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Extracellular Vesicles in Viral Infections: Mechanisms, Diagnostics, and Therapeutic Perspectives for Pandemic Preparedness (SDG 3)

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Abstract

Extracellular vesicles, specifically exosomes, are released by virus-infected cells and are readily absorbed by other cells. Drugs based on cell-to-cell communication can reduce morbidity and mortality, supporting WHO's "One Health" approach. Consequently, addressing diseases like cardiovascular issues, pulmonary and renal complications, autoimmune syndromes, prion diseases, neurodegenerative conditions, COVID-19, osteoporosis, and cancers is essential for achieving the UN-SDG Agenda 2030. This review on exosomes and their function in viral infections focuses on their purification, patho-physiological pathways, genetic biomarkers, and immunological features.. This review outlines precision diagnostics, elimination strategies, and future research directions for viral eradication therapies. The biogenesis of exosomes and how they can inhibit virus replication are critical for advancing viral eradication strategies, particularly for HIV and SARS-CoV-2. This review highlights key clinical implications and emphasizes the need for continuous monitoring of host responses to enhance physician-led management and reduce global mortality.

Keywords: extracellular vesicles, exosomes, viral infections, mechanisms, markers, purification, precision diagnosis, eradication/elimination therapy.

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Introduction

Extracellular vesicles (EVs) are lipid-bound vesicles secreted by cells into the extracellular space [1]. Exosomes were first described in eukaryotic systems 50 years ago [2]. The discovery of exosomes was linked to epiphyses, a thin cartilage layer in mice, first described by H. Clarke Anderson and colleagues in 1969. Copious reports have addressed the term "exosome" in use as an alternative to "extracellular vesicles"[2]. Exosomes are secreted into the extracellular space and function as membrane-bound vesicles. Exosomes underline the basis for cellular, morphological and developmental changes corresponding to cancer cells, immune cells, epithelial cells, and virus-infected cells, as well as biological fluids, such as blood, urine, sperm, saliva, and cerebrospinal fluids [3]. According to the International Society for Extracellular Vesicles (ISEV), extracellular vesicles are non-replicative, lipid bilayer vesicles spontaneously released from various cells [4-5]. Cellular exosomes generate a diverse range of lipid-bound vesicles in the extracellular lumen, regulated by the plasma membrane or endosomes [6]. Based on their size, function, composition, and biogenesis processes, exosomes are subtypes of EV formed by an endosomal route and are typically 50–150 nm in size [7]. Microvesicles measure 100–1000 nm, and apoptotic bodies 500–4000 nm [8].

Originally believed to be cell waste, exosomes are released under normal and pathological conditions. In contrast to extracellular vesicles, which develop inside the lumen of multi-vesicular bodies, micro-vesicles are released from the plasma membrane by budding and shedding [9, 10]. These structures eventually facilitate cell-to-cell signaling, immune evasion, inflammation, and modulation of recipient cell behavior [11-12]. The

present review describes the latest updates on extracellular vesicles' main role in disease pathogenesis, spreading, and mitigation of several viruses. Precision diagnostics using biomarkers and immunological signatures can significantly improve detection and control of major viral infections. An outline perspective on some prominent virus-EVs relationships and the associated impacts are deciphered in this review. The systemic mechanisms in extracellular vesicles production, purification, genetic biomarkers, EV-biogenesis, and viral infectivity are addressed. This review provides a comprehensive overview of extracellular vesicles in viral infections, highlighting their roles in biogenesis, diagnostics, and therapeutic applications—particularly for HIV, SARS-CoV-2, and Epstein–Barr virus—within the broader context of pandemic preparedness and sustainable health (SDG 3).

Mechanism of Extracellular vesicles production

Extracellular vesicles (EVs) are minuscule membrane-bound vesicles which facilitate cell-to-cell communication through the transport of biomolecules from the cytoplasm of donor cells to recipient cells across extracellular environments [131]. Hence, the cellular counterparts comprise of endocytosis, cell outer surface, internal luminal endocytic compartment, or fusion with plasma membranes, respectively [13].

Exosomes vary significantly in their formation, release, transport, and absorption by cells. Current research primarily focuses on the biosynthesis mechanisms of exosomes derived from virus-infected host cells [14]. The uptake of exosomes by recipient cells depends on specific proteins and glycoproteins present on both the vesicle and target cell surfaces [15]. These vesicles facilitate the transfer of key macromolecules, including proteins, lipids, genetic material, mRNA, and miRNA, which vary based on the host cell origin [16-17].

