



Exosomes in viral infection: Effects for pathogenesis and treatment strategies

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Abstract: Exosomes are small vesicles that carry molecules from one cell to another. They have many features that make them interesting for research, such as their stability, low immunogenicity, size of the nanoscale, toxicity, and selective delivery. Exosomes can also interact with viruses in diverse ways. Emerging research highlights the significant role of exosomes in viral infections, particularly in the context of diseases like COVID-19, HIV, HBV and HCV. Understanding the intricate interplay between exosomes and the human immune system holds great promise for the development of effective antiviral therapies. An important aspect is gaining clarity on how exosomes influence the immune system and enhance viral infectivity through their inherent characteristics. By leveraging the innate properties of exosomes, viruses exploit the machinery involved in exosome biogenesis to set replication, facilitate the spread of infection, and eliminate immune responses. They can either help or hinder viral infection by modulating the immune system. This review summarizes the recent findings on how exosomes mediate viral infection and how they can be used for diagnosis or therapy. This could lead to new clinical applications of exosomes in disease management.

Introduction

Exosomes are tiny sacs with two layers of membrane that measure 100 nm across, but can range from 30 to 150 nm (Sheykhhasan *et al.*, 2022; Sheykhhasan *et al.*, 2021b; Khoei *et al.*, 2020). They can be found in many kinds of body fluids, such as blood, saliva, urine, bile, cerebrospinal fluid, semen, breast milk, pleural effusion, and amniotic fluid (Zhou *et al.*, 2021; Yu *et al.*, 2022). Exosomes carry over 1600 different proteins that have various functions in living cells, such as regulating metabolism and maintaining

structure (Kalluri and LeBleu, 2020). Exosomes are involved in intercellular communication via transferring molecules between cells (Fayazi *et al.*, 2021). They also have the great potential to serve as drug delivery vehicles, due to their high stability in circulation, low immunogenicity/toxicity, high penetration ability to blood brain barrier and selective targeting of cell types (Fig. 1) (Murphy *et al.*, 2019; Sil *et al.*, 2020).

Exosomes have different functions depending on where they come from. For example, exosomes from tumor cells help them to spread and invade other tissues (Deb *et al.*, 2021). Some cells, such as those that are infected by viruses, can produce exosomes that have different proteins on their surface or inside them. These proteins are derived from the virus that infects the cell. The exosomes can then carry these viral proteins to other cells and infect them with the same virus. This is one way that exosomes can mediate viral

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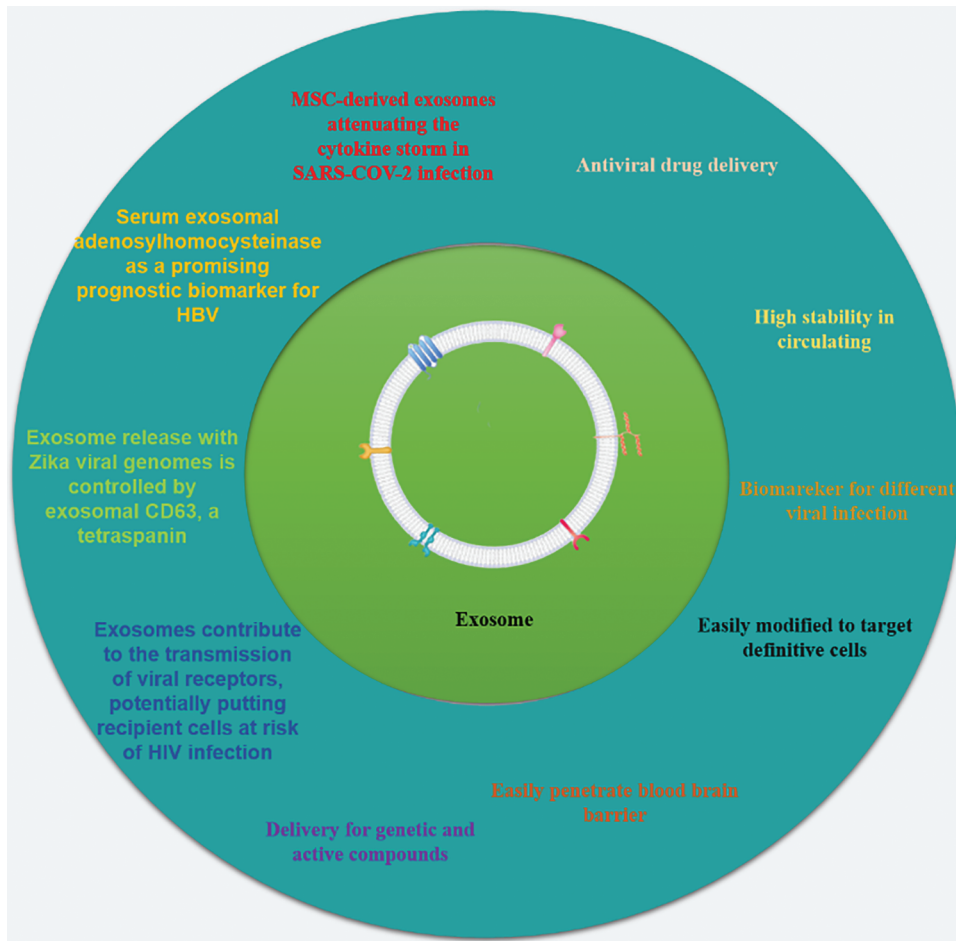


