induce high levels of neutralizing antibodies. Virology 2007;382:26–37.

90. Kim SH, Bianco NR, Shufesky WJ, Morelli AE, Robbins PD. MHC class II+ exosomes in plasma suppress inflammation in an antigen-specific and Fas ligand/Fas-dependent manner. J Immunol 2007;179:2235–2241.

91. Pisitkun T, Johnstone R, Knepper MA. Discovery of urinary biomarkers. Mol Cell Proteomics 2006;5:1760–1771.

92. Hoorn EJ, Pisitkun T, Zietse R, Gross P, Frokiaer J, Wang NS, Gonzales PA, Star RA, Knepper MA. Prospects for urinary proteomics: exosomes as a source of urinary biomarkers. Nephrology (Carlton) 2005;10:283–290.

93. Delcayre A, Estelles A, Sperinde J, Roulon T, Paz P, Aguilera B, Villanueva J, Khine S, Le Pecq JB. Exosome Display technology: applications to the development of new diagnostics and therapeutics. Blood Cells Mol Dis 2005;35:158–168.

94. Katzmann DJ, Stefan CJ, Babst M, Emr SD. Vps27 recruits ESCRT machinery to endosomes during MVB sorting. J Cell Biol 2003;162:413–423.

95. Teo H, Perisic O, Gonzalez B, Williams RL. ESCRT-II, an endosome-associated complex required for protein sorting: crystal structure and interactions with ESCRT-III and membranes. Dev Cell 2004;7:559–569.