Editorial

An immunoregulatory role of dendritic cell-derived exosomes versus HIV-1 infection: take it easy but be warned

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Introduction

Dendritic cells (DCs) are professional antigen-presenting cells capable to initiate and then drive T cell responses. Naturally, DCs sense various pathogens and their products in order to present those to immune cells and in turn initiate immune reaction. In a case of wounding, DCs recognize products released by damaged cells and then contribute to the induction of inflammation associated with further clearance of necrotic and apoptotic cells (1). In addition to DC subtypes that initiate inflammatory reaction, there are DC subsets, which exert tolerogenic properties directed to dampen extensive inflammation and promote switching to wound healing (2).

During HIV-1 infection, host DCs (especially mucosal DCs) can sense virus through innate cytosolic immune receptors and then initiate antiviral responses associated with production of type 1 interferon (IFN) and up-regulation of IFN-stimulated genes (3). Type 1 IFN induces death of infected T cells by bystander effect and suppresses viral replication (4). Furthermore, in some individuals, HIV-1-dependent activation of DCs leads to the induction of highly potent antiviral response related to the appearance of virus-specific CD8⁺ T cells capable to cause natural, drug-free resistance to the infection and support effective HIV-1 control. There is a reciprocal interaction between DCs and HIV-1-specific T cells that involves innate major histocompatibility complex (MHC) class I receptors from the Ig-like receptor family and facilitates HIV-1 control (5).

However, this virus can overturn the protective function of DCs and use these cells for invasion. HIV-1 enters the organism mostly through vaginal and rectal ways whose submucosa contains high numbers of residential DCs. Compared to infected CD4⁺ CCR5⁺ T cells where the rapid burst of infection is observed (6), it is difficult to detect HIV-1-infected DCs. Mucosal DCs catch HIV-1 through the endocytic mechanism after binding to C-lectins such as DC-SIGN, langerin, DC immunoreceptor (also known as C-type lectin domain 4A or CLEC4A), etc. (7). Langerhans cells, i.e., resident DCs of skin and mucosa, are more resistant to HIV-1 infection compared with other DC subsets and can efficiently degrade viral particles in Birbeck granules (subdomains of the endosomal recycling compartment) after internalization (8). However, at higher concentrations, virus is able to infect Langerhans cells and then be transferred to CD4⁺ T cells (9).

DCs transfer virus to CD4⁺ T cells in secondary lymphoid tissues. Moreover, DCs can serve as a depot for virus and support HIV-1 replication at low levels (10). Viral transmission from infected DCs to T cells employs
formation of infectious synapse (IS) between a DC and a T cell (11,12) or exosome secretion pathway (13,14). Upon IS formation, captured viral particles are switched from the transfer to the endolysosomal pathways towards trafficking to the synapse (15). The viral trafficking to IS uses a pathway of tetraspanin sorting to the immunological synapse between a DC and naive T cell thereby impairing the mechanism of the immunological synapse formation (16). In the exosome-dependent pathway, virus exploits the exosome antigen-dissemination pathway for transmission (14). HIV-1-containing exosomes are released by both immature and mature DCs. Interestingly, on a per-particle basis, exosome-derived viral particles were 10-fold more infectious than cell-free HIV-1 particles (17). In addition, exosome-associated virus can be targeted by virus-specific immune response significantly less efficiently suggesting for an avenue for virus escape.

In DCs, poor HIV-1 replication can be explained by the existence of cell-protective restriction mechanisms. In myeloid cells and DCs, sterile α-motif and HD domain 1 (SAMHD1), a dNTP triphosphohydrolase, decreases the intracellular dNTP pool essential for virus reverse transcription and synthesis of cDNA. This in turn blocks HIV-1 replication after virus entry to a DC (18). Tripartite motif-containing protein 5 (TRIM5) acts as a receptor able to sense retroviral capsid lattice and then induce the innate immune response through up-regulation of the intracellular innate immune signaling (19). DNA dC- > dU-editing enzymes belonging to the APOBEC3 family provide retroviral resistance by inducing G-to-A hypermutation in the provirus that initiates further degradation of viral transcripts (20).

