EXOSOMES: COMPOSITION, BIOGENESIS AND FUNCTION

Clotilde Théry, Laurence Zitvogel‡ and Sebastian Amigorena**

Exosomes are small membrane vesicles of endocytic origin that are secreted by most cells in culture. Interest in exosomes has intensified after their recent description in antigen-presenting cells and the observation that they can stimulate immune responses *in vivo*. In the past few years, several groups have reported the secretion of exosomes by various cell types, and have discussed their potential biological functions. Here, we describe the physical properties that define exosomes as a specific population of secreted vesicles, we summarize their biological effects, particularly on the immune system, and we discuss the potential roles that secreted vesicles could have as intercellular messengers.

**INSERM U520, Institut Curie, 12 rue Lhomond, 75005 Paris, France.*
‡ERIT-M 0208 INSERM, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif, France.
Correspondence to C.T.
e-mail: Clotilde.Thery@curie.fr
doi:10.1038/nri855

Exosomes were described initially as microvesicles containing 5′-nucleotidase activity that were released from neoplastic cell lines[1]. A few years later, other groups reported the secretion of vesicles of endocytic origin by cultured reticulocytes. These small vesicles (~50 nm in diameter), which were present inside large multivesicular endosomes, contained transferrin receptors — a marker that is used to follow endocytosis and the recycling of cell-surface proteins — that had been internalized from the plasma membrane[2,3] (FIG. 1). They seemed to form by invagination and budding from the limiting membrane of late endosomes, resulting in vesicles that contained cytosol and that exposed the extracellular domain of transferrin receptors at their surface. Using electron microscopy, these authors observed fusion profiles of multivesicular endosomes with the plasma membrane, leading to the secretion of the internal vesicles into the extracellular environment. These vesicles were purified by centrifugation of the reticulocyte-culture supernatant, and called 'exosomes'[4]. At the time, late endosomes were considered to be predegradative compartments — their content was destined for full degradation in lysosomes and was not recycled back to the extracellular environment. Therefore, these results were controversial for other authors, who thought that the vesicles that were purified by ultracentrifugation were membranes shed by dying cells in culture.

Several recent electron-microscopy studies, however, have established the existence of fusion profiles between multivesicular late endosomes and the plasma membrane in living cells of haematopoietic origin, such as cytotoxic T lymphocytes (CTLs)[5], Epstein–Barr virus (EBV)-transformed B cells[6], mastocytes[7], dendritic cells (DCs)[8,9] and platelets[10]. These cell types, and perhaps every cell that contains multivesicular endocytic compartments, could potentially secrete exosomes. Indeed, lipid vesicles purified from the culture supernatant of various haematopoietic cells[6–9,11–15], tumours of haematopoietic or non-haematopoietic origin[16], or epithelial cells[17] have been described in several studies. Compiling data from these studies can now help to define the characteristic properties of exosomes that distinguish them from vesicles that originate from other cellular locations, such as the plasma membrane. Several recent studies have also addressed the potential functions of exosomes, both *in vitro* and *in vivo*.

Exosome composition

Physical properties and purification of exosomes. The most common procedure to purify exosomes from cell-culture supernatants involves a series of centrifugations to remove dead cells and large debris, followed by a final high-speed ultracentrifugation to pellet exosomes[6,11]. Such a procedure, however, does not discriminate between exosomes and other small vesicular structures,

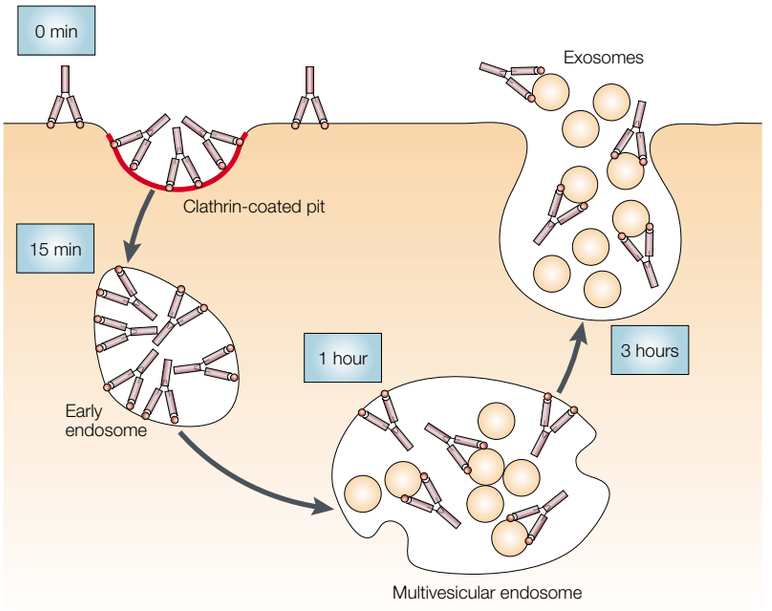

Figure 1 | **Summary of the observations that led to the first description of exosomes.** The secretion of vesicles present inside multivesicular endocytic compartments was reported in 1983 by Harding *et al.*[3], and confirmed in 1985 by Pan *et al.*[2]. Using immunoelectron microscopy, the authors visualized the fate of an internalized anti-transferrin-receptor antibody in reticulocytes. In fixed cells (0 min), the antibody binds to the cell surface and is found in some clathrin-coated pits. In cells that have been incubated at 37°C for 15 min after antibody binding, the label is found in large 'empty' vesicles, which probably correspond to early endosomes. After one hour at 37°C, the internalized anti-transferrin-receptor antibody is found in endosomes containing internal vesicles (multivesicular endosomes), and is localized mostly at the surface of the internal vesicles. After three hours, fusion of some of these multivesicular compartments with the plasma membrane is observed, showing that vesicles bearing transferrin receptors that were internalized from the plasma membrane at time 0 are released into the extracellular environment. These vesicles were later called 'exosomes'[4]. Adapted from REF. 2.

or large protein aggregates. Other criteria must be used to identify exosomes.

Exosomes float on sucrose gradients, as do all lipid vesicles, and their density ranges from 1.13 g ml^{-1} (for B-cell-derived exosomes) to 1.19 g ml^{-1} (for intestinal-cell-derived exosomes)[6,9,10,16,17]. Contaminating material, such as protein aggregates or nucleosomal fragments that are released by apoptotic cells, are separated readily from exosomes by flotation on sucrose gradients[12].

When analysed by whole-mount electron microscopy, exosomes have a characteristic 'saucer-like' morphology — a flattened sphere that is limited by a lipid bi-layer. Generally, they are between 30 and 100 nm in diameter (too small, therefore, to be observed by photon microscopy), with B-cell-derived exosomes being the most homogeneous in size (60–80 nm)[6]. These characteristics are consistent with the observed size and morphology of internal vesicles in multivesicular endosomes[6].

Exosomes are not the only type of secreted membrane. Larger membrane structures can be purified from the supernatants of prostate cells (prostasome-like granules)[18], platelets[10], activated neutrophils (so-called 'ectosomes')[19], chinese-hamster ovary cells and mononuclear cells cultured at high concentration in serum-free medium[20]. Similar structures released by activated human monocytic cell lines[21] can be observed by confocal microscopy. Such vesicles are greater than 100 nm in diameter (up to 1 μm), and, therefore, do not fit with the definition of exosomes. They probably derive from the plasma membrane.

Because other membrane vesicles can be secreted, it is essential to purify exosomes accurately from cell-culture supernatants. Filtration of the cell-culture supernatant through 0.22-μm filters, followed by direct high-speed ultracentrifugation[12], reduces the contamination of exosome preparations with larger vesicles that are shed from the plasma membrane. In addition, because exosomes are present in serum (W. Stoorvogel, personal communication; C.T. and S.A., unpublished observations), it is crucial to avoid contamination with bovine exosomes from the fetal calf serum (FCS) that is used to culture the exosome-producing cells[9]. For this reason, cells are cultured in medium in which FCS is replaced with insulin–transferrin–sodium-selenite supplement[13] or with bovine serum albumin (BSA)[17]. Alternatively, culture medium that contains up to 20% FCS can be depleted from endogenous exosomes by overnight high-speed ultracentrifugation[9,15]. In previous studies, the purification of exosomes from metabolically radiolabelled cells and analysis of the radioactive exosomal proteins was used to show that exosomes originated from the cells, rather than from the FCS[6,9,16].

