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Endosomal vesicles as vehicles for viral genomes

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Abstract

The endocytic pathway is the principal cell entry pathway for large cargo and pathogens. Among the wide variety of specialized lipid structures within endosomes, the intraluminal vesicles formed in early endosomes and transferred to late endosomal compartments are emerging as critical effectors of viral infection and immune recognition. Various viruses deliver their genomes into these intraluminal vesicles, which serve as vehicles to transport the genome to the nuclear periphery for replication. When secreted as exosomes, intraluminal vesicles containing viral genomes can infect permissive cells, or activate immune responses in myeloid cells. We therefore propose that endosomal intraluminal vesicles and exosomes are key effectors of viral pathogenesis.

Keywords

intraluminal vesicle (ILV); enveloped RNA virus cell entry; virus egress by exocytosis; endosomal carrier vesicle; infectious exosomes; exocytic vaccine

Virus entry through the endocytic pathway

Clathrin-mediated endocytosis is arguably the major entry pathway for large cargo and pathogens. As endocytosis proceeds, endocytic compartments acquire a wide variety of membrane structures including invaginations, tubules and intraluminal vesicles (ILVs) [1–4], each of which has specific functions in cargo selection, intracellular trafficking, cell signaling, or metabolism (Box 1). Many viruses utilize cellular receptors directly or indirectly associated with clathrin or clathrin adaptors to enter the host cell through the endocytic pathway. In enveloped viruses, the genome is enveloped in a lipid membrane, which must be fused to a cellular membrane to deliver the viral genome or nucleocapsid into the cytoplasm. The viral envelope glycoproteins catalyze this membrane fusion event with conformational changes triggered by environmental cues [5]. The set of physicochemical requirements required to trigger the fusogenic conformational change—such as pH, lipid

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composition, and post-translational modifications—determines where and when in the endocytic pathway viral membrane fusion occurs.

Box 1

The endocytic system

Endocytosis is the mechanism for internalization of molecules of all sizes, plasma membrane components, and nanoparticles by the invagination of the plasma membrane and the formation of vesicles through membrane fission (see Figure I; reviewed in Ref. [2]). Clathrin-mediated endocytosis is the major pathway for vesicles to bud from the plasma membrane, but several clathrin-independent pathways also contribute to endocytosis including the caveolin-dependent pathway. Endocytic vesicles fuse with early endosomes (EEs) in the peripheral cytoplasm. EEs are the main sorting station in the endocytic pathway. As they accumulate extracellular cargo from endocytic vesicles, some proteins and membrane components are recycled to the plasma, while others are transferred to the Golgi apparatus. However, most cargo is retained inside EEs. Intraluminal vesicles (ILVs) bud from the endosomal membrane, beginning in EEs, though the timing of when ILVs begin to form remains to be clarified. EEs and the ILVs they contain are transported towards the nucleus on microtubules (MT). During this time, EEs mature into late endosomes (LE). LE maturation involves changes in lipid and protein composition and in the pH of the lumen. Additionally, vesicles bud from the outer (limiting) membrane of the maturing endosome to form ILVs. As maturation proceeds, LEs undergo homotypic fusion, grow in size, acquire more ILVs, and receive newly synthesized components from the secretory pathway. Some ILVs can be secreted at the cell surface through scission of an LE fragment and subsequent fusion of the fragment with the plasma membrane. The fusion of an LE with a lysosome generates a transient hybrid organelle, the endolysosome (EL), in which active degradation takes place. Endolysosomes then mature to lysosomes, storage organelles for hydrolases and membrane components. Proteins from the Rab family of GTPases define many of the functional attributes of endosomes by regulating the biological activities of effector proteins, which include the ESCRT machinery responsible for budding and sorting of endosomes and ILVs.

