Dendritic Cell Dysfunction: A Key to HIV Spread and Persistence

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INTRODUCTION

Dendritic cells (DC) are both widely dispersed in the body and are potent activators of effector immune response, which stresses their important role in HIV infection [1,2]. DCs are present at sites of HIV entry, the oral and vaginal mucosa [3,4] or circulating blood [5], and at sites of preferential HIV infection and maintenance in the lymph nodes. It has been shown that HIV-infected DCs mediate viral infection of CD4 T cell co-cultures [6,7] and it is believed that DCs transmit HIV to CD4 T cells in the lymphoid tissues in vivo [2,8], through utilization of DC-derived exosomes or an 'immunological synapse' [9]. Thus, DCs are important in initial encounter and response to the virus in the mucosa and blood. They are also key players in spread of infection via trans-CD4 T cell infection (or in cis), and stimulation of adaptive immune responses in attempts to eliminate virus. HIV uptake by DCs to mature, as witnessed during HIV uptake, however, leads to attenuation of antigen-specific immune responses and may significantly contribute to induction of T cell regulatory responses. These regulatory responses typically can be a consequence of low co-stimulatory molecule expression (e.g. CD40, CD80/86) or increased co-inhibitory molecule expression (e.g. PD-L1, PD-L2) on DCs and production of soluble immune regulatory mediators (e.g. IDO, IL-10, TGFβ) by DCs or instructed T cells. The failure of DCs to properly process and present HIV antigens and stimulate specific adaptive responses can prevent viral clearance and aid in establishment of viral reservoirs, two of the main obstacles to a therapeutic cure.

ABBREVIATIONS

DC: Dendritic Cell; CTL: Cytotoxic Lymphocyte; DC-SIGN: Dendritic Cell-Specific ICAM-3 Grabbing Non-integrin; HIV: HIV type 1.

Keywords

• Immune regulation
• Dendritic cells
• HIV immunity

the main sites of early HIV interaction, are present in an immature phenotype [13]. In this state, DCs are highly adept at endocytosis and express lower levels of MHC class I and II molecules, as well as co-stimulatory molecules [14]. Immature DCs possess a wide variety of receptors, such as Fc receptors, lectins, and langerin, which allow them to efficiently bind and internalize antigen. This wide range of receptors allows for efficient receptor mediated phagocytosis of many foreign antigens. Many antigens that are internalized by DCs are routed to MHC-II expressing lysosomal vesicles where they are degraded and processed to form MHCII-peptide complexes for T cell stimulation.

The DC-specific lectin receptor, DC-specific ICAM-grabbing non-integrin (DC-SIGN), rapidly internalizes ligands and forms DC-SIGN rich vesicles which are targeted to late endosomes and lysosomes [15]. DC-SIGN is highly expressed on DCs and binds the heavily glycosylated HIV surface glycoprotein gp120, which facilitates HIV capture DCs and enhances transmission to T cells [16]. While HIV can be transmitted independently of DC-SIGN, this molecule is of particular interest as it promotes DC migration to T cell-rich areas of lymph tissue and has also been revealed as a mechanism to subvert immune responses [17-19].

In the initial step of uptake of HIV by DCs, endocytic vesicles may play an important role in HIV trans-infection, but it is still unclear if this is a separate pathway of transmission or plays a role in synapse-mediated HIV transfer. HIV that is endocytosed by DCs may be degraded through lysosomal fusion, avoiding degradation and shuttling to an ‘infectious synapse’, or may be released as an infectious exosome. HIV has been shown to be associated with exosomes and bud from primary macrophages [20,21], but it is unclear if this process can also facilitate DC-mediated trans-infection [22,23].

**DC-mediated HIV transmission**

In contrast to CD4 T cell infection [8], viral replication in DCs is relatively low and the frequency of HIV-infected DCs are low [24,25]. Blood DCs [26,27] and Langerhans cells [28,29] from healthy individuals are infected at low frequency with HIV in vitro [30] and in vivo [31]. This has been attributed to significantly less HIV receptor expression [32,33], intracellular routing and HIV killing [2,34], and replication-restricting host factors [35-40]. However, one study using a rhesus macaque SIV cervical infection model found that mucosal DCs accounted for 90% of SIV dissemination [41]. This highlights the importance of even a low productive infection of DCs. Through HIV uptake and dissemination the virus is able to utilize multiple pathways to transfer from DC to CD4 T cells.

The ability of DCs to trans-infect T cells has been known for some time now [42,43]. This process involves the capture of HIV on the surface of DCs [32], mobilization of DCs [44], and the clustering and polarization of both HIV particles and T cell interacting receptors on the DC surface [45]. This allows DCs to interact with CD4 T cells via this ‘immunological synapse’ and transfer HIV without necessarily becoming productively infected themselves [46]. HIV trans-infection is mediated by numerous receptors, such as C-type lectins DC-SIGN [47-49] and Langerin [50,51], but independent trans-infection models exist and this could be based on the plasticity of DC subsets mediating the HIV transfer or on HIV itself [52]. DC-SIGN, in particular, binds HIV gp120 with high affinity [53] and enhances HIV infection of CD4 T cells [54,55], but elimination of DC-SIGN has shown that trans-infection is not entirely dependent on this interaction [56].

Notably, DCs have many receptors that are believed to facilitate trans-infection to CD4 T cells. These multiple pathways quite possibly stem from the distinct expression patterns of receptors with overlapping functions in DC subtypes [57]. The emerging lectin DC immunoreceptor (DCIR) [58], mannose receptor [57], BDCA-2, and peroxisome proliferator-activator receptor (PPAR)γ [59,60] are all expressed at a varying degree based on DC subtype and differentially regulate HIV binding and transfer [32,50,61,62]. These HIV-binding and transfer pathways have been shown to occur in vitro, but more *in vivo* studies are needed to conclude the importance and magnitude of each response.

The actual transfer of HIV to CD4 T cells is cell contact-dependent and occurs through the ‘infectious synapse’. This involves a normal junction of T cell activation molecules required for APC stimulation of T cells, at which point HIV can be transferred [63,64]. Again, the cell surface makeup and subtype of DC contributes to this transfer, but these interactions have not been fully identified. HIV and its associated receptors, however, are found to be highly concentrated at the synapse. Establishment of mechanisms and dominance of contact-dependent HIV transfer *in vivo* are needed as this interaction facilitates viral spread and preferential CD4 T cell infection [6,65].

Interestingly, long-term DC-mediated transmission of HIV to CD4 T cells is shown to occur through virus production by DCs [34,66,67]. Immature DCs can maintain HIV for up to one week [49, 68, 69], but a majority of HIV can be degraded within one day [70,71]. This suggests that HIV may be replicated *de novo* in DCs and late virus transmission is from progeny virus [72]. Initial investigations have shown that establishment of productive infection of DCs leads to spread of mainly progeny virus into CD4 T cells [73]. Also, HIV gp120 binding to DC-SIGN triggers Raf1-initiated phosphorylation of p65 and facilitates HIV transcription [74,75]. While not as abundant as CD4 T cell infections, this suggests that DCs may serve as a viral reservoir to some degree *in vivo*. *Trans-* and *cis*-infections are not likely to be independent and exclusive, but rather overlap and contribute to HIV infection and spread *in vivo*.

Follicular DCs (FDC) are not a typical DC which are found mainly in germinal centers and B cell follicles of lymphoid tissues [76]. While FDCs are not able to process and present antigen using MHC-restricted pathways, they are capable of capturing robust numbers of pathogens. FDCs are shown to trap and maintain large amounts of HIV in the follicles during infection, and thus believed to be one of the main reservoirs of infectious virus [77,78]. Importantly, FDCs have close proximity to CD4 T cells in these tissues and this juxtaposition correlates to a high concentration of productively infected follicular helper T cells [79]. In a similar fashion, it is believed that typical DCs can maintain persistent infection for long periods [80] and contribute to both cellular reservoirs and follicular helper T cell preferential infection.
HIV-driven DC dysfunction

In both the blood and tissues DCs are present in an immature state that allows them to survey their local environment for antigen and respond to danger signals from nearby cells [81]. After antigen capture and activation, DCs downregulate immature surface receptors and lose their antigen capture capacity. This coincides with DCs gaining the ability to migrate to secondary lymphoid tissues [82] and present antigen to lymphocytes to drive antigen-specific adaptive immunity [13]. The presentation of peptides on MHC molecules is the first signal in stimulating effector responses. The first signal alone is not enough to drive robust effector responses. Rather, a second signal from DCs is also associated with co-infections and this is believed to advance progressive HIV infection [108-110]. This is due in part to constant immune activation, which is one of the indicators of HIV progression. Mucosal [111,112] and blood DCs [113,114] have been shown to be dysregulated by microbial pathogens, which may assist in chronic immune activation and HIV spread through DC-mediated dissemination [115]. Specifically, gut mucosal DCs have high rates of inflammatory mediators [116] and blood DCs infected with an oral pathogen upregulate co-receptor expression and migratory capacity [117,118]. Prolonged type-I IFN production due to chronic inflammation has been correlated with disease progression and may contribute to immune exhaustion [119]. Bacterial uptake and activation of pyroptosis can also trigger IL-1β production and further extend inflammatory conditions [120]. Co-infection can result in a further alteration of DC activity or contribute to the chronic inflammatory response. Patients with both HIV infection and opportunistic infections have upregulated TLR 2 and 4 on the surface of DCs, further contributing to immune imbalances [121]. Microbial pathogens, their translocation, and imbalance in blood DC numbers are believed to support chronic HIV infection [108,115,122-124].

The frequencies of DCs are immediately lowered during acute HIV infection and maintained throughout chronic infection [125-127]. These reductions correlate with increased viral load and a continual dampening of effector cell activity. Type I IFN is produced during HIV infection, which can negatively influence DC numbers through inhibition of DC differentiation in vivo [128]. In an acute SIV infection model, rhesus macaques were observed to have an influx of DCs from blood into lymphoid tissues at viral set point. This correlated with high rates of DC apoptosis [129], thus resulting in reduction of DCs in blood and tissues [130,131]. Blood DCs have also been found to have pro-apoptotic phenotypes that correlate with reduced blood DCs and increased viral load [132].

It should be noted that many of the immunomodulatory effects of HIV-infected DCs is thought to be produced through faulty T helper cell priming, weak T cell proliferation, and cytokine production. In contrast, HIV-infected DCs are thought to have the ability to efficiently activate cytotoxic lymphocytes (CTL) [133,134], albeit lower levels of CTL have been described [135]. The HIV regulatory protein Nef has been shown to downregulate MHC class I molecules on DCs, thus potentially contributing to inhibition of CTL response [136,137]. This highlights an area in need of further studies, as inefficient accessory molecule expression may be subverted for CTL activation.

Role of immature dendritic cells in immune regulation

Another emerging consequence of faulty DC maturation is the production of regulatory mediators and development of regulatory T cells (Treg) [138,139]. Most importantly, immature DCs have been found to be sources of the cellular proliferation regulating enzyme, indoleamine 2,3- deoxyxygenase (IDO) [140,141] and the important regulatory T cell cytokines IL-10 [87,142] and TGFβ. HIV-infected DCs have been shown to produce IDO [143-145], at least partially dependent on DC-SIGN interaction [146], resulting in stimulation of T cells to produce IL-10 and TGFβ in co-culture [87,147]. This promotes regulatory responses, which are thought to facilitate dampening of immune
responses in progression from acute to chronic infection. Hence, a productive DC infection can undermine effector T cell responses to eliminate HIV.

Effector cell exhaustion and regulatory immune responses frequently occur during chronic inflammatory conditions. Type-I IFN leads to increasing expression of programmed-death ligand 1 (PD-L1) on DCs [148], which is a key mediator of effector cell senescence [149]. This is a reversible mechanism as blockade of PD-L1 on DCs can promote T cell proliferation and activation [150,151]. Additionally, these DCs can upregulate IL-10 production and downregulate IFNγ production in T cells. The production of IDO during inflammatory conditions can facilitate the conversion of Th17 cells to Treg cells [152,153]. In addition, inflammatory cytokines can upregulate TNF-related apoptosis-inducing ligand (TRAIL) or death receptors on DCs that contribute to CD4 T cell depletion [154,155].

Tregs are increased in the blood of HIV-infected individuals [156-158]. Also, Tregs increase in the lymph nodes during HIV progressions [159-161]. Tregs are potent inhibitors of HIV-specific CD4 and CD8 T cell responses [162] and this is likely a mechanism that contributes to decreased CTL response and to maintenance of HIV-infected reservoirs in the lymphoid tissues [163]. These increased Treg populations arise from both direct and indirect production of IL-10 and TGFβ during HIV infection and the production of IDO during HIV or other infections. IDO regulates proliferation and activation of T cells, but also coordinates differentiation of Tregs. These regulatory molecules either drive naïve T cell populations to differentiate into Tregs or drive conversion of existing effector cells to take on a regulatory phenotype. Production of immunoregulatory mediators directly from DCs or production from T cells stimulated by dysregulated DCs leads to production of a regulatory immune phenotype that favors HIV persistence and replication.

In addition to increased Tregs in the blood and lymphoid tissues of HIV-infected individuals, the Tregs showed heightened levels of activation [164]. These Tregs express increased levels of the contact inhibitor molecules CTLA-4 [165,166] and GITR [161], as well as secrete higher levels of the regulatory cytokine TGFβ [167]. This increase in activity not only makes Tregs more efficient in inhibiting effector molecule function, but also further promotes Treg differentiation. This positive feedback further disrupts effector cell response and promotes HIV persistence. Another mechanism of immune regulation is through adenosine deaminase activity [168]. These surface receptors play a role in HIV outcomes [169,170]. Further studies elucidating the exact viral and host components generating regulatory responses are needed to provide counteracting measures.

CONCLUDING REMARKS

As a result of intricate studies that have been performed in the last few years it is now clear that DCs play a crucial role in HIV progression. They are involved in every stage of HIV infection, from initial recognition and transmission, to effector immune response, and to chronic immune activation. Due to the dysregulation and manipulation of DCs by HIV and other co-infections, DCs are believed to contribute to establishment of viral reservoirs and immunoregulatory responses that favor chronic infection. Further investigations to determine specific mechanisms of HIV and DC interactions will provide the framework to reverse this manipulation and harness the ability of DCs to mount a robust, protective immune response. This will be beneficial in finding new targets for HIV therapeutic approaches and, as DCs are ideal for cell-based vaccines, move research closer to the overall goal of an effective HIV vaccine.

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