FIGURE 1. The potential exosomal properties and their use in various viral treatments. The advantageous features of exosomes, including their ability to efficiently transport genetic material, proteins, and medications are summarized. Exosomes also possess the ability to target specific cells, exhibit biocompatibility and non-immunogenic properties, and can be readily absorbed by cells. The application of exosomes in treating various viral infections, such as HBV, HIV, and SARS-CoV-2 are showcased. The diagram was adapted from Saad *et al.* (2021) with some modifications and updates.

infection (Peng *et al.*, 2023). Exosomes and viruses have many things in common, such as how they are made and what they carry. Exosomes that are linked to viruses can also affect the immune system in different ways. They can either help the virus to escape the immune system or activate the immune

system to fight the virus. This means that exosomes can regulate the immune response in both directions (Peng *et al.*, 2023) (Fig. 2). The aim of this review is to summarize the multifunctional role of exosomes in the infection of SARS-CoV-2, hepatitis, and nervous system viruses.

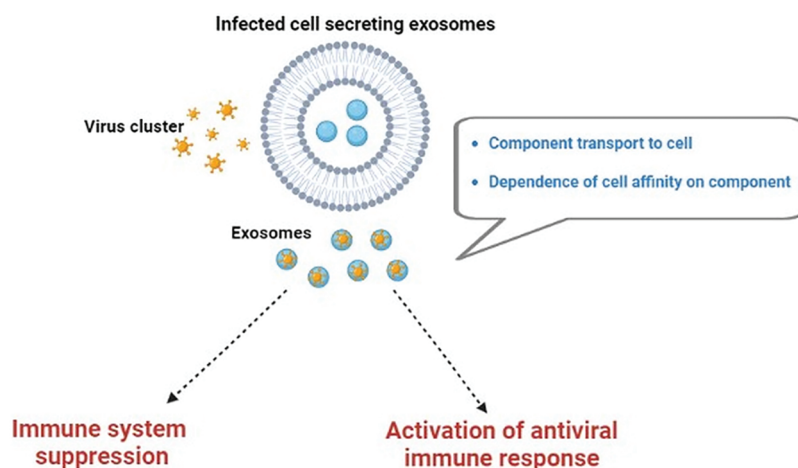


FIGURE 2. The dual function of exosomes in viral infection. Viruses employ the exosome biogenesis machinery to start replication, propagate infection, and circumvent the immune system. However, exosomes also take part in defense measures by activating the innate immune system.

Search Strategy

The PubMed and Medline databases were systematically searched. The inclusion criteria were studies that were published up to April 2023. The Medical Subject Headings (MeSH) terms “exosome” and “viral infection” were utilized. The phrase “exosome” or “COVID-19” or “Hepatitis virus” or “Zika” or “HIV” was also used.

Similarities between viral infection and exosomes

Viruses can use exosomes to make themselves more infectious. For example, exosomes can carry transcription factors, which are proteins that help viruses make more copies of themselves (Subra *et al.*, 2007).

Exosomes and viral infection can deliver their molecules, such as proteins, RNA, or DNA, to other cells and alter their gene expression and function. Their molecules have a key role for attaching to the cell membrane (for example, tetraspanins are a family of proteins that are involved in cell adhesion and signaling) (Andreu and Yáñez-Mó, 2014). In addition, the cell affinity to bind viral protein are mostly determined by its secreted exosome. Furthermore, the affinity of the cell to bind viral protein is largely influenced by the type and amount of exosome that it secretes. However, this phenomenon has only been investigated *in vitro* studies experimentally (Urbanelli *et al.*, 2019) and it could be an interesting topic for future research. Therefore, there is a bidirectional interaction between viruses and exosomes in the human body that could affect the occurrence or development of various virus-related diseases (Fig. 2).

The COVID-19 definition, diagnosis, and immunopathology

The initial symptoms of SARS-CoV-2 infection are often mild and resemble those of other respiratory virus diseases, such as dry cough, tiredness, sore throat, anorexia, high fever, myalgia, and nasal congestion (Ashour *et al.*, 2020; Krajewska *et al.*, 2020; Mohammadi *et al.*, 2022; Sheykhhasan *et al.*, 2020). However, these symptoms may worsen over time and cause respiratory distress that requires hospitalization (Al Balushi *et al.*, 2020). It has been estimated that about 80% of symptomatic patients do not need medical assistance or hospitalization, but the mortality rate among patients admitted to the ICU varies from 39% to 72% (Verity *et al.*, 2020). Furthermore, the COVID-19 virus particles can impair the function of sensory receptor cells (blocking olfaction) by binding to the angiotensin-converting enzyme 2 or ACE2 receptors, which are highly expressed in the olfactory epithelial cells and oral mucosa (Fotuhi *et al.*, 2020).

As expected, old adults with COVID-19 infection tend to have higher levels of production of cytokines, including IL-2, IL-6, IL-7, IL-10, IP-10, and TNF α , than young or healthy adults (Palanques-Pastor *et al.*, 2020; Zhong *et al.*, 2020). Furthermore, excessive levels of pro-inflammatory cytokines can cause higher blood viscosity and fatal thromboembolism in some elderly adults with COVID-19 infection (Soy *et al.*, 2020). In these regards, multiple organ dysfunction may result from hyper-activation of pro-inflammatory markers and vascular damage in the heart, brain, kidney, and liver. Besides, the COVID-19-infected patients experience

neurological symptoms such as anosmia or stroke (Ahmad and Rathore, 2020).

Several molecular tests have been approved for *in vitro* diagnostics of COVID-19, such as RT-PCR (Allan *et al.*, 2020; Wang *et al.*, 2020), Next-Generation Sequencing (NGS) (Chen *et al.*, 2020), and loop-mediated isothermal amplification (LAMP) (Jiang *et al.*, 2020). RT-PCR can detect high levels of SARS-CoV-2 viral RNA in patients with asymptomatic and pre-symptomatic SARS-CoV-2 infections (Nile *et al.*, 2020).

SARS-CoV-2 antibodies that recognize prior infection and immunity are useful for ongoing surveillance, epidemiologic/vaccine studies, and estimating the risk of health care workers (Cheng *et al.*, 2020; Huang *et al.*, 2020; Krammer and Simon, 2020). Specifically, SARS-CoV-2 IgG appears \sim 10 days post-infection and peaks at day 49, while IgM antibodies appear \sim 7 days after symptom onset and peak at day 28 (Huang *et al.*, 2020).

The mechanisms and functional roles of exosomes in the COVID-19 pandemic

It is known that the immune system is a natural defense mechanism that fights against invasive microbial infections. Exosomes mediate various types of biological processes during micro-organism infections and stimulate immune responses (Chitra and Harikumar, 2018). Briefly, immunosomes are formed by the budding of sub-cellular endosomal membranes inward from immune effector cells (Wu *et al.*, 2019) and delivered extracellularly to various cell types, such as T and B cells, dendritic cells (DCs), platelets, NK cells, mast cells, and epithelial cells (Admyre *et al.*, 2007). Recent studies suggested that exosomes can be a promising specimen for the detection of COVID-19. SARS-CoV-2-infected cells can produce exosomes with more virus particles that these exosomes that transport viral and self-antigens, triggering the host immune response (Wan *et al.*, 2020). It was proved that exosomes are important not only for sorting ACE2, but also for sorting proteins, miRNAs, and other cargo that can be transmitted to healthy cells like other viruses (Gunasekaran *et al.*, 2017). Exosomes also alter the cargo of proteins involved in the pro-inflammatory, coagulopathy, and endothelial damage. Furthermore, it was reported that some exosomes can transfer ACE2 to receipt cells and facilitate the SARS-CoV2 internalization and infection (Wang *et al.*, 2020), suggesting a link between exosomes and COVID-19 diagnosis (Hassanpour *et al.*, 2020). The treatment of COVID-19 infection with antibody-mediated exosomes involves targeting the spike protein with monoclonal antibodies and internalizing them into the endosomes, then fusing the membranes to transfer genetic materials and prevent viral replication (Salvatori *et al.*, 2020; Lee *et al.*, 2021). It has been shown that exosomes and other extracellular vesicles (EVs) are released by infected cells and can adjust the immune response (Gurunathan *et al.*, 2021). Barberis *et al.*, 2021 found that SARS-CoV-2 infection changes exosome content and affects their role in disease progression and their potential application as biomarkers of disease severity (Barberis *et al.*, 2021). For example, Fujita *et al.* (2021) found three potential early COVID-19 biomarkers and found that COPB2 extracellular vesicle had

the optimal predictive value for the severe deterioration of COVID-19 patients (Fujita *et al.*, 2021). It has been demonstrated that exosomes produced by SARS-CoV-2-infected cells contain specific proteins, such as fibrinogen, complement C1r subcomponent, and serum amyloid P-component, that act as important disease biomarkers (Barberis *et al.*, 2021). Moreover, exosomes from virus-infected cells can release cytokines and chemokines from human monocytes and/or airway epithelial cells, which then trigger an innate immune response (Chahar *et al.*, 2018).