Finally, DCs employ a set of receptors capable to recognize viral particles and viral RNA/cDNA. In addition to HIV-1-sensing C-type lectin receptors, there are Toll-like receptors (TLRs). However, virus can use TLR8 and D-SIGN for infection and replication in DCs (21). Cytosolic 3’ repair exonuclease (TREX1) can hide virus from cytoplasmic viral DNA sensors by binding to HIV-1 DNA and degrading excessive viral DNA (22). By down-regulation of IFN-stimulated genes, this nuclease suppresses the anti-HIV-1 innate immune response and also limits expansion of the lysosomal compartment (23). In contrast, another cytosolic DNA sensor, cyclic GMP-AMP synthase (cGAS), by binding to viral DNA, synthesizes cyclic GMP-AMP, an intracellular messenger, that triggers production of type 1 IFN and other cytokines through STING/TRAF family member-associated NF-kB activator (TANK-1)-dependent stimulation of transcription factors NF-kB and IFN regulatory factor 3 (IRF3) (24,25).

A great capacity to recognize viral products and then induce specific anti-viral immune response along with a relative resistance of DCs to HIV-1 infection served as a basis for the development of a rationale for use dendritic cells in anti-HIV immunotherapy. Loading of autologous virus-free DCs with a HIV-1-specific antigen with subsequent vaccination of a HIV-1 infected individual with antigen-activated DCs was suggested to boost the restoration of the host anti-viral immune response (26). Basically, any viral product such as a whole autologous heat- or chemically-inactivated virus particles, viral peptides (such as pol and gag) or viral RNA transcripts may serve as an antigen for generation of DC-based vaccines (27).

In this review-comment, we will consider the impact of DC-based immunotherapy on the treatment of HIV-1 infection and a role of exosomes in the control of DC vaccine-induced antiviral immune response.

### DC-based immunotherapy of HIV-1 infection

In the context of HIV-1 infection, the main purpose of DC-based vaccines is the activation of cytotoxic CD8+ T cells since CD4+ T cells can be widely infected by the virus and therefore become ineffective or impaired (28). In chronically HIV-1-infected patients, function of CD8+ T cells and DCs are also defective. Gag-specific CD8+ T cells were shown to produce IFN-γ but failed to produce reliable amounts of perforin and IL-2. These cells are unable to expand in co-culture with gag-specific DCs suggesting for the loss of recall memory to HIV-1 protein gag. DCs from HIV-1 patients can effectively present antigens to other viruses such as influenza, Epstein Barr virus, and cytomegalovirus but lack the capacity to expand gag-specific CD8+ T cells (29).

The development of DC-based vaccines is a multi-step process. After antigen loading, DCs should be matured by supplementation of culture medium with cytokines and/or growth factors. For example, in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF), antigen-loaded DCs mature to conventional (i.e., “classical”) CD4+ DCs with propensity to the proinflammatory activation of naive T cells. When exposed to type I IFN and macrophage colony-stimulating factor (M-CSF), DCs mature to plasmacytoid-like DCs characterized by a potent production
of type 1 IFN (30). After vaccination, immune response in DC-immunized subjects can be monitored with help of enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot (ELISPOT), flow cytometry, and their combinations.

To date, over 15 clinical trials have been performed in order to assess the efficiency of DC vaccines in HIV-1 therapy. In these trials, there were various clinical settings. In some clinical studies, asymptomatic HIV-1-infected untreated subjects were recruited (31-36), while patients on antiretroviral therapy were enrolled in other studies (37-41). The trials were small since they involved only from 4 up to 56 subjects.

Investigators also used various protocols for development of DC-based vaccines. In some studies, DCs were pulsed with a variable mixture of peptides derived from HIV-1 proteins Gag, Env, Pol, Nef, Vif, Vpu, and gp160 (31,34,36,37). Other researchers loaded DCs with a whole inactivated virus (32,33,38). Yet other investigators used viral mRNA encoding Gag, Nef, Ref, Vpr, and Tat for electroporation of DCs (39,40). Macatangay et al. (41) reported development of DC-based vaccine loaded with apoptotic bodies released by dying autologous HIV-1-infected T cells. An interesting approach was implicated by Norton et al. (42) to obtain DCs constitutively expressed HIV-1 specific epitopes. For transduction of DCs, Norton et al. (42) used lentiviral vectors encoding HIV-1 epitopes fused through self-cleaving peptide to CD40L that helps to release peptide to the endoplasmic reticulum after entry. The vectors contain Vpx, a lentiviral protein, which neutralize SAMHD1-dependent inhibition of viral replication and support long-lasting expression of viral epitopes in DCs (42).

The protocols used for the maturation of antigen-loaded DCs were also different. Generally, in most studies, investigators supplemented the culture medium with cytokines as follows: GM-CSF, IFN-α, interleukin-1β (IL-1β), IL-4, IL-6, and tumor necrosis factor (TNF)-α (27). In all studies, vaccines were injected percutaneously in different doses varying from 2×10⁶ (33,36) to 1.2×10⁷ (40) cell per dose. Vaccination regimens significantly varied. A total of 3 to 6 doses of a vaccine were injected with a periodicity ranging from every 2 weeks to 1 month (i.e., 4 weeks).

