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A mechanistic overview of dendritic cell-mediated HIV-1 *trans* infection: the story so far

Suzanne DG Kijewski¹ and Suryaram Gummuluru^{1,*}

¹Department of Microbiology, Boston University School of Medicine, Boston, MA 02118, USA

Abstract

Despite progress in antiretroviral therapy, HIV-1 rebound after cessation of antiretroviral therapy suggests that establishment of long-term cellular reservoirs of virus is a significant barrier to functional cure. There is considerable evidence that dendritic cells (DCs) play an important role in systemic virus dissemination. Although productive infection of DCs is inefficient, DCs capture HIV-1 and transfer-captured particles to CD4⁺ T cells, a mechanism of DC-mediated HIV-1 *trans* infection. Recent findings suggest that DC-mediated *trans* infection of HIV-1 is dependent on recognition of GM3, a virus-particle-associated host-derived ligand, by CD169 expressed on DCs. In this review, we describe mechanisms of DC-mediated HIV-1 *trans* infection and discuss specifically the role of CD169 in establishing infection in CD4⁺ T cells.

Keywords

CD169; cell-associated virus transfer; dendritic cells; GM3; HIV; *trans* infection

HIV: global health threat/dissemination

It has been over 30 years since the causative agent of AIDS was discovered. In that time approximately 78 million people have been infected with HIV and 39 million people have died of AIDS-related illnesses [1]. There are 35 million people infected worldwide, ranking HIV a serious global health issue [1]. While the infection rates worldwide have decreased 38% since 2001, primarily because of significant improvements in antiretroviral therapy, there is no viable vaccine or cure for HIV infection.

On the front lines of HIV infection there is an arms race between establishment of virus infection and the immune system's ability to react and inhibit virus infection and dissemination. It is now understood that once HIV infects an individual, a reservoir of latently infected, long-lived memory CD4⁺ T cells is quickly established, creating a virus stronghold, that is maintained throughout the course of disease. Considerable research effort has been expended on finding methods to inhibit the initial establishment of virus infection

* Author for correspondence: Tel.: +1 617 414 8075; Fax: +1 617 638 4286; rgummulu@bu.edu.

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and the subsequent depletion of the immune system. This review will focus on the role of dendritic cells (DCs) in the systemic dissemination and establishment of virus infection, and, in particular, we will discuss the mechanism of DC-mediated *trans* infection of CD4⁺ T cells.

DCs & their role

In 1974, Ralph Steinman identified a new cell type in lymphoid organs of mice. Steinman and colleagues named this new cell type 'dendritic' cell due to the cell's stellate or tree-like morphology [2]. Since their discovery, DCs have been found to populate all of the peripheral mucosal tissues and are at the front line of defense for the immune system. They are not only able to launch innate immune responses upon pathogen detection but also contribute to modulating subsequent adaptive immune responses. Located in mucosal tissues, these sentinel cells sample and patrol their environment for foreign pathogens and have the ability to traffic to secondary lymph organs where they present antigens to naive CD4⁺ T cells to initiate adaptive immune responses [3].

DCs express a number of pattern recognition receptors (PRRs), which they use to detect viral and bacterial invaders. PRRs recognize common pathogen-associated molecular patterns (PAMPs) and this recognition is essential for triggering proinflammatory genes, maturation of DCs and subsequent migration to the secondary lymph nodes. DCs form immunological synapses with T cells presenting antigens through MHC complexes and signaling through costimulatory molecules [4]. Due to these features, DCs are known as professional antigen-presenting cells (APCs) that link the innate and adaptive immune responses [3]. Therefore, DCs have a central role in pathogen recognition and directing the immune response. Not surprisingly, viruses such as HIV-1 have evolved to subvert DC functions to facilitate dissemination. An example of HIV-1 subversion of DC function is the use of immunological synapses to facilitate efficient cell-associated virus spread to target CD4⁺ T cells and evade detection by PRRs within DCs. DCs have been shown to be integral in the dissemination of transmitted HIV by either transferring *de novo* replicated virus to CD4⁺ T cells or by capturing HIV-1 particles and retaining them in an infectious state that are then subsequently transmitted to CD4⁺ T cells, a mechanism of DC-mediated *trans* infection [5,6]. Transfer of captured HIV-1 particles across DC–T-cell synaptic junctions, also called 'virological synapses,' primarily because of the presence of virus particles at the DC–T-cell contact site, is a robust mechanism for establishment of productive infection in CD4⁺ T cell [7–9]. In this way HIV-1 appears to exploit DCs for dissemination. This review will focus on the role of DCs in the HIV-1 *trans* infection pathway.

