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RESEARCH ARTICLE

Antigen Presenting Cell-Mediated HIV-1 *Trans* Infection in the Establishment and Maintenance of the Viral Reservoir

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ABSTRACT

Despite potent antiretroviral therapy (ART), an HIV-1 reservoir persists that represents a major barrier to a cure. Understanding the mechanisms by which the HIV-1 reservoir is established and maintained is critical for the discovery of effective treatments to significantly reduce or eliminate the viral reservoir. In addition to *cis* infection, in which HIV-1 directly infects target $CD4^+$ T cells, cell-to-cell transmission, or *trans* infection, can also occur. HIV-1 *trans* infection is significantly more efficient than *cis* infection, mostly due to the occurrence of multiple infections per cell during transfer. Additionally, *trans* infection is efficient even in the presence of ART and/or neutralizing antibodies. Cell-to-cell transmission is mediated by $CD4^+$ T cells and professional antigen presenting cells (APC). Here we focus on APC, i.e., myeloid dendritic cells, B lymphocytes, and monocytes/macrophages, that bind, internalize, and transfer HIV-1 to target $CD4^+$ T cells via various proposed mechanisms. We assess the potential impact of *trans* infection on the establishment and maintenance of the HIV-1 reservoir including its role in disease progression. We consider the natural interactions between APC and $CD4^+$ T cells *in vivo* that HIV-1 may hijack, allowing for the highly efficient *trans* infection of $CD4^+$ T cells, maintaining the viral reservoirs in tissue despite undetectable plasma viral loads in peripheral blood. We propose that these modes of viral pathogenesis need to be addressed in potential cure strategies to ensure eradication of the viral reservoir.

Introduction

Following the advent of antiretroviral therapy (ART), people living with HIV-1 can suppress plasma viremia to undetectable levels and live relatively normal and healthy lives. However, reservoirs of persistent HIV-1 infection endure and are thought to represent a major barrier to a cure.[1-9] Typically, the HIV-1 reservoir is defined as latently infected resting CD4+ T cells, which are impervious to ART.[1,2,4] This latent HIV-1 reservoir is formed early during viral infection likely via one of two (or both) mechanisms: (1) some HIV-1 infected activated CD4+ T cells survive and subsequently return to a memory phenotype[2,3,5,7,10-12]; and/or (2) HIV-1 directly infects resting memory CD4+ T cells, particularly in the presence of the CCR7 ligands CCL19 or CCL21.[13] These chemokines, which are constitutively expressed in lymphoid organs, do not increase expression of T cell activation or proliferation markers but increase the permissiveness of these cells to HIV-1 infection.[13] The latent HIV-1 reservoir in resting CD4+ T cells is maintained due to the long half-lives of these cells and via homeostatic proliferation.[1,5,6] While CD4+ T cells represent a major reservoir of HIV-1 infection, other cellular and anatomical reservoirs exist, including macrophages, myeloid dendritic cells and astrocytes, as well as the central nervous system, gastrointestinal tract, lymphatics and urinary tract, respectively.[5,8,9,14] Reservoirs in these privileged or complex sites need to be acknowledged in potential cure strategies as many clinical studies are focused only on the circulating immune cells in the peripheral blood.

Maintenance of the HIV-1 reservoirs during suppressive ART may also occur as a result of suboptimal ART levels in some anatomical sites, allowing for cryptic viral replication and therefore new infections, although this hypothesis is controversial.[6,8,15,16] Cell-to-cell transfer of HIV-1, or *trans* infection, has also been implicated in the establishment and maintenance of the latent viral reservoir.[6,7,17-20] Compared to direct, *cis* infection, *trans* infection can be 10- to 1000-fold more efficient and may lead to multiple infections per cell likely due to a high multiplicity of infection (MOI) at the site of cell-to-cell contact.[17,18,20-22] Cell-to-cell transmission of HIV-1 utilizes the natural interactions between immune cells to allow for HIV-1 to be transferred into uninfected cells leading to virus infection.[6,20] Of note, HIV-1 *trans* infection is insensitive to antiviral drugs and neutralizing antibodies.[6,7,17,21,23] Cell-to-cell HIV-1 transmission has been shown between infected and uninfected CD4+ T cells as well as being mediated by mast cells and basophils.[19,24,25] Infection of CD4+ T cells can also be mediated by professional antigen presenting cells (APC), i.e., dendritic cells (DC), B lymphocytes, and monocytes/macrophages, and is termed APC-mediated *trans* infection.[26-29] In this review, we focus on DC-, B cell-, and macrophage-mediated *trans* infection of CD4+ T cells and consider the impact that these events have on the establishment and maintenance of the viral reservoirs. Additionally, we speculate as to how APC-mediated *trans* infection may be involved in disease progression, emphasizing the importance of addressing this mechanism of HIV-1 pathogenesis when investigating potential cure strategies.