Molecular composition of exosomes. The presence of known cellular proteins in exosome preparations from various cellular sources has been analysed by western blotting[6–8,15,16,22] and by fluorescence-activated cell sorting (FACS) analysis of exosome-coated beads[12,15,23,24]. More-extensive analyses, involving trypsin digestion and mass spectrometry, to identify unknown or unexpected cellular proteins that are present in exosomes have been carried out also, on exosomes derived from DCs[9,12], mast cells[13] and intestinal epithelial cells (enterocytes)[17].

The available proteomic studies define a subset of cellular proteins that are targeted specifically to exosomes. We analysed exosomes that are produced by mouse[9,12] and human (M. Boussac, C.T., J.-B. LePecq and S.A., unpublished observations) DCs — 80% of the proteins that are contained in the exosomes are conserved between the two species. FIGURE 2 is a schematic representation of DC-derived exosomes. Most of the proteins, or protein families, that are shown have been described also in exosomes that are produced by other cell types. TABLE 1 gives a summary of these conserved exosomal proteins. The function of most of these proteins in exosomes is unknown at present. Importantly, these studies also showed that exosomes are clearly distinct from the microvesicles that are produced by apoptotic cells and they are only secreted by living cells.

Both ubiquitous and cell-specific proteins might be targeted selectively to exosomes. The former are most probably involved in exosome biogenesis and, perhaps, in some unknown common exosome functions. They include cytosolic proteins — such as tubulin, actin and actin-binding proteins (that is, cytoskeletal components)

ANNEXINS
A family of cytosolic proteins that have phospholipid-binding domains, the association of which with intracellular membranes is regulated by Ca^{2+}. Several annexins are involved in membrane-fusion events between intracellular compartments.

RAB PROTEINS
Cytosolic proteins that have GTPase activity, which, in their GTP-bound form, associate with membranes. Different RAB proteins associate with different intracellular compartments — for example, RAB5 associates with early endosomes, RAB7 with late endosomes and RAB11 with recycling endosomes.

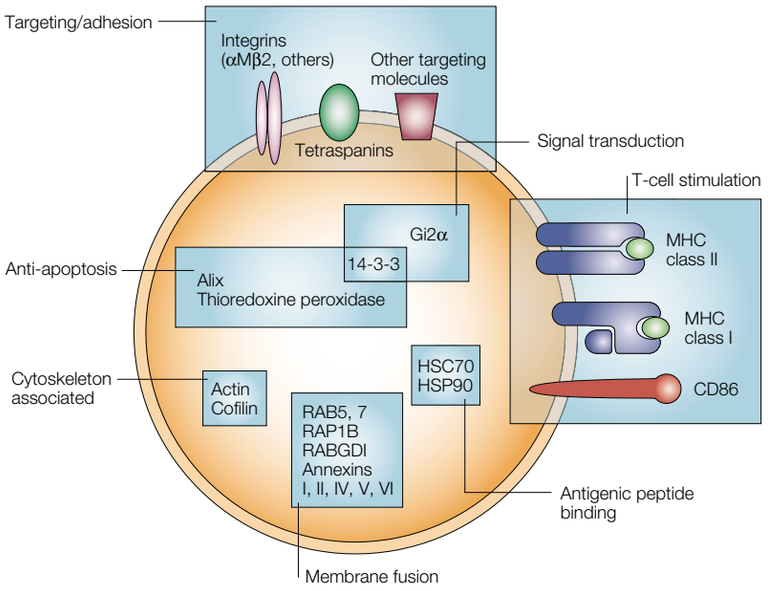

Figure 2 | **Schematic representation of exosomes produced by dendritic cells.** The proposed structure of an exosome — as a vesicle that is delimited by a lipid bi-layer, which contains cytosol from the producing cell and exposes the extracellular domain of various transmembrane proteins at its surface — derives from the original observations of reticulocytes[2] and from other experimental evidence (see text). The proteins that are shown here were identified by mass spectrometry and other means in exosomes from both mouse[12] and human (M. Boussac, C.T., J.-B. LePecq and S. A., unpublished observations) dendritic cells. The proteins are arranged in categories, according to their known functions in the cell.

— as well as ANNEXINS and RAB PROTEINS (which are involved in intracellular membrane fusions and transport). They also include molecules that are involved in signal transduction (such as protein kinases, 14-3-3 and heterotrimeric G proteins). Various metabolic enzymes (such as peroxidases, pyruvate and lipid kinases, and enolase-1) are found in exosomes from enterocytes and human DCs (M. Boussac, C.T., J.-B. LePecq and S.A., unpublished observations). Exosomes also contain HEAT-SHOCK PROTEINS, such as constitutive isoforms of HSP70 and HSP90. These ubiquitous proteins are involved in antigen presentation, as they can bind antigenic peptides and participate in loading peptides onto MHC molecules[25]. MHC class I molecules are also present in exosomes from most cell types. Finally, one of the most abundant protein families that is found in exosomes comprises the TETRASPANINS. Several members of this family — including CD9, CD63, CD81 and CD82 — are highly enriched in exosomes from virtually any cell type. Tetraspanins interact with many protein partners — including MHC molecules and integrins — which indicates that they are involved in the organization of large molecular complexes and membrane subdomains.

Exosomes also contain proteins that are involved in specific cell functions. Exosomes from antigen-presenting cells (APCs) have been analysed in most detail. MHC class II molecules are very abundant in exosomes from all cells that express MHC class II. Exosomes from DCs also contain CD86, which is an important co-stimulatory molecule for T cells. T-cell receptors are also specifically enriched on T-cell-derived exosomes. Exosomes contain a series of cell-specific transmembrane proteins — including α- and β-chains of integrins (such as αM on DCs, β2 on DCs and T cells, and α4β1 on reticulocytes), immunoglobulin-family members (such as intercellular adhesion molecule 1 (ICAM1)/CD54 on B cells, A33 antigen on enterocytes and P-selectin on platelets) or cell-surface peptidases (such as dipeptidylpeptidase IV/CD26 on enterocytes and aminopeptidase N/CD13 on mastocytes). Milk-fat-globule EGF-factor VIII (MFGE8)/lactadherin[26] — a milk-fat-globule protein that is expressed by DCs and some tumour-cell lines — is also very abundant in exosomes that are produced by these cells[9]. These proteins probably 'address' exosomes to target cells.

In terms of the lipid composition of exosomes, limited data are available so far. The general composition of reticulocyte-derived (or leukocyte-derived) exosomes is similar to that of the plasma membrane of the producing cell[4]. The presence of lyso-bis-phosphatidic acid, a lipid that is enriched in late endocytic compartments, has been reported in B-cell-derived exosomes[27]. Phosphatidylserine (PS) — a lipid that is present normally at the cytosolic side of the plasma membrane — is also present, but at low levels, at the surface of exosomes that are derived from platelets[10] and DCs (P. Véron, C.T. and S.A., unpublished observations). Finally, internal vesicles of late endosomes and exosomes of EBV-transformed B cells are rich in cholesterol, as are the plasma-membrane microdomains known as 'lipid rafts'[28]. The lipid composition of exosomes from other cell types is not known yet. Together, these studies analysing the molecular composition of exosomes define this population of vesicles as a *bona fide* secreted sub-cellular compartment.

Exosome biogenesis

Endosomal origin of exosomes. When analysing the protein composition of exosomes, the first striking observation is that the range of proteins is rather limited — exosome preparations do not contain any proteins of nuclear, mitochondrial, endoplasmic-reticulum or Golgi-apparatus origin. All of the exosomal proteins that have been identified are found in the cytosol, in the membrane of endocytic compartments or at the plasma membrane. Direct comparison of exosomes with the cell surface of the cells from which they are derived by FACS analysis shows that they are not simply fragments of the plasma membrane, because they lack some abundant cell-surface proteins — such as Fc receptors in DC-derived exosomes[12]; CD28, CD40L and CD45 in T-cell-derived exosomes[15]; and transferrin receptor in B-cell-derived exosomes[6,24]. Furthermore, some exosomal proteins that were thought to be present only at the cell surface — such as CD9 or the αMβ2 integrin — are also present in endocytic compartments in DCs[12]. Finally, many of the cytosolic proteins that are found in exosomes have been found in the endocytic pathway — such as annexin II[29], RAB5/RAB7 (REF. 29) and the tumour-susceptibility protein TSG101, which was shown recently to be important for the transport of

HEAT-SHOCK PROTEINS (HSPs). A family of proteins that are involved in the binding of other misfolded proteins, and transporting them to the cellular degradation machinery. Several HSPs are synthesized only in conditions of stress, such as heat shock, but a few family members — such as endoplasmic-reticulum-resident gp96, and cytosolic HSC70 and HSP84 — are expressed constitutively.

