

MINIREVIEW

Microvesicles and Viral Infection[∇]

David G. Meckes, Jr., and Nancy Raab-Traub*

Department of Microbiology and Immunology, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Cells secrete various membrane-enclosed microvesicles from their cell surface (shedding microvesicles) and from internal, endosome-derived membranes (exosomes). Intriguingly, these vesicles have many characteristics in common with enveloped viruses, including biophysical properties, biogenesis, and uptake by cells. Recent discoveries describing the microvesicle-mediated intercellular transfer of functional cellular proteins, RNAs, and mRNAs have revealed additional similarities between viruses and cellular microvesicles. Apparent differences include the complexity of viral entry, temporally regulated viral expression, and self-replication proceeding to infection of new cells. Interestingly, many virally infected cells secrete microvesicles that differ in content from their virion counterparts but may contain various viral proteins and RNAs. For the most part, these particles have not been analyzed for their content or functions during viral infection. However, early studies of microvesicles (L-particles) secreted from herpes simplex virus-infected cells provided the first evidence of microvesicle-mediated intercellular communication. In the case of Epstein-Barr virus, recent evidence suggests that this tumorigenic herpesvirus also utilizes exosomes as a mechanism of cell-to-cell communication through the transfer of signaling competent proteins and functional microRNAs to uninfected cells. This review focuses on aspects of the biology of microvesicles with an emphasis on their potential contributions to viral infection and pathogenesis.

The compartmentalization of molecules within the cell into specialized organelles is necessary to carry out many cellular processes. These dynamic structures exchange components within the cell in response to various biological events. Although the importance of organelles within the cell is well established, it has only recently been appreciated that membrane vesicles containing proteins and RNAs represent a new class of extracellular organelles named microvesicles (MV), which exhibit intriguing biological functions (62, 94, 103, 137). It is clear that some viruses utilize vesicle secretion pathways during infection. Therefore, understanding the interplay between viruses and microvesicles may contribute to the development of novel therapeutics and vaccines to control viral infections.

Biological fluids surrounding the extracellular space of cells and tissues contain various types of membrane-enclosed microvesicles. The variety of vesicles released from cells and the methods used to isolate them have led to confusion in the nomenclature. For the purpose of this review, all extracellular membrane vesicles released from cells will be termed microvesicles. This class of organelles currently includes exosomes, shedding microvesicles (SM) and apoptotic bodies (AB), which have been grouped based on biophysical properties (Table 1). Depending on their cellular origin, microvesicles contain specific molecules and have several virus-like characteristics, including their physical properties and their ability to

transport biologically active macromolecules between cells. Their functional properties include the modulation of angiogenesis (69, 77), cell proliferation (68, 138), cell invasion (59, 66, 106), gene regulation (68, 125, 157), and immune regulation (19, 33, 129).

EXOSOMES

The best-characterized microvesicles are exosomes, which are 40- to 100-nm endosome-derived vesicles that exhibit a uniform cup-like morphology when visualized by electron microscopy (following negative staining) or as round vesicles when observed by transmission electron microscopy (TEM) and cryo-electron microscopy (cryo-EM) (36, 155) (Table 1). Exosomes are essentially intraluminal vesicles (ILVs) released from cells that were generated by inward budding of endosomal multivesicular bodies (MVBs) (143). The ILVs of MVBs can be targeted for degradation through lysosomal pathways, or the MVB may traffic to the plasma membrane, where the ILVs are released into the extracellular space by fusion of the MVB membrane with the plasma membrane (Fig. 1). Interestingly, exosomes are the only known secreted cellular vesicles that originate from internal membranes. Exosomes have been found in many biological fluids, including urine (37, 116, 122), plasma (117), ascites (8), saliva (111), breast milk (3), bronchoalveolar lavage liquid (124), and amniotic fluid (85), making them ideal candidates for diagnostic biomarkers.

The mechanism that determines the specific fate of ILVs remains unknown; however, it is well established that the endosomal sorting complex required for transport (ESCRT) machinery is important for the sorting of ubiquitinated cargo into ILVs and for ILV formation (167). Therefore, it is not surpris-

* Corresponding author. Mailing address: Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill NC 27599. Phone: (919) 966-1701. Fax: (919) 966-9673. E-mail: nrt@med.unc.edu.