DC-mediated HIV-1 *trans* infection

Myeloid DC express the HIV-1 receptor CD4 and the co-receptors CCR5 and CXCR4, and therefore can be productively infected by both R5- and X4-tropic HIV-1 strains.[28,30-32] Due to higher levels of CCR5 expression, R5-tropic HIV-1 strains are more efficient at infecting myeloid DC.[30] While DC can be productively infected, HIV-1 replication in these cells is much lower, as is the frequency of HIV-1 infected DCs *in vivo* compared to CD4+ T cells.[30,31,33] However, myeloid DC can bind HIV-1 gp120 independent of CD4 and CCR5/CXCR4 and subsequently *trans* infect CD4+ T cell targets.[28,30,32,34-38] C-type lectin receptors on DC, including the DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN), Langerin, DC-Immunoreceptor (DCIR), and mannose receptor (MR), can facilitate this process.[28,30-32,38-41] DC-SIGN, which has been studied extensively for its role in DC-mediated *trans* infection, binds and internalizes HIV-1 via endocytosis into early endosomes before it is transferred to target CD4+ T cells.[6,18,28,30-32,38,39,41-43] With high levels of DC-SIGN expression on their surface, monocyte-derived immature DC (iDC) can bind HIV-1 through gp120, which has a higher affinity for DC-SIGN compared to the CD4 receptor.[39,44,45] Of note, iDC derived from CD14+ monocytes cultured with granulocyte-macrophage colony-stimulating factor (GM-CSF) and recombinant human interleukin-4 (IL-4) can *trans* infect CD4+ T cells following incubation with a sub-infectious dose of HIV-1_{BaL} (CCR5-tropic HIV-1 strain), i.e., a multiplicity of infection (MOI) that is not able to productively infect CD4+ T cells in *cis* (MOI 10^{-3}). However, these iDC, while able to *trans* infect total CD4+ T cells, do so at a significantly lower efficiency than B cells. Additionally, while they can *trans* infect central memory CD4+ T cells (T_{CM}), they are inefficient at *trans* infecting naïve CD4+ T cells (T_N), unlike B cells, which we discuss in more detail below.[27]

Mature DC (mDC) have also been implicated in *trans* infection where they store HIV-1 in endosomal compartments or invaginations of the plasma membrane before transfer to $CD4^+$ T cell targets.[43,46,47] Similar to iDC, CD40L-matured DC are inefficient at mediating HIV-1 *trans* infection of T_N cells.[27] While DC-SIGN expression is reduced upon DC maturation[22,43,48,49], HIV-1 capture and transfer efficiency is increased.[37,49] Unlike HIV-1 capture by iDC via DC-SIGN, capture by mDC does not require viral envelope glycoproteins.[43,50,51] Instead, the sialic-acid binding immunoglobulin-like lectin 1 (Siglec-1) receptor is implicated in mDC-mediated *trans* infection. Siglec-1, which is highly expressed on mDC, recognizes sialyllactose-containing membrane gangliosides on HIV-1, specifically GM3, a virus particle-associated ligand. Siglec-1 expression correlates with HIV-1 capture and *trans* infection of $CD4^+$ T cells while anti-Siglec-1 antibodies inhibit HIV-1 capture.[7,18,22,32,36,37,49,52-55] Furthermore, it was found that Siglec-1 is upregulated on myeloid DCs matured by lipopolysaccharide (LPS) or type I interferon, and using infectious HIV-1, LPS-matured mDC capture significantly more virus than iDC.[37,43,49]