TETRASPANINS
A family of transmembrane proteins that have four transmembrane domains and two extracellular domains of different sizes, which are defined by several conserved amino acids in the transmembrane domains. Their function is not known clearly, but they seem to interact with many other transmembrane proteins and to form large multimeric protein networks.

Table 1 | **Protein families present in exosomes from various cell types**

Exosome protein	Cell type	References
Antigen presentation		
MHC class I	B cells	68
	Dendritic cells	8
	Enterocytes	17
	Tumours	16
	T cells	15
MHC class II	B cells	6
	Dendritic cells	8
	Enterocytes (IFN-γ-treated)	17
	Mastocytes	7
	T cells	15
Integrins		
α4β1	Reticulocytes	69
αMβ2	Dendritic cells	9
β2	T cells	15
αLβ2	Mastocytes	13
Immunoglobulin-family members		
ICAM1/CD54	B cells	22
	Dendritic cells	24
	Mastocytes	13
P-selectin	Platelets	10
A33 antigen	Enterocytes	17
Cell-surface peptidases		
Dipeptidylpeptidase IV/CD26	Enterocytes	17
Aminopeptidase n/CD13	Mastocytes	13
Tetraspanins		
CD63	B cells	22
	Dendritic cells	8
	Enterocytes	17
	Platelets	10
	T cells	15
	Mastocytes	31
CD37, CD53, CD81, CD82	B cells	22
CD9	Dendritic cells	9
Heat-shock proteins		
HSC70	Reticulocytes	4
	Dendritic cells	9
	Tumours	16
HSP84/90	Dendritic cells	9
	Enterocytes	17
Cytoskeletal proteins		
Actin	Dendritic cells	9
	Enterocytes	17
	Mastocytes	13
Actin-binding proteins (cofilin)	Dendritic cells	12
Tubulin	Dendritic cells	12
	Enterocytes	17
Membrane transport and fusion		
Annexins I, II, IV, V, VI	Dendritic cells	12
Annexin VI	Mastocytes	13
RAB7/RAP1B/RABGDI	Dendritic cells	12
Signal transduction		
Gi2α/14-3-3	Dendritic cells	12
CBL/LCK	T cells	15
Metabolic enzymes		
Enolase-1	Enterocytes	17
Thioredoxine peroxidase	Dendritic cells	12

HSP, heat-shock protein; ICAM1, intercellular cell-adhesion molecule 1; IFN-γ, interferon-γ.

membrane proteins in the endocytic pathway[30]. Together, these results strengthen the hypothesis of the endosomal origin of exosomes. However, the possibility cannot be excluded that some of the exosome preparations that have been studied contain a mixture of vesicles of endosomal and plasma-membrane origin.

Only a subset of the endosomal/lysosomal proteins are contained in exosomes — exosomes do not contain any lysosomal proteases or other soluble endocytic residents, or any subunits of the v-ATPase. In addition, exosomes that are secreted by B cells exclude the invariant chain (CD74; which associates with MHC class II molecules in the endoplasmic reticulum and targets them to the endocytic pathway), the non-polymorphic MHC class II molecule HLA-DM (a lysosomal resident that is involved in the formation of complexes between polymorphic MHC class II molecules and peptides) and the lysosomal marker lysosomal-associated membrane protein 2 (LAMP2)[22]. The invariant chain is absent also from DC[8]- and mastocyte[7]-derived exosomes. By contrast, LAMP2 is detected readily in DC-derived exosomes[8]. The presence of another lysosomal marker, LAMP1, in exosomes from EBV-transformed B cells and mastocytes is controversial. In these two cell types, exosomes were found to be positive for LAMP1 by immuno-electron microscopy[6,7], but negative by western blotting[22] and FACS analysis[31]. Exosomes from tumour cells contain LAMP1 (REF. 16). The presence of endocytic proteins in exosomes most probably reflects their targeting to the internal vesicles of multivesicular endosomes, which might vary in different cell types.

The mechanisms for sorting proteins into the internal vesicles of multivesicular compartments and, hence, to exosomes are still poorly understood. Recent observations indicate that ubiquitylation of the cytosolic domain of selected membrane proteins is involved[32,33]. A ubiquitin ligase, c-CBL, was found in T-cell-derived exosomes[15]. Furthermore, the ubiquitin-binding ability of Vps23 — the yeast homologue of tsg101 (a protein that is present in DC-derived exosomes[12]) — is necessary for sorting proteins into internal vesicles of late endosomes[32]. These observations, however, are far from showing that ubiquitylation is involved in exosome formation, and other, ubiquitin-independent mechanisms could also occur[34].

Budding at the limiting endosomal membrane. The biogenesis of exosomes determines their membrane orientation. If exosomes form by inward budding from the limiting membrane of endosomes, then they should contain cytosol and expose the extracellular domains of transmembrane proteins (FIG. 3). Indeed, antibodies that recognize the cytosolic proteins that are found in exosomes (such as HSC70 and annexin II) do not label whole-mounts of exosomes in immuno-electron microscopy[9], whereas antibodies that are directed against the extracellular domains of MHC class II molecules, CD9 and the αMβ2 integrin do label these mounts[6,9]. In addition, beads that are coated with antibodies that are specific for MHC class II molecules bind exosomes in cell-culture supernatants[24], and anti-CD63-coated beads

bind purified exosomes[31]. Although they are not conclusive, these observations are consistent with the proposed membrane orientation and 'inward-budding' model of the biogenesis of exosomes.

The budding event that is involved in exosome formation occurs in a reverse membrane orientation compared with most intracellular budding events — transport vesicles that carry cargo between intracellular compartments expose the cytosolic domains of transmembrane proteins (FIG. 3). 'Reverse-budding' events occur, for example, during apoptosis, when membrane vesicles of different sizes (including microvesicles and blebs) are shed from the plasma membrane[35]. They also occur in live cells when milk-fat globules are released from mammary-gland epithelial cells[36], or when plasma-membrane-derived vesicles are shed after the activation of platelets[10] or monocytes[21]. In apoptotic cells, exposure at the cell surface of PS is observed. Surface exposure of PS is also observed transiently in monocytes that are stimulated with ATP, which results in the shedding of microvesicles from the cell surface[21]. A 'flip' of PS from the inner to the outer leaflet of membranes might, therefore, be required for reverse-budding events. Consistent with this idea, PS was detected at the surface of milk-fat globules, and is responsible for the association of MFGE8/lactadherin with these vesicles[37]. Similarly, PS (P. Veron, C.T. and S.A., unpublished observations) and MFGE8/lactadherin[9] are present at the surface of DC-derived exosomes. Plasma-membrane vesicles and, to a lesser extent, exosomes that are released by platelets also expose PS[10].

It is most probable that all of these vesicles that are generated by reverse-budding events require a common cytosolic machinery. The assembly of virions in infected cells, and budding of the plasma membrane or other internal membranes, also requires a reverse-budding event. Remarkably, tsg101, a protein that is involved in the budding of HIV-1 (REFS 38–40) and in the biogenesis of multivesicular endosomes[32], is found in DC-derived exosomes also[12].

Secretion of exosomes. As mentioned above, exosomes are secreted by the fusion of late endocytic compartments with the plasma membrane. The fusion of endocytic compartments with the cell surface is constitutive in many cell types, such as EBV-transformed B cells, immature DCs and epithelial cells. In certain haematopoietic cells, such as T cells and mast cells, late endocytic vesicles (often referred to as 'secretory lysosomes') fuse with the plasma membrane after activation in a Ca^{2+}-dependent manner[41]. Secretory lysosomes also contain internal vesicles. Exosome secretion follows the same rules as excretory-lysosome fusion — it is constitutive in EBV-transformed B cells, DCs and epithelial cells[6,9,17], and is regulated in mast cells and T cells[7,15].

Very little is known about the molecular machinery that is involved in the fusion of multivesicular compartments or secretory lysosomes with the plasma membrane. Most intracellular membrane-fusion events are determined by a specific protein machinery — which includes soluble factors (such as N-ethylmaleimide-sensitive factor, NSF, and soluble NSF-attachment protein, SNAP) and membrane complexes (such as SNAP-attachment protein receptor, SNARE)[42]. Both membranes that are involved in the fusion event need to bear specific SNAREs (known as vesicle or v-SNARE and target or t-SNARE). Particular v- and t-SNAREs dictate the specificity of intracellular membrane-fusion events. In mast cells, the fusion machinery that is used for DEGRANULATION has been analysed in detail[43–45]. SNAP23, and syntaxin-3 and -4 (three members of the t-SNARE family), and VAMP7 and VAMP8 (two v-SNAREs) are involved. In fibroblasts, synaptotagmin VII positively controls the Ca^{2+} dependence of lysosome fusion with the cell surface[46], whereas in mastocytes, synaptotagmin II is a negative regulator[47]. However, at present, it is unclear whether these fusion complexes are also responsible for exosome secretion.