DC subsets

DCs are found throughout the body, in the bloodstream and are known to traffic to secondary lymph nodes. Three main DC subsets have been described in human blood: plasmacytoid DCs (pDCs; CD11c-CD123⁺) and two types of CD11c⁺ myeloid DCs (mDCs) divided into the major BDCA1⁺ and a minor BDCA3⁺ DC subsets [10,11]. In addition to peripheral blood, DCs have also been described in mucosal tissues, such as the tissue resident Langerhans cells (LCs; CD1a⁺ E-cadherin⁺ langerin⁺) [12–14]. mDCs are a

relatively short-lived DC subset, which constantly circulate between blood and tissues, monitoring viral and bacterial pathogens. Immature mDCs are highly phagocytic and express diverse Toll-like receptors (TLRs) 2, 3, 4, and 7 that sense both extra-cellular and phagocytosed PAMPs, and upon binding their ligands trigger cytokine release that shape T-cell responses to a Th1 or Th2 phenotype [12,14].

In contrast to mDCs, pDCs are mainly tissue resident, have a longer life span and are known for their rapid response to viral pathogens by producing type I interferon [12]. Expression profile of TLRs in pDCs is divergent from that observed in mDCs. Specifically, pDCs express TLR7 and 9, which are triggered by sensing viral RNA and DNA respectively in endosomal compartments [12]. pDCs may play a key role in the immune system's control of HIV-1 infection with early IFN- α expression upon detection of viral RNA and subsequent inhibition of virus production. However, these cells may also contribute to the chronic inflammation that leads to HIV persistence [15]. The chronic inflammation seen in HIV infection may be due to continued unchecked production of IFN- α and apoptotic ligand TRAIL by activated pDCs, which has been shown to trigger apoptosis in CD4⁺ T cells during the course of infection [15,16]. While type I IFN production can result in the induction of expression of interferon-stimulated genes (ISGs) that restrict HIV-1 infection, it might also result in chronic production of chemokines, such as CXCL9 and CXCL10, that result in persistent recruitment of activated cells to tissue sites of virus infection [17]. Interestingly, pDC dysfunction has been associated with rapid disease progression in HIV-1-infected individuals [15].

Finally, LCs are DCs found in the epidermis. They are characterized by the expression of unique organelles called Birbeck granules, endosomal compartments that are exclusively expressed in LCs and are thought to be involved in antigen presentation [18–20]. LCs sample the microenvironment in the genital mucosa and in the luminal space by extending dendrites across the epithelial barrier [21]. Epidermal LCs in the genital mucosa have been shown to harbor lentiviral genomes early after transmission [21] and transfer virus to T cells [22,23]. Furthermore, productive infection of LCs *in vitro* has also been observed [24–29].

Although the above three DC subsets can be isolated from either blood or tissue samples, the cell yield is limiting for *in vitro* experiments. Due to ease of isolation and cell yield, most of the research on DCs is performed with monocyte-derived dendritic cells (moDCs) by differentiating blood-derived monocytes (CD14⁺) in the presence of the cytokines, GM-CSF and IL-4, as an *in vitro* model of conventional mDCs [30].

DCs & HIV: *cis* infection

DCs have long been implicated in facilitating HIV transmission and dissemination at mucosal surfaces [6,31–32] via mechanisms that have yet to be clearly defined. Although moDCs are susceptible to infection by HIV-1, productive infection of moDCs *in vitro* and *in vivo* is 10–100-fold lower than CD4⁺ T cells [6]. There are many pre- and postentry restrictions that lead to this deficiency. The first of these restrictions is the low levels of entry receptors CD4 and CCR5 on DCs [33]. As a result, virions are less able to attach, fuse and enter moDCs. The virions that are able to fuse and enter are confronted with many IFN-