iDC have long been implicated in the formation of the viral reservoir during transmission events as they can capture HIV-1 via DC-SIGN at the site of infection, i.e., rectal or vaginal mucosa after sexual exposure, and subsequently travel to secondary lymphoid tissues to mount an immune response where they have many interactions with target $CD4^+$ T cells.[6,22,37-39,41-43,45] Similarly, mDC in lymphoid tissues have numerous interactions with uninfected $CD4^+$ T cells which densely populate these areas. Lymph nodes (LN) removed from an HIV-1-infected individual before and after initiation of ART showed Siglec-1 positive cells in the perivascular, sub-capsular, and perifollicular areas with a similar pattern before and after ART.[36] Knowing that cell-to-cell HIV-1 transmission can occur even during ART and that there may be lower ART concentrations in lymphoid tissues suggests that mDC-mediated *trans* infection via Siglec-1 may occur *in vivo*. Importantly, DC may be able to *trans* infect multiple $CD4^+$ T cells during exploratory contacts as they search for their cognate interaction.[49] Additionally, DC-mediated *trans* infection that occurs during a cognate interaction with antigen-specific $CD4^+$ T cells is highly efficient.[56] Considering lymphoid tissues are a major site of HIV-1 replication and that DC migrate to these tissues where they repeatedly establish interactions with $CD4^+$ T cells implies the role DC-mediated HIV-1 *trans* infection can play in both the establishment and maintenance of the viral reservoir.

B cell-mediated HIV-1 *trans* infection

Unlike DC and macrophages, B cells cannot be productively infected with HIV-1. While they express high levels of CXCR4, they express only low levels of CD4 and lack CCR5 expression.[57,58] B cells were first implicated in *trans* infection of $CD4^+$ T cells through binding of HIV-1 immune complexes (HIV-1 IC) via CD21 (Complement Receptor-2). Through HIV-1 IC and CD21 binding, virus remained on the cell surface but was infectious for target $CD4^+$ T cells with *trans* infection being significantly reduced when anti-CD21 antibodies were used before HIV-1 IC incubation.[59-63] As previously described, DC can bind HIV-1 via DC-SIGN and subsequently *trans* infect $CD4^+$ T cells. Interestingly, a subset of B cells from the blood and tonsils of uninfected individuals express DC-SIGN and expression is increased after stimulation with CD40L and IL-4. These activated B cells can also bind and internalize both CCR5- and CXCR4-tropic HIV-1 via DC-SIGN and mediate *trans* infection of $CD4^+$ T cells in a similar manner to DC. When DC-SIGN is blocked prior to HIV-1 incubation, *trans* infection is significantly inhibited.[58] This work provides an HIV-1 IC-independent mechanism for *trans* infection of $CD4^+$ T cells by B cells.

T_N cells were previously thought to contribute minimally to the viral reservoir due to their significantly lower frequency of infection, as measured by HIV-1 DNA copies, compared to the memory $CD4^+$ T cell subsets.[1,64] However, Zerbato *et al* recently showed, both *in vitro* and *ex vivo*, that despite harboring less HIV-1 DNA than T_{CM} cells, T_N cells produce as much if not more virus after reactivation with latency reversing agents.[65,66] A possible explanation for this finding is that the contribution of intact HIV-1 proviruses in T_N cells is greater than in T_{CM} cells.[67,68] Additionally, the proviral sequences in T_N cells are unique, with the number of clones increasing as cells differentiate from T_N to effector memory, in which they were mainly clonal, suggesting T_N cells repopulate the effector memory viral reservoir.[68,69] These findings suggest that T_N cells contribute more to the reservoir than initially thought. Furthermore, although T_N cells are extremely resistant to *cis* infection with CCR5-tropic HIV-1 *in vitro* due to minimal CCR5 expression, HIV-1 DNA is detectable in T_N cells of viremic and virus-suppressed individuals and CCR5-tropic virus has been recovered from

them.[65,67,68,70,71] Since CCR5-tropic HIV-1 is responsible for the vast majority of transmission and early stages of infection, the mechanism by which CCR5-tropic HIV-1 can infect T_N cells is of importance, especially considering the impact infection of this $CD4^+$ T cell subset has on the viral reservoir.[72,73]

To explain this phenomenon, it was hypothesized that B cells can mediate *trans* infection of T_N cells with CCR5-tropic HIV-1, bypassing the need for CCR5-co-receptor expression. While both B cells and iDC can mediate HIV-1_{BaL} *trans* infection of T_{CM} and bulk $CD4^+$ T cells, only B cells, compared to iDC and mDC, can efficiently *trans* infect T_N cells *in vitro*.[27] Again, it is important to note that in this model APC are incubated with a sub-infectious dose of HIV-1_{BaL}, i.e., an MOI that is not able to productively infect $CD4^+$ T cells in *cis* (MOI 10^{-3}), emphasizing the efficiency of *trans* infection compared to *cis* infection. Additionally, this finding was consistent whether the T cell targets were previously activated with phytohemagglutinin (PHA) and IL-2 or exposed to CCL19 which does not induce global T cell activation.[13] Furthermore, co-culture of B cells with T_N cells does not affect the T_N phenotype, with the T_N cell subset remaining predominately $CCR5^-$.[27]