Generally, DC vaccination was safe and well-tolerated by patients. Side effects of immunization were minimal or moderate including erythema, local inflammation, or subcutaneous bleeding at the injection site, and asymptomatic enlargement of peripheral lymph nodes. Adverse side effects of vaccination included thrombocytopenia, neutropenia (38), and severe pruritus (42).

The vaccination led to marked decrease of plasmatic HIV-1 virus load up to 4–5 copies per mL of blood (35,37,40) mainly due to the DC-dependent activation of antigen-specific cytotoxic T cells and enhancement of the anti-viral immune reaction mostly mediated by CD8+ T lymphocytes. Induced cytotoxic T cells perform the destruction of infected T cells thereby limiting cell sources for virus replication and storage. DC vaccination also led to increase in numbers of circulating CD4+ T cells and functional CD4+ T cells, reactivation of latent HIV-1 thereby reducing the reservoir of latent virus in affected patients (42). Andrés et al. (43) replicated these results suggesting for the clinical value of DC vaccination for inducing virus-specific T cell subsets capable to control HIV-1 replication and intracellular viral reservoir.

The immunization with DCs also resulted in enhanced production of inflammatory cytokines (IFN-γ, IL-17), IL-21 (essential for proliferation of cytotoxic T cells and natural killer cells), and IL-2 (essential for differentiation of naïve T cells to regulatory T cells and differentiation of antigen-activated T cells to effector T cells and memory T cells (36). Notably, after completing vaccination course, patients were able to stay off the antiretroviral therapy for a long time. For example, Allard et al. (39) reported a 96-week patients’ withdrawal from the antiretroviral treatment for 6 of 17 participants after DC vaccination. Lu et al. (32) showed long-term inhibition of virus load by >90% with stable T cell counts for at least 1 year after completing of DC vaccination.

However, not all but only a part of HIV-1 patients responded to vaccination. Monocyte-derived DCs from HIV-1 patients that undergo the antiretroviral therapy were shown to secrete low levels of IL-12 (essential for differentiation of naïve T cells to proinflammatory Th1 cell) after induction of CD40L (44). Reduced IL-12 correlated with the lack of post-vaccination viral load control (41). However, DCs from patients subjected to the antiretroviral therapy after exposure to IFN-γ and CD40L able to produce more IL-12 (44). Accordingly, CD8+ T cells become more potent IFN-γ producer after activation by DCs treated with CD40L and poly (I:C), a synthetic mimic of viral double-stranded RNA and TLR3 ligand (45). Indeed, pre-vaccination conditions are important for the
control of DC function.

There is a significant heterogeneity in the magnitude of DC vaccine-induced immunoproliferative responses in vaccinated HIV-1 patients. Gandhi et al. (38) detected no significant immunoproliferative response while other studies showed modest or transient responses and increase in CD8+ T cell counts that correlated with partial virus load control (33-35). However, other researchers observed a prominent increase of CD8+ T cell mediated antiviral response associated with reduced viral load (36,38).

The variability of DC vaccination-induced responses may possibly arise from the difference between experimental design of clinical studies and selection criteria for participants. Indeed, vaccine preparation methods and clinical vaccination protocols (at least key points such as vaccine response criteria) are needed to be standardized. Standardization is also necessary for the assessment of vaccine efficiency since naïve, untreated patients and subjects under the antiretroviral therapy respond to DC vaccine in a different way. Probably, standardization of selection criteria for patients may be also helpful. Asymptomatic subjects with early chronic HIV-1 infection may be more relevant for evaluation of DC-based vaccines since they have a pretty normal immune response, virus is not subjected to strong immunological pressure, and viral reservoir is still small.

Combination of DC-based immunotherapy with other antiviral pharmaceutical agents may be beneficial and result in the reciprocal enhancement of therapeutic effect. For example, combined treatment with Vacc-4x (a four-HIV-1-specific peptide vaccine), recombinant GM-CSF, and romidepsin (a histone deacetylase inhibitor) resulted in decrease of total HIV-1 DNA by nearly 40%, virus reactivation, and significant decrease of the size of latent HIV-1 reservoir (46). Administration of the peptide vaccine and GM-CSF before treatment with romidepsin was directed to recover DC-mediated anti-viral immune response. Therefore, DC vaccination before pharmaceutical medication may be preferential in order to enhance the efficiency of the anti-HIV-1 therapy.