dependent and independent viral restrictions that block virus replication at early steps in the viral life cycle [34–38]. For instance, IFN- α -inducible APOBEC3G can block virus replication at the reverse transcription step via its cytidine deaminase activity that results in the introduction of G to A mutations in HIV genomes [37], a result of which is the introduction of stop codons or nonsense mutations in the viral open reading frames. HIV-1 encodes a viral protein called viral infectivity factor, Vif, which counteracts APOBEC3G restriction by recruiting cullin5-elonginB/C ubiquitin ligase complex and inducing polyubiquitylation and degradation of Apobec3G [39–41]. In 2011, two independent groups discovered an additional restriction factor of HIV-1 named SAMHD1 [42,43] that is constitutively expressed in monocytes, resting T cells, moDCs and macrophages [42–45]. Furthermore, expression of SAMHD1 can also be induced upon exposure to type I IFNs [46–48]. SAMHD1 restricts HIV-1 replication by hydrolyzing deoxyribonucleotides in cells, thus reducing the pool of nucleotides available for reverse transcription [42,43]. In addition to its dNTPase activity, SAMHD1 has also been recently described to encode RNase activity, which may also contribute to its ability to restrict HIV-1 infection in moDCs by degrading the incoming viral genomic RNA [49]. Interestingly, HIV-2 and primate lentiviruses encode Vpx, which through the CUL4/DCAF1 E3 ubiquitin ligase complex targets SAMHD1 for proteasomal degradation, allowing for productive infection of DCs [43].

Despite the pre- and postentry restrictions to virus replication, low levels of productive HIV-1 infection of moDCs and mDCs have been reported *in vitro* [50–52]. Furthermore, *de novo* synthesized HIV-1 particles can be transferred to CD4⁺ T cells with high efficiency across viro-logical synapses [52]. Interestingly, it has been hypothesized that establishment of productive infection of moDCs and *de novo* expression of Gag in moDCs triggers activation of a yet-to-be identified cytoplasmic sensing mechanism that results in type I IFN response and maturation of moDCs [53]. Whether these late antiviral responses also inhibit virus replication and dissemination is of active research interest [53–55] and remains unclear. Therefore, due to the listed restrictions to virus replication, *cis*-infection of moDCs by HIV-1 presumably occurs at a low efficiency and possibly leads to immune detection. However, the moDC-mediated *trans* infection pathway might play a key role in HIV-1 dissemination.

First evidence of *trans* infection

In 1992, Cameron and colleagues made the first observation of moDC-mediated HIV-1 *trans* infection and the role of DCs as a catalyst of virus dissemination [56]. Interestingly, the authors were able to demonstrate that while moDCs themselves were not infected, they however played a key role in transferring infectious virus particles to CD4⁺ T cells. To prove virus-exposed but uninfected DCs were able to transfer virus to T cells, the authors used xenogeneic cocultures of either murine bone marrow derived DCs or human moDCs pulsed with HIV-1 and combined with either murine or human CD4⁺ T cells. Interestingly, both human and murine DCs transferred HIV-1 to human CD4⁺ T cells [56]. These data suggested that HIV-1, an obligate intracellular parasite, might exploit DCs to spread efficiently and evade immune sensing by not infecting DCs themselves.

Receptors

After the discovery of this new role of moDCs, identification of the receptor/ligand interaction responsible for moDC-mediated transfer of virus to CD4⁺ T cells was of major research interest. HIV-1 primarily interacts with its environment via the viral envelope, the outermost structure of the virion, which consists of projecting glycoprotein (gp120) spikes on a membrane derived from an infected host cell. Using cryoelectron microscopy, Zhu and colleagues discovered that on average wild-type HIV-1 particles express 7–14 glycoprotein spikes on its surface [57]. Much smaller in number than previously hypothesized, these limited number of spikes leave a large portion of the virion membrane devoid of virus-encoded determinants. Rather, numerous host-derived factors are incorporated in the lipid bilayer of the virion and have been hypothesized to play important roles in HIV-1 interactions with host cells [58,59]. Thus, receptors that recognize both viral and host-derived determinants have been hypothesized to mediate virus interactions with moDCs. First, we will focus on receptors that interact with HIV-1 gp120.

Beyond the primary receptor CD4 and coreceptors CCR5 and CXCR4 that mediate virus fusion and entry, there are a multitude of receptors that can act as attachment factors for HIV-1 gp120 (**Table 1**) and enhance virus capture without triggering virus particle fusion. Many of these attachment factors are C-type lectins, or calcium-dependent glycan binding proteins. These receptors are transmembrane proteins that are commonly involved in cell-to-cell adhesion and immune signaling [60]. Additionally, this family of receptors all contain carbohydrate recognition domains (CRDs), which facilitate the high affinity binding of high mannose oligosaccharides found on glycoproteins of enveloped viruses [60].