B cells and $CD4^+$ T cells interact regularly *in vivo* in and around B cell follicles in secondary lymphoid organs (SLO). These interactions are important for B cell and $CD4^+$ T follicular helper cell (T_{FH}) differentiation and the formation and maintenance of antibody responses.[74-77] Importantly, T_{FH} cells have also been implicated as a major reservoir of HIV-1 infection in humans and in simian immunodeficiency virus (SIV) infection in macaques, harboring high levels of viral DNA and RNA.[78-83] Additionally, T_{FH} cell populations, along with germinal center (GC) B cells, increase during HIV-1 infection compared to uninfected persons, and these increases are not normalized by ART, providing a long-term reservoir for HIV-1 infection.[81,83] Regarding CCR5 expression, macaque and human T_{FH} cells predominately lack CCR5 expression, but do include a precursor subset termed pre-T_{FH}. These cells are present during the proliferation and differentiation phase following initial activation of a T_N cell by a DC, but before interaction with their cognate B cell, which is categorized by some CCR5 expression. Like T_N cells, despite there being only minimal CCR5 expression on T_{FH} cells, they are infected *in vivo* with CCR5-tropic virus, thus their mode of infection is also of importance.[78,80,81] Similar to B cell-mediated HIV-1 *trans* infection of T_N cells, we hypothesize that B cells can also mediate *trans* infection of T_{FH} cells in SLO, aiding in the establishment and maintenance of the viral reservoir.

Macrophage-mediated HIV-1 *trans* infection

Macrophages express CD4, CCR5 and CXCR4, and therefore can be productively infected with R5 and X4-tropic HIV-1.[26,84-86] Due to higher CCR5 expression they are more efficiently infected by CCR5-tropic HIV-1. However, how monocytes are differentiated into macrophages also impacts their co-receptor expression.[84] Differentiation of monocytes with macrophage colony stimulating factor (M-CSF) causes an increase in both CCR5 and CXCR4 expression, while differentiation with GM-CSF causes an upregulation in CCR5 expression, but a down regulation in CXCR4 expression.[57] In addition to *cis* infection, monocyte-derived macrophages (MDM) have also been implicated as mediators of *trans* infection of $CD4^+$ T cells through binding HIV-1 via DC-SIGN, Siglec-1, and MR.[26,84,87-92] Moreover, DC-SIGN is upregulated on MDM exposed to IL-13.[91,92] These MDM can *trans* infect $CD4^+$ T cells with HIV-1_{BaL} and *trans* infection efficiency is associated with DC-SIGN expression. Additionally, blocking DC-SIGN on MDM prior to virus exposure reduces *trans* infection by 87% by day 12 post co-culture.[26] Another study found that in those MDM that lack DC-SIGN expression, 60% of HIV-1 binding is MR-mediated and that blocking MR reduces *trans* infection of $CD4^+$ T cells up to 80%.[89] Activating MDM with IFN-α causes an upregulation in Siglec-1 expression, although to a lesser extent than on DC. However, MDM can still mediate *trans* infection of $CD4^+$ T cells via Siglec-1 binding of HIV-1 and when Siglec-1 is blocked, it largely inhibits *trans* infection.[36] Like DC, macrophages are one of the first cells to come in contact with HIV-1 during sexual transmission in mucosal tissue.[87-89] As APC, macrophages have many transient adhesive contacts with $CD4^+$ T cells in lymphoid organs where HIV-1 can also be transferred, implicating these cells in both the formation and maintenance of the viral reservoir.[88]

APC-mediated HIV-1 *trans* infection's impact on disease progression

While most HIV-1-infected individuals will progress to AIDS in the absence of ART (progressors; PR), there is a small percentage of individuals who naturally control disease progression in the absence of ART and are broadly termed non-progressors (NP). These NP can be further classified into elite controllers (EC), viremic controllers (VC), and long-term NP (LTNP). The criteria for these subgroups are

as follows: EC= undetectable viral load >7 years post infection, VC= at least two viral load measures below 2,000 copies HIV-1 RNA/mL, and LTNP= $CD4^+$ T cell counts >500 cells/mm^3 over 7 years post infection.[27,93] Understanding how NP naturally control HIV-1 infection may provide insight for new therapeutics that could mimic this control in PR.