Exosome function

If they do mediate intercellular communication, exosomes or other secreted membrane vesicles, could provide the organism with a mechanism to achieve things that might otherwise be difficult to realize. First, exosomes could bear combinations of ligands that would engage different cell-surface receptors simultaneously. This would be similar to what happens during the interaction between two cells, but without the need for direct cell–cell contact. Second, exosomes could bind to target-cell membranes, which would then bear 'new' surface molecules and, potentially, acquire new adhesion properties. Third, exosomes could fuse with target cells and, so, exchange membrane proteins and cytosol between two cell types. Of course, if any of these processes do occur — which remains to be shown even *in vitro* — they would need to be highly regulated, as for all other modes of intercellular communication. Several studies have analysed the biological activities of exosomes *in vitro*, but very little is known about their possible functions *in vivo*. Twenty years after the initial description of exosomes, their physiological relevance remains unclear. In this section, we attempt to summarize recent studies that have analysed exosome functions *in vitro* and discuss their possible biological functions *in vivo*.

Membrane exchange between cells. Whether they fuse with target cells or not, if exosomes mediate any kind of biological function, they must transfer membrane material between different cells. Membrane transfer has been reported often *in vitro*, in systems that either required, or did not require direct cell–cell contact.

Recently, several groups have reported the transfer of membrane proteins during direct cell–cell contact. $CD8^+$ T cells acquire MHC class-I–peptide complexes and other cell-surface molecules of APCs during the antigen-presentation process[48–51]. The transfer of MHC class I molecules occurs through internalization by the T-cell receptor (TCR), and the transfer of other surface molecules occurs through their corresponding specific receptors. During this transfer, T cells become sensitive to lysis by neighbouring CTLs that are specific for the

DEGRANULATION
In mastocytes and cytotoxic T lymphocytes, this term refers to the activation-induced fusion of secretory granules with the plasma membrane, and to the subsequent release of the content of these granules into the extracellular space.

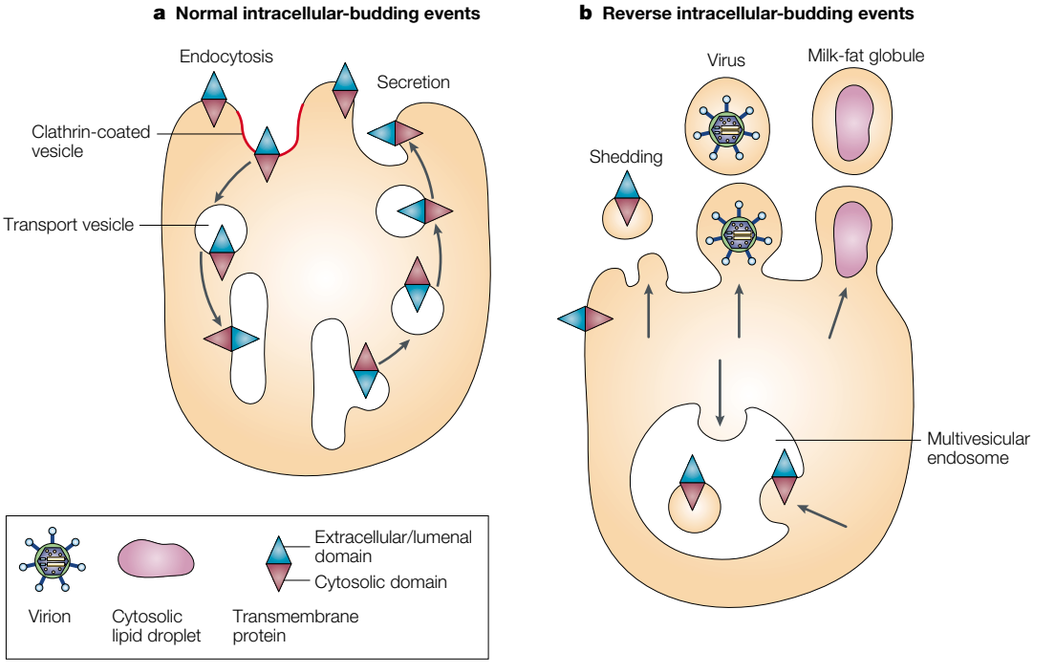

Figure 3 | **Orientation of various budding events occuring in a cell. a** | Normal budding events. The transport of cargo proteins between intracellular compartments occurs by means of vesicles budding off a compartment and travelling through the cytosol towards another compartment (transport vesicles), or towards the cell surface (secretion). These vesicles contain proteins from the lumen of the donor compartment, and expose at their surface the cytosolic part of the transmembrane proteins that they carry. For the endocytosis of cell-surface receptors (clathrin-coated vesicles), vesicles that have the same membrane orientation form. **b** | Reverse budding events. Budding events in which the vesicle that forms contains cytosol and exposes the extracellular or lumenal domain of receptors can occur also. This is the case at the plasma membrane during virus budding, the secretion of milk-fat globules or the shedding of membrane vesicles, or during the formation of the internal vesicles of multivesicular endosomes.

same MHC class-I–peptide complexes. This process could contribute to the progressive elimination of CTLs when they have interacted with many targets, thereby downregulating immune responses. $CD4^+$ T cells acquire membranes that bear MHC class-II–peptide complexes and other transmembrane proteins while migrating through endothelial-cell sheets[52]. Conversely, allogeneic MHC molecules from melanoma cells or T cells are captured by DCs, which then become sensitive to CTLs that are specific for the allogeneic molecules[53]. The transfer of membrane molecules between T cells and APCs could, therefore, help to downmodulate immune responses. In B cells, the B-cell receptor also mediates the efficient capture of membrane antigens that are expressed at the surface of target cells[54]. Antigens that are acquired by B cells are then efficiently processed and used to stimulate specific T cells. In these types of transfer, the requirement for direct cell–cell contact indicates that if exosomes are involved, they must be secreted into the intercellular space (that is, the immunological synapse in the case of T-cell–APC interactions).

In other cases, the transfer of membrane molecules might occur in the absence of direct contact. Once activated, $CD4^+$ T cells capture, in a receptor-independent manner, membrane vesicles that are released into the supernatant by APCs[55,56]. DCs acquire transmembrane molecules that are released by other DCs[57,58] and, so, become more efficient at stimulating T cells in mixed-lymphocyte reactions. Although these two sets of studies have not characterized the exchanged membrane vesicles extensively, they seem to share some properties with exosomes. For example, as for the secretion of exosomes by DCs, the secretion of these exchanged membranes does not seem to require any particular stimulus. Furthermore, like these membranes, DC-derived exosomes can be addressed specifically to both T cells and DCs, through the membrane molecules that are present at their surface[9] — MAC1 (the αMβ2 integrin) on exosomes binds ICAM1 expressed by T cells and DCs, and MFGE8/lactadherin present on exosomes binds αvβ3/5 integrins expressed by DCs. These correlations, however, do not necessarily show that exosomes are involved in the membrane transfers that are described in these systems.

The most convincing example of the importance of membrane transfer between cells has been provided recently. Large vesicles bearing CC-chemokine receptor 5 (CCR5) are released by chinese-hamster ovary cells and blood mononuclear cells[20]. These vesicles transfer CCR5 to monocytes, $CD4^+$ T cells and endothelial cells that do not express this receptor, rendering them sensitive to infection with macrophage-tropic HIV-1 virus.

In vivo also, early studies of bone-marrow transplantation reported the transfer of membranes between host and donor cells. In 1981, Sharrow *et al.* observed that thymocytes from the donor acquired MHC molecules

of host origin[59]; conversely, in 1991, Gray *et al.* observed that FOLLICULAR DENDRITIC CELLS (FDCs) of host origin acquired peptide-loaded MHC molecules from the donor[60]. In the latter case, a recent study by Denzer *et al.*[27] indicates that B-cell-derived exosomes are the source of the MHC molecules that are acquired by FDCs. More recently, the transfer of membranes between cells *in vivo* has been reported in *Drosophila* embryos[61]. This transfer mediates the diffusion throughout the tissue of membrane-associated morphogens — that is, molecules that determine the future identity of precursor cells in the embryonic tissue. The term 'argosome' has been used to refer to these membrane vesicles that transfer from one cell to the next by endocytosis. Argosomes have not been characterized biochemically or morphologically so far.

