Dendritic Cells Enhance HIV Infection of Memory CD4⁺ T Cells in Human Lymphoid Tissues

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Abstract

Dendritic cells (DCs) play a key role in controlling infections by coordinating innate and adaptive immune responses to invading pathogens. Paradoxically, DCs can increase HIV-1 dissemination *in vitro* by binding and transferring infectious virions to CD4⁺ T cells, a process called transinfection. Transinfection has been well characterized in cultured cell lines and circulating primary T cells, but it is unknown whether DCs enhance infection of CD4⁺ T cells *in vivo*. In untreated HIV infection, massive CD4⁺ T-cell infection and depletion occur in secondary lymphoid tissues long before decline is evident in the peripheral circulation. To study the role of DCs in HIV infection of lymphoid tissues, we utilized human tonsil tissues, cultured either as tissue blocks or as aggregate suspension cultures, in single-round infection experiments. In these experiments, addition of monocyte-derived DCs (MDDCs) to the cultures increased T-cell infection, particularly in CD4⁺ T cells expressing lower levels of HLA-DR. Subset analysis demonstrated that MDDCs increased HIV-1 infection of central and effector memory T-cell populations. Depletion of endogenous myeloid DCs (myDCs) from the cultures decreased memory T-cell infection, and readdition of MDDCs restored infection to predepletion levels. Using an HIV-1 fusion assay, we found that MDDCs equally increased HIV delivery into naïve, central, and effector memory T cells in the cultures, whereas predepletion of myDCs reduced fusion into memory T cells. Together, these data suggest that resident myDCs facilitate memory T-cell infection in lymphoid tissues, implicating DC-mediated transinfection in driving HIV dissemination within these tissues in untreated HIV/AIDS.

Introduction

DENDRITIC CELLS (DCs) are key mediators of innate and adaptive immune responses to invading pathogens. In the case of HIV-1 infection, DCs are capable of eliciting a robust immune response against the virus.^{1–7} However, in *in vitro* experiments, mature DCs (mDCs) transinfect CD4⁺ T cells, augmenting the amount of CD4⁺ T cells that are infected by HIV-1, compared with CD4⁺ T cells infected in the absence of mDCs.^{8–12} In transinfection, mDCs transfer intact infectious virus to CD4⁺ T cells, without becoming infected themselves.^{13,14} Transinfection is possible because mDCs concentrate HIV-1 in an invagination of the plasma membrane that is still accessible to the surface of the cell at the infectious synapse, the site of contact between them and the CD4⁺ T cells.^{8,15–17}

Transinfection is greatly increased upon DC maturation with cytokines or bacterial products.¹⁰ In the canonical model of DC maturation, immature DCs phagocytose the antigens they encounter by macropinocytosis and receptor-mediated endocytosis.¹⁸ The antigens are cleaved into antigenic pep-

tides that can be loaded into MHC-II molecules for antigen presentation. As the DCs develop a mature phenotype, they increase the expression of costimulatory molecules, such as CD80 and CD86, as well as MHC-II molecules and chemokine receptors, notably CCR7. CCR7 binding to its ligands, CCL19 and CCL21, results in DC migration toward the lymphoid tissues.¹⁹ DC maturation is also accompanied by a decrease in phagocytosis of viruses and bacteria and consequent decline in *de novo* antigen presentation.^{20–23} Decreased internalization of pathogens is accompanied by increased transinfection delivered from virus-containing compartments formed from plasma membrane invaginations on the DC surface.¹⁵

Transinfection has been described and studied using cultured peripheral blood cells and cell lines; however, it is not known whether it plays a role within the lymphoid tissues that HIV infects. In this study, we employed human tonsil cultures, either as tissue blocks or as suspension cultures, to assess the contribution of DCs in the infection of the CD4⁺ T cells in these sites.^{24–27} We found that addition of monocyte-derived DCs (MDDCs) to the cultures increased the level of

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infection, especially in CD4⁺ T cells expressing lower levels of HLA-DR, a marker of T-cell activation. This suggested to us that DCs could be aiding the infection of the T cells that may not be immediate HIV targets otherwise. MDDCs augmented the infection of memory T cells, especially effector memory T cells (T_{EM}). Depletion of myeloid DCs (myDCs) from tonsillar tissue resulted in a decrease in the productive infection of memory T cells. Depletion of tonsillar DCs also resulted in a reduction in HIV fusion (entry) into CD4⁺ T cells, and the addition of monocyte-derived DCs to myDC-depleted cultures restored fusion to predepletion levels. Together, these experiments suggest that DCs can mediate HIV fusion and infection of memory T cells in lymphoid tissues and implicate resident myDCs in initiating and sustaining HIV infection in lymphoid tissues.