In 1992, Curtis *et al.* used an expression cloning strategy to screen a placental cDNA library for gp120 binding proteins independent of CD4 and identified a new C-type lectin [71]. Following this initial finding, Geijtenbeek and colleagues described a C-type lectin expressed in DCs that bound HIV-1 and assigned it the name DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin) [61]. Interestingly, the sequence of DC-SIGN was identical to the C-type lectin Curtis *et al.* had previously identified [61]. Using both a Raji B cell line expressing DC-SIGN and antibodies to block DC-SIGN function in DCs, the authors went on to demonstrate the ability of DC-SIGN to specifically capture HIV-1 particles by binding mannosylated gp120 and transfer virions to CD4⁺ T cells [61]. While DC-SIGN was originally believed to be the essential DC-specific receptor necessary for capture and *trans* infection of HIV-1 to CD4⁺ T cells, subsequent findings suggest that other C-type lectin receptors such as mannose receptor, langerin, and DC immune receptor (DCIR; also called Lectin-like immunoreceptor [LLIR]) expressed on dermal DCs, LCs, and blood mDCs could also mediate HIV-1 *trans* infection [21,63–64]. From all of these findings (summarized in **Table 1**), it is clear that many C-type lectins expressed on varied DC subsets have the capability to bind mannosylated gp120, though the relative contribution of these lectins to HIV-1 capture and *trans* infection *in vivo* remains to be determined.

Beyond C-type lectins there are additional HIV-1 attachment factors on DCs that can capture and transfer HIV-1 to CD4⁺T cells. Syndecan-3 is a heparin sulfate proteoglycan

that binds gp120, presumably via charge-based interactions [66]. In addition, galactosylceramide (GalCer), expressed on both mDCs circulating in blood and mucosal tissue resident DCs, has also been shown to play a role in mediating virus transfer from DCs to autologous CD4⁺ T cells [68]. Thus, despite the relatively low expression of gp120 on the virus surface, it is evident that sentinel DCs have many receptors that have the ability to specifically bind HIV-1 gp120. Notwithstanding the above list of HIV-1 attachment factors, there must be a hierarchy of physiologically relevant interactions that determine the ultimate fate of the virus particles upon capture by DCs.

While it has been hypothesized that the virus captured by gp120-binding receptors might play a role in evasion of HIV-1 from innate immune sensing mechanisms and trafficking of captured virus particles to T cells [61], capture of HIV-1 by DC-SIGN can result in virus particle trafficking to a degradative endosomal compartment and enhanced MHC I presentation of viral antigens [72,73]. Furthermore, capture of virus by langerin on LCs can result in virion targeting to Birbeck granules for degradation [74]. Alternatively, it has been shown that binding of gp120 by CLRs including DC-SIGN can trigger an IL-10 response, which may play a role in immune evasion by downregulating MHC-II and costimulatory molecules [75–77]. Thus, as the sole virus determinant exposed on the surface, gp120 is subject to detection by a number of receptors that might impact innate responses. Whether these receptors that capture HIV-1 particles in a gp120-dependent manner enable virus dissemination or play an integral role in DC-intrinsic innate responses remains to be determined. In contrast to virus-encoded determinants, incorporation of host determinants might facilitate virus capture by DCs and result in evasion from immune sensing or degradation of virus particles.

GM3/gangliosides

The majority of known receptors that capture virus do so through an interaction with HIV-1 gp120 or gp41 (**Table 1**). However, capture of HIV-1 particles by DCs can occur even in the presence of inhibitors that block all of the gp120-dependent interactions [78]. Furthermore, it has long been appreciated that while maturation of DCs downregulates cell-surface expression of DC-SIGN and phagocytosis, capture of HIV-1 particles is dramatically enhanced upon maturation of moDCs [78–80]. Interestingly, gp120-deficient HIV-1 particles can also be captured by moDCs at efficiency similar to that observed with gp120-containing virus particles, and capture of gp120-deficient HIV-1 particles was enhanced upon maturation of both moDCs and blood mDCs [78–81]. These findings suggested that in addition to gp120, host determinant(s) incorporated into the lipid bilayer of HIV-1 particles can also mediate efficient virus interactions with DCs. Surprisingly, the host determinant necessary for HIV-1 capture by DCs is a glycosphingolipid (GSL) [79,81]. GSLs comprise one of three families of complex sphingolipids derived from a common ceramide backbone [82]. While these molecules make up approximately 5% of the overall plasma membrane lipid composition, they are highly enriched in lipid rafts and are selectively incorporated in HIV-1 particles as they bud from the host cell [82,83].