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TABLE 1. Properties of microvesicles

Parameter	Properties of:		
	Exosomes	Shedding microvesicles	Apoptotic bodies
Size	40–100 nm	100–1,000 nm	1–5 μM
Density	1.10–1.19 g/ml	1.16 g/ml	1.24–1.28 g/ml
Shape	Homogeneous	Variable	Variable
Isolation	Differential centrifugation 100,000–200,000 g Sucrose gradient Immunoisolation	Differential centrifugation 10,000–20,000 g Immunoisolation	Established protocol lacking Sediment at 1,200 g, 10,000 g, and 100,000 g
Markers	Tetraspanins (CD63, CD9), ALIX, TSG101	Integrins, MMPs, ^a tissue factor	DNA content, histones
Reference(s)	62, 126	32, 35	153

^a MMPs, matrix metalloproteinases.

ing that exosomes are enriched in ESCRT components such as TSG101 and Alix (Fig. 2) (103). However, the exact function of ESCRT proteins in exosome production is unclear. Depletion of an ESCRT-0 component, Hrs, that is important for cargo transport to MVBs, decreased exosome secretion in dendritic cells stimulated with ovalbumin and calcium ionophore (149). However, in oligodendrocytes, exosome secretion occurs through an ESCRT-independent mechanism involving the lipid ceramide (155). Ceramide has been proposed to create clustering of membrane microdomains and induce membrane curvature, promoting invaginations in lipid

bilayers. ILV formation and exosome release are reduced when neutral sphingomyelinase, the enzyme required to make ceramide, is inhibited (155). Exosomes are also enriched for other lipid components, including sphingomyelin, cholesterol, and the glycolipid GM3 (41, 170) and lipid raft resident proteins, including caveolins and flotillins (97, 141) (Fig. 2). The distinct markers suggest that, dependent on specific cargo or cellular stimulation, separate pathways contribute to MVB formation and exosome production. Multiple stimuli and different cellular environments have been shown to induce vesiculation, including differentiation (47), activation (64), senescence (95),

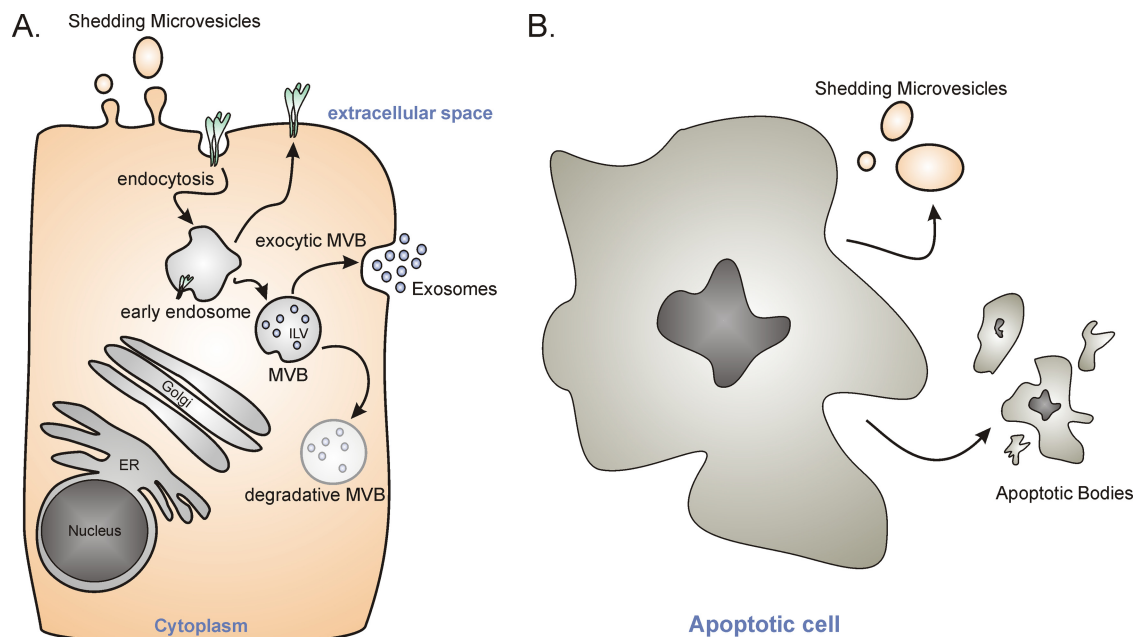


FIG. 1. Microvesicle biogenesis pathways. (A) Endocytosed proteins on the plasma membrane traffic to early endosomes where they can be sorted back to the plasma membrane or to multivesicular bodies (MVBs). MVBs contain intraluminal vesicles (ILVs) that are generated by budding from the limiting membrane of endosomes. Distinct MVB populations exist, a degradative MVB that leads to lysosomal destruction of MVB content or an exocytic pathway that traffics to the plasma membrane and, following membrane fusion, releases ILVs from the cell in the form of exosomes. Vesicles can also actively be released directly from the plasma membrane requiring a budding mechanism. These vesicles have been termed shedding microvesicles. ER, endoplasmic reticulum. (B) Dying or apoptotic cells release shedding microvesicles in the early stages of apoptosis and larger apoptotic bodies at later times that contain nuclear and cytoplasmic remnants of the degrading cell.