Materials and Methods

Viruses

HIV-1 strain, NL43-GFP-IRES-Nef (NL43-GFP-Nef), which expresses GFP and Nef on a bicistronic Nef mRNA,²⁸ was a kind gift from David Levy. Virus stocks were prepared by CaPO₄ transfection of HEK-293T cells as described.²⁹ BlaM-Vpr containing HIV-1 (NL43-GFP-BlaM) was produced by cotransfecting 1 µg BlaM-Vpr plasmid (NIH AIDS Reagent Program)³⁰ for every 10 µg of NL43-GFP-Nef provirus. The virus supernatants were concentrated by centrifugation at 32,000×g for 2 h at 4°C through a 10% sucrose/phosphate buffered saline (PBS) pad and resuspended in RPMI +10% fetal bovine serum (FBS). Infectivity of the stocks was quantified using LuSIV LTR-Luciferase indicator cells.³¹

Tonsil histocultures

Deidentified human palatine tonsils were obtained from the Histology Tissue Procurement Facility from University Hospitals of Cleveland following IRB-approved protocols for collection of discarded surgical tissues. Tonsil blocks were sliced into 2-mm blocks using a McIlwan tissue chopper and placed on organotypic inserts (EMD Millipore) in a six-well plate. Culture medium [RPMI without phenol red (Gibco/Life Technologies) supplemented with 10% pooled human serum (HyClone), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% L-glutamine] was added to the well so that the blocks were cultured at the air/liquid interface. For flow cytometry analysis of the block cultures, single-cell suspensions were prepared by physical disruption of the infected tonsil blocks. Human lymphoid aggregate cultures (HLACs) were prepared as previously described³² by dissociation of tonsil tissue using a mixture of collagenases (Liberase™ Thermolysin Medium; Roche) at 37°C in a rotating incubator for 90 min and passed through a cell strainer to remove debris. Dead cells were removed by MACS® dead cell removal kit (Miltenyi Biotech) and cultured at a density of 10⁷ cells/ml (0.1 ml) in 96-well round bottom plates. For depletion experiments, myDCs were selected using a CD1c isolation kit according to the manufacturer (Miltenyi Biotech). The flow-through from all steps was collected, pooled (excluding myDCs), and cultured as above.

Monocyte-derived DCs

CD14⁺ monocytes were isolated from healthy donor PBMCs using CD14⁺ beads (Miltenyi Biotech) and cultured

in RPMI 1640 (Life Technologies), supplemented with 10% FBS (HyClone), 100 U/ml penicillin, and 100 µg/ml streptomycin, and supplemented with 100 ng/ml IL-4 and 50 ng/ml GM-CSF (Miltenyi Biotech). MDDCs were matured by overnight stimulation with 100 ng/ml LPS (Sigma) 5 to 6 days after initiation of the cultures. Mature MDDCs were pelleted, washed, and resuspended in fresh HLAC medium.

Tonsil infection

Tonsil blocks were infected by adding concentrated HIV-1 stocks directly onto the blocks (13 ng p24/block). Infection was analyzed 3 days postinfection. HLACs (10⁶/well) were infected by adding virus (32 ng p24) directly to the culture media without or with MDDCs (5 × 10⁴/well or 5 × 10³/well where noted). At 24 h postinfection, half the media were replaced and antiretroviral drugs (T20-Enfuvirtide 0.1 µM, Raltegravir 5 µM) were added to prevent second round of infection. Cells were analyzed by flow cytometry 2 days postinfection.

HIV fusion assay

HLACs were infected with NL43-GFP-BlaM for 6 h at 37°C. The cultures were then washed and loaded with CCF2-AM (coumarin cephalosporin fluorescein 2-acetoxymethyl, Life Technologies) for 1 h at room temperature, washed again, and resuspended in HLAC medium supplemented with 25 mM HEPES and 2.5 mM probenecid (Sigma) for 18 h at room temperature. Cells were then washed and processed for flow cytometry.

Flow cytometry

Cells were stained with the following antibodies: CD3-BV650 (OKT3) and CD4-APC (OKT4) (Biolegend), CD45RO-ECD (UCHL1) (Beckman-Coulter), CD3-PE-Cy5 (HIT3A), HLA-DR-APC (G46-6) and CCR7 (2H4) (Becton Dickinson), and PE-labeled anti-mouse IgM (Invitrogen). All stains were performed at 4°C for 20 min. Cells were analyzed using either a BD FACSCalibur or a BD LSRII flow cytometer and analyzed using Flowjo software (Flowjo, LLC). Dead cells were excluded using a live/dead stain (Life Technologies) and gated based on size by forward and side scatter. Plotting the forward scatter area versus forward scatter height indicated that cell doublets, including DC-T cell conjugates, were rare, less than 0.001% of the events counted. To calculate the percentage of infected CD4⁺ T cells, both CD3⁺/CD4⁺ and CD3⁺/CD4⁻/GFP⁺ were included to account for CD4 downmodulation in HIV-1-infected cells. Fusion analysis was performed on the CD3⁺/CD4⁺ population 6 h postinfection before HIV gene expression and CD4 downregulation. Naive T cells (T_N) were identified as CCR7⁺/CD45RO⁻, central memory T cells (T_{CM}) as CCR7⁺/CD45RO⁺, and effector memory T cells (T_{EM}) as CCR7⁻/CD45RO⁺. HLA-DR was used as a marker for T-cell activation.