As APC-mediated *trans* infection is an extremely efficient means of viral dissemination and is potentially important for HIV-1 reservoir seeding and maintenance, the efficiency of B cell- and DC-mediated *trans* infection in seronegative (SN), PR, and NP samples from the Multicenter AIDS Cohort Study and the Women's Interagency HIV Study (MWCCS) was examined to determine if there was a link between *trans* infection efficiency and disease progression. Interestingly, both DC and B cells from NP cannot mediate *trans* infection of $CD4^+$ T cells, unlike PR and SN APC.[93] Of note, $CD4^+$ T cells from NP are efficiently HIV-1 *cis*-infected *in vitro*. Additionally, B cells and DC from NP and SN revealed no difference in DC-SIGN expression or their ability to bind HIV-1. Cholesterol is essential for DC- and macrophage-mediated *trans* infection as when DC are treated with peroxisome proliferator-activated receptor γ (PPARγ) and liver X receptor (LXR), HIV-1 *trans* infection is inhibited by an increase in cholesterol efflux through ATP-binding cassette transporter A1 (ABCA1) activity.[94,95] Thus, it was hypothesized that increased cholesterol efflux is related to the inability of APC from NP to *trans* infect $CD4^+$ T cells. Indeed, this lack of *trans* infection is linked to lower cholesterol levels and an increase in ABCA1 levels in APC from NP. Additionally, NP APC's ability to *trans* infect is restored by cholesterol reconstitution and siRNA knockdown of ABCA1.[93] It is also important to note that this trait is present in APC from NP before HIV-1 infection, indicating it is hereditary.[93] This work was followed by an analysis of macrophage-mediated *trans* infection by SN, PR, and NP.[26] Similarly, MDM from NP cannot *trans* infect $CD4^+$ T cells, while MDM from SN and PR are efficient mediators of *trans* infection. However, in contrast to B cells and DC, *trans* infection efficiency is linked to the number of DC-$SIGN^+$ MDM with significantly fewer NP MDM expressing DC-SIGN. Additionally, cholesterol levels, lipid rafting, and virus internalization into early endosomes is significantly lower in NP MDM than PR MDM.[26]

As previously stated, B cells can mediate CCR5-tropic HIV-1 *trans* infection of predominately $CCR5^-$ T_N cells.[27] If this mechanism of T_N cell infection is a major mechanism by which infection occurs, then NP may have a reduced or absent level of HIV-1 DNA in this $CD4^+$ T cell subset. HIV-1 DNA levels were quantified in T_N and total $CD4^+$ T cells from 7 NP not under ART and 7 PR on ART. Interestingly, HIV-1 DNA was not detected in T_N cells from the EC tested, while a low number of HIV-1 DNA copies were detected in the other NP (LTNP and VC). Importantly, T_N cells from NP harbor significantly lower copies of HIV-1 DNA than T_N cells from PR.[27] Consistent with these data, Pinzone *et al* performed proviral sequencing on $CD4^+$ T cell subsets from 5 EC not on ART and 5 PR on ART and found that T_N cell infection was barely detectable in EC and was significantly lower than in T_N cells from PR.[69] Importantly, these EC had predominately CCR5-tropic HIV-1 proviruses. Additionally, EC had overall less HIV-1 infection compared to PR, as seen by significantly lower levels of HIV-1 DNA in total $CD4^+$ T cells, as well as in T_N and memory subsets, consistent with other findings of smaller reservoirs in $CD4^+$ T cells of EC compared to PR.[69,96-98] This work supports the role of APC-mediated *trans* infection in disease progression, specifically in its potential impact on the viral reservoir size and T_N cell infection.