Biological functions of exosomes. As has been pointed out, early studies of reticulocytes indicated that exosomes are secreted to discard membrane proteins, such as transferrin receptors, that have become useless in mature red blood cells[62]. So, exosomes would be an alternative to lysosomal degradation. This pathway could be effective, for example, in eliminating proteins that are resistant to degradation by lysosomal proteases. It is tempting to speculate that this was the primary function of exosomes, which was then extended to other haematopoietic cells, where lysosomes have functions other than degradation (for example, antigen presentation). The working hypothesis in the field is that exosomes have some, as yet unknown, physiological functions.

Most of the studies that examine exosome function use exosomes that are derived from APCs. In a pioneering study, Raposo *et al.* showed that exosomes that are secreted by EBV-transformed B cells stimulated human $CD4^+$ T-cell clones in an antigen-specific manner[6]. The T-cell response that was induced by the exosomes was not as potent as the response that was induced by EBV-transformed B cells themselves — exosomes produced by 5×10^5–5×10^6 B cells were required to obtain significant levels of T-cell proliferation *in vitro* (similar to the stimulation that is observed using 1×10^4–5×10^4 B cells). The lower efficacy of T-cell stimulation by exosomes *in vitro* might be due to the relative amounts of peptide-loaded MHC class II molecules in exosomes compared with B cells, or to other differences, such as protein composition, or size or topology of exosome surfaces compared with B-cell surfaces. T-cell stimulation by exosomes produced by rat mast cells that were engineered to express mouse or human MHC class II molecules has also been reported recently[31]. These studies showed, for the first time, that exosomes might have a function other that discarding unwanted proteins — that is, T-cell stimulation.

We showed in 1998 that exosomes that are produced by mouse DCs pulsed with tumour peptides induce the rejection of established tumours[8]. Tumour rejection was tumour-peptide specific and required T cells. *In vivo*, the exosomes produced by between 0.5×10^6 and 1×10^6 DCs were as potent (or more so) as similar numbers of DCs for the induction of anti-tumour immune responses. These observations showed that DC-derived exosomes stimulated T-cell-mediated anti-tumour immune responses *in vivo*. We found recently that DC-derived exosomes bearing an H-Y peptide activated specific naive T cells *in vivo* (C.T., L. Duban, O. Lantz and S.A., unpublished observations). Interestingly, exosomes could not induce naive T-cell proliferation *in vitro*, unless mature DCs were present also. Similarly, human DC-derived exosomes loaded with MHC class-I-restricted peptides activate the corresponding $CD8^+$ T-cell clones, provided that DCs are present in the cultures (D. Hsu and L.Z., unpublished observations). DC-derived exosomes might, therefore, transfer MHC–peptide complexes between different DCs, thereby increasing the number of peptide-bearing APCs (FIG. 4). Because immature DCs produce more exosomes than mature DCs[9], exosomes could be produced in peripheral tissues and sensitize DCs before their migration to lymph nodes. It is, of course, possible that exosome production occurs also after DC migration to lymph nodes.

Exosomes might also transfer antigens from tumour cells to DCs[16]. Like DC-derived exosomes, exosomes that are produced by melanoma tumour cells contain MHC class I molecules. Interestingly, tumour-cell-derived exosomes also contain tumour antigens, such as melan-A/MART1 in the case of melanoma tumour cells. DCs that expressed the appropriate restriction element (that is, the MHC class I molecule that is recognized by the T-cell clone) induced effective T-cell activation *in vitro* when 'fed' with exosomes that were derived from tumour cells expressing the corresponding antigen, but not the restriction element. Tumour-cell-derived exosomes might, therefore, provide a source of antigen for CROSS-PRESENTATION by DCs. *In vivo*, DCs loaded with tumour-cell-derived exosomes induced tumour rejection. Surprisingly, this effect was not entirely tumour specific, as exosomes from certain tumours can protect against allogeneic tumours, whereas irradiated tumour cells protect against tumour challenge in a strictly tumour-specific manner. The reason for this 'cross-protection' is unclear, but it could be due to an enrichment of shared tumour antigens in exosomes.

Altogether, these results show that exosomes can transfer antigens to DCs. DCs, depending on their maturation state, might induce T-cell priming or tolerance. Interestingly, exosomes that are derived from DCs or tumour cells do not induce DC maturation *per se*. Although exosomes were immunogenic in the experimental settings reported above, it remains to be determined directly whether T-cell stimulation by exosomes might induce tolerance also. Indeed, 40-nm vesicles produced by rat intestinal epithelial cells that were cultured in the presence of interferon-γ (IFN-γ) — to induce MHC class-II expression — and predigested ovalbumin induced some degree of antigen-specific tolerance[63]. Interestingly, vesicles purified from the serum of rats previously fed an ovalbumin diet also induced some tolerogenic responses to ovalbumin in delayed-type-hypersensitivity assays. The vesicles that were used in these studies, named 'tolerosomes', were not characterized fully.

FOLLICULAR DENDRITIC CELLS (FDCs). Cells with a dendritic morphology that are present in the lymph nodes, where they present intact antigens held in immune complexes to B cells. FDCs are of non-haematopoietic origin, and are not related to dendritic cells.

CROSS-PRESENTATION
This term refers to the ability of certain antigen-presenting cells (APCs) to load peptides that are derived from exogenous antigens onto MHC class I molecules. This property is atypical, as most cells present exclusively peptides from their endogenous proteins on MHC class I molecules. Cross-presentation is essential for the initiation of immune responses against viruses that do not infect APCs.