Fluorescence microscopy

Tonsil blocks were imaged using a Deltavision widefield fluorescence imaging system (Applied Precision, Inc.) and analyzed using the Deltavision Softworks analysis software. MDDCs were labeled using Celltracker Red™ (Life

Technologies) according to the product protocol. Tonsil blocks were imaged through the entire depth of the tissue and projected as maximum intensity volume images to visualize all labeled cells in the blocks. Multiple adjacent fields were collected and stitched into a single image after volume projections were generated.

Statistical analysis

p-Values were calculated using paired *t*-test analysis (two-tailed) with GraphPad Prism software using the values from four to seven experiments, as indicated. Computations and graphing were performed using a combination of Microsoft Excel and GraphPad Prism software.

Results

DCs enhance HIV infection in tonsillar histocultures

To test whether DCs increase HIV infection in lymphoid tissues, human tonsillar tissues were dissected and cultured as intact blocks on organotypic filter inserts, providing an air/liquid interface similar to previously described floating block cultures.^{24,25} The blocks were infected with NL43-GFP-Nef, an engineered HIV-1 that expresses all HIV proteins along with GFP, encoded on a bicistronic Nef transcript.²⁸ This CXCR4-tropic virus was utilized because a high percentage of CD4⁺ T cells in tonsillar tissue express CXCR4, whereas CCR5 expression is highly restricted. The use of CXCR4-tropic HIV has become predominant when tonsillar cultures are used as a model of HIV infection.^{24,33–37} Infections were performed in the absence or presence of labeled LPS-activated MDDCs and analyzed 3 days later by fluorescence microscopy. Cultures infected in the presence of MDDCs had substantially more GFP-positive infected cells than cultures without MDDCs (Fig. 1). To quantify the impact of the DCs, the blocks were dissociated and analyzed by flow cytometry, demonstrating that MDDCs increased productive infection of T cells in the blocks by approximately fourfold over cell-free HIV (2.6% and 0.7% infected T cells, respectively, Fig. 1B).

To facilitate sample throughput, tonsil blocks were dissociated and cultured as cell suspensions, referred to as human lymphoid aggregate cultures (HLACs),³² before infection. HLACs were infected with NL43-GFP-Nef in the absence or presence of MDDCs as above and analyzed by flow cytometry 2 days later using HLA-DR expression to assess the T-cell activation status (Fig. 2). The CD4⁺ T cells in the HLACs were identified as HLA-DR^{high} and HLA-DR^{low} based on the expression level in the highly activated lymphoblast population (HLA-DR^{high}). Cells expressing increased HLA-DR were expected to support increased virus replication because activated T cells are favored targets of productive infection.^{38–40} Indeed, in cell-free infection, HLA-DR^{high} T cells were the primary target of infection in the HLACs. Surprisingly, addition of MDDCs not only increased overall T-cell infection but T cells expressing lower levels of HLA-DR were also particularly impacted (Fig. 2). In these experiments, the majority of increased infection in MDDC cocultures was in the HLA-DR low T cells (Fig. 2B), suggesting that MDDCs can aid the infection of less activated T cells in lymphoid tissues.

FIG. 1. MDDCs increase HIV-1 infection in tonsil block histocultures. Tonsil blocks ($\sim 0.5 \text{ mm}^3$) were cultured on membrane inserts overnight and infected with NL43-GFP-Nef without (*left*) and with (*right*) MDDCs ($10^4/\text{block}$). (A) Representative tonsil blocks were imaged 3 days postinfection through the entire depth of the tissue and projected as single maximum intensity volume images to visualize all GFP-positive cells in the blocks. Multiple adjacent fields were collected and stitched into a single image after volume projection. Scale bar = $400 \mu\text{m}$. (B) Three tonsil blocks per condition were collected and dissociated into single-cell suspensions 3 days postinfection and analyzed by flow cytometry for CD3 (T cells) and GFP expression (HIV infection).

MDDCs increase the productive infection of memory T cells in HLACs

To address whether DCs differentially impact infection of T cell subsets, we analyzed naïve central memory and effector memory CD4⁺ T-cell populations (T_N , T_{CM} , and T_{EM})⁴¹ in infected HLACs (Fig. 3B). Overall, MDDCs increased T-cell infection approximately twofold on average; however, within the T-cell subsets, the effector memory cell population was most significantly impacted (4.0 ± 0.6 -fold), followed by the central memory cells (2.5 ± 0.3 -fold) (Fig. 3C). Infection of naïve T cells was essentially undetectable in either condition.