In 2012, using independent experimental approaches, the Gummuluru and Martinez-Picado laboratories identified GSLs containing terminal α -2,3 sialic acid residues, such as GM3

(trisialotetrahexosylganglioside), as the host determinant(s) incorporated within virus particle membrane, necessary for gp120-independent HIV-1 capture by mature moDCs [84,85]. Specific requirement for GM3 was demonstrated by competitive inhibition of HIV-1 capture by mature moDCs only by virus-sized liposomes or giant unilamellar vesicles bearing α -2,3 sialylated GSLs but not by α -2,6 or α -2,8 sialylated or asialo-GSLs [84,85]. Additionally, specific depletion of GM3, from virus particle membrane by deriving HIV-1 particles from virus producer cells with targeted deficiency in GM3 synthase, significantly reduced virus capture and mature moDC-mediated *trans* infection [85]. Therefore, mature DC capture and *trans* infection of HIV-1 are independent of gp120 and dependent on GM3 expression on the viral membrane. GM3 is expressed in the plasma membranes of macrophages and CD4⁺ T cells [86,87], the primary virus reservoirs *in vivo*. Interestingly, GM3 expression in plasma-membrane lipid rafts is upregulated upon T-cell or macrophage activation [83], resulting in increased levels of GM3 incorporated into budding virions derived from these cells [85]. Enhancement of GM3 levels in HIV-1 virions can result in significant increases in virus capture by mature DCs [83,85] with subsequent enhancements to mature DC-mediated *trans* infection of T cells.

Importantly, incorporation of host-cell-derived gangliosides, such as GM3, within the virus particle membrane is dependent on HIV-1 Gag-directed budding from GSL-enriched lipid raft plasma membrane microdomains [88,89]. Choice of plasma membrane microdomains preferentially accessed for virus assembly is governed by sequences in the matrix (MA) domain of HIV-1 Gag. Hence, mutations in the N-terminal basic region or deletion of the membrane-targeting domain of HIV-1 matrix (MA) that alters virus assembly site to intracellular membranes result in reduced GM3 incorporation in MA-deficient viruses and diminished virus capture by mature moDCs [90]. Interestingly, diverse enveloped viruses including Nipah and Hendra hemorrhagic fever viruses (paramyxoviruses), and murine leukemia virus (MLV; a retrovirus), incorporate sialylated GSLs in the virus particle membranes and are captured by mature moDCs [69,90–91], suggesting that GSL-dependent interactions with mature moDCs might be a conserved mechanism of DC-mediated enveloped virus dissemination [90].

CD169

Identification of GM3 as the virus-particle-associated ligand necessary for virus capture by moDC and mDCs was critical to the rapid discovery of CD169 or Siglec1 as the receptor responsible for DC-mediated HIV-1 *trans* infection [69,70]. CD169 satisfied all the requirements of DC-associated HIV-1 attachment factor: CD169 is expressed exclusively on myeloid cells, such as macrophages and DCs [92,93]. While peripheral blood monocytes do not express CD169, expression is induced upon differentiation of monocytes into macrophages or moDCs [69]; CD169 preferentially binds to glycoconjugates with terminal α -2,3 sialic-acid residues [93]; and expression of CD169, a type I IFN-inducible protein, is enhanced on mature moDCs, thus accounting for the dramatic increase in HIV-1 capture by moDCs matured by TLR ligands that initiate TRIF-dependent signaling cascades resulting in type I IFN production over that observed with immature moDCs [69].

CD169, also called sialoadhesin or Siglec 1, a member of the immunoglobulin-like lectin superfamily [30], was first characterized as a sialic-acid-dependent receptor of sheep erythrocytes [92,94] expressed on mouse bone marrow derived and tissue-resident macrophages. CD169 is a myeloid-cell-specific activation marker, whose expression is enhanced on inflammatory monocytes and tissue-resident macrophages in chronic inflammatory diseases [92,95–98]. All Siglecs, including CD169, have been implicated to play roles in cell-to-cell adhesion and initiate cell signaling to dampen or activate immune responses [99]. Furthermore, Siglecs have a homologous amino-terminal immunoglobulin (Ig)-like V-set domain that contains the sialic acid binding site and a variable number (2–17) of Ig-like C2 set domains. CD169 is the largest member of the Siglec family with 17 Ig-like domains in the amino terminus, but unlike other members of the Siglec family, lacks signaling domains in the cytoplasmic tail [100,101]. It has been speculated that the function of the 17 Ig-like domains is to extend the sialic-acid binding site of the molecule away from the plasma membrane, favoring cell–cell interactions [92]. The extended extracellular domain of CD169 is also hypothesized to favor cell–pathogen interactions in *trans* [93].