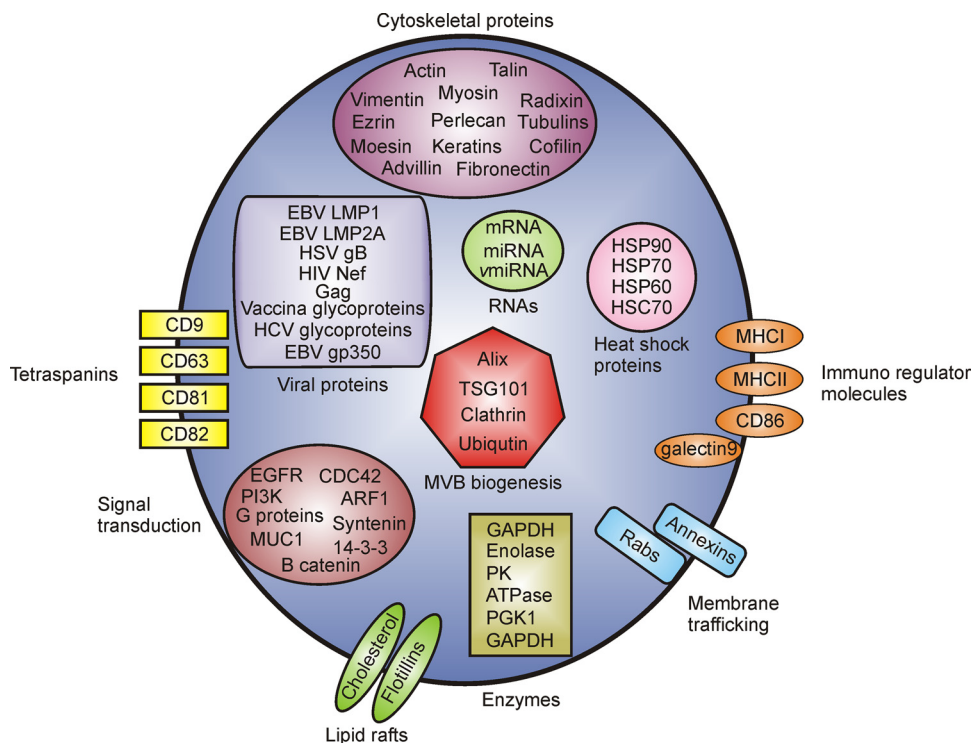


FIG. 2. Molecules found in exosomes. Proteomic and biochemical analysis of purified exosomes have identified many specific proteins and RNAs present within these structures that are often in different abundances than those of their intracellular counterparts. Molecules presented here have been grouped based on functions or protein classes. vmiRNA, viral miRNA; PK, protein kinase; MHCI and MHCII, MHC class I and II, respectively; PGK1, phosphoglycerate kinase 1; MUC1, mucin 1; ARF1, ADP-ribosylation factor 1.

stress (93), stimulation with cytokines (139), exposure to ATP (17), cell death (61), hypoxia (119), transformation (45), expression of oncogenes (6, 27, 78), and viral infection (11, 88, 102, 140, 162). Thus, in addition to the two defined MVB populations, one destined for a lysosomal degradation and the other for exosomal secretion, it is likely that additional MVB populations produce exosomes with distinct compositions.

Tetraspanins, which are heavily enriched in exosomes, have been proposed to have a major function in exosome formation (103) (Fig. 2). The human genome contains at least 32 tetraspanins. These proteins have four transmembrane domains and conserved motifs, including a CCG motif and two disulfide bonds within the second extracellular loop (65). Tetraspanins form complexes in membrane microdomains termed tetraspanin-enriched membrane domains (TEMs) through interactions between themselves and other transmembrane proteins (172). The tetraspanin-interacting complexes are often referred to as the tetraspanin web. The tetraspanin web may be important for trafficking of exosome components to the ILV of MVBs and therefore exosome biogenesis. In support of this hypothesis, expression of the tetraspanins, CD9 or CD82, induced exosomal sorting and secretion of β -catenin from cells (29). Additionally, tetraspanins CD63 and CD81 have been shown to bind components of the ESCRT machinery (52).

Rabs, small GTPases which participate in vesicle docking and membrane fusion events, are also commonly detected in exosomes (109, 153). Rabs form complexes with proteins involved in membrane trafficking through the endocytic system and are routinely used as markers of various endocytic com-

partments. For instance, Rab4 localizes to the early/recycling endosomes, Rab5 to the plasma membrane and early endosomes, and Rab7 to late endosomes (142). As expected from their role in vesicular trafficking, Rabs are important for exosome formation and release from cells. It has recently been shown that inhibition of Rab35 function with a dominant-negative construct induces accumulation of endosomal vesicles and a decrease in exosome release from cells (73). In the same study, Rab35 knockdown slowed the movement of vesicles to the plasma membrane, suggesting that Rab35 is important for MVB movement to the plasma membrane. Using an RNA interference (RNAi) screen, Ostrowski et al. discovered that Rab27a and Rab27b are important for MVB docking at the plasma membrane and therefore exosome secretion from HeLa cells (118).