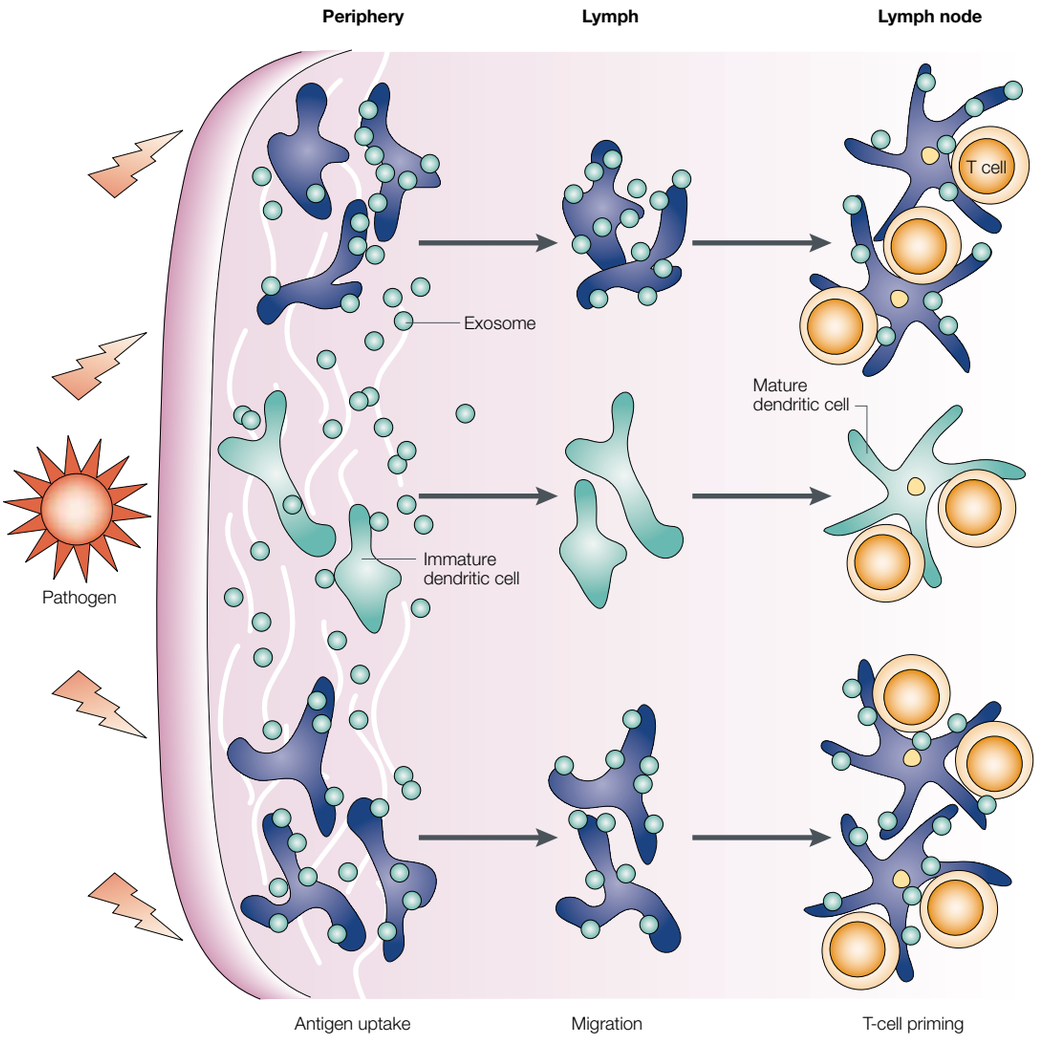

Figure 4 | **Working model for the role of exosomes in immune responses.** After the uptake of incoming pathogens in the periphery, immature or maturing dendritic cells (green) generate peptide–MHC complexes. Some of these complexes could be secreted on exosomes, and locally sensitize other dendritic cells (blue) that have not encountered the pathogen directly. As a result of the effects of inflammation, all of these dendritic cells migrate out of the tissue towards the draining lymph nodes. Although maturing dendritic cells seem to secrete fewer exosomes than immature cells[9], an exchange of exosomes inside the lymph nodes between newly arrived (and not fully mature) and resident dendritic cells could take place also. Therefore, exosome production would increase the number of dendritic cells that bear the relevant peptide–MHC complexes, and thereby amplify the magnitude of immune responses. In the absence of inflammation, spontaneous migration of exosome-bearing dendritic cells could contribute to tolerance induction.

Although properly characterized exosomes have only been shown so far to induce T-cell priming, it is still possible that the outcome of the immunological response that they induce depends on their cellular origin, the target cells that they encounter *in vivo* or the 'immunological' context (for example, inflammation).

In addition to these antigen-specific effects of exosomes, several groups have reported antigen-independent effects. Mast-cell-derived exosomes incubated *in vitro* with autologous spleen cells induce their activation, which results in blast formation, cell proliferation and the production of IL-2 and IFN-γ[13]. They also induce the activation of T cells in the spleen and mesenteric lymph nodes after *in vivo* injection. The mechanisms of this antigen-independent effect are unclear, but could involve CDC25, a protein that is active in the mitotic process and is found in mast-cell-derived exosomes. In the case of these antigen-independent effects, it is very difficult to exclude any possible contamination of the exosome preparations (with endotoxin, for example), which could affect the experimental systems analysed.

By contrast, exosomes might have antigen-independent immunosuppressive effects. The presence of viral latent membrane protein 1 — a protein that inhibits phytohaemagglutinin- or anti-CD3/-CD28-induced

T-cell proliferation — in exosomes that are produced by EBV-transformed B cells indicates that these exosomes could have immunosuppressive effects[64]. This study, however, did not test the immunosuppressive effect of exosomes directly. Phytohaemagglutinin- or anti-CD59-stimulated T cells secrete microvesicles that induce T-cell apoptosis, due to the presence of FASL and/or APO2L/TRAIL at the microvesicle surface[65]. Indirect observations indicate, but do not show conclusively, that these vesicles are exosomes. In this case, exosomes would have a role in spreading activation-induced T-cell-death signals, to prevent potential autoimmune damage. Finally, melanoma cells also secrete FASL-bearing exosome-like vesicles that have pro-apoptotic activity on T cells[66]. In this case, exosome secretion could help tumour cells to escape destruction by T cells.

In spite of these *in vitro* and *in vivo* functional studies, the physiological relevance of exosomes remains questionable. Indeed, all of these studies have used exosomes that were produced by cells in culture, and it is still unclear to what extent exosomes are produced physiologically. The best evidence for exosome production *in vivo* remains the observation by Denzer *et al.* that FDCs have exosome-like vesicles at their surface *in vivo*[27]. These exosomes express MHC class II molecules and other exosome markers that are absent from the FDC plasma membrane. This observation is particularly interesting because FDCs bear MHC class II molecules but do not synthesize them[60]. Indeed, B-cell-derived exosomes bind to FDCs, but not to other cell types, *in vitro*. Exosomes might, therefore, transfer MHC class II molecules from B cells to FDCs. Two independent observations also indicate the production of exosomes *in vivo*. First, exosome-like vesicles can be purified from human serum (M.-P. Caby and C. Bonnerot, unpublished observations). It is, however, very difficult to be certain that these vesicles actually correspond to exosomes, as opposed to other shed membranes or lipid structures. Second, high levels of exosomes accumulate in tumour ascites or pleural effusions in patients who have various types of non-immunogenic tumour — for example, breast, ovarian or mesothelioma[67]. The ascites-derived exosomes loaded onto DCs induced autologous tumour-specific CTLs *in vitro*.

Importantly, it is still unknown whether exosomes that are produced *in vivo* have any specific function. Exosomes could, for example, be a by-product of cell activation, or function exclusively to eliminate unused proteins. The experimental demonstration of exosome function *in vivo* will undoubtedly be the main challenge for future research in this field.

Exosomes and immunotherapy

Regardless of their putative physiological role, exosomes have the potential to be used as vectors for vaccination. Indeed, DC-derived exosomes express high levels of functional MHC class-I– and class-II–peptide complexes, together with CD86 molecules, and could, theoretically, substitute for DCs to elicit MHC class-I- and class-II-restricted T-cell responses and tumour rejection. Also, tumour-derived exosomes are a source of tumour MHC class I molecules, constitutive heat-shock proteins and cytosolic candidate tumour antigens, which could be used for DC loading in cancer immunotherapy. Here, we discuss our preclinical data that indicate the functionality of exosomal MHC–peptide complexes and their immunogenicity in mouse tumour models, highlighting their potential for the immunotherapy of cancer.

DC-derived exosomes and tumour rejection. The original observation was that exosomes secreted by bone-marrow-derived DCs pulsed with tumour peptides elicited T-cell-dependent rejection of P815 established tumours, and significant retardation of growth of TS/A mammary tumours. Exosome-mediated tumour rejection was tumour-peptide specific, and long-term protection was tumour specific. In mice that had rejected their tumour, tumour-specific CTLs could be recovered after *in vitro* restimulation of splenocytes. A single subcutaneous injection of exosomes secreted from 1×10^6 DCs could efficiently substitute for whole DCs pulsed with acid-eluted tumour peptides in preventing tumour growth[8]. The precise cellular and molecular mechanisms that account for the immunogenicity of exosomes secreted by DCs remain to be studied. Importantly, human DC-derived exosomes are very similar to mouse DC-derived exosomes, in terms of both protein composition (M. Boussac, C.T., J.-B. Le-Pecq and S.A., unpublished observations) and function (D. Hsu and L.Z.; C.T., L. Duban, O. Latz and S.A., unpublished observations).

Phase I trials using human DC-derived exosomes. On the basis of these results, the use of exosomes for the immunotherapy of cancer patients was undertaken. A 'good manufactoring process' (GMP) has been successfully used to harvest exosomes that are secreted by monocyte-derived DCs (H. Lamparski, unpublished observations). In such GMP preclinical conditions, large quantities of exosomes that bear functional MHC class I and II molecules can be recovered reproducibly.

A Phase I clinical trial involving the administration of DC-derived exosomes loaded with MAGE3 epitopes has been started in France (Institut Gustave Roussy and Institut Curie) in *HLA-A1* or *B35/HLA-DP.04* patients who have MAGE3-expressing metastatic melanoma tumours.

Fifteen patients have been enrolled so far. The primary endpoint of safety has been achieved, and objective clinical regressions in skin and lymph-node tumour sites were observed (Anosys, Inc., unpublished observations). A second clinical trial of DC-derived exosomes in unresectable non-small-cell lung carcinoma was conducted at Duke University. Immunological studies aimed at monitoring CTL precursor frequencies specific for MAGE3 epitopes before and after immunization with exosomes, as well as correlating tumour regressions with CTL clonal expansion, are ongoing. Preliminary results have shown prolonged disease stabilization in this heavily pre-treated population of patients. In addition,

multi-centre Phase II studies of DC-derived exosomes are planned for patients with advanced melanoma and non-small-cell lung cancer.