As seen in **Table 1**, CD169 is the one attachment factor that has been reported to bind both sialylated HIV-1 envelope gp120 and sialylated GSLs [85,97,102]. While gp120-dependent capture of HIV-1 particles by CD169 was primarily observed on macrophages [102], knockdown of CD169 strongly abrogated capture of virus-like particles (VLPs), gp120-deficient HIV-1 and infectious HIV-1 by mature moDCs, pointing to the importance of CD169 in gp120-independent capture of HIV-1 particles by mature DCs [69]. Importantly, mature moDC-mediated *trans* infection to CD4⁺ T cells was attenuated only when capture of HIV-1 by CD169 was blocked by usage of either shRNAs to knock down CD169 expression or when CD169 function was blocked by antibodies [69]. Furthermore, reduction in GM3 content of HIV-1 particles by targeting virus assembly to GM3-deficient intracellular membranes [90] or by pharmacologic manipulation of GSL biosynthesis in virus producer cells [69,70] resulted in reduced virus capture by CD169 and reduced access to CD169-mediated *trans* infection pathway. These findings suggest that GM3 (GSL)-dependent recognition of HIV-1 particles by CD169 is the critical interaction that drives mature DC-mediated dissemination of HIV-1 infection to CD4⁺ T cells (see **Figure 1**).

HIV-1 trafficking in DCs & *trans* infection

In 2003, McDonald *et al.* used live-cell microscopy to demonstrate recruitment of both captured virus and receptors to the junction between mature DCs and T cells [103], called a virological synapse due to the structural similarity to an immunological synapse [9]. There is ongoing debate and investigation into whether HIV-1 virions are internalized postcapture within endosomal compartments prior to trafficking to virological synapses and transfer to CD4⁺ T cells. A number of previous studies have tried to address this question by treating virus-exposed moDCs to proteases to determine whether captured virions are at the surface and remain sensitive or were internalized and protected from protease treatment [81,103–106]. Though these tests demonstrated that moDC-associated virus is partially protected from protease treatment, the nature of the compartment harboring captured virus has proven elusive. Additionally, immunofluorescence microscopy approaches suggest that virus particles are routed to a tetraspanin (CD81⁺, CD82⁺ and CD9⁺)-rich compartment with a pH

of 6.2 following virus capture [107], prior to retrograde transfer to the mature DC–T-cell virological synapse. Interestingly, virus capture by CD169 results in localization of HIV-1 particles within minutes in a CD169⁺ CD81⁺ pocket-like structure at the cell periphery in mature moDCs, and upon initiation of mature DC–T-cell contacts is trafficked to the DC–T-cell junctions [69,70]. Additionally, this CD81⁺ pocket-like structure, harboring virus in mature moDCs, appears to be induced by interaction between HIV-1 and CD169 rather than be constitutively present. The current hypothesis is that HIV-1 traffics to an invaginated pocket which appears internal but is actually accessible to the surface of the mature moDC [Akiyama H *et al.*, Unpublished Data] [108]. This pocket appears to be accessible to small HIV-1 neutralizing agents such as soluble CD4 (sCD4) [Akiyama H *et al.*, Unpublished Data] or Fab fragments of anti-gp120 broadly neutralizing antibodies (bNAbs) [109] but restricts entry to larger sized antibodies. Therefore, we hypothesize that virus is kept protected from degradation within nonacidic compartments in an invagination contiguous with the mature DC surface but protected from detection and neutralization by anti-gp120 bNAbs (modeled in **Figure 1**). These findings suggest that CD169 might also be responsible for routing captured virus particles to localization within the CD81⁺ compartment and to mature DC–T-cell virological synapse. Interestingly, CD169 has no known cytoplasmic tyrosine-based signaling or endocytic motifs. Future studies will need to address the molecular details of CD169-mediated HIV-1 trafficking in mature DCs.