There currently is no direct evidence that exosomes can also bud directly from the plasma membrane, but domains within the plasma membrane have been shown to be enriched in exosomal proteins, lipids, and carbohydrates and are termed endosome-like domains (48). These domains may be a mechanism for trafficking of cargo from the plasma membrane back to MVBs. Alternatively, these domains may function in vesicle budding from the plasma membrane similarly to what has been described for certain enveloped viruses (74). Additional support that exosomes or exosome-like vesicles may bud from the plasma membrane is the observation that vesicles with the typical size of exosomes (50 to 100 nm) have been found budding from the plasma membrane (20). However, since exo-

somes by definition are derived from ILVs of MVBs, these vesicles are a distinct population of vesicles secreted from cells.

SHEDDING MICROVESICLES

Shedding microvesicles (SMVs) are an additional class of microvesicles that are released from the plasma membrane instead of internal membranes and contain different protein components (Fig. 1). SMVs are larger and more heterogeneous in size than exosomes, ranging from 100 nm to 1 μ m (62, 64) (Table 1). The release of SMVs from the cell surface is a regulated process that is induced by cellular stimulus-like infection, activation, transformation, and stress. SMVs have been found to contain proteins, RNAs, and microRNAs (miRNAs) (25). Like exosomes, SMVs contain cholesterol-rich microdomains, or lipid rafts, and common lipid raft-associated proteins, such as flotillin-1 and tissue factor (42). SMVs may also contain specific integrins, cytokines, chemokines, metalloproteinases, and higher levels of phosphatidyl serine (PS) exposed on the outer-membrane leaflet (6, 43, 55, 94, 123, 135). Less is known about SMV vesiculation than exosome biogenesis; however, it is understood that the enzymes involved in this process, at least for vesiculation in platelet cells, include aminophospholipid translocase, scramblase, floppase, and calpain (121). In microglia cells, the enzyme acidic sphingomyelinase, which is distinct from neutral sphingomyelinase that is important for exosome formation, is essential for SMV release following ATP stimulation (17); Bianco et al. proposed that ATP released from dying cells acts as a stimulus for microglia to secrete SMVs containing proinflammatory cytokines. In addition to these enzymes, it is likely that clustering of SMV cargo into membrane microdomains drives the SMV budding process, as the addition of membrane targeting motifs to highly oligomeric cytoplasmic proteins was sufficient to induce their secretion from the plasma membrane into SMVs (136).

APOPTOTIC BODIES

Apoptotic bodies (AB), which are released from cells during the later stages of programmed cell death, are another type of membrane-enclosed vesicle. AB are large, ranging from 1 μ m to 5 μ m in size, and contain exposed PS and many cellular remnants, including fragmented DNA and cellular organelles (72, 153) (Table 1). AB can also transfer cargo, such as oncogenes and DNA, between cells and have been shown to be important in antigen presentation and immunosuppression (12, 14, 34, 67). Compared to their MV counterparts, AB have been understudied, as an effective isolation procedure is lacking and they are the by-product of cell death (62). Further study is warranted, as it is likely that AB participate in important cellular communication events that may be distinct from those described for exosomes or SMVs.

FUNCTIONS OF MICROVESICLES

The discovery of MV dates back to 1967, when Peter Wolf first described the generation of dust particulates by activated blood platelets that were later termed exosomes (156, 168). An ever-increasing number of recent studies support the role of microvesicles in a novel mechanism of cell-to-cell communi-

cation through the transfer of soluble and insoluble factors (5, 110, 120, 138, 157). Much of our current understanding of the functions of microvesicles comes from the study of various cancers, for which the process of tumorigenesis leads to an increase in microvesicle secretion (127). Cancer patients have an elevated number of MV circulating in their blood that correlates with a poor prognosis (90). Microvesicles have been implicated in multiple aspects of cancer biology and disease progression, including transformation, (9) tumor growth (84, 138), tumor microenvironment remodeling (151), invasion (66), angiogenesis (77), cell migration (119) metastasis (70), immune evasion and response (154), and differentiation (158, 165). Microvesicles also have functions in antigen presentation (128), neuronal communication (49), coagulation (13, 42), pathogen spread (50, 75, 166), sperm maturation (145), and pregnancy (150). The process of vesiculation may also participate in cellular waste removal for unwanted proteins as a cellular defense response to regulate overactive signaling complexes, particularly those induced by oncogenic proteins (1, 29). Although there are considerable data supporting the role of microvesicles in various pathological processes, there are a limited number of studies documenting their function *in vivo*.

Microvesicles exert their biological functions through interactions with recipient cells or the transfer of molecular components following membrane fusion events. One unique feature of microvesicles is the ability to transfer multiple effector molecules at once and shuttle proteins between cells, which would otherwise not be possible through alternative secretion mechanisms like those used by soluble growth factors (5, 110, 138). For instance, microvesicles can contain and transfer transmembrane proteins, insoluble proteins, and proteins that do not possess a classical signal sequence for exocytosis (5, 110). Microvesicles can also transfer activated receptors that are signaling competent following target cell uptake (5). This type of transfer may contribute to a more sustained cellular response.