Exosome internalization occurs through multiple mechanisms, including phagocytosis, macropinocytosis, lipid raft-mediated endocytosis, caveolin-dependent uptake, and clathrin-dependent or independent endocytosis [18-19]. Additionally, receptor-mediated endocytosis plays a crucial role in their uptake [20]. While exosome degradation and molecular interactions have been well-documented [21-22], their inherent pathways contribute to tumor-stroma interactions, endothelial permeability, metabolism, and pre-metastatic niche formation in cancer progression [23]. Furthermore, miRNA modulation influences the compatibility of recipient cells, affecting miRNA uptake and foreign DNA incorporation [24-25].

Extracellular vesicles are known as "small vesicles" in the domain of life of various organisms (prokaryotes, eukaryotes, and archaea), being surface-enclosed by lipid plasma membrane layers [26]. A key function of EVs is to protect their cargo proteins from degradation [27-28]. Exosomes originate from endosome-derived vesicles, also known as multivesicular bodies (MVBs). Genetic engineering and various biogenesis pathways, such as RAB (Ras-related proteins in the brain)-dependent mechanisms, play a crucial role in regulating exosome formation by controlling ubiquitination domain site blocking and release [29-30]. Additionally, living cells continuously secrete exosomes into the extracellular space, contributing to intercellular communication and various physiological processes [31-32]. For example, since they have similar buoyant densities and morphologies, exosomes from HIV- or HCV-infected cells cannot be easily differentiated or identified from infectious virus particles using standard biophysical methods [33].

Exosomes can form either spontaneously or in response to physiological and pathological processes such as cell death, hypoxia, neurological diseases, senescence, stress, and other major conditions. Their release varies depending on the biological context and the type of originating cell [34-35]. For exosome characterization, there are only very few standard methods for visualization, such as transmission electron microscopy (TEM), nanoparticle tracking analysis [36], asymmetric field-flow fractionation [37], ATR-FTIR [38] (attenuated total reflection FT-IR) and resistance pulse sensing [39]. A NanoSight nanoparticle tracking analysis system and immunoblot analysis of universal exosome protein markers (CD63, CD81, HSP69, and TSG101) was described by [40].

Genetically confirmed biomarkers for extracellular vesicles

Exosomes are confined to the extracellular space, interacting semi-fluidly with various organelles and modulating cellular responses. EVs interact with nearby and distant target cells. The also have a role as a biomarker in several diseases, such as metabolic syndrome, cancer, neurological, and genetic inheritance [41-42]. Due to their size, EVs move around freely and accumulate in various body fluids. Therefore, EVs serve as carriers of essential biomolecules, including proteins, lipids, and nucleic acids. Exosomes provide non-invasive liquid biomarkers with diagnostic and therapeutic functions [43]. Exosomes with immunomodulatory functions have been reported to be involved in the occurrence and progression of various autoimmune diseases [44].

DNA associated with EVs plays a role in both communicable and non-communicable diseases, including cancer and its prognosis [45]. The interaction of nucleosides between cells and extracellular fluids is involved in regulating cellular processes, contributing to tumorigenesis and metastasis [46].Exosomes carry several biomarkers linked to the ESCRT complex, including CD63, VAMP3, and ARF6 (ESCRT = Endosomal Sorting Complex Required for Transport, CD63 = Cluster of Differentiation 63, VAMP3 = Vesicle-Associated Membrane Protein 3, and ARF6 = ADP-Ribosylation Factor 6), highlighting their critical role in cellular communication and disease progression. Additionally, microvesicles have been found to contain over 52,000 proteins, along with 16,000 mRNAs, 12,500 miRNAs, and 350 lipids, based on extensive database screenings [47].

Purification of extracellular vesicles from viruses

EVs are lipid-bound vesicles released by host cells and contain cargo such as proteins, nucleic acids and genetic material (DNA and RNA), which are translocated to the recipient cells. Historically, different centrifugation and ultracentrifugation

techniques have been widely used for concentrating EVs from viruses [48-49]. Viruses enveloped in particles were increasingly used for therapeutic vaccines. To date, the purification of virus-like particles (VLPs) remains in its "embryonic" phase relying upon virus purification platforms. Depending on the particle density gradient, centrifugation, filtration, and various chromatographic approaches are applied [50].

The purification of EVs for release remains a challenge; several techniques have been tested and developed for virus separation and for the preparation of exosomes by fractionation through chromatography [51-52]. Examples are size exclusion chromatography, affinity chromatography, ultracentrifugation, and immune-affinity techniques, and also anion exchange chromatography. Clustering of EVs can lead to cell aggregation and interfere with recipient cell uptake [53]. Thus, nanoscale flow cytometry (nano-FACS) was suggested [54-55].