Mechanisms of APC-mediated HIV-1 *trans* infection

Various mechanisms for HIV-1 transfer from APC to $CD4^+$ T cells have been proposed with one being exosomes.[35,99-103] HIV-1 infected monocyte derived iDCs generate fibronectin and galactin-3 containing exosomes which are 4-fold more infective than cell free HIV-1. Additionally, anti-fibronectin antibodies and β-lactose, a galectin-3 antagonist, significantly block DC exosome-mediated HIV-1 *trans* infection of $CD4^+$ T cells.[35] Similarly, mDC have also been implicated in *trans* infecting $CD4^+$ T cells via exosomes in an envelope glycoprotein-independent manner.[99,104] This mDC-mediated *trans* infection via exosomes also allows for efficient *trans* infection of resting $CD4^+$ T cells.[100] Another mechanism for viral transfer is filopodia, which are actin-rich membrane protrusions.[7,18,19,105] Filopodia are induced in iDC after DC-SIGN-mediated HIV-1 binding which then allows for *trans* infection of $CD4^+$ T cell targets.[18,19,105-107] Other membrane protrusions, namely tunneling nanotubes (TNT), have also been implicated as a mode of viral transfer in APC-mediated *trans* infection. Macrophages produce TNT after HIV-1 infection through involvement of Nef, with HIV-1 trafficking through endocytic compartments in TNT.[7,18,19,108-111] Additionally, DC matured by type 1 immunity mediators produce TNT-like structures in response to CD40L with these structures able to traffic HIV-1 like particles from

donor to recipient cells.[112] In regard to reservoir formation and maintenance, TNT are of importance as they allow for protection of HIV-1 from immune surveillance and a mechanism of efficient spreading.[7,18,111]

The virological synapse is frequently used to define viral cell-to-cell transmission and is similar to the immunological synapse formed between APC and T cells during antigen presentation.[18] There can be recruitment of cell adhesion molecules, such as the lymphocyte function associated antigen-1 (LFA-1) and its ligand, intercellular adhesion molecule-1(ICAM-1), and of receptors including CD4, CCR5, and CXCR4, as well as actin rearrangement.[18,113,114] However, the virological synapse is commonly described for $CD4^+$ T cell-to-$CD4^+$ T cell transfer, and modes of cell-to-cell HIV-1 transmission not requiring HIV-1 receptors/co-receptors and adhesion molecules have also been shown.[18,27,113-115] Virological, or infectious, synapses have been implicated in DC-mediated *trans* infection.[7,19,22,116] Interestingly, Akiyama *et al* found that not only can DC bind HIV-1 through Siglec-1, but that after virus is internalized, the cytoplasmic tail of Siglec-1 allows for HIV-1 trafficking and *trans* infection to $CD4^+$ T cells. Additionally, this mechanism of *trans* infection was not susceptible to neutralizing antibodies.[55] The varying hypotheses on requirements for viral transfer likely depend on the specific cells involved in *trans* infection and are not "one size fits all". Immunological synapses between APC and $CD4^+$ T cells could provide HIV-1 with the ability to hijack these natural, frequent interactions allowing for highly efficient viral spread. Also, an increase in TNT or filopodia due to the presence of HIV-1 in turn can increase cell-to-cell contact, thus increasing the likelihood of *trans* infection.

Conclusions

While $CD4^+$ T cells are the major reservoir for HIV-1 infection, the processes by which they can be infected in anatomical reservoirs such as the brain/CNS, gastrointestinal tract, lymphatics, and urinary tract is of importance.[5,8,9,14] Notably, APC-mediated HIV-1 *trans* infection is significantly more efficient than *cis* infection due to the transfer of multiple virus particles and the high MOI at the site of cell-to-cell contact, thus increasing the likelihood that virus escapes immune responses.[17,18,20-22] Additionally, higher copies of HIV-1 DNA in $CD4^+$ T cells isolated from tissue compared to peripheral blood indicates *trans* infection as a likely means of $CD4^+$ T cell infection as it can lead to multiple infections per cell.[17,18,20,117] At the site of sexually transmitted HIV-1 infections, macrophages and iDC are some of the first cells to interact with HIV-1. Macrophages and iDC in anal and vaginal mucosa can bind and internalize HIV-1 via DC-SIGN and Siglec-1. Subsequently they could *trans* infect activated $CD4^+$ T cells present in the submucosa, establishing the HIV-1 reservoir very early in infection (**Figure 1A**).[118] Alternatively, iDC that bind HIV-1 via DC-SIGN, can mature and travel to the draining LN via the CCL19/21 chemokine gradient through their CCR7 expression for antigen presentation to $CD4^+$ T cells (**Figure 1B, E**).[119-121] In the LN paracortex, mDC survey $CD4^+$ T cells for their cognate T_N cell. In addition to T_N cells, there are T_{CM} cells present that home to SLO due to CCR7 expression, as well as activated $CD4^+$ T cells that transiently interact with mDC, potentially allowing for HIV-1 *trans* infection (**Figure 1E**).[120,122] Additionally, iDC migrate through SLO and thus if they have bound HIV-1 through DC-SIGN, could also *trans* infect the $CD4^+$ T cells in the paracortex of LN (**Figure 1E**).[122] Pathogens are also transported to draining LNs through lymphatic vessels where they enter the subcapsular sinus (SCS). The SCS is lined with SCS macrophages that express Siglec-1 through which they can bind antigens.[121,123,124] As the SCS also borders the T cell zone (paracortex), SCS macrophages that have bound HIV-1 through Siglec-1 may be able to *trans* infect $CD4^+$ T cells they encounter (**Figure 1C**).