In addition to protein cargo, microvesicles may contain both mRNA and miRNAs (110, 120, 138, 157). A seminal paper in the field demonstrated the functional transfer of mRNA through exosomes to cells from which new protein products were produced (157). This was the first demonstration that exosomes could transfer functional RNA to recipient cells and thus participate in a novel mechanism of cell-to-cell communication through epigenetic reprogramming of target cells. In the same study, a specific miRNA profile within the exosomes, differing from that of the intracellular levels, was also identified. Subsequent studies have shown that the transferred miRNAs were actually capable of silencing targets in recipient cells.

Very little is known about the mechanisms of microvesicle-mediated cargo transfer. It has been proposed that direct contact with target cell surface receptors, cleavage of surface proteins, fusion with the cell membrane, and endocytosis may all contribute to entry and effects on recipient cells (Fig. 3) (25, 76). Interestingly, an ingenuity pathway analysis (IPA) of MV cargo revealed that many of the molecules found in MV have been implicated in virus binding and entry (62). As many viruses enter through endocytic routes, MV may also enter through endocytic pathways (46, 112). The diversity of MV

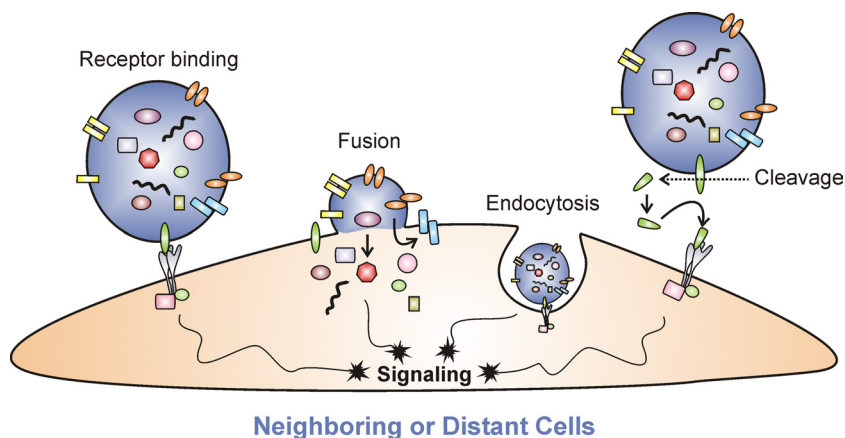


FIG. 3. Potential mechanisms of microvesicle-mediated intercellular communication. Proteins present on the microvesicle surface can bind to receptors on the target cell membrane, activating signaling pathways within that cell (Receptor binding). Microvesicles may fuse with target cell membranes and release their contents at the plasma membrane (Fusion) or internal membranes following uptake into endocytic vesicles (Endocytosis). Deposited cargo can activate signaling pathways, silence target gene expression, and produce new proteins following transcription of mRNA. Microvesicles may also interact with molecules present within endocytic vesicles that induce signal transduction cascades in the absence of vesicle fusion. Proteases located in the extracellular space may cleave proteins exposed on the microvesicle surface. The potential ligands generated could bind to cell surface receptors on the target cell and initiate signaling events (Cleavage).

populations and components suggests that MV enter cells through numerous mechanisms similar to the multiple pathways identified for viruses (101).

MICROVESICLES AND VIRUS INFECTION

Microvesicles produced during many types of viral infection were identified in early studies, and some of their properties have been documented. For example, L-particles produced in herpesvirus infection were shown to enhance viral replication, while the high levels of secretion of hepatitis B virus (HBV) surface antigens were thought to function as immune decoys. The further study of subviral particles and cellular exosomes will continue to clarify the effects of their production on viral pathogenesis.

Retroviruses. The similarity between microvesicle and enveloped virus biogenesis and entry into cells is thought-provoking and has led to the Trojan exosome hypothesis of HIV assembly and cell-cell spread (57). This theory postulates that retroviruses have evolved to utilize a preexisting host exosome biogenesis pathway for the formation of infectious virus and can also utilize a nonviral mode of exosome uptake that is independent of the viral Env protein. However, recent data unequivocally shows that HIV buds from the plasma membrane and not internal MVB membranes (18). The virus does recruit components of the host ESCRT machinery to the site of budding and possesses some similarities to shedding microvesicles. Other enveloped viruses also utilize the cellular machinery of vesiculation for their formation and cell-to-cell spread (63, 80, 131). Viruses that interact with or require the ESCRT pathway for release include rhabdoviruses, filoviruses, arenaviruses, paramyxoviruses, herpesviruses, HBV, and hepatitis C virus (HCV) (30, 63, 113). Additional evidence that HIV budding is distinct from exosome formation is that inhibition of ceramide synthesis, which blocks exosome shedding, does not affect HIV budding, although a decrease in infectivity was observed (21, 75). However, HIV may utilize raft microdo-

main rich in tetraspanins for virus assembly as Gag proteins from both HIV and human T cell lymphotropic virus type 1 (HTLV-1) interact with tetraspanins (60, 104, 105). CD81 and CD63 participate in HIV budding, cell-to-cell spread, and infectivity, demonstrating the overall importance of this protein family in retrovirus biology (60, 75, 79, 134). The investigation of virus budding and cellular microvesicle biogenesis will likely provide additional insight into these processes and may identify novel targets to inhibit microvesicle or virus release from cells.