Nano-FACS is a powerful tool for separating viruses and EVs from contaminating substances. Other precipitation methods were reported; poly-ethylene glycol (PEG) has been used for many years to precipitate macromolecules such as proteins from viruses [56]. PEG is used as the main active compound in several commercial precipitation reagents for EVs. However, PEG precipitation is usually a more time-consuming method for exosomes precipitation and subsequent centrifugation [57]. In a recent example of flow filtration technology (AF4 technology), the separation of different exosome populations, such as Exo-S and Exo-L, could be achieved [58-59]. However, the selection of EVs separation technology strongly depend on their intended use. Thus, density, size and virus species specificity need to be robustly assessed for technical outcomes, often leading to a combination of techniques [60] (**Table 1**). Functional studies of EVs have demonstrated that infected viral host cell release does not infect virions (single virus particles outside cells). Virions have a similar size, density, and biochemical composition, as viral proteins are present both in EV and virions to different extents [71]. The separation of virus-like particles (VLP, which resemble viruses but are not infectious) can be handled by a combination of flow-through chromatography, micro- and ultra-filtration steps, and size exclusion chromatography [72, 73]. The approach is not suitable to separate different types of vesicles [74, 75].

EV isolation protocols involve concentration methods incorporating ultra-centrifugation, crowding reagent precipitation (PEG), cross-flow filtration, column chromatography, affinity purification and nano-FACS [76]. Flow-field-flow fractionation, electrophoresis, dielectrophoresis, filtration-based techniques, ion-exchange techniques, affinity-based techniques, and size exclusion chromatography are also enlisted for EVs isolation [77].

HIC (hydrophobic interaction chromatography) involving exosomes isolation with hydrophobicity profiles (exosome-spiked human plasma/urine, and cell lines involving PET (poly (ethylene terephthalate)) and C-CP (capillary- channeled polymer) are further purification methods [78]. Immunotherapy relying on exosomes has been proven as an effective strategy in combating various diseases (viral infections, auto-immune diseases, and cancers) emphasizing novel cell-free immune regeneration [79]. Thus, exosomes represent a promising avenue for biomedical research aimed at fighting viral infections with high mortality rates.

The biogenesis and molecular mechanisms of extracellular vesicles

A key question in EV biogenesis and molecular pathways revolves around the formation of late endosomes, particularly through the inward budding of the multi-vesicular body (MVB) membrane, which aligns with the "Trojan exosome" hypothesis [80]. Large MVBs generate intraluminal vesicles (ILVs) as a result of membrane invagination within late endosomes [81].

EVs, including exosomes, are enclosed within lipid bilayer membranes, formed through the outward shedding of microvesicles and the inward budding of the plasma membrane [82]. Endosomal compartments have been identified as central to exosome formation [83]. Additionally, some viruses acquire their envelope by utilizing the host cell membrane during capsid assembly.[84] reported that extracellular vesicles have been very closely linked to tumorigenesis and the spread of viruses and pathogenic agents such as enveloped viruses (HIV, dengue, coronaviruses, hepatitis A virus (HAV), norovirus, and rhinovirus), as well as being involved in the propagation of protein aggregate disarray [85]. EVs and retroviruses share the same cellular vesiculation machinery, which accounts for their remarkable similarities [85]. This resemblance is particularly evident in their high lipid composition and protein content, including glycosylphosphatidylinositol (GPI)-anchored proteins and cytosolic proteins, which are integral to their structure and function.

The Trojan exosome theory suggests that retroviruses exploit the exosome biogenesis pathway for virion production, allowing viral particles to be absorbed by recipient cells through a receptor-independent mechanism [86, 87]. This enables viruses to hijack the natural exosomal communication system, facilitating infection and immune evasion. In contrast to retroviruses, Pelchen-Matthews et al. [85] found minimal evidence that EVs actively modify target bioactive proteins, nucleic acids, lipids, DNA, and RNA. An essential part of viral delivery and pathogenicity is the extracellular vesicle-mediated transport of viruses from host cells. Thus, the expansion of the natural tropism of viruses to include target cells that lack canonical viral receptors adds up to the Trojan exosome theory [88]. EV-associated miRNAs and mRNAs were enriched with specific cell-sorting motifs [89].

The parallelism in the production of viruses and EVs is demonstrated by the functional transfer of exosomes linked to key molecules to target cells. According to Kumar et al. [14], recipient cells were impacted by the delivery of the EVs-associated virulence molecules since it made them more susceptible to viral infections. Thus, viral proteins can cause non-involved immune cells to die, leading to the loss of immune cells in the early phases of viral infections [90]. By inhibiting the forma-

tion of antibodies in white blood cells, viral surface receptors on EVs help evade the host's immune response and render immune cells vulnerable to viral infections (**Fig. 1**) [91-92]. Hence, EV biogenesis is corroborated for drug resistance and signal transduction cascades for future insights.

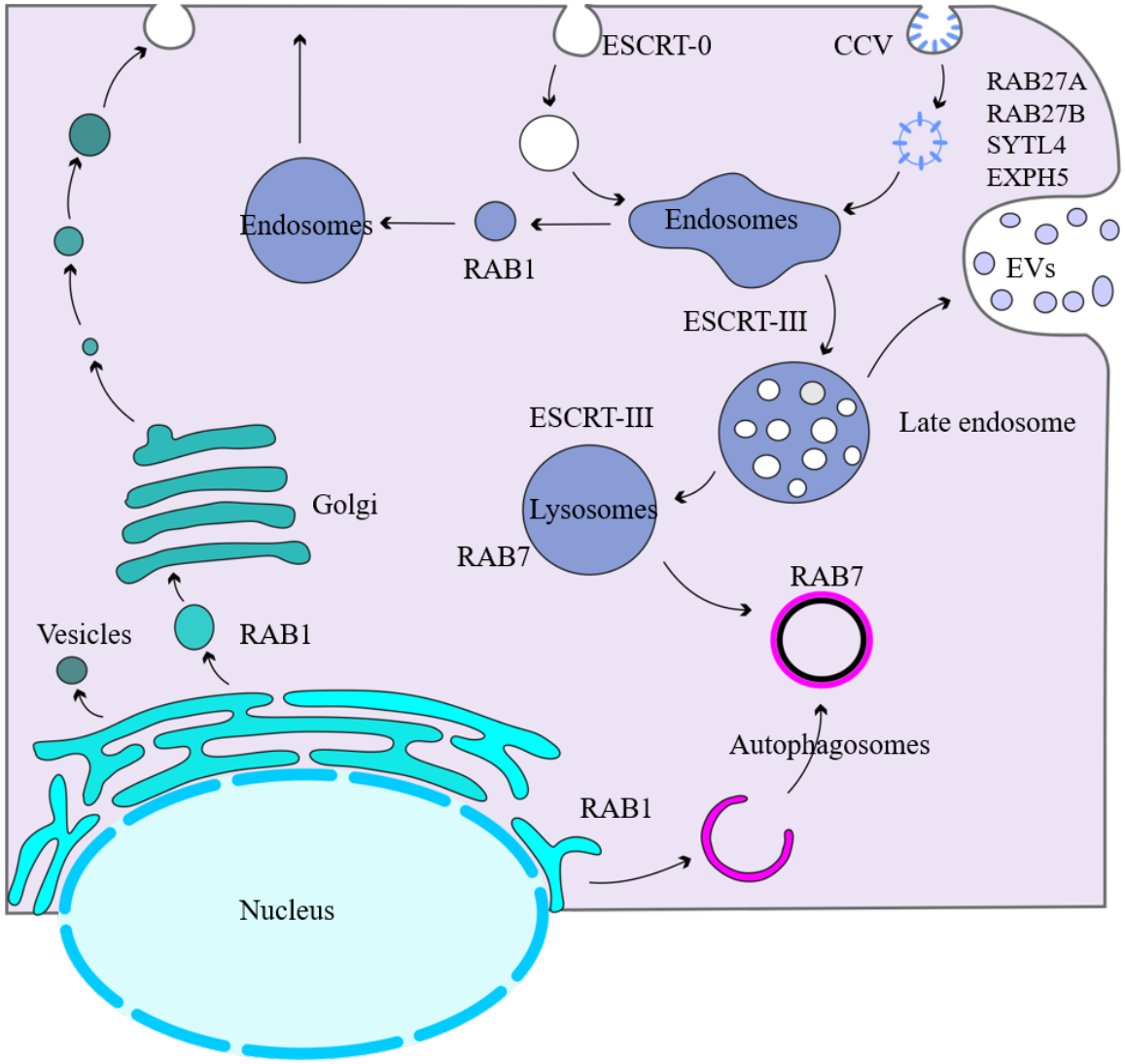

Figure 1. Modulation of the extracellular vesicle biogenesis pathway during a viral infection.

Extracellular vesicle from HIV and Epstein–Barr virus infection

Humans infected with HIV have been shown to release EVs [14]. However, the functional properties of these EVs have not been clearly linked to treatment outcomes, even though individuals with significant numbers and larger sizes of HIV-containing EVs exhibit viral suppression [93-94]. Additionally, a lower CD4 count is associated with an increased abundance of EVs, which has been found to negatively correlate with blood EV levels [14].

HIV-infected cells can incorporate viral envelope proteins into EVs, potentially influencing viral spread and immune responses [95]. In cell culture studies, EVs derived from HIV-infected cells have been observed to increase susceptibility to viral infections. Further, research indicates that HIV-infected U1 macrophages isolated from smokers exhibit elevated levels of cytochrome P450 enzymes (CYP1A1 and CYP1B1) and IL-6, whereas HIV-infected individuals have reduced levels of antioxidant enzymes (AOEs) such as superoxide dismutase (SOD-1) and catalase (CAT) [95-97].

Extracellular vesicles that contain viral proteins are released by B-cells infected with Epstein-Barr virus (EBV). The EBV, a gamma-herpes virus, is a major human pathogen identified in human tumor samples.

Notably, major EBV oncoproteins, including latent membrane protein 1 (LMP1), have been identified in EVs secreted from EBV-infected cell lines [98]. LMP1 is involved in the cellular transformation, contributing to EBV-driven oncogenesis. Also, they had been found in serum of mice with nasopharyn-

geal cancer, highlighting their possible influence on disease progression, but also their suitability as biomarkers for early disease discovery.

CD63 may contribute to the selective incorporation of LMP1 into EVs [99-100]. The lack of an immune response may result from the excessive secretion of EVs during Epstein-Barr virus (EBV) infection, indicating their role in viral immune evasion (**Fig.2**). This affirms novel immune mechanisms for associated viral pathophysiology comparing EBV, HIV and SARS-COV for a definitive common mechanism.

corona viruses are a family of encapsulated RNA viruses that infect people and domestic animals and cause illness [106-107]. Worldwide, coronavirus infections in domestic animals, such as porcine epidemic diarrhea virus (PEDV) in pigs and infectious bronchitis virus (IBV) in domestic hens, have led to significant economic losses in the livestock and poultry industries [108]. Up to one-third of adult cases of the common cold are caused by coronavirus infection, such as human coronavirus 229E (HCV-229E; HCV-OC43), which has historically been linked to moderate upper respiratory symptoms [109].

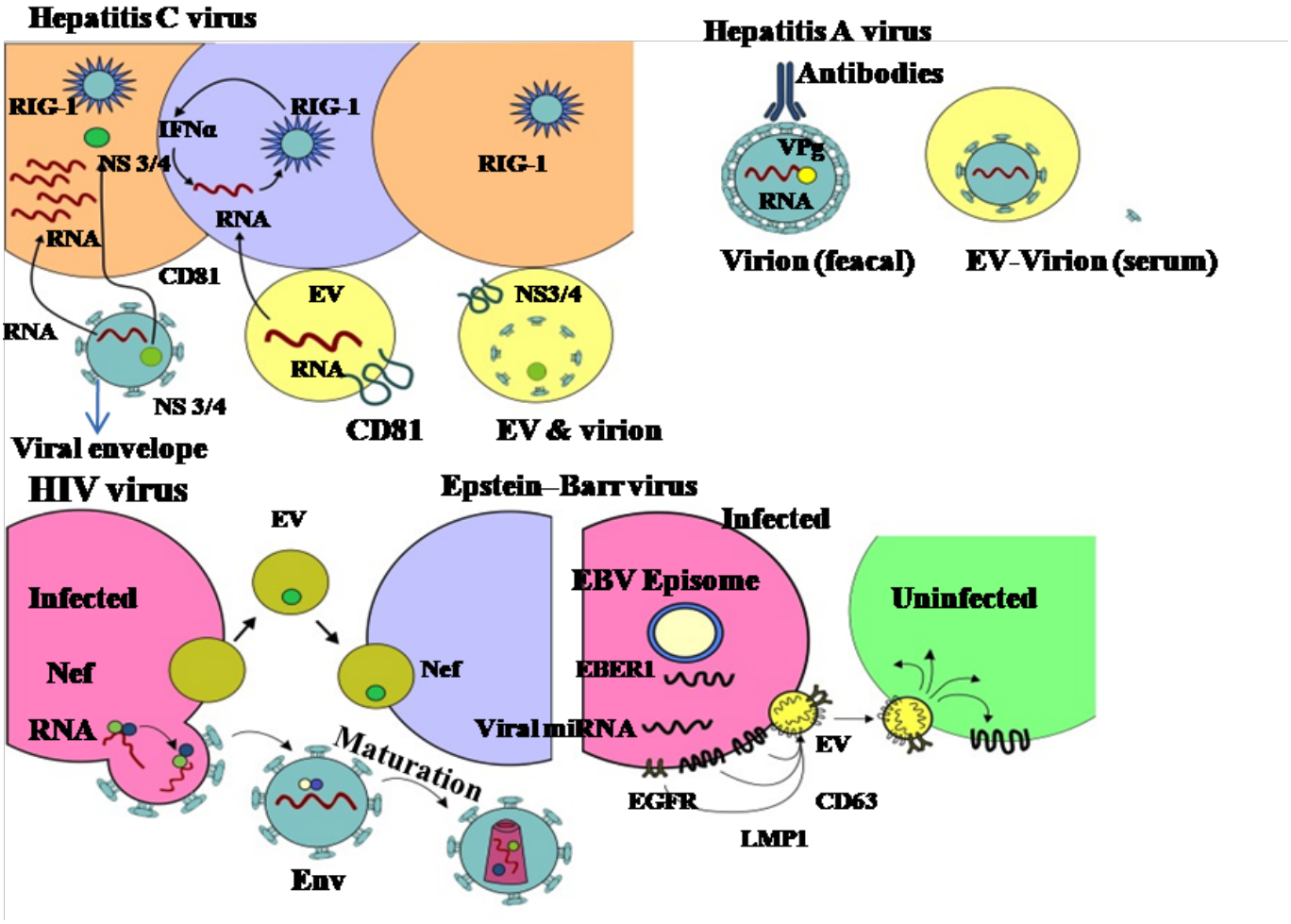

Figure 2. Interactions between viruses and EVs (for Human immunodeficiency virus-HIV and Epstein–Barr virus (EBV).

Extracellular vesicle and coronavirus infection

Exosomes in transmission and manifestation of SARS-CoV-2 are derogated for exosome-mediated therapeutics involving drug delivery mechanisms [101]. Furthermore, N-protein has been found in EVs isolated from coronavirus-infected individuals, suggesting the presence of coronavirus particles in EVs. Moreover, EVs regulate additional viral entry mechanisms [102]. The corona virus S-proteins may also be transported into exosomes using intracellular proteins and enhanced viral infection [103-104]. According to Cavanagh et al. [105], Envelope protein (E), nucleocapsid protein (N), spike protein (S), and membrane protein are among the structural proteins found in their viral genome [132]. Eventually, spike glycol protein is translated into precursor protein (S0) and non-covalent proteins like S1 and S2 [110].

The infection process of COVID is facilitated by a spike protein present on virions. Similar to the envelope proteins of HIV or the hemagglutinin of influenza viruses, coronavirus spike proteins belong to the class I fusion proteins [19]. Coronaviruses' distinctive surface spikes comprise S molecule trimers

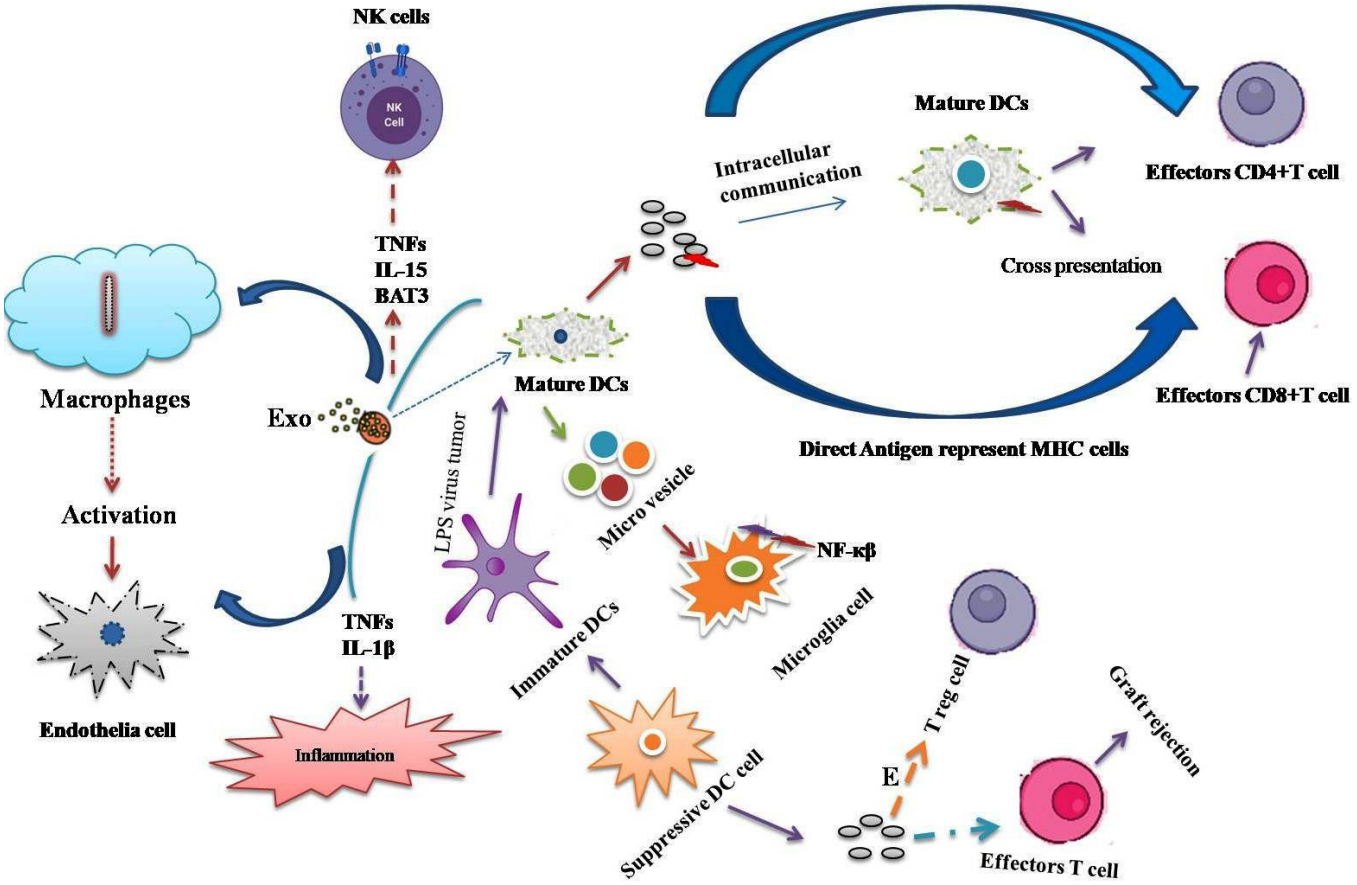

Figure 3. Schematic representation of Innate and Acquired immune responses regulated by DC-derived exosomes. Exosomes secreted by immature DCs can become immune suppressive, promote the activation of T-reg cells, and induce apoptosis of effectors T cells.

[111]. S is a class I viral fusion protein that is bound to host cell receptors and cell communication mediators [112]. S proteins induce cell-cell fusion late in infections [112]. A transmembrane protein weighing 128 and 165 kDa is encased in the S monomer structure by a very large N-terminal and a small C-terminal endodomain and ectodomain, respectively [113]. After being broken down, these proteins are carried as signal peptide molecules into the endoplasmic reticulum. The protein's 40 kDa is increased by N-linked glycosylation [114]. In beta-and gamma-coronaviruses, including infectious bronchitis virus (IBV), bovine coronavirus (BCoV), and mouse hepatitis virus (MHV), a furin-like host cell protease cleaves the S protein into two additional polypeptides, S1 and S2, which are of the same size [115]. The N and C terminals are identified as S1 and S2, respectively, and S protein cleavage happens downstream of a high basic Penta peptide motif and undetectable cleavage of mature S proteins [116]. However, some coronavirus strains display S1 and S2 cleavage late in the virion assembly and release process from host-infected cells [117]. Thus, the structural and biochemical data support the initial hypothesis that S is the influenza HA protein's function **Table 2.** Hence, we postulate the alternative hypothesis for EVs and SARS-CoV2 infections.

Function of extracellular vesicles in Immunological ways

The acquired immune response is now influenced by immune cell-derived EVs. In the mechanism of antigen production, Antigen presentation cells (APCs - macrophages or dendritic cells) release MHC-1 and MHC-II molecules that can activate native CD8 and CD4 T cells through co-stimulatory molecules on their surface [124-125]. As a result, EVs can transport foreign components; for example, *Mycobacterium tuberculosis*-infected macrophages release bacterial antigens [126]. The majority of the signaling for host cells that the surface-enveloped virus can enter is done by primary and secondary receptors and viral ligands; in contrast, HIV-1 initiates binding by using the CD_4 host cell surface receptor and other co-receptors, such as the CCR5 and CXCR4 chemokine receptors [127,128].

EVs containing double-stranded DNA (dsDNA) from virus-infected cells trigger an immune response (**Fig. 3**). Dendritic cells (DC)-derived EVs further activate innate immune pathways by inducing the release of tumor necrosis factor

Table 1. Comparison of Extracellular vesicle separation technologies

Extracellular vesicle separation chromatography techniques	Particle size (nm)	Markers	Advantage	Disadvantage	References
Anion exchange chromatography (IEX)	200 nm	CD63 and CD81	High recovery or high purity	Complicated and time-consuming.	[61]
Affinity chromatography	200 nm	CD9, CD63 and CD81	High purity of exosomes.	Separation of exosomes and target proteins.	[62]
Size exclusion chromatography	400 nm	Blood-based EV-associated biomarkers	Separation of proteins and exosomes.	Protein, antibody, and virus purification	[63]
Ultracentrifugation techniques	50-100 nm	CD63, TSG101, MHC class II	Gold standard, suitable for large-volume samples, cheap and mature.	Time-consuming, cumbersome operation, low yield may damage exosomes.	[64,65]
nanoFACS methods	200 nm	-	EV and viruses based on their fluorescence allows for the recovery of a functional product.	In certain flow cytometers calibrated for nanoFACS, the sample typically needs to be diluted.	[66]
PEG Precipitation	-	Expression of CD63, CD9 and CD81exosomal markers	Low purity, highest particle yield and miRNAs expression.	Precipitates larger macromolecules/EV contamination material.	[67]
Flow field filtration (AF4)	90-120 nm	Hsp40, G-proteins, JAK1 and TGFBRs	Fast, low cost; easy automation and Integration; high portability.	AF4 is highly reproducible, fast, simple, label-free, and has low sample capability.	[68]
Sucrose gradient centrifugation	100 nm	CD9 and CD81	Highest purity of the exosomes.	Centrifugation needs time; Overlapping densities between viruses and exosomes.	[69,70]