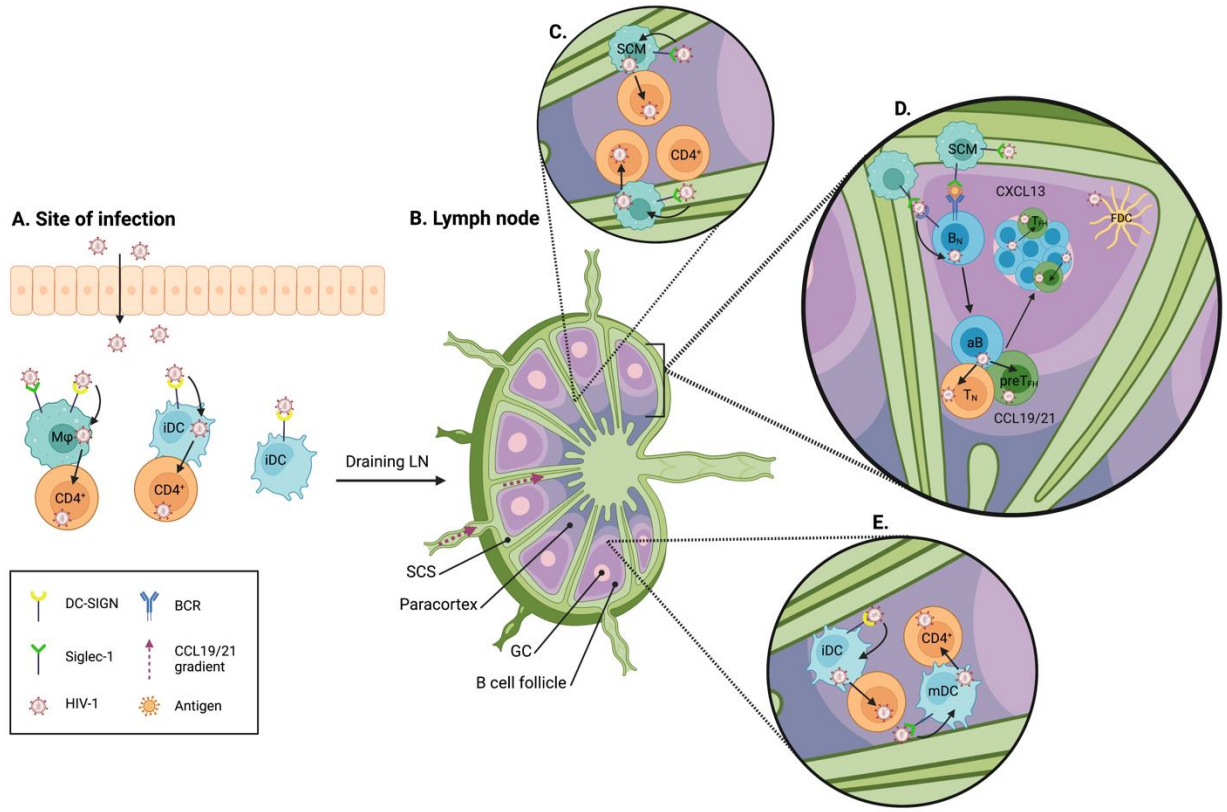

Figure 1. Schematic depicting APC and CD4$^+$ T cells interactions with proposed HIV-1 *trans* infections. (**A**) At the site of infection in sexually transmitted cases, macrophages (Mφ) and iDC bind HIV-1 via DC-SIGN or DC-SIGN and Siglec-1, respectively, which they transfer to target CD4$^+$ T cells. (**B**) Lymph node areas defined. CCL19/21 chemokine gradient guides DC to paracortex (T cell zone). (**C**) Subcapsular macrophages (SCM) in the SCS bind HIV-1 via Siglec-1 and *trans* infect CD4$^+$ T cells in the paracortex. (**D**) Naïve B cells (B_N) survey SCM for their cognate antigen and bind HIV-1 presented on SCM via DC-SIGN. Once activated, B cells upregulate CCR7 and travel to the B:T cell border by the CCL19/21 chemokine gradient. At the B:T cell border, B cells survey CD4$^+$ T cells. B cells may transfer virus to T_N cells while surveying or to pre-T_{FH} cells during their antigen-specific interaction. B cells and now fully differentiated T_{FH} cells can subsequently form GC where further B cell-mediated *trans* infections occur. (**E**) In the paracortex, mDC that have internalized HIV-1 survey CD4$^+$ T cells for their cognate T_N cell and transfer virus during these interactions. iDC that migrate into SLO bind HIV-1 via DC-SIGN and *trans* infect CD4$^+$ T cells. *Figure created with BioRender.com.*

In the B cell follicles of LN, naïve B cells survey SCS macrophages and follicular DC (fDC) for their cognate antigen. Extracellular HIV-1 accumulates on fDC and can survive for long periods of time, remaining infectious even in the presence of neutralizing antibodies, and SCS macrophages bind HIV-1 via Siglec-1.[79] B cells survive in part through the TNF family cytokine BAFF, which is secreted by resident stromal cells, with production upregulated during HIV-1 infection. This B cell survival factor can also induce expression of C-type lectins, such as DC-SIGN on B cells.[58,125,126] These circumstances allow B cells to bind and internalize HIV-1 via DC-SIGN as they survey for their cognate antigen. Once B cells encounter their cognate antigen they upregulate markers including CCR7, which allows them to migrate to the B:T cell border through the CCL19/21 chemokine gradient. Here B cells will form an antigen-specific interaction with pre-T_{FH} cells that previously encountered the same antigen. Subsequently, B cells and now fully differentiated T_{FH} cells can form GC where more antigen-dependent interactions occur that give rise to antibody class switching and affinity maturation.