The study of microvesicles secreted during virus infection is complicated by the similar size and density of microvesicles and infectious virus particles, making it challenging to separate the two populations (Table 2). HIV particles have biophysical properties almost identical to those of the exosomes secreted from the same cells, including the ~100-nm size, a buoyant density of 1.13 to 1.21 g/liter, and functional effects on immune cell activation (26, 153, 164, 170) (Table 2). In early studies, it was difficult to purify HIV particles free of exosomes complicating the analysis of the composition and function of the distinct types of particles (15, 56, 115). Recent purification strategies utilizing iodixanol density gradients and immunoaffinity isolation have successfully separated purified HIV virions free of exosomes (26, 31).

The potential functions of exosomes secreted during HIV infection are just beginning to emerge. Exosomes released from infected cells have been shown to contain coreceptors for HIV which can enhance virus entry into cells (99, 132). Expression of the viral Nef protein alters the endosomal system by increasing the number of endosomes, lysosomes, and multivesicular bodies (MVBs) (38, 100, 133, 144). HIV Nef induces massive vesicle secretion from infected and noninfected cells and can be detected in the serum of infected individuals (4, 114). Recently, Nef has been found in exosomes secreted from cells, and these Nef-containing exosomes induced apoptosis in CD4⁺ T cells (24, 96). Therefore, exosomal Nef may

TABLE 2. Particles released from virus infected cells

Virion	Particle type and characteristics					Reference(s)
	Size (nm)	Density (g/ml)	Microvesicle	Size (nm)	Density (g/ml)	
HIV	~100	1.13–1.21	HIV exosomes	~100	1.13–1.21	26
HCV	35–100	1.10–1.14	HCV exosomes	~100	~1.08	54
HBV	44	1.20–1.28	HBV subviral particles			
			Quasi-spherical	22	1.20	82
			Filaments	up to 1,200		22
HSV	150–200	1.07–1.26	HSV L particles	80–200		10, 146, 147
HCMV	150–200	1.18–1.22	HCMV exosomes	20–50	1.08–1.17	16, 162, 169
EBV	150–200	~1.20	EBV exosomes	40–100	1.08–1.22	87, 159
			EBV shedding microvesicles	>100		128, 160
Vaccinia virus	360 by 270 by 250	1.13–1.21	Vaccinia virus microvesicles	50–200	1.25	39, 140

contribute to HIV pathogenesis by facilitating the depletion of CD4⁺ T cells. Nef has also been shown to be transported from infected macrophages to neighboring uninfected B cells through a contact-dependent mechanism requiring nanotubes. This transfer impaired antibody class switching and humoral immunity (171). The transfer of Nef or other viral components through intracellular and extracellular vesicles may represent an important mechanism for immune evasion by viruses.

In a classic example of host-pathogen relationships, it also appears that the host cell machinery utilizes vesicle secretion for its defense against viral infection. Secreted vesicles may present viral antigens and activate immune cells during a cellular response (2, 19, 91, 128, 154, 162). Microvesicles may also contribute to the innate immune defense mechanism. For example, APOBEC3G, a cytidine deaminase that is part of the cellular antiviral system against retroviruses, can be packaged and transferred to adjacent cells through exosomes to inhibit HIV replication (89). The contribution of exosomes to the innate immune response is also suggested by the finding that exosomes released from airway epithelial cells neutralize influenza virus (88).

Hepatitis C virus. Three distinct types of particles have been shown to be secreted from HCV-infected cells *in vitro* (54). The most abundant membrane-enclosed vesicle released from HCV-infected cells is intact virus, which is an ~60-nm population with a membrane bilayer, an internal capsid structure, and high infectivity (Table 2). An ~45-nm population does not possess a lipid bilayer and has a higher density and a lower infectivity-to-HCV RNA ratio. Larger vesicles (~100 nm) that resembled cellular exosomes observed by cryo-EM were also secreted during infection but had barely detectable infectivity. Interestingly, HCV structural proteins are detected in exosomes circulating in HCV-infected patients, suggesting a potential contribution of these vesicles to viral pathogenesis (102). However, the composition and properties of these microvesicles released during HCV infection remain to be elucidated.

Hepatitis B virus and poxviruses. Since almost all cell types are capable of vesiculation, it would not be surprising if microvesicles are secreted from all cells during active viral infection. HBV infection results in the massive release of noninfectious subviral particles containing the viral surface antigens (28). These particles can be spherical, resembling exosomes, or filamentous and similar in size to larger shedding mi-

crovesicles. Subviral particles are very abundant in patient serum and can reach a 10,000-fold-higher concentration than that of infectious virus particles (53). Again, the composition and function of these particles have been largely unexplored yet have been hypothesized to function in immune evasion strategies. It has been suggested that subviral particles distract the immune system from the few infectious particles. Similarly, cells infected with vaccinia virus also produce particles that contain viral glycoproteins but lack other viral cargo (140). Although it is possible that the production of these vesicles by infected cells is a by-product of cell lysis and virion assembly, the many proposed functions and identified properties of secreted microvesicles suggest that they likely contribute to the pathogenesis of both hepadnavirus and poxvirus infections.

Herpes simplex virus. Herpes simplex virus (HSV)-infected cells also secrete microvesicles, originally called L-particles, that are noninfectious as they lack the viral capsid and DNA (98, 108, 130, 148). These particles contain viral tegument proteins and glycoproteins and, similar to HSV virions, likely contain many cellular factors (98, 108, 130). Interestingly, HSV L-particles are comparable in size to exosomes, are formed on internal membranes, and are capable of delivering functional cargo to uninfected cells (40, 107). Since the purpose of some of the tegument proteins is to prime the cell for infection (81, 86), at least one function of HSV L-particles could be in enhancing viral infectivity or replication (107). This is supported by the observation that L-particles enhance the ability of transfected viral DNA to form infectious foci or plaques (40). HSV may also utilize the exosomal pathway for immune evasion. Expression of HSV glycoprotein B (gB) in a human melanoma cell line, Mel JuSo, altered the major histocompatibility complex (MHC) class II antigen-processing machinery by shuttling HLA-DR to the exosomal secretion pathway instead of the cell surface (152). Additionally, extracellular vesicles secreted from these cells are rich in the tetraspanin CD63, a late endosome marker and exosome component, and contained gB in a complex with HLA-DR. Based on our current knowledge of microvesicle functions, it is likely that HSV L-particles contribute to viral pathogenesis within the infected host by enhancing virion infectivity and providing immune evasion functions.

Epstein-Barr virus. The study of Epstein-Barr virus (EBV) microvesicles has also expanded our understanding of the cell biology of microvesicles, and additional potential functional

properties have been identified. The latent membrane protein 1 (LMP1), the major oncoprotein of the virus, is required for EBV-induced B cell immortalization and is sufficient to transform rodent fibroblasts *in vitro* (83, 163). The secretion of this protein into exosomes was first described for transformed B cells (51) and later for epithelial cells grown in culture (27). LMP1 is also detected abundantly in the serum of patients with nasopharyngeal carcinoma (NPC) and is present in exosomes isolated from the serum of mice carrying NPC tumors (71, 110). The sorting of LMP1 into exosomes is not completely understood; however, a recent report indicated that the interaction of LMP1 with the tetraspanin CD63 was important for LMP1 trafficking to MVBs and may participate in LMP1 exosomal secretion (161). Interestingly, the localization of LMP1 to lipid rafts or ubiquitination, two proposed mechanisms for MVB targeting, did not appear to be important for LMP1 exosome-mediated secretion. Immunoelectron microscopy of EBV-infected cells has also detected LMP1 in vesicles budding from the plasma membrane (160), and LMP1 is present in distinct fractions isolated from extracellular media, consistent with its presence in other microvesicle populations in addition to exosomes (87). Further study is needed to understand LMP1 intracellular trafficking and its secretion into potentially separate microvesicle populations.

Significantly, the secretion of LMP1, a potent signal transduction protein, was shown to affect uninfected recipient target cells. This was first suggested when purified LMP1 protein and exosomes secreted from B cells containing LMP1 were shown to inhibit T cell proliferation and NK cytotoxicity (44). More recently, exosomes produced by EBV-infected NPC cells were shown to contain an immune modulator protein, galectin 9, that contributed to the observed immunosuppressive effects (87, 92). The secretion of immunosuppressive exosomes containing LMP1 and other virally induced proteins from EBV-infected cells is likely important in viral pathogenesis and may also contribute to tumor progression. Interestingly, EBV-infected NPC tumors are heavily infiltrated with T cells that are unable to clear the tumor (23). Exosomes may therefore represent part of an immune evasion strategy utilized by the virus and tumor.

Our recent studies of tumor cell-derived exosomes provide additional evidence for the functional delivery of LMP1 to target cells (110). LMP1 was found to be abundantly secreted from NPC cells infected with EBV, and LMP1 expression alone was sufficient to induce its secretion from a noninfected epithelial cell line. The uptake of LMP1-containing exosomes by uninfected target cells induced growth-stimulating signaling pathways in recipient cells. These findings suggest that through exosomal transfer of LMP1, EBV can manipulate the growth characteristics of neighboring cells. This may be especially important in the pathogenesis of NPC, since not all NPC cells express detectable levels of LMP1. Release of LMP1 from a rare expressing cell could have wide-ranging effects on the entire cell population. Intriguingly, LMP1 expression also altered the composition of exosomes and increased the levels in exosomes of two important signaling molecules frequently activated in cancers, phosphatidylinositol 3-kinase (PI3K) and the epidermal growth factor receptor (EGFR). Similar results were also described for the increased secretion of fibroblast growth factor 2 (FGF2) from LMP1-expressing cells (27).

LMP1 may modulate the selective sorting of proteins into the exosomal pathway, suggesting that EBV manipulates these pathways for intercellular communication.

In addition to LMP1, virally encoded miRNAs that can be transferred to uninfected recipient cells have also been shown to be contained in exosomes secreted from EBV-infected cells (58, 110, 120). The delivery of these miRNAs specifically silenced known miRNA targets, providing the first evidence of functional delivery of miRNAs through exosomes (120). Additionally, uninfected B cells isolated from patients harboring EBV contained viral miRNAs, suggesting that miRNA transfer can occur *in vivo*. We have also shown that NPC exosomes are enriched for certain viral miRNAs relative to their intracellular levels (110). This suggests a selective and specific secretion of miRNAs from infected cells that may contribute to their potential functions outside the infected cell. Evidence for functional miRNA delivery *in vivo* is currently lacking; however, a recent study utilized neuronal targeted exosomes to deliver small interfering RNA (siRNA) to achieve specific target gene knockdown (7). When these findings are taken together, it appears as though EBV utilizes the exosome pathway for the selective secretion of viral and cellular proteins and miRNAs that likely participate in cell-cell communication in the absence of virus production and potentially modulate cell function.

CONCLUSIONS

The field of virology has contributed immensely to our understanding of microvesicle biology. The study of enveloped viruses is mature compared to the microvesicle field. Some of the seminal discoveries in the area of microvesicle research were made first through the study of virally infected cells. These include the function of microvesicles as immune activators (2, 128), in intracellular communication (107), in immune suppression (44), and for the transfer of functional miRNAs (120). It is likely that techniques previously and currently being developed to study viruses will continue to advance the study of microvesicles. Additionally, our understanding of the complex process of viral entry may elucidate requirements that affect the specificity of exosome uptake. Moreover, since many virally infected cells secrete microvesicles in addition to infectious virus particles, the further study of virally modified microvesicles will clarify their role in infection.

The microvesicle transfer of viral and cellular factors, particularly in the case of persistent infections such as those of the herpesviruses, would enable the manipulation of neighboring uninfected cells, which could be beneficial both to the virus and to the host, as this could potentially reduce viral replication to a minimum. Microvesicle-mediated communication would allow the virus to respond to or control the cellular microenvironment in the absence of viral replication. The current findings suggest that viruses utilize the cellular vesiculation pathway for virus budding/assembly, immune evasion, and intercellular communication. Our current mechanistic understanding of microvesicle biology and function, especially in regard to virus infection, is in its infancy. However, the obviously broad biological and medical implications of microvesicles make them a significant and exciting area of re-

search with potential high impact on our understanding of pathogenesis within the infected individual.

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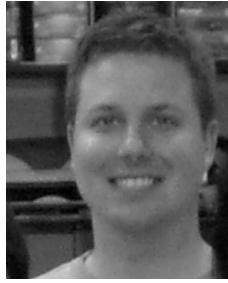
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David Meckes (Ph.D., 2008) obtained his B.S. from the University of the Sciences in Philadelphia and his Ph.D. from The Pennsylvania State University College of Medicine. His Ph.D. work in the laboratory of Dr. John Wills was on herpes simplex virus assembly and viral protein-protein interactions. He is now an American Cancer Society postdoctoral fellow at the University of North Carolina Lineberger Comprehensive Cancer Center in the laboratory of Dr. Nancy Raab-Traub. He currently studies the biological properties of microvesicles secreted from Epstein-Barr virus latently infected cells and the signaling and trafficking properties of the viral oncoprotein latent membrane protein 1 (LMP1).



Nancy Raab-Traub (Ph.D., 1980) received her B.S. from the University of Michigan and her Ph.D. from the University of Chicago in the laboratory of Dr. Elliott Kieff, where she participated in the original cloning and mapping of the EBV genome. She was a postdoctoral fellow with Dr. Joseph Pagano at the University of North Carolina, where she joined the faculty and is now the Sarah Graham Kenan Professor of Microbiology and Immunology. Her work has focused on the molecular biology and pathogenesis of the Epstein-Barr virus and its contribution to the development of nasopharyngeal carcinoma (NPC). Her studies identified the viral genes expressed in NPCs, including a family of RNAs that are the template for multiple viral miRNAs. She has characterized the transforming properties of the viral proteins in epithelial cells and the activation of unique signaling pathways by the viral oncoprotein latent membrane protein 1 (LMP1).