During endocytosis, it was assumed that enveloped RNA viruses and some enveloped DNA viruses fuse with limiting endosomal membranes, membranes that separate the cytoplasm from the endosomal lumen. This assumption was challenged when vesicular stomatitis virus (VSV) was found to fuse with ILVs in early/intermediate endosomes, releasing the nucleocapsid into the lumen of ILVs. These ILVs are then transported along microtubules to late endosomes near the nucleus. VSV was the first virus reported to utilize ILVs as transport vehicles in this manner. In late endosomes, ILVs fuse with the limiting endosomal membrane to deliver the nucleocapsid into the cytosol [6], a process known as “back-fusion.” Back-fusion of ILVs to the limiting membrane is dependent on BMP (bis(monoacylglycero)phosphate), PI(3)P (phosphatidylinositol-3-phosphate) and ESCRT (Endosomal Sorting Complexes Required for Transport) components in the limiting membrane. Back-fusion is regulated by Hrs, an ESCRT-0 component, and the ESCRT accessory protein Alix [6–9]. The availability of ILVs in the early endosomal compartment in which VSV undergoes its initial fusion event has been questioned [10], however other pathogens have been shown to utilize a similar ILV back-fusion pathway to enter the host cell. Lethal toxin from *Bacillus anthracis* utilizes ILV back-fusion to deliver its toxin

subunit into the cytoplasm [11], and some members of the flavivirus genus, which includes important human pathogens such as dengue, West Nile and Japanese encephalitis viruses, were recently shown to utilize a similar pathway [7]. Moreover, recent studies revealed that ILVs containing genomic RNA from hepatitis C virus (HCV) can be secreted as exosomes, which proceed to infect human cells [12, 13] or activate plasmacytoid dendritic cells [14]. The recent discovery that ILVs are used to transport the nucleocapsids of several different viruses, and perhaps shield them from degradation in the endosomal lumen, suggests that ILV-dependent cell entry pathways are more common and important for viral pathogenesis than previously appreciated. We review these and other recent advances and their implications for the prevention and treatment of viral diseases.

Physicochemical requirements of membrane fusion direct some viruses to ILVs

Membrane lipid composition determines the site of virus entry

Early and late endosomal membranes, and the wide variety of membraneous structures they contain, are comprised of distinct lipid compositions (Figure 1) [1–4]. Early endosomes, like the plasma membrane from which they originate, are rich in cholesterol, phosphatidylserine (PS) and phosphatidylinositols [15–17]. The lipid substructures that originate from early endosomes, including invaginations, tubules, and ILVs, initially have the same lipid composition (lipids can subsequently undergo sorting). As membranes progress along the endocytic pathway their cholesterol content decreases. Cholesterol is replaced with ceramide in late endosomes and lysosomes, where it maintains membrane fluidity [18]. The anionic, acid-resistant lipid BMP (also known as lysobisphosphatidic acid or LBPA) is enriched in the internal membranes of late endosomes and lysosomes including the ILVs derived from them, but not in ILVs transported from early endosomes [15, 16]. Hence ILVs derived from early or late endosomes form distinct pools. BMP increases the fusogenicity of vesicle membranes at pH <6 and induces internal vesiculation in liposomes resembling multivesicular endosomes found *in vivo* [8, 19]. Autoantibodies against BMP result in autoimmune disorders such as antiphospholipid syndrome and Niemann-Pick type C syndrome. Anti-BMP antibodies cause dysfunctional sorting and trafficking in late endosomes [20, 21], suggesting a critical role for BMP in late endosome function and dynamics.

Recent evidence suggests that these changing lipid compositions in endosomes, combined with the pH threshold of fusion, may direct virus entry to specific points along the endocytic pathway. Indeed, certain enveloped RNA viruses require a specific lipid composition in their target cellular membrane for efficient fusion. For example, cholesterol and pH <6–6.5 are necessary for fusion of alphaviruses, such as Semliki Forest virus, with early endosomal membranes, where cholesterol is abundant [22]. In another example, flaviviruses including dengue virus require PS or other similar anionic phospholipids in the target membrane for efficient fusion [7, 23]; cholesterol, though not strictly required, further enhances fusion [24, 25].

Although it is clear that viruses have requirements for different membrane types, the mechanisms underlying this specificity are only emerging. In the context of flaviviruses, virus particles from at least two species bind to PS with high affinity and specificity [7]. PS is normally restricted to the cytoplasmic leaflets of the plasma membrane and early endosomal membranes. However, when calcium is released into the cytoplasm—a common cellular distress signal during infection, apoptosis or cancer—phospholipid transporters known as scramblases translocate PS to the extracellular and luminal leaflets [7, 26–28]. PS in the extracellular leaflet serves as an “eat me” signal for phagocytosis of distressed, dying or cancerous cells and activation of myeloid cells [29–31]. Notably, recent evidence suggests that the redistribution of PS towards extracellular or luminal leaflets following intracellular calcium release may be important for flavivirus infectivity. Indeed, certain flaviviruses trigger cytoplasmic calcium release and activate PI(3)P signaling during cell entry [7], and chelation of intracellular calcium inhibits their infection [7]. The presence of PS on the outer plasma membrane leaflet of insect cells even in the absence of calcium release [32], in contrast to mammalian cells, provides an explanation for why dengue virus can fuse with the plasma membrane of insect but not mammalian cells in an acidic environment [23].