(TNF) cytokines, including FAS ligands and TNF-related apoptosis-inducing ligand (TRAIL) [129,130-131].

EVs contribute to immunological surveillance and host defense mechanisms. Additionally, mature DCs facilitate the circulation of exosomes that deliver peptides or antigens via endogenous MHC molecules, enhancing antigen presentation and adaptive immune responses.

Conclusion and future perspectives

Extracellular vesicles (EVs), particularly exosomes, play critical roles in the pathophysiology of viral infections by transporting viral components such as proteins, nucleic acids, and signaling molecules. Their involvement in processes like immune modulation, viral spread, and biomarker transport underscores their

Table 2. Extracellular vesicles in virus pathogenesis

Virus	Types	Specific Action	Action	References
HIV	Viral protein	Nef	HIV-infected cell-derived exosomes carrying negative regulatory factor (Nef), HIV-infected microglia, macrophages, and CNS cells, induce inflammation and secrete viral proteins.	[95]
	Pro-inflammatory markers	IL-6, TNF-β, IL-8	IL-8 serves as a biomarker for HIV patients with altered immune function due to alcohol and tobacco abuse	[96]
	miRNA	vmiR-88 and vmiR-99	Triggers endosomal toll-like receptor (TLR) 8 and nuclear factor-κB (NF-κB) signaling, stimulating the release of TNFα by delivering EV to bystander macrophages and may contribute to chronic immune activation inducing HIV replication.	[118]
Influenza virus	Protein	Epithelial mucins MUC1, MUC4, and MUC16	Human airway-derived exosome-like vesicles containing mucins neutralize human influenza virus infection.	[119]
	miRNA	miR-483-3p, hsa-miR-1975	Anti-viral and inflammatory response to influenza virus infection; suppresses influenza virus replication.	[120]
Corona virus	Viral Protein	spike S proteins (SARS-CoV)	Vaccine candidates for immunotherapy.	[121]
Human papilloma virus (HPV)	mRNAs	E6 and E7	Contribute to viral immune evasion and act in concert to promote tumor development through the interaction with multiple cellular proteins.	[122]
	miRNA	miR-9, -20b, and let-7b	Cancer-associated, cellular pathways are targeted by these miRNAs.	[123]
	Proinflammatory mediators	CCL2 and TNFα	Possesses anti-apoptotic properties and inflammatory immune mediators.	[20]

relevance in both the diagnosis and treatment of viral diseases, including HIV, Epstein–Barr virus, and SARS-CoV-2.

This review has highlighted key aspects of EV biogenesis, molecular mechanisms, diagnostic potential, and therapeutic strategies. Advances in separation and characterization techniques, such as nanoFACS and asymmetric flow field-flow fractionation, are accelerating the development of EV-based diagnostics and therapeutics. Moreover, the overlap between viral particles and EVs offers unique opportunities but also poses technical challenges in ensuring specificity and reproducibility. Moving forward, integrating EV research with clinical applications requires standardized isolation protocols, better biomarker validation, and interdisciplinary approaches combining molecular biology, nanotechnology, and computational methods. The potential of EVs in vaccine development, targeted drug delivery, and immunotherapy remains promising but demands

rigorous clinical validation.

In the context of global health and Sustainable Development Goal 3 (Good Health and Well-being), EV-based strategies offer a non-invasive, scalable, and innovative platform for addressing current and future pandemics. Continued research into EV biology and its translational potential will be instrumental in advancing precision medicine and strengthening pandemic preparedness.

Author contributions

Conceptualization, Writing—original draft preparation, Visualization and Supervision: **R.K.G.** Writing—review, Editing, Visualization, Validation, Software: **R.K.G**, **M.F.R**, **M.L, R.M.Z, M.A.K, M.Q.W, M.T, B.K.K,** and **R.M.D**.; Project Administration: **R.K.G., M.L;** All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that they have no known or perceived competing financial interests, nor personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request. As this is a review article, no novel data were generated.

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