Use of sophisticated microscopy techniques such as ion abrasion scanning electron microscopy, electron tomography and super-resolution light microscopy have provided fine structural details of the mature moDC–T-cell virological synapse [110]. These approaches have provided stunning visual evidence of sheet-like dendrites emanating from mature moDC surface enveloping CD4⁺ T cells during the establishment of the virological synapse [110]. With the ion abrasion SEM technology, Felts *et al.* were also able to image filopodial extensions from T cells which were shown to extend into the mature moDC folds containing virus, and virions ‘surfing’ on these filopodial extensions to reach T-cell surface [110]. This conformation of folds and filo-podia provides a pocket or microenvironment, which previously was thought to be internal.

The importance of the virus path centers on the search for effective neutralizing antibodies in disrupting cell-to-cell transmission. The search for effective bNAbs has been at the crux of designing a successful HIV vaccine. Large strides have been made in designing effective bNAbs by mapping the origin of antibodies produced in HIV⁺ patients [111]. Antibodies targeting particular areas of the HIV-1 envelope, specifically the variable regions 1 and 2, have recently shown great promise in both preventive and therapeutic approaches [112]. While most studies have demonstrated potent efficacy of bNAbs against cell-free virus infections, neutralization potency of bNAbs is attenuated in cell-associated virus challenges [109,113,114] presumably because bNAbs are restricted from accessing HIV-1 particles transmitted across virological synapses [109]. Since cell-to-cell transmission of HIV-1 likely accounts for significant viral spread *in vivo*, higher concentration of neutralizing antibodies might be required to achieve significant suppression of ongoing virus replication. To this end, the Baltimore laboratory has described an adeno-associated virus vector strategy for achieving high concentrations of bNAbs *in vivo*, with demonstrated efficacy against HIV-1

mucosal transmission in a humanized mouse model of infection [115,116]. Further understanding of the virus trafficking and localization within DCs will allow for intelligent design of inhibitors to target DC-mediated HIV-1 *trans* infection that will complement bNAb therapeutic. In particular, these inhibitors will need to be directed at the virus capture receptor, CD169, or be rationally designed to access the mature DC–T-cell virological synapses and block *trans* infection.

Ongoing challenges: *in vivo*

There are ongoing challenges in determining the *in vivo* relevance of DC-mediated *trans* infection and how much this pathway contributes to virus dissemination. Determining the level of *trans* infection *in vivo* is critical for developing new therapies to potentially inhibit capture and *trans* infection of virus by DCs. The studies cited throughout this review have used *in vitro* studies to model what may occur *in vivo*. Current options to investigate *in vivo* relevance are animal models including experimental infections of humanized mice and nonhuman primates (Asian macaques) as well as human tissue explants. Each of these model systems has their limitations in recapitulating HIV-1 pathogenesis in humans. Most humanized mouse models fail to incorporate human myeloid cells in significant numbers and are thus not suitable for systematically testing the role of DCs in HIV-1 pathogenesis [117]. Experimental infection of Asian macaques (which are not natural hosts for primate lentiviruses) by SIV strains have shown that DCs in vaginal epithelium and lamina propria are infected early in infection and could possibly migrate to T-cell-rich areas [118]. Other macaque studies have found lack of infection of DCs in the genital mucosa, but point to the likelihood that DCs will capture virus without being infected and subsequently migrate to lymph nodes [119]. Notwithstanding the ability to induce an AIDS-like illness in Asian macaques with select SIVmac strains, there is significant sequence diversity between SIVmac and HIV-1 and the presence of additional open reading frames within the viral genome (SIVmac encodes an additional protein, Vpx, that is not present in HIV-1 genome) that might differentially impact virus interactions with host cells. A limiting factor with tissue explants is that DCs tend to migrate out of explant tissues making it difficult to investigate later time points postvirus inoculation.

Interestingly, in a recently published clinical study, DCs from HIV-1 infected nonprogressors were unable to mediate HIV-1 *trans* infection of CD4⁺ T cells [120]. This deficiency was mapped to increased expression of the reverse cholesterol transporter, ABCA1, in DCs resulting in reduced cellular levels of cholesterol [120]. These *in vivo* findings nicely corroborate previous *in vitro* observations that demonstrated robust decreases in HIV-1 capture and *trans* infection by DCs upon cholesterol depletion [121,122]. It is entirely possible that lowering cholesterol levels in DCs either reduces cell-surface CD169 expression, prevents clustering of CD169 in plasma membrane microdomains for optimal virus interactions or reduces the probability of formation of DC–T-cell virological synapses. Future *in vitro* studies will need to address the molecular basis for the observed clinical benefit of lowering cholesterol levels in DCs.