At the distance, DCs were shown to communicate to each other by releasing exosomes that assisted in the propagation of the immune response and enhance responses against pathogens. DC-derived exosomes also serve as a communication tool with other immune cells such as T cells and B cells (47).

**DC-derived exosomes: role in immune regulation**

Exosomes are extracellular vesicles surrounded by a lipid bilayer and capable to transfer a variety of molecules such as proteins, DNA, mRNA, microRNA (miRNA), lipids, etc. There are several types of extracellular vehicles that are differentiated by size and origin. Exosomes (or microvesicles) are released by budding from the cell surface. By contrast, exosomes (size range, 30–150 nm) are generated by invagination budding of the internal endosomal membrane followed by formation of multivesicular bodies, their fusion the plasma membrane, and liberation of exosomes to the extracellular space (48).

Exosomes are highly enriched with specific proteins like tetraspanins (CD63, CD81, and CD914) and 70 kDa heat shock protein (HSP70), which can be used as exosomal markers. The exosomal membrane also contains receptors and other proteins that are necessary for targeting of recipient cells and assisting in exosome internalization by endocytosis or phagocytosis and further release of the exosomal cargo into the cytoplasm of the acceptor cell (49). Delivery of the exosomal material to recipient cells can influence intracellular signaling and induce changes in cell function and behavior (50). The exosomal composition and exosome-mediated biological effects depend on the exosome-releasing donor cell and local microenvironment. Changes in inflammation, infection or transformation influence the exosomal content. Exosomes can play protective or pathogenic role (51). As mentioned above, DC-derived exosomes can serve as a vehicle for HIV-1 propagation and infection of CD4+ T cells. Exosomes secreted by tumor cells transfer the biomaterial that can induce malignant changes in the normal recipient cells upon delivery (52).

As known, DCs as immune cells, which recognize, process and present antigens, represent a link between the innate and adaptive immunity. DCs present processed antigens bound either to MHC class I or MHC class II molecules to naïve CD8+ T cells or CD4+ T cells respectively, which then transform to effector cells or memory cells. Effector cells are actively mediate the immune response while memory cells are responsible for storage of immunological antigen-specific information to be reactivated in case of a repeat infection of the pathogen that contains this antigen (53).

Exosomes are extensively involved in the transport and presentation of antigen-MHC complexes to CD8+ and
CD4⁺ T cells. DC-derived exosomes can present antigens directly or through the mechanism of cross-presentation. In direct presentation, T cells directly accept an antigen that leads to their activation. In cross-presentation, DCs accept antigens transferred by exosomes, additionally process antigens and then present to T lymphocytes (54). In addition, DC-derived exosomes promote cytotoxic effects mediated by CD8⁺ T cells (55). Exosomes also up-regulate antibody production against bacterial pathogens (56) and humoral immunity in overall (57). Finally, exosomes released by DCs stimulate immune responses against neoplastic cells (58).

Exosomal miRNAs are an important component of the immunoregulatory properties of DC-derived chromosomes. The formation of the functional immunological synapse leads to a significantly more intensive exchange by miRNA-containing exosomes between DCs and T cells (59). DCs were also shown to transfer functional miRNAs to each other via exosomes (60). MiRNA are involved in post-translational control of gene expression and silencing of a wide range of mRNA targets. However, in HIV-1 infection, miRNAs are likely to play a minimal role in antiviral fight. HIV-1 protein Nef was observed to bind to Ago2, a key factor in miRNA biogenesis that leads to the inhibition of any RNA interference (61). Again, for example, the virus can stimulate overproduction of miR-29b and cause neural complications through exosome-dependent transport of this miRNA to astrocytes and subsequent down-regulation of expression of platelet-derived growth factor-B (PDGF-B), a growth factor of healing and amplification of neural cells (62). Furthermore, the virus can rule from infected cells the exosome-dependent trafficking of trans-activation response element RNA, an essential activating product for HIV-1 replication (63).

**DC-derived exosomes and anti-HIV-1 therapy**

In fact, HIV-1 is a trick virus that mimics the human nature to survive. A set of viral mutations was detected to explain how HIV-1 can to reorganize the local immune microenvironment to be effective for replication and further invasion. We are quote sceptic on the use of DC-derived exosomes to heal that viral infection. First, three patients only were in that little examination (63).

Exosomes are possibly used as a tool of immune regulation during the dendritic cell-based immune therapy against HIV-1. Second, using autologous DCs is a challenge for HIV-1 infection because of an easy adaptation of the virus to novel microenvironment and these particles may be back-infected.

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**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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