Tumour-derived exosomes and cross-presentation. Ideally, immunotherapy strategies aimed at immunizing the host should be able to elicit T-cell-based immune responses specific for a broad repertoire of tumour-rejection antigens. Although mature DCs seem to be the most potent natural adjuvants, suitable methods leading to efficient antigen uptake, processing and cross-presentation onto MHC class I molecules are still awaited. Several approaches involving the use of whole tumour RNA, tumour lysates, apoptotic or necrotic debris, or fusion with tumour cells, are now under investigation. Wolfers *et al.*[16] reported that: melanoma-cell-line-derived exosomes contain differentiation tumour antigens; tumour exosomes loaded onto DCs transfer shared tumour antigens, which triggers the activation of MHC class-I-restricted T-cell clones *in vitro*; and tumour exosomes are a source of tumour-rejection antigens, because tumour exosomes promote T-cell-dependent cross-protection against syngeneic and allogeneic tumours in mice. Tumour-derived exosomes are not only released *in vitro* by tumour cell lines in culture supernatants. We found recently that malignant effusions from cancer patients also contain abundant membrane vesicles, which have certain exosome characteristics[67]. Tumour-specific lymphocyte populations could be expanded efficiently from peripheral-blood cells using autologous monocyte-derived DCs pulsed with ascites-derived exosomes.

Conclusions

The recent analyses of exosome composition in different cell types indicate the following minimal requirements for defining a membrane vesicle as an exosome: a size of 30–90 nm diameter; a density in sucrose gradients of 1.13–1.19 g ml^{-1}; and an endocytic origin and enrichment with tetraspanin molecules. The past few years have witnessed a renewed interest of immunologists in exosomes, mostly as a result of the demonstration of their immuno-stimulating effects *in vivo*. Although these studies have prompted the clinical use of exosomes, they have not addressed the mechanisms of biogenesis, or established the physiological relevance, of exosomes. Recent advances in the analysis of the formation of multivesicular compartments will most probably unravel the mechanisms of exosome generation. Improvements in the methods of exosome purification and the characterization of their protein composition should provide us with tools to interfere directly with exosome production and function *in vivo*. Indeed, uncovering the physiological role of this entirely new mode of cell–cell communication that exosomes might use will be a very exciting goal.

1. Trams, E. G., Lauter, C. J., Salem, N. Jr & Heine, U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochim. Biophys. Acta* **645**, 63–70 (1981).
2. Pan, B. T., Teng, K., Wu, C., Adam, M. & Johnstone, R. M. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J. Cell Biol.* **101**, 942–948 (1985).
3. Harding, C., Heuser, J. & Stahl, P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. *J. Cell Biol.* **97**, 329–339 (1983).
References 2 and 3 describe very detailed and careful electron-microscopy analyses of the fate of an endocytosed protein in reticulocytes, and point out, for the first time, the possible secretion of the content of multivesicular late endosomes into the extracellular space.
4. Johnstone, R. M., Adam, M., Hammond, J. R., Orr, L. & Turbide, C. Vesicle formation during reticulocyte maturation. Association of plasma-membrane activities with released vesicles (exosomes). *J. Biol. Chem.* **262**, 9412–9420 (1987).
5. Peters, P. J. *et al.* Molecules relevant for T-cell–target-cell interaction are present in cytolytic granules of human T lymphocytes. *Eur. J. Immunol.* **19**, 1469–1475 (1989).
6. Raposo, G. *et al.* B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.* **183**, 1161–1172 (1996).
This study shows, for the first time, the secretion of vesicles that are derived from multivesicular late endosomes by an antigen-presenting cell, and the presence of functional MHC class II molecules on the released exosomes.
7. Raposo, G. *et al.* Accumulation of major histocompatibility complex class II molecules in mast-cell secretory granules and their release upon degranulation. *Mol. Biol. Cell* **8**, 2631–2645 (1997).
8. Zitvogel, L. *et al.* Eradication of established murine tumors using a novel cell-free vaccine: dendritic-cell-derived exosomes. *Nature Med.* **4**, 594–600 (1998).
This study shows that exosomes can be secreted by dendritic cells, and that such exosomes can induce antigen-dependent T-cell-mediated immune responses in mice.
9. Thery, C. *et al.* Molecular characterization of dendritic-cell-derived exosomes. Selective accumulation of the heat-shock protein hsc73. *J. Cell Biol.* **147**, 599–610 (1999).
10. Heijnen, H. F., Schiel, A. E., Fijnheer, R., Geuze, H. J. & Sixma, J. J. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and α-granules. *Blood* **94**, 3791–3799 (1999).
11. Davis, J. Q., Dansereau, D., Johnstone, R. M. & Bennett, V. Selective externalization of an ATP-binding protein structurally related to the clathrin-uncoating ATPase/heat-shock protein in vesicles containing terminal transferrin receptors during reticulocyte maturation. *J. Biol. Chem.* **261**, 15368–15371 (1986).
12. Thery, C. *et al.* Proteomic analysis of dendritic-cell-derived exosomes: a secreted subcellular compartment distinct from apoptotic vesicles. *J. Immunol.* **166**, 7309–7318 (2001).
A detailed analysis, together with reference 9, of the protein composition of dendritic-cell-derived exosomes, and a demonstration that they are distinct from apoptotic-cell-derived microvesicles.
13. Skokos, D. *et al.* Mast-cell-dependent B- and T-lymphocyte activation is mediated by the secretion of immunologically active exosomes. *J. Immunol.* **166**, 868–876 (2001).
14. Martinez-Lorenzo, M. J. *et al.* Activated human T cells release bioactive Fas ligand and APO2 ligand in microvesicles. *J. Immunol.* **163**, 1274–1281 (1999).
15. Blanchard, N. *et al.* TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/ζ complex. *J. Immunol.* **168**, 3235–3241 (2002).
16. Wolfers, J. *et al.* Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nature Med.* **7**, 297–303 (2001).
17. van Niel, G. *et al.* Intestinal epithelial cells secrete exosome-like vesicles. *Gastroenterology* **121**, 337–349 (2001).
18. Nilsson, B. O., Lennartsson, L., Carlsson, L., Nilsson, S. & Ronquist, G. Expression of prostasome-like granules by the prostate cancer cell lines PC3, Du145 and LnCaP grown in monolayer. *Ups. J. Med. Sci.* **104**, 199–206 (1999).
19. Hess, C., Sadallah, S., Hefti, A., Landmann, R. & Schifferli, J. A. Ectosomes released by human neutrophils are specialized functional units. *J. Immunol.* **163**, 4564–4573 (1999).
20. Mack, M. *et al.* Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. *Nature Med.* **6**, 769–775 (2000).
21. MacKenzie, A. *et al.* Rapid secretion of interleukin-1β by microvesicle shedding. *Immunity* **15**, 825–835 (2001).
22. Escola, J. M. *et al.* Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B lymphocytes. *J. Biol. Chem.* **273**, 20121–20127 (1998).
23. Rabesandratana, H., Toutant, J. P., Reggio, H. & Vidal, M. 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 (1998).
24. Clayton, A. *et al.* Analysis of antigen-presenting-cell-derived exosomes, based on immuno-magnetic isolation and flow cytometry. *J. Immunol. Methods* **247**, 163–174 (2001).
25. Srivastava, P. Interaction of heat-shock proteins with peptides and antigen-presenting cells: chaperoning of the innate and adaptive immune responses. *Annu. Rev. Immunol.* **20**, 395–425 (2002).
26. Stubbs, J. D. *et al.* cDNA cloning of a mouse mammary epithelial cell surface protein reveals the existence of epidermal growth factor-like domains linked to factor VIII-like sequences. *Proc. Natl Acad. Sci. USA* **87**, 8417–8421 (1990).
27. Denzer, K. *et al.* Follicular dendritic cells carry MHC class-II-expressing microvesicles at their surface. *J. Immunol.* **165**, 1259–1265 (2000).
The first direct observation of exosomes in a human tissue (tonsils) in the absence of any *in vitro* culture step. Most of the other data in this paper, however, are obtained after the *in vitro* culture of B cells.
28. Mobius, W. *et al.* Immunoelectron microscopic localization of cholesterol using biotinylated and non-cytolytic perfringolysin O. *J. Histochem. Cytochem.* **50**, 43–55 (2002).
29. Gruenberg, J. & Maxfield, F. R. Membrane transport in the endocytic pathway. *Curr. Opin. Cell Biol.* **7**, 552–563 (1995).
30. Babst, M., Odorizzi, G., Estepa, E. J. & Emr, S. D. Mammalian tumor susceptibility gene 101 (TSG101) and the yeast homologue, Vps23p, both function in late endosomal trafficking. *Traffic* **1**, 248–258 (2000).
31. Vincent-Schneider, H. *et al.* Exosomes bearing HLA–DR1 molecules need dendritic cells to efficiently stimulate specific T cells. *Int. Immunol.* **14**, 713–722 (2002).
32. Katzmann, D. J., Babst, M. & Emr, S. D. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* **106**, 145–155 (2001).

33. Hicke, L. A new ticket for entry into budding vesicles — ubiquitin. *Cell* **106**, 527–530 (2001).
34. Reggiori, F. & Pelham, H. R. Sorting of proteins into multivesicular bodies: ubiquitin-dependent and -independent targeting. *EMBO J.* **20**, 5176–5186 (2001).
35. Aupeix, K. *et al.* The significance of shed membrane particles during programmed cell death *in vitro*, and *in vivo*, in HIV-1 infection. *J. Clin. Invest.* **99**, 1546–1554 (1997).
36. Mather, I. H. in *The Mammary Gland* (eds Neville, M. C. & Daniel, C. W.) 217–267 (Plenum, New York, 1987).
37. Peterson, J. A., Patton, S. & Hamosh, M. Glycoproteins of the human milk fat globule in the protection of the breast-fed infant against infections. *Biol. Neonate* **74**, 143–162 (1998).
38. Garrus, J. E. *et al.* Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* **107**, 55–65 (2001).
39. VerPlank, L. *et al.* Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc. Natl Acad. Sci. USA* **98**, 7724–7729 (2001).
40. Martin-Serrano, J., Zang, T. & Bieniasz, P. D. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nature Med.* **7**, 1313–1319 (2001).
41. Blott, E. J. & Griffiths, G. M. Secretory lysosomes. *Nature Rev. Mol. Cell Biol.* **3**, 122–131 (2002).
42. Pelham, H. R. SNAREs and the specificity of membrane fusion. *Trends Cell Biol.* **11**, 99–101 (2001).
43. Paumet, F. *et al.* Soluble NSF attachment protein receptors (SNAREs) in RBL-2H3 mast cells: functional role of syntaxin 4 in exocytosis and identification of a vesicle-associated membrane protein-8-containing secretory compartment. *J. Immunol.* **164**, 5850–5857 (2000).
44. Hibi, T., Hirashima, N. & Nakanishi, M. Rat basophilic leukemia cells express syntaxin-3 and VAMP-7 in granule membranes. *Biochem. Biophys. Res. Commun.* **271**, 36–41 (2000).
45. Guo, Z., Turner, C. & Castle, D. Relocation of the t-SNARE SNAP-23 from lamellipodia-like cell-surface projections regulates compound exocytosis in mast cells. *Cell* **94**, 537–548 (1998).
46. Martinez, I. *et al.* Synaptotagmin VII regulates Ca^{2+}-dependent exocytosis of lysosomes in fibroblasts. *J. Cell Biol.* **148**, 1141–1149 (2000).
47. Baram, D. *et al.* Synaptotagmin II negatively regulates Ca^{2+}-triggered exocytosis of lysosomes in mast cells. *J. Exp. Med.* **189**, 1649–1658 (1999).
48. Hwang, I. *et al.* T cells can use either T-cell receptor or CD28 receptors to absorb and internalize cell-surface molecules derived from antigen-presenting cells. *J. Exp. Med.* **191**, 1137–1148 (2000).
49. Huang, J. F. *et al.* TCR-mediated internalization of peptide–MHC complexes acquired by T cells. *Science* **286**, 952–954 (1999).
50. Hudrisier, D., Riond, J., Mazarguil, H., Gairin, J. E. & Joly, E. Cutting edge: CTLs rapidly capture membrane fragments from target cells in a TCR signaling-dependent manner. *J. Immunol.* **166**, 3645–3649 (2001).
51. Stinchcombe, J. C., Bossi, G., Booth, S. & Griffiths, G. M. The immunological synapse of CTL contains a secretory domain and membrane bridges. *Immunity* **15**, 751–761 (2001).
52. Brezinschek, R. I., Oppenheimer-Marks, N. & Lipsky, P. E. Activated T cells acquire endothelial cell-surface determinants during transendothelial migration. *J. Immunol.* **162**, 1677–1684 (1999).
53. Russo, V. *et al.* Acquisition of intact allogeneic human leukocyte antigen molecules by human dendritic cells. *Blood* **95**, 3473–3477 (2000).
54. Batista, F. D., Iber, D. & Neuberger, M. S. B cells acquire antigen from target cells after synapse formation. *Nature* **411**, 489–494 (2001).
55. Patel, D. M., Arnold, P. Y., White, G. A., Nardella, J. P. & Mannie, M. D. Class II MHC/peptide complexes are released from APC and are acquired by T-cell responders during specific antigen recognition. *J. Immunol.* **163**, 5201–5210 (1999).
56. Arnold, P. Y. & Mannie, M. D. Vesicles bearing MHC class II molecules mediate transfer of antigen from antigen-presenting cells to $CD4^+$ T cells. *Eur. J. Immunol.* **29**, 1363–1373 (1999).
57. Bedford, P., Garner, K. & Knight, S. C. MHC class II molecules transferred between allogeneic dendritic cells stimulate primary mixed leukocyte reactions. *Int. Immunol.* **11**, 1739–1744 (1999).
58. Knight, S. C., Iqball, S., Roberts, M. S., Macatonia, S. & Bedford, P. A. Transfer of antigen between dendritic cells in the stimulation of primary T-cell proliferation. *Eur. J. Immunol.* **28**, 1636–1644 (1998).
59. Sharrow, S. O., Mathieson, B. J. & Singer, A. Cell-surface appearance of unexpected host MHC determinants on thymocytes from radiation bone-marrow chimeras. *J. Immunol.* **126**, 1327–1335 (1981).
60. Gray, D., Kosco, M. & Stockinger, B. Novel pathways of antigen presentation for the maintenance of memory. *Int. Immunol.* **3**, 141–148 (1991).
61. Greco, V., Hannus, M. & Eaton, S. Argosomes: a potential vehicle for the spread of morphogens through epithelia. *Cell* **106**, 633–645 (2001).
This study shows a visualization *in vivo* in embryonic tissues of the transfer of membrane structures that are labelled with a fluorescent lipid between adjacent cells. The membrane structures that are involved have not been characterized.
62. Pan, B. T. & Johnstone, R. M. Fate of the transferrin receptor during maturation of sheep reticulocytes *in vitro*: selective externalization of the receptor. *Cell* **33**, 967–978 (1983).
63. Karlsson, M. *et al.* 'Tolerosomes' are produced by intestinal epithelial cells. *Eur. J. Immunol.* **31**, 2892–2900 (2001).
64. Dukers, D. F. *et al.* Direct immunosuppressive effects of EBV-encoded latent membrane protein 1. *J. Immunol.* **165**, 663–670 (2000).
65. Monleon, I. *et al.* Differential secretion of Fas ligand- or APO2 ligand/TNF-related apoptosis-inducing ligand-carrying microvesicles during activation-induced death of human T cells. *J. Immunol.* **167**, 6736–6744 (2001).
66. Andreola, G. *et al.* Induction of lymphocyte apoptosis by tumor-cell secretion of FasL-bearing microvesicles. *J. Exp. Med.* **195**, 1303–1316 (2002).
67. André, F. *et al*. Malignant ascitis accumulate immunogenic tumor-derived exosomes: novel approach for cancer immunotherapy. *Lancet* (in the press).
68. Kleijmeer, M. J. *et al.* Antigen loading of MHC class I molecules in the endocytic tract. *Traffic* **2**, 124–137 (2001).
69. Rieu, S., Geminard, C., Rabesandratana, H., Sainte-Marie, J. & Vidal, M. Exosomes released during reticulocyte maturation bind to fibronectin via integrin $\alpha4\beta1$. *Eur. J. Biochem.* **267**, 583–590 (2000).

Acknowledgements

We thank C. Hivroz and P. Benaroch for helpful comments on the manuscript. S.A. and L.Z. were supported by European Community grants.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez: http://www.ncbi.nlm.nih.gov/Entrez/
EBV

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
A33 | αM | annexin I | annexin II | annexin IV | annexin V | annexin VI | APO2L/TRAIL | β2 | CBL | CCR5 | CD3 | CD9 | CD13 | CD26 | CD28 | CD37 | CD40L | CD45 | CD53 | CD59 | CD63 | CD74 | CD81 | CD82 | CD86 | CDC25 | cofilin | enolase-1 | FASL | HLA-DM | HSC70 | HSP70 | HSP90 | ICAM1 | IFN-γ | IL-2 | LAMP1 | LAMP2 | LCK | MAGE3 | MART1 | MFGE8 | NSF | P-selectin | RAB5 | RAB7 | RAP1B | SNAP | SNAP23 | synaptotagmin II | synaptotagmin VII | syntaxin-3 | syntaxin-4 | TSG101 | VAMP7 | VAMP8

FURTHER INFORMATION

Anosys, Inc.: http://www.anosys.com/

Access to this interactive links box is free online.