Depletion of resident myDCs from HLACs decreases productive infection of memory CD4⁺ T cells

To assess the impact on HIV-1 infection by DCs present within the lymphoid tissues, we performed a series of depletion and readdition experiments. myDCs (CD1c⁺ myDC) and CD123⁺ plasmacytoid DCs (pDCs) are the major DC subtypes present in lymphoid tissues and can be identified by their differential expression of CD1c⁺ (myDC) and CD123⁺

FIG. 2. MDDCs increase HIV-1 infection of HLA-DR^{low} T cells in tonsil cell suspension cultures. HLACs were prepared from collagenase-treated tonsil tissue, followed by dead cell removal using Annexin-IV bead capture. Cells (10^6 /well) were infected with NL43-GFP-Nef in the absence or presence of MDDCs (5×10^4 /well) and analyzed by flow cytometry 2 days postinfection. Dead cells were excluded by dye uptake and gated on forward and side scatter to identify lymphocyte and lymphoblast populations. CD4⁺ T cells (CD3⁺/CD4⁺ and CD3⁺/CD4⁺/GFP⁺-infected cells) were analyzed for HLA-DR expression. (A) Flow cytometry dot plot of a representative experiment; % GFP⁺ cells are indicated in the HLA-DR^{high} and HLA-DR^{low} populations. DC-mediated fold increase in infection is indicated in the right column. (B) Combined lymphocyte and lymphoblast populations were analyzed by flow cytometry in three independent experiments and expressed as average fold increase in infected cells in +MDDC cultures $\pm$ SEM. DC, dendritic cell; HLACs, human lymphoid aggregate cultures.

(pDC) in lineage-negative lymphocytes.^{42,43} myDCs isolated from the blood have been previously shown to be highly proficient at transinfection, whereas pDCs are not.^{15,44} myDCs have been reported to comprise less than 0.5% of tonsillar lymphoid cells^{45,46}; in these HLACs, they were approximately 0.4% of the lymphoid population (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid).

To test whether resident myDCs influenced T-cell infection in the HLACs, myDCs were depleted from the cultures by magnetic bead separation (Miltenyi Biotech); in a typical experiment, CD1c⁺ myDCs were reduced by $\sim 75\%$ (Supplementary Fig. S1). Depletion of myDCs resulted in a significant decrease in overall T-cell infection (Fig. 4). Because the myDCs selected from the HLACs were of very low viability, readdition to the depleted cultures did not restore infection (not shown); however, addition of MDDCs to the cultures restored infection to predepletion levels (Fig. 4A). In subset analysis, myDC depletion resulted in reduction of HIV infection of both central and effector memory T cells, and addition of MDDCs resulted in restoration of infection levels in those populations (Fig. 4B). Together, these experiments suggest that lymphoid resident myDCs drive increased infection of memory T-cell populations.

MDDCs increase HIV fusion into naïve and memory T cells in HLACs

We next asked whether DCs preferentially deliver HIV virions to the T-cell subsets in the HLACs using a Beta-lactamase (BlaM)-based fusion assay. The assay relies on the incorporation of a BlaM-Vpr chimeric protein into the virion and its subsequent delivery into the target cell by HIV glycoprotein-mediated fusion. Fusion is monitored by the enzymatic cleavage of CCF2, a fluorescent dye substrate of β -lactamase that is loaded into the target cells.^{30,35} As expected, coculture of the HLACs with MDDCs increased HIV fusion into CD4⁺ T cells (Fig. 5A, B). Subset analysis revealed that MDDCs enhanced fusion twofold on average into each of the T-cell subsets analyzed, although the amount of

FIG. 3. MDDCs increase HIV-1 infection of memory T cells in tonsil histocultures. HLACs (10^6 /well) were infected with NL43-GFP-Nef without or with MDDCs (5×10^4 cell/well) and analyzed 2 days postinfection by flow cytometry. (A) Total CD4⁺ T-cell infection (% GFP⁺). Addition of MDDCs increased infection 1.9 ± 0.3 -fold. (B) CD4⁺ T cells were subdivided into naïve (T_N, CD45RO⁻/CCR7⁺), central memory (T_{CM}, CD45RO⁺/CCR7⁺), and effector memory (T_{EM}, CD45RO⁺/CCR7⁻) subsets. Representative dot plot of T-cell subset distribution in HLACs. (C) MDDCs increased infection of T_{CM} (2.5 ± 0.3 -fold) and T_{EM} (4.0 ± 0.6 -fold). Data in (A, C) are compiled from seven independent experiments. T_{CM}, central memory T cells; T_{EM}, effector memