Exosomes may play a crucial role in HIV dendritic cell immunotherapy

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The human immunodeficiency virus (HIV) is a lentivirus that infects cells of the immune system, resulting in cell death and loss of cell-mediated immunity. When the number of immune cells drops below a critical population, HIV can result in the acquired immunodeficiency syndrome (AIDS), wherein opportunistic infections and cancers flourish in the absence of immune surveillance. Worldwide, there are over 30 million people infected with HIV, resulting in a significant global health burden (1). The current standard of care for HIV is combined antiretroviral therapy (cART), and while this effectively prevents disease progression and death, treatment must be continued for the rest of the patient's life (2). Therefore, new therapies that can eradicate and prevent the disease are a crucial area of research in global health.

HIV enters the body through the mucus membranes. The first cells it encounters are immature dendritic cells (iDCs), which act as sentries and recognize foreign microorganisms. Upon contact with pathogens, iDCs endocytose the microorganism, resulting in iDC activation and migration to the secondary lymphoid organs. In the lymph nodes, the now mature dendritic cells (mDCs) present the epitopes derived from internalized microorganisms to CD4+ and CD8+ T lymphocytes (CD4TL, CD8TL), priming them for the active immune response (3). However, when an iDC presents the HIV particle to CD4TL, rather than activating them for the immune response, it results in the infection of CD4TL through a process called trans-infection (described as the "Trojan horse" hypothesis) (4). Despite this observed mechanism, it has also been demonstrated that the HIV particle is rapidly degraded within the iDC. Therefore, it is likely that CD4TL infection occurs through two different methods, both through trans-infection and through the production and secretion of *de novo* viral particles by infected iDCs (3).

One of the most compelling novel therapeutic strategies for HIV is that of dendritic cell immunotherapy (DC-IT). In this treatment, patients' myeloid iDCs are removed and treated with an inactivated form of the HIV particle *ex vivo*. This stimulates DCs to mature, and upon reintroduction into the body the mDCs stimulate a CD4TL response to HIV, resulting in an effective immune response (1). To date, 13 clinical trials using DC-based immunotherapy have been completed with varying degrees of success (5). While DC-IT has been used to induce a persistent immune response, the heterogeneous factors involved in a patient's HIV infection, including host/viral genome, specific infection characteristics, and level of HIV-mediated immune suppression, result in differing responses to DC-IT (2). Therefore, it is critical that we continue to identify the intricate biomarkers and signaling mechanisms associated with HIV infection and DC function.

One such mechanism is that of exosome secretion. Exosomes are minute extracellular vesicles 30–100 nm

in size that are generated through the budding of the inner endosomal membrane and fusion with the plasma membrane. Exosomes are highly heterogeneous, with diverse biological functions depending on their molecular contents. They can contain most kinds of macromolecules, and have been identified as a mechanism by which cells communicate with each other through the transfer of proteins, mRNAs, and non-coding RNAs (6). In addition to their functional effects, the use of exosomes as pathologic biomarkers is a burgeoning field of study. As exosome contents differ based on cell of origin and cell state, profiling of exosomes isolated from a patient's blood may be a useful method of non-invasively examining a cells' pathologic state (7).

DC derived exosomes have been shown to regulate nearly all aspects of the immune system. Depending on the maturity status of the parent DC, DC-exosomes may contain major histocompatibility II complexes, allowing them to act as antigen-presenting bodies and stimulate the adaptive immune response, including both CD4TL and CD8TL. They have also been shown to induce antigen-specific humoral immunity, activate natural killer cells, promote the allergenic response, and aid in the immune system's response to tumor cells (5).

In a recent issue of *Medical Hypothesis*, Ellwanger *et al.* described their hypothesis based on preliminary data collected by Pontillo *et al.*, and examined exosome secretion and the CD4TL response to DC-IT (8). Using monocytes and monocyte-derived DCs collected from six phenotypically matching patients enrolled in a DC-IT trial, the authors analyzed the expression of eighty-four genes involved in the anti-HIV response, as well as the expression of the *TSG101* gene, an exosomal marker. Pontillo *et al.* separated the patients into two groups based on whether the genes were predominantly downregulated (group A) or predominantly upregulated (group B) compared to control monocytes. While the patients' diseases were phenotypically similar, group A exhibited higher levels of CD4TL than group B, indicating that these patients may be more responsive to DC-IT. They also found that the expression of *TSG101* negatively correlated with anti-HIV response genes (increased in group A, decreased in group B). This may indicate that the production and secretion of exosomes impacts the HIV response (5,8).

These early findings highlight interesting aspects of exosome signaling in HIV. Exosomes are believed to often act in support of the immune response through antigen presentation and activation of the secondary immune response (6). However, the down-regulation of *TSG101* combined with an increase in CD4TL implies that in HIV and DC-IT, exosomes may be having an opposite effect. It is possible that these data support what has been termed the "Trojan exosome hypothesis". First described by Gould *et al.*, this hypothesis proposes that retroviruses utilize the exosomal biogenesis and secretion pathway for replication of their viral particles. This is founded in the observation that exosomes and HIV particles share a similar protein and lipid composition, although similar findings have also been reported using other retroviruses (4).

However, recent data indicate that while HIV and exosomes use similar pathways within the cell, they appear to be separate particles. Subra *et al.* successfully separated HIV particles from exosomes using ultracentrifugation and immunocapture. They found that after pulsing DCs with HIV, DC-exosomes induced apoptosis in CD4TL, and were not capable of spreading the HIV infection. On the other hand, HIV particles secreted by DCs and separated from exosomes were infectious without inducing apoptosis (9). A recent study from the same research group by Mfunyi *et al.* identified the dependence of DC exosome release on dendritic cell immunoreceptor (DCIR), the same receptor which DCs and CD4TL use to bind the HIV virion. Inhibition of this receptor prevented exosome secretion, solidifying the parallels between the cellular mechanisms used by HIV and exosomes (10). Further work demonstrated that exosomes from HIV stimulated DCs contained the pro-apoptotic protein DAP-3, whereas exosomes from non-stimulated DCs did not (10).

While the full parallels between exosomes and HIV particles remain undefined, it is possible that HIV is utilizing exosomal biogenesis as an additional mechanism of trans-infection. Exosomes produced by fully matured antigen presenting mDCs can serve as additional antigen presenting bodies, activating immature iDCs and CD4TL (6). Upon infection, HIV may hijack these processes in DCs, resulting in the production of HIV containing exosomes that can infect other DCs and CD4TL, greatly increasing the spread of HIV infection (3). However, based on the findings of Mfunyi *et al.* and Subra *et al.*, it appears likely that HIV is merely hijacking the exosomal biogenesis process for creation of discrete HIV particles, separate from HIV containing exosomes.

Ellwanger *et al.* drew two potential conclusions from their findings. First, since they observed that group A patients with decreased expression of *TSG101* also had increased numbers of CD4TL, it is possible that

exosomes have a negative effect on HIV immunity. This is likely through HIV-DC exosome-mediated apoptosis of CD4+T cells. Therefore, the DC-IT patients whose DCs produced fewer exosomes resulted in increased numbers of apoptotic CD4TL. However, patients in group A also exhibited down-regulation of anti-HIV response genes, implying that DC-IT is decreasing HIV resistance. On the other hand, group B patients had higher expression of HIV response genes after DC-IT, as well as increased *TSG101* expression. Based on these results, it is possible that the increased secretion of exosomes seen in group B patients may correlate to increased release of the HIV viral particle, thereby activating HIV response genes in additional DCs (5).

While these preliminary results are inconclusive, they highlight the continued need for investigation of exosomal functions in the immune system. Based on the manuscript by Ellwanger *et al.* and the previous findings of Mfunyi *et al.* and Subra *et al.*, it appears likely exosomes are playing a critical role in the DC HIV response. Modulating exosome expression, potentially through DCIR, may be a mechanism of HIV immunity both in conjunction with DC-IT and alone. However, these findings must be further examined. The hypothesis by Ellwanger *et al.* is based on minimal data, with two patient groups containing three members each. Moreover, they did not examine actual exosome release, merely the expression of exosome related gene *TSG101*. Further verification of these findings that build off the data presented by Mfunyi *et al.* will provide important insights into exosome and HIV biogenesis, immunity, and treatment.

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Footnote

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