Endosomal ILVs deliver viral genomes into the cytosol via back-fusion

The lipid and pH requirements for fusion of flaviviruses to a host cell membrane usually result in fusion to early-to-intermediate endosomal compartments, which contain a multitude of ILVs [1] (except with insect cells under acidic conditions when fusion may occur at the plasma membrane- see above). Due to the excess of ILV membranes over the limiting endosomal membrane, there is a higher probability that flaviviruses will fuse with ILVs. The enrichment of PS resulting from the release of cytoplasmic calcium during flavivirus entry may further increase the likelihood of the virus to fuse with an ILV membrane [7]. As discussed above, back-fusion of ILVs requires PI(3)P and BMP, which is specific to the limiting membrane of late endosomes. Indeed, BMP is required for delivery of flavivirus nucleocapsids into the cytoplasm, but not for the initial fusion of virions to ILVs [6, 7]. In addition, inhibition of PI(3)P kinase prevents formation of ILVs in early endosomes and inhibits flavivirus infectivity [6, 7]. These findings suggest flaviviruses enter the host cell by delivering their nucleocapsids to the interior of an ILV, where they remain trapped until the ILV membrane is fused back to a limiting endosomal membrane, delivering the nucleocapsid into the cytoplasm [7]. A similar sequential cell entry mechanism was previously proposed for VSV [6]; however, VSV does not impact PI(3)P signaling or trigger intracellular calcium release [33–35].

ILVs: transporters of viral genomes to the nuclear periphery

Early endosomes, containing ILVs, travel along microtubules to the perinuclear region, where they fuse with late endosomal membranes, delivering the ILVs into the late endosomal lumen (Figure 2) [2]. Viral nucleocapsids contained in endosomal ILVs follow the same path. ESCRT proteins in late endosomes mediate back-fusion of ILVs to the limiting membrane [1, 6], but the cytosolic localization of ESCRT proteins implies that their effect on back-fusion may be indirect. Back-fusion allows recycling of various cellular components including major histocompatibility complex class II (MHC II), mannose-6-

phosphate receptors and tetraspannin proteins, all of which are enriched in ILVs [2, 15]. The ILV-dependent cell entry pathways proposed for VSV and flaviviruses [6, 7] lead us to propose that ILVs can serve as efficient vehicles for viruses to deliver their genetic material to the nuclear periphery. We speculate that reaching the perinuclear region is advantageous for those enveloped DNA viruses that must replicate their genome in the nucleus such as herpesviruses [36], and for enveloped RNA viruses that assemble their replication complexes in perinuclear organelles such as the endoplasmic reticulum (ER) or ER-derived compartments, as is the case for flaviviruses [37] and HCV [38].

ILVs as exosomes: Another route for infection

In addition to serving as transport vehicles to the nuclear periphery, recent evidence suggests that ILVs provide a route for cell-to-cell transmission. As late endosomes mature, they mostly fuse with lysosomal compartments, however a fraction of late endosomes can be induced to fuse with the plasma membrane [2, 39]. Fusion of late endosomes with the plasma membrane releases endosomal ILVs into the extracellular milieu (Figures 2 and 3). ILVs secreted in this manner are referred to as exosomes, which are small (40–100 nm), unilamellar vesicles. The molecular mechanisms of exosome biogenesis and secretion are poorly understood. Exosome secretion reportedly depends on ESCRT function (although ESCRT-independent mechanisms may also exist) and is regulated in part by Rab GTPases [2, 39] (Box 1). Upon release in the bloodstream, lymphatic system or neural synapse, exosomes can be endocytosed by another cell. If exosomes undergo back-fusion with a late endosomal membrane, their cargo can deliver important signals to the cell on the status of surrounding cells [2, 39].