The relevance of CD169 capture and *trans* infection has been supported by multiple studies that investigated the level of CD169 on circulating monocytes in HIV-1 infected patients

[97,98]. Individuals with high viral loads and those that had progressed to AIDS had increased expression of CD169 on their circulating monocytes compared with patients with undetectable viral loads [97,98]. The authors point to upregulation of CD169 on monocytes due to early induction of type I IFN responses and suggest the possibility that CD169⁺ monocytes are enhancing systemic dissemination of virus [97]. In a recent study, TGF- β , the most abundant cytokine found in semen, was shown to increase expression of CD169 on moDCs resulting in increased virus capture suggesting an *in vivo* role in sexual transmission [123]. Additionally, CD169 expression in lymphatic tissues is conserved across species and has been shown to be prevalent in secondary lymphoid organs, tissue sites that are integral to HIV-1 dissemination [92]. Future studies using better animal models for infection will have to examine whether blocking CD169 is able to diminish spread of HIV-1 to CD4⁺ target cells and decrease establishment of infection *in vivo*.

Conclusion

HIV-1 exploits DC biology to evade the immune system and disseminate virus to target CD4⁺ T cells. This CD169-dependent DC-mediated *trans* infection pathway might be an efficient method of HIV-1 dissemination and contribute significantly to viral pathogenesis.

Future perspective

With approximately 35 million people living with HIV and an additional two million new infections per year, it is essential to find strategies to inhibit virus dissemination. In order to combat viral infection and slow its attack on the immune system, it is essential to understand the techniques HIV-1 uses to parasitize DC functions and disrupt these strategies.

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EXECUTIVE SUMMARY

- Despite progress in antiretroviral therapy over the past 30 years, there is no cure or vaccine for HIV and it continues to be a global health issue.
- Although dendritic cells (DCs) are not infected at high levels, they play an important role in HIV-1 dissemination to CD4⁺ T cells.
- Though there are a myriad of receptors expressed on DCs which bind HIV-1 gp120, contribution of these receptors to DC-mediated trans infection pathway is minimal.
- trans infection of HIV-1 from DCs to T cells is dependent on GM3 - CD169 interactions, a gp120-independent virus–host cell interaction.
- Capture of virus by CD169 results in HIV-1 trafficking to a surface-accessible invaginated pocket prior to transfer to CD4⁺ T cells across virological synapses, but requires more study.
- Targeting HIV trans infection may be an important strategy to slow HIV dissemination and establishment of a latent reservoir.

Figure 1. Differences in HIV-1 capture and *trans* infection by immature and mature dendritic cells

Immature DCs express high levels of DC-SIGN and other C-type lectin receptors relative to mature DCs. Most of the virus particles captured by immature DCs are targeted to lysosomal compartments for degradation. By contrast, mature DCs express high levels of CD169 and GM3-dependent HIV-1 capture by CD169 results in virus particle trafficking to nonlysosomal compartments at the cell periphery that remain contiguous with the extracellular milieu. HIV-1 particles captured by CD169 on mature DCs are transferred to CD4⁺ T cells across virological synapses. DC: Dendritic cell; DCIR: Dendritic cell immune receptor; DC-SIGN: Dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin; GSL: Glycosphingolipid; HSPG: Heparan sulfate proteoglycan.

Table 1

Receptors that capture HIV-1.

Receptor	Ligand	Subset	Ref.
DC-SIGN	HIV Env gp120	Subepithelial rectal and lamina propria DCs	[61,62]
Mannose	HIV Env gp120	Dermal DCs	[63]
Langerin	HIV Env gp120	Langerhans cells in the skin in genital epithelia	[21,63]
DCIR	HIV Env gp120	Immature moDCs	[64]
HSPG	HIV Env gp120	Immature moDCs	[65]
Syndecans	HIV Env gp120	Immature moDCs	[66,67]
GSL (galactosylceramide)	HIV Env gp41 and gp120	moDCs, myeloid DCs, mucosal tissue resident DCs	[68]
CD169/Siglec1	GM3	Mature moDCs	[69,70]

DC: Dendritic cell; DCIR: Dendritic cell immune receptor; DC-SIGN: Dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin; GSL: Glycosphingolipid; HSPG: Heparan sulfate proteoglycan; moDC: Monocyte-derived dendritic cell.