[74-77] Additionally, as B cells survey for their cognate T_{FH} cell, they engage in other CD4$^+$ T cell interactions. We postulate that these interactions, along with the antigen specific B:T_{FH} cell interactions at the B:T border and in GC, result in HIV-1 *trans* infection of CD4$^+$ T cells (**Figure 1D**).

During HIV-1 infection, the majority of viral replication occurs in SLO, even during ART-treated infections, presenting an important source of viral reservoirs that need to be addressed.[79,80,83,117] The enrichment of infected cells in these areas is likely

mediated by several factors. B cell follicles provide a sanctuary for the virus to escape $CD8^+$ T cell-mediated elimination, as the frequencies of these cells are lower in this area. Additionally, a chronic state of inflammation during HIV-1 infection can enhance viral production from infected cells, allowing replenishment of the reservoir.[79,117] Viral persistence in SLO during ART despite viral suppression in peripheral blood has also been linked to lower drug concentrations in these tissues.[8,15,17] While in their active form HIV-1 infected T_{FH} cells may not be considered part of the latent reservoir, after the GC response has ended a large proportion of T_{FH} cells survive as memory cells in SLO. Thus, this subset is a major reservoir of HIV-1 that largely does not express the CCR5 co-receptor needed for *cis* infection.[81,127,128] The chemokine CCL19, which is constitutively expressed in SLO for DC and T cell trafficking, enhances the permissiveness of $CD4^+$ T cells to HIV-1 infection and allows for efficient B cell-mediated *trans* infection of T_N cells.[13,27] In addition to T_N cells being able to be *trans* infected, it is suggested that T_N cells repopulate the effector memory $CD4^+$ T cell reservoir.[68,69] Finally, through DC-SIGN and Siglec-1 expression, macrophages as well as iDC and mDC are efficient mediators of HIV-1 *trans* infection of $CD4^+$ T cells. In sum, we hypothesize that HIV-1 hijacks the natural, frequent interactions between APC and $CD4^+$ T cells. This allows for highly efficient *trans* infection to occur *in vivo* which provides a means for both establishment and maintenance of the HIV-1 reservoir in $CD4^+$ T cells. To this end, these mechanisms need to be addressed in any potential cure strategies.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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