Exosomes can also enhance antiviral responses in infected cells by various mechanisms (Rezabakhsh *et al.*, 2022). For example, the secretion and synthesis of interferons can be increased by the transfer of interferon-stimulated genes (ISGs) through exosomes (Yao *et al.*, 2018). Studies indicate that MSC-Exo contains high amounts of anti-inflammatory cytokines such as IL-10 and TGF- β (Rezabakhsh *et al.*, 2022). MSC-Exo can produce specific growth factors, including vascular endothelial growth factor and basic fibroblast growth factor, that can restore the function of the alveolar-blood barrier and limit the infiltration of immune cells into the infected areas (Rezabakhsh *et al.*, 2022). Exosome has cytoprotective properties against viral infection and high levels of antibacterial components, according to various studies. Exosome may have antibacterial effects by inhibiting bacterial growth and promoting phagocytosis in local immune cells (Russell *et al.*, 2020). It appears that intranasal administration of exosome at the early stages of COVID-19 may reduce the replication and dissemination of certain SARS-CoV-2 strains into lower regions (Barberis *et al.*, 2021). In fact, intranasal administration of exosomes is a more effective route for treating pulmonary disease and neurodegenerative disorders, as this way delivers more exosomes to the target site, while intravenous injection reduces the number of exosomes significantly within a few hours (Moss *et al.*, 2021). However, intravenous administration of purified exosomes may reduce the need for invasive mechanical ventilation for respiratory failure in those patients with severe COVID-19 (Tsuchiya *et al.*, 2020).

In an earlier study, exosomes derived from BM-MSCs were safe in improving pneumonia parameters, attenuating the cytokine storm, and restoring COVID-19 patients' immunity (Sengupta *et al.*, 2020). However, recent reports suggested further clinical research on exosomes derived from MSCs and ADSCs for the treatment of COVID-19 patients (Rogers *et al.*, 2020; Mahida *et al.*, 2020; Worthington and Hagoood, 2020).

Hepatitis Virus Infection

Hepatitis is a type of viral infection that induces inflammation of the liver and can cause serious health complications and death. The infection caused by the hepatitis virus continues to be major public health challenge globally, resulting in a high number of deaths worldwide (approximately 2 billion peoples) (Matičić *et al.*, 2020; Razavi-Shearer *et al.*, 2018). According to the World Health Organization, hepatitis B and C are the most common types of viral hepatitis. It is estimated that over 350 million people worldwide have

contracted the hepatitis B virus (HBV) annually (Pungpapong *et al.*, 2007). Chronic hepatitis B (CHB) is a lifelong infection caused by the HBV, which can damage the liver and increase the risk of liver cancer. CHB can be transmitted through even unaware contact with blood, semen, and/or other body fluids from an infected person. The prevalence of hepatitis B infection varies widely across different regions of the world, depending on the modes of transmission. The World Health Organization classifies regions as high, intermediate, or low prevalence based on the percentage of people with chronic hepatitis B infection. Those high prevalence regions (>8%) include most of sub-Saharan Africa and some parts of Asia. Intermediate prevalence regions (2%–7%) include North Africa, Middle East, Europe, Latin America, and South Asia. Low prevalence regions (<2%) include North America, Australia, New Zealand, and Japan (<https://www.who.int/westernpacific/health-topics/hepatitis/regional-hepatitis-data>) (Razavi-Shearer *et al.*, 2018).

A comprehension of the existing diagnostic tests for HBV is essential to effectively screen, diagnose, and provide treatment to patients with HBV. The condition of viral pathogenesis is mainly caused by alterations in the growth factors and cytokines that regulate the extracellular matrix (ECM). These changes enhance the survival of the ECM and prevent its degradation, leading to liver damage over time (Devhare *et al.*, 2017). This work underlines the role of the miR-222/transferrin receptor axis in liver fibrosis and offers fresh perspectives on therapeutic treatment choices. Hepatitis C virus (HCV) is a member of hepatitis virus family that affects around 130–170 million people worldwide. HCV can lead to serious health problems such as cirrhosis and hepatocellular carcinoma (HCC) (Kim *et al.*, 2016). Recent studies have shown that HCV can spread by mechanisms that bypass the receptors. Therefore, the receptor-mediated therapeutic strategies (antibodies) have become less effective (Yin *et al.*, 2022).

Exosome as carrier for hepatitis virus

Exosomes are small vesicles that can carry substances from infected cells to uninfected cells and facilitate the transmission of HBV. Most liver cells, including hepatocytes and Kupffer cells, have the ability to release or be targeted by exosomes (Jiao *et al.*, 2021).

Ramakrishnaiah *et al.* (2013) were the first to demonstrate in 2013 that exosomes from HCV-infected hepatoma cells could contain viral particles (Ramakrishnaiah *et al.*, 2013). On this subject, exosome markers (such as the tetraspanin CD63) have a crucial role in the formation and distribution of HBV particles (Ninomiya *et al.*, 2021). In fact, exosomes act as a protective shield for the viral components to evade the immune system (Aydin *et al.*, 2021). In this respect, exosomes can shield HBV particles from being destroyed. This, in turn, facilitates the infection/dysfunction of hepatocytes and immune cells such as NK cells (Yang *et al.*, 2017). One of the mechanisms for weakening the body's ability to fight viruses is that non-coding RNA (e.g., miR-155, miR-221, and miR-192) present in HBV-infected exosomes can directly target the human IL-21 gene and subsequently impair the function of T cells,

which are essential for antiviral immunity (Enomoto *et al.*, 2017). Wu *et al.* (2023) demonstrated the presence of a significant mechanism involving the hepatitis B virus (surface antigen) and Na⁺-taurocholate (co-transporting polypeptide) on the surface of exosomes derived from HBV-expressing cells (Wu *et al.*, 2023). Exosome-encapsulated HBV was able to initiate infection in susceptible cells, demonstrating its capability for transmission. However, non-permissive cells exhibited a significantly lower efficiency in absorbing exosomal HBV (Wu *et al.*, 2023). In a study conducted by Zhang *et al.* (2023), it was discovered that liver fibrosis is exacerbated by exosomal miR-222 derived from HBV-infected hepatocytes, which occurs through the blockade of the transferrin receptor and the induction of ferroptosis (Zhang *et al.*, 2023). Recent findings reveal that exosomes derived from serum infected with HBV have been found to diminish NK cell function. These exosomes adversely affect various aspects of NK cell activity, such as interferon (IFN) production, lytic function, proliferation, and viability. Moreover, they also impede cellular responses to poly (I:C) stimulation (Yang *et al.*, 2017). In HBV transgenic mice, exosomes produced by HBV hindered the immune response and expedited the progression of chronic hepatitis B (CHB). The presence of HBV-derived exosomes resulted in the inhibition of HBV-replicating cell elimination in HBV-infected mice, thereby exacerbating the course of the disease. These findings indicate that HBV infection leads to alterations in exosomal contents, which have a significant impact on disease progression (Liu *et al.*, 2023). In recent years, there have been significant advancements in the discovery of diagnostic and prognostic biomarkers for various types of viral infection (Bodaghi *et al.*, 2023; Tribolet *et al.*, 2020; Hasham *et al.*, 2020; Kramvis *et al.*, 2022). Notably, microRNAs, lnc RNAs, and crRNAs in exosomes have emerged as promising biomarkers. A recent study explored the possibility of serum exosomal adenosylhomocysteinase as a novel predictive biomarker for (HBV-induced) liver cirrhosis (Tong *et al.*, 2021a), shedding light on the importance of identifying and utilizing new biomarkers in the diagnosis and prognosis of diseases. In individuals with chronic hepatitis B who have not undergone prior treatment, there exists a favorable correlation between serum exosome HBV-miR-3 and markers such as HBV DNA, pgRNA, and notably, hepatitis B e antigen (Gan *et al.*, 2022). Moreover, the utilization of exosomal HBV-DNA has been identified as beneficial for individuals exhibiting a high level of suspicion for HBV infection, despite yielding negative results in serum HBV-DNA tests (Xu *et al.*, 2023). The serum exosomal AHCY level has been identified as a potential unique predictive biomarker in patients with HBV-LC. Its presence holds significant implications for the prognosis of HBV-LC patients (Tong *et al.*, 2021a). Furthermore, research findings have indicated the potential of exosomes in treating HBV infection by inhibiting HBV transcription and replication (Liu *et al.*, 2023).

HCV infection stimulates increased production of miR-155 in liver tissues, leading to abnormal growth of liver cells and the formation of tumors (Zhang *et al.*, 2012).

According to recent findings, it has been observed that RNA present in exosomes infected with HCV has the ability

to stimulate immune cells, particularly monocytes, to increase the production of Galactin-9 (gal-9). Gal-9 is a molecule that binds to T cells and facilitates the persistence of HCV infection within the body (Mengshol *et al.*, 2010). The above discovery holds great potential in the early diagnosis and treatment of HCV infection. Adenosylhomocysteinase, a crucial enzyme in transmethylation reactions and a component of serum exosomal, emerges as a promising prognostic biomarker for liver cirrhosis induced by HBV (Tong *et al.*, 2021b). In addition, it has been observed that individuals with chronic HCV exhibit elevated levels of CD81 protein in their serum exosomes. This finding suggests a potential link between CD81 protein, inflammation, and the severity of fibrosis (Welker *et al.*, 2012).

Interferon- α (IFN- α) holds significant importance in HBV treatment strategies as it possesses inhibitory potential against the hepatitis B surface antigen. As a result, it is widely considered a valuable candidate for HBV therapy. Furthermore, the utilization of exosomes loaded with IFN- α has emerged as a promising approach to sustain the drug's efficacy. These exosomes facilitate the delivery of IFN- α from healthy liver cells to the infected ones, aiding in the continuity of the treatment process (Wu *et al.*, 2021). Exosomes have demonstrated effective capabilities in antigen presentation for recombinant vaccines. A notable example is the utilization of exosomes derived from CD4⁺ T cells, which have shown the ability to enhance B cell activity and improve the effectiveness of the HBsAg vaccine (Lu *et al.*, 2019; Jesus *et al.*, 2018). Pradhan *et al.* (2022) discovered a significant increase in miR-375 levels within exosomes isolated from individuals with cirrhosis and hepatocellular carcinoma (HCC). Further evidence supporting the involvement of miR-375 in HCV pathogenesis comes from observations of inhibited cell migration and proliferation in HCV-infected cells when miR-375 is depleted. Additionally, exosomes derived from HCV-infected cells have the ability to release miR-375, which can then be transferred to uninfected Huh7.5 cells, resulting in enhanced proliferation and migration capabilities (Pradhan *et al.*, 2022).

Viral infection of nervous system and exosomes involvement

Moreover, the weakened immune system in immunosuppressed people may make it easier for viruses to infect different parts of the body, such as the nervous system (Kennedy, 2021). Of this, Zika virus (ZIKV) was first identified as a yellow fever virus in the Zika Forest of Uganda. In 2015, ZIKV reemerged as a potential cause of severe developmental problems in the central nervous system (CNS) (Dick *et al.*, 1952). ZIKV can impair the formation of new neurons in the CNS. For example, in an experimental model, Zika virus caused neuronal death and small brain size by activating a stress-induced response to misfolded proteins (Gladwyn-Ng *et al.*, 2018). Especially, it is essential to monitor pregnant women who were exposed to ZIKV to detect any possible neurological problems early (Marbán-Castro *et al.*, 2021). On the other hand, ZIKV infection was found to cause autonomic neuropathy and meningoencephalitis complications in the adult nervous system (Acosta-Ampudia *et al.*, 2018).

Exosomes can pass through the BBB, which means they can carry viral signals to the brain. This is important for viral

infection of the nervous system. By studying exosomes from infected cells, researchers found that exosomes are associated with non-structural protein 1 (NS1), which is a viral toxin (El Safadi *et al.*, 2022). Moreover, ZIKV infections can affect the miRNAs in exosomes from host neural cells (such as miR-4792), which are involved in biological processes like oxidative stress (Tabari *et al.*, 2020). Neutral Sphingomyelinase SMPD3 is a crucial molecule for exosome production or release in neural cells. Zhou *et al.* (2019) reported that ZIKV can change the activity of this molecule, which may help it to spread through exosomes and cause severe neuronal damage (Zhou *et al.*, 2019). Zika virus-infected cells secrete exosomes with specific viral protein profiles, and more exosomes are released during ZIKV infection than in noninfected cells. Also, the exosome release with Zika viral genomes is controlled by CD63, a tetraspanin that is abundant in exosomes (York *et al.*, 2021).

Exosome in HIV

Among viruses, the most widely recognized retrovirus is HIV-1 (Lu *et al.*, 2011; Poondla *et al.*, 2023; Sheykhhasan *et al.*, 2021a). Exosomes play a role in facilitating the transmission of HIV-related proteins to target cells, thereby accelerating the spread of infection by rendering recipient cells more susceptible to HIV (Arenaccio *et al.*, 2015). Specifically, the protein Nef is transmitted via exosomes, which subsequently activate cells and heighten their vulnerability to HIV infection upon reaching latent cells (Arenaccio *et al.*, 2015). Furthermore, Nef possesses the capacity to inhibit the production of CD4⁺ exosomes by T cells, thereby diminishing the immune cells' capability to identify viruses (Lu *et al.*, 2019). Previous studies have demonstrated that the presence of Nef in exosomes leads to the apoptosis or senescence of CD4⁺ T cells (Lenassi *et al.*, 2010; Arenaccio *et al.*, 2014). Based on Schaefer *et al.* (2008), it has been observed that macrophages release exosomes containing Nef, which hinder T cell function by breaking down MHC-I and CD4⁺ molecules through a beta-COP-dependent mechanism (Schaefer *et al.*, 2008). Furthermore, it has been observed that Nef⁺ exosomes possess the capability to hinder the adaptive immune response by decreasing the production of IgA and IgG in B cells. This evasion mechanism employed by the virus allows it to evade the humoral immune response, as documented (Xu *et al.*, 2009). In addition, Dubrovsky *et al.* (2022) conducted research revealing that when human monocyte-derived macrophages, generated from monocytes pre-treated with extracellular vesicles containing the HIV-1 protein Nef (exNef), were stimulated by lipopolysaccharide, they exhibited an increased release of pro-inflammatory cytokines (Dubrovsky *et al.*, 2022; Rezaie *et al.*, 2021). Also, it was observed that the quantity of plasma extracellular vesicles significantly increased during the onset of an acute infection, correlating with the viral load. Following the simian immunodeficiency virus (SIV) or macaque model of HIV infection, specific microRNAs such as miR-181a, miR-342-3p, and miR-29a, which are present in extracellular vesicles, exhibited a decrease and remained downregulated during the latency phase (Huang *et al.*, 2023).

Exosomes released from HIV-1-infected cells carry viral receptors, potentially rendering target cells more vulnerable to infection. Notably, the transmission of CCR5 to CCR5 null cells through exosomes derived from CCR5⁺ ovary cells and peripheral blood mononuclear cells (PBMCs) has been observed, resulting in an increased susceptibility to HIV-1 infection (Mack *et al.*, 2000). According to research conducted by Rozmyslowicz *et al.* (2003), exosomes produced by megakaryocytes and platelets contain HIV co-receptors CXCR4. This presence of CXCR4 makes CXCR4-null cells more susceptible to X4-HIV infection (Rozmyslowicz *et al.*, 2003). These findings indicate that exosomes contribute to the transmission of viral receptors, potentially putting recipient cells at risk of HIV infection. However, the impact of these findings on animal models remains uncertain (Rezaie *et al.*, 2021).

Furthermore, it has been observed that viral nucleic acids can be packaged into exosomes, leading to the transfer of the viral genome to healthy cells and consequently enhancing the rate of infection. For example, exosomes derived from HIV-infected cells have been found to transmit the specific transactivation response element (TAR) RNA (Zhou *et al.*, 2019). TAR RNA, located at the 5' end of HIV transcript copies, interacts with the Tat protein to generate viral RNAs. The presence of TAR-RNA in exosomes can suppress a Bcl-2 interacting protein, finally promoting viral development and resistance to apoptosis (Zhou *et al.*, 2019; Marbán-Castro *et al.*, 2021). In fact, TAR plays a crucial role in preventing apoptosis, thereby facilitating the production of the virus within infected cells (Tabari *et al.*, 2020).

Exosomes derived from infected cells can potentially exert an antiviral influence. Specifically, exosomes from infected cells contain APOBEC3G molecules, which serve as antiviral proteins that impede virus formation. These proteins effectively convert cytosine residues to uracil in the negative strand of viral DNA during reverse transcription. Notably, the exclusion of Vif, an opposing viral protein, from exosomes enhances this advantageous trait (Khatua *et al.*, 2009). Additionally, the exosomes released by infected cells contain cGAMP, a molecule capable of triggering the production of interferon and activating innate immune responses. This, in turn, leads to the initiation of an antiviral defense mechanism (Bridgeman *et al.*, 2015; Gentili *et al.*, 2015). Based on the research conducted by Bernard *et al.*, it has been found that certain HIV-related miRNAs, such as miRNA-88 and miRNA-99, play a role in activating the endosomal NFκB and TLR8 signaling pathways. These signaling pathways, in turn, stimulate the immune system's response to combat HIV by inducing the production of TNF-α in macrophages (Bernard *et al.*, 2014). Based on research findings, it has been observed that when NLRP3, a specific protein, is silenced in microglia, it leads to the production of Tat-microglia-derived-EVs. These EVs have shown to possess protective effects on various aspects of neuronal function, including miniature excitatory postsynaptic currents, spine density, and synaptic proteins. While the role of NLRP3 in inflammation is widely acknowledged, an intriguing discovery has suggested that

targeting NLRP3 could be beneficial in treating HIV-associated neurological disorders, as it plays a role in EV-mediated neuronal injury (Muthukumar *et al.*, 2022).

The potential of exosome-derived miRNA-21 as a predictive biomarker was proposed in studying HIV-1-infected elite controllers' patients. This proposition arose from the observation that exosomes obtained from a specific subgroup of these patients demonstrated a decrease in miRNA-21 levels. This decrease correlated with the progression of HIV infection, as reflected by a decline in T CD4 lymphocyte count (Ruiz-de-León *et al.*, 2019).

Kodidela *et al.* (2018) conducted a proteome analysis on exosomes derived from the plasma of HIV-positive individuals who were frequent smokers, drinkers, or both. Their findings revealed different concentrations of hemopexin, alpha-2-macroglobulin, properdin, IL-8, and IL-10 in the exosomes of the HIV-positive groups who smoked and drank. These altered exosomal proteins and chemokines hold potential as biomarkers for identifying HIV-positive individuals with substance dependence (Kodidela *et al.*, 2018; Kodidela *et al.*, 2020). A research study conducted by Poveda *et al.* (2023) demonstrated the significant effectiveness of IL-18 levels in distinguishing individuals with HIV from uninfected controls. It also proved to be a reliable differentiator between elite controllers and those receiving antiretroviral therapy (ART), as well as between individuals with durable control and those with transitory control (Poveda *et al.*, 2023). Exosomes have been discovered to carry immunologically activating proteins and contribute to oxidative stress. They also exhibit immunomodulatory effects on myeloid cells, and in HIV patients receiving antiretroviral therapy (ART), they may potentially have pro-inflammatory and redox effects during pathogenesis (Chettimada *et al.*, 2018). Marques de Menezes' study revealed a correlation between EVs exhibiting activation of monocyte and neural markers and HIV-related cognitive impairment. This discovery suggests that specific subsets of EVs could serve as novel biomarkers for assessing brain damage in HIV infection. Furthermore, the study highlighted a potential interaction between circulating platelet EV levels and monocyte activation, indicating a previously unrecognized aspect in the pathophysiology of HIV-induced cognitive impairment (de Menezes *et al.*, 2022). Based on research findings, it has been observed that in individuals with HIV who are using cART (combination antiretroviral therapy), there is a connection between extracellular vesicles expressing monocyte markers and carotid intima-media thickness (cIMT). Moreover, EV fractions derived from HIV-positive individuals have been found to induce endothelial cell death through necrotic pathways. These discoveries suggest that EVs could potentially serve as both indicators and therapeutic targets in understanding the development of cardiovascular disease (CVD) within the context of treated HIV infection (Menezes *et al.*, 2022).

Exosomes and important issues

Exosomes come from inside the body, so they can be used for therapeutic purposes such as delivering proteins, peptides, RNA, genes, vaccines, and immunotherapy. The main

challenge for using exosomes is to find ways to isolate and purify them. Different uses need different levels of recovery and specificity. Standard methods are needed for separating and purifying exosomes. The methods should be fast, efficient, specific, viable, and reproducible. Exosomes from inside the body are stable in the blood and can avoid being eaten by phagocytes and macrophages. Exosomes only last in the body for 2 to 20 min, though. The existing content inside exosomes limits how much external drug can be loaded, making it less effective (Chaudhari *et al.*, 2022).

Various techniques have been employed to load drugs into exosomes, such as preloading and post-loading; however, these techniques require further optimization to achieve optimal outcomes. Large-scale production of exosomes entails serious concerns regarding aspects such as purity, potency, scalability, variability, and repeatability. It will entail considerable expenses to produce on a massive scale and introduce it to clinical settings. To elucidate how to utilize exosomes in biomedical applications, the function of exosomes must be investigated thoroughly (Chaudhari *et al.*, 2022).

Exosomes that are loaded with drugs or not may have different effects and behaviors in living organisms. The pharmacokinetics and pharmacodynamics of therapeutic exosomes, which are the processes of how the drug moves and acts in the body, may differ from those of endogenous exosomes, which are the ones that are naturally present in the body (Chaudhari *et al.*, 2022). The influence of exosomes on the exposure of drugs *in vivo*, which is the amount and duration of drug presence in the body, has to be extensively evaluated through long-term safety and toxicity studies. The impact of drug-loaded exosomes on the recipient cells, which are the cells that take up the exosomes, the absorption process, which is how the exosomes enter the cells, the intracellular and extracellular behavior, which is how the exosomes act inside and outside the cells, and the drug unloading mechanisms, which are how the exosomes release the drug, can all be studied using *in vitro* models, which are experiments done outside living organisms. The PK of therapeutic exosomes can be further investigated using additional *in vivo* methods, which are experiments done inside living organisms, and physiologically based pharmacokinetic models, which are mathematical models that simulate how the body works (Chaudhari *et al.*, 2022). The creation of artificial exosomes is a promising solution for translational medicine, which is the process of applying research findings to clinical practice, because it can address the critical issues listed above and result in homogeneous exosomes, which are exosomes that have consistent properties and quality. Exosome-based biological applications have a bright future as synthetic exosomes are currently being developed (Chaudhari *et al.*, 2022).

Discussion

The present study offers a detailed analysis of the role of exosomes, a subtype of extracellular vesicles, in the pathogenesis of viral infections. Exosomes are nanoscale, bilayered vesicles that originate from the endosomal compartment of most eukaryotic cells. They serve as

important regulators and mediators of intercellular communication, as they can deliver their cargo of proteins, lipids, and nucleic acids to recipient cells. The structure and composition of exosomes depend on the tissue and cell type of origin, and they can also vary in response to different clinical conditions. Several viruses have the ability to exploit exosomes and manipulate their functions for various objectives, such as immune evasion, viral dissemination, and host cell reprogramming (Saad *et al.*, 2021).

Viruses exploit the biogenesis mechanisms of the exosomes they hijack to facilitate their capsid assembly, virion production, and viral particle secretion. They also employ exosomes as nano-carriers to transport viral proteins and/or viral miRNAs to uninfected cells, thereby modulating their cellular functions. Exosomes have multiple roles in biological processes, such as mediating intercellular communication, regulating immune responses, and transferring various materials from one cell to another. SARS-CoV-2, similar to other viruses, utilizes exosomes as a viral vehicle for intra-host transmission and viral amplification. Exosomes can be a potential option for designing various viral vaccines for the treatment and prevention of pandemic diseases, including COVID-19 (Saad *et al.*, 2021).

The various biological functions of exosomes are largely influenced by their cellular origin and cargo composition. Exosomes can serve as important carriers of viruses, enhancing viral infection and antiviral immunity in different host cells. They are expected to offer a novel research platform for the diagnosis and treatment of viral disease.

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