Although exosomes usually contain cytoplasm from the cell from which they originated, VSV and flaviviruses can deliver their genomes directly into endosomal ILVs [6, 7], as discussed in the previous sections. If such ILVs are secreted as exosomes and then enter the endocytic pathway of a different cell, the viral nucleocapsid inside the vesicles could, in principle, be delivered into the cytoplasm of a recipient cell permissive to infection; thereby providing a mechanism for intercellular transmission of viral infection. Indeed, just such a mechanism was recently reported for HCV. Exosomes isolated from HCV-infected human hepatoma cells were shown to contain full-length viral RNA, along with core and envelope proteins [13], and were capable of infecting naive cells [12]. Notably, exosomes containing HCV RNA were partially resistant to antibody neutralization, suggesting that HCV may utilize transmission via exosomes as an immune evasion mechanism [12]. Consistent with infectious exosomes being derived from endosomal ILVs, the ESCRT-0 component Hrs is critical for release of HCV nucleocapsid through exosomes [13]. Hrs depletion not only reduces exosome production, but also inhibits HCV replication and cell-to-cell transmission [13], suggesting that exosomes are a major route for HCV transmission.

It could be expected that any virus that delivers its genome into endosomal ILVs may also undergo cell-to-cell transmission via exosomes, similar to HCV. While flaviviruses and VSV have not yet been reported to form infectious exosomes, we can speculate that these viruses are indeed capable of exosome-mediated infection. In support of this notion, anthrax lethal toxin, which also utilizes ILV back-fusion to deliver its toxin subunit (lethal factor)

into the cytoplasm [11], was recently reported to act on uninfected cells via toxin-containing exosomes originating from endosomal ILVs [40]. These exosomes delivered lethal factor to the cytosol of naive cells without the lipid and pH requirements needed for normal anthrax toxin receptor-mediated cell entry. In addition, transport of the toxin in ILVs sheltered it from the extracellular milieu and the immune system [40]. We conclude that cell-to-cell transmission of microbial agents encapsulated in exosomes may be a general and important mechanism of pathogenesis that occurs to some extent with all viruses that deliver their nucleocapsids into endosomal ILVs.

Induction and evasion of immune responses by exosomes containing viral RNA

Immune cell activation of by nucleocapsid-containing exosomes

Soluble and membrane-bound cargo in exosomes derived from endosomal ILVs is thought to perform important functions in intercellular communication, although this has yet to be confirmed in an animal model. For example, when antigen-loaded dendritic cells (DCs) interact with cognate antigen-specific CD4⁺ T cells, the DCs transfer exosomes containing MHC II and CD9 to the interacting T cells, thereby inducing T-cell activation [41, 42]. Therefore, the question arises, can exosomes containing viral nucleocapsids be transferred to immune cells and activate them in a similar manner? An early indication that this might be possible came when cells infected with HCV were found to activate non-permissive DCs. This activation was dependent on cell-to-cell contact and high HCV-RNA levels in the infected cells [43]. A recent study confirmed that the DC cell activation by HCV-infected cells occurs through transfer of HCV RNA-containing exosomes during cell-to-cell contact. Furthermore, purified RNA-containing exosomes are sufficient to activate DCs [14]. Cell-to-cell transfer of these exosomes requires ESCRT components and annexin A2, an RNA-binding protein involved in vesicle trafficking [14]. Thus, the activation of non-permissive DCs by exosomal export of viral RNA from infected cells may proceed through a similar mechanism as the activation of T cells by transfer of MHC II-containing exosomes from antigen-loaded DCs.

Notably, the efficiency of antigen transfer by MHC II-containing exosomes is enhanced when the exosomes also contain a viral glycoprotein that binds to proteins or glycans on the surface of the T cell [44]. It remains to be determined whether similar receptor-binding effects promote immune recognition of exosomes containing viral components. For example, fusion of dengue virus with ILVs could generate exosomes bearing the dengue envelope glycoprotein, E, on their surface. Since E binds the DC-SIGN receptor on DCs [45, 46], one might expect particularly efficient transfer of dengue exosomes to DCs. However, since DCs can be permissive to dengue infection, in contrast to HCV, exosome transfer to DCs may promote dengue infection rather than fight it. Future studies are required to elucidate the possible roles of endocytic compartments in innate immune sensing of flavivirus infection.

Exosomes as reservoirs of virus latency

In contrast to the induction of immune responses by nucleocapsid-containing exosomes described above, there is also evidence that some viruses harness exosomes to avoid immune recognition in the bloodstream, or as reservoirs of virus latency. Hepatitis A virus (HAV), a non-enveloped virus, was found to be encapsulated into vesicles derived from endosomal membranes [47]. These enveloped HAV particles were fully infectious. They resembled exosomes, and their biogenesis was dependent on ESCRTs and ESCRT effectors [47]. Their membrane cloak protected them from antibody neutralization, and virus-specific antibodies appear only after 3–4 weeks of infection. Membrane hijacking by HAV blurs the classical distinction between enveloped and non-enveloped viruses and has broad implications for immune recognition and vaccine design [47].

In addition to the direct effects of exosomes on infection and immune recognition, the exocytic pathway can also contribute indirectly to viral pathogenesis. Certain enveloped viruses enter antigen-presenting cells (APCs) via the endocytic pathway without fusing with ILVs, and are subsequently secreted back to the cell surface via the same pathway as exosomes. For example, HIV particles endocytosed by DCs via the DC-SIGN receptor can be efficiently transferred through a pathway similar to exosome transfer to interacting T cells, which are subsequently infected [48]. Moreover, antibodies bound to HIV enhance endocytosis by DCs via Fc receptors on the cell surface, and certain neutralizing antibodies can be released in endosomes, restoring infectivity to the virus [49]. In cases such as these, the APCs serve as transport vehicles, or virus reservoirs, in the infected organism while providing shelter from immune recognition. This could account for the failure of certain vaccine candidates, particularly since immunization generates antibodies that may promote entry of the virus into the carrier APCs.

Concluding remarks

The recent advances reviewed above suggest that endosomal ILVs and exosomes perform critical functions in cell entry, cell-to-cell transmission and immune detection of many different viral pathogens. The discovery that these functions are conserved across unrelated virus families suggests that they are more common and generally important for viral pathogenesis than previously appreciated. This new perspective has potentially significant implications for the design of antiviral vaccines and therapeutics. First, compounds that interfere with ILV traffic may offer novel therapeutic strategies against viral infection. The biogenesis, sorting and transport of ILVs within the endocytic pathway depend on a large set of effectors and regulatory factors. Back-fusion of ILVs to the limiting membrane depends on BMP and PI(3)P lipids in the target limiting membrane. The ESCRT components Hrs and Alix are required for the regulation of BMP biosynthesis and for ILV formation [8, 9]. The trafficking of ILVs is also regulated by PI(3)P-dependent signaling via the PI(3)P-binding protein Snx16 [6]. Moreover, cell entry of flaviviruses amplifies PI(3)P kinase activity and the associated signaling response [7]. The transfer of HCV RNA-containing exosomes to DCs requires annexin A2, an RNA-binding protein involved in vesicle trafficking [14]. Future studies are needed to investigate whether compounds that interfere with ILV biogenesis and traffic may offer a therapeutic window (Box 2).

Box 2**Outstanding questions**

- Endosomes contain distinct types of intraluminal vesicles (ILVs) derived from different endosomal membranes with different lipid and protein compositions. Several questions arise regarding the diversity of ILVs in the endosomal pathway:
 - How is BMP incorporated into late endosomal ILVs?
 - What are the functional differences between the PI(3)P-rich and BMP-rich pools of ILVs?
 - Which specific types of ILVs do flaviviruses, hepatitis C virus (HCV), vesicular stomatitis virus (VSV), and other viruses that fuse with ILVs target?
 - Do ILVs undergo homo- or heterotypic fusion? If so, how does this type of fusion impact virus entry?
- Does HCV deliver its genomic RNA into early endosomal ILVs like flaviviruses? If so, do HCV RNA-containing exosomes originate from these ILVs?
- Do flaviviruses such as dengue virus generate exosomes containing viral nucleocapsid like HCV? Does VSV generate infectious exosomes?
- How is the signal for the secretion of exosomes containing viral nucleocapsids initiated? Are specific innate immune sensors required?
- Which cellular factors sort ILV traffic to either the plasma membrane (for secretion as exosomes) or to LEs (for back-fusion with the limiting LE membrane and delivery of ILV cargo into the cytoplasm)?
- How important are the roles of endosomal ILVs and exosomes in viral pathogenesis? Which other enveloped and non-enveloped viruses utilize ILVs or exosomes to promote infection or evade immune recognition?
- Can factors directing ILV traffic be successfully targeted by antiviral therapeutics?
- Can the exocytic pathway be successfully harnessed to develop vaccines that induce robust T-cell immunity?

The second antiviral strategy that is emerging stems from the discovery that exosomes containing viral components can induce T-cell responses. Exosomes can be transferred from DCs or other APCs to T cells via cell-to-cell contacts to induce robust T-cell responses. This effect can be enhanced when the exosomes contain viral glycoproteins that bind to T cells [44]. Moreover, purified exosomes containing viral RNA are sufficient to activate DCs [14]. Hence exosomes containing viral RNA and glycoproteins, such as those generated after fusion of an enveloped virus to an endosomal ILV, are excellent candidates for vaccine

antigens capable of inducing T-cell immunity (Box 2). Exosome vaccines are of particular importance in fighting viruses that have proven resistant to the more conventional strategies of antibody-mediated immunity, such as HIV and HCV. In summary, we propose that endosomal ILVs play an important role in viral pathogenesis, and that preventative vaccines or therapeutic compounds focused on ILV traffic hold promise as novel antiviral strategies.

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Highlights

Intra-endosomal vesicles are key effectors of viral infection and immune recognition

ILVs serve as vehicles to carry the viral genome to the nuclear periphery for replication

When secreted as exosomes, ILVs containing viral genomes can infect permissive cells

Secreted ILVs containing viral genomes can activate immune responses in myeloid cells

Figure 1.

The lipid composition of the limiting and internal membranes of compartments in the endocytic pathway. (i) Endocytic intraluminal vesicles (ILVs) bud inward from the plasma membrane, (ii) fuse with early endosomes (EE), which are transported along microtubules (MT), (iii), while maturing into late endosomes (LE). (iv) Late endosomes fuse with each other, and (v) with lysosomes to produce endolysosomes (EL). Cholesterol (Chol) decreases in concentration in the endocytic pathway, especially in limiting endosomal membranes, and is replaced by ceramide (Cer), which is also present in microdomains in the plasma membrane. Phosphatidylserine (PS) is present mainly in the cytoplasmic leaflets of the

plasma and EE membranes. Bis(monoacylglycero)phosphate (BMP) is specific to the internal membranes of late endosomal compartments. The distinct lipid compositions of different endosomal membranes direct intracellular trafficking and virus entry.

Figure 2.

Cell entry and egress pathways of enveloped viruses involving endosomal intraluminal vesicles (ILVs). ILVs are formed in early endosomes, which are then transported along microtubules to late endosomes with which they fuse. Some endosomes are recycled to the cell surface, releasing any ILVs that they contain into the extracellular milieu as “exosomes”. Various enveloped-RNA viruses deliver their nucleocapsids (yellow) into ILVs, which can either deliver the nucleocapsids into the perinuclear cytoplasm or transmit the nucleocapsids to another cell via exocytosis. ILV-dependent virus entry and egress depends on several membrane fission and fusion events: (i) budding and fission of an

endocytic vesicle from the plasma membrane followed by fusion of the vesicle with an early endosome; (ii) fusion of the viral and endosomal ILV membranes; (iii) budding and fission of an ILV from the early endosomal membrane and (iv) subsequent back-fusion of the ILV to the limiting late endosomal membrane; (v) fusion of an endosome to the plasma membrane to secrete ILVs as exosomes.

Figure 3.

Key membrane fusion events in the transport of viral components by endosomal intraluminal vesicles (ILVs). **(a)** Virus-ILV membrane fusion. The virus nucleocapsid (yellow) is delivered into the interior of the ILV. Viral glycoproteins are delivered into the ILV membrane, with the glycosylated ectodomains facing the endosome lumen. This fusion event is catalyzed by a conformational change in the viral envelope protein (dark blue). **(b)** Back-fusion of ILVs to the late endosomal limiting membrane. The fusion event delivers the ILV contents and viral nucleocapsid into the cytoplasm. Specific phospholipids and cellular effectors are required for back-fusion. **(c)** Exocytic fusion of the endosome to the plasma membrane. Any ILVs within the endosomal compartment are released into the extracellular milieu upon fusion. Exosomes released in this manner can infect adjacent or distant cells, or can be transmitted to a dendritic cell or T cell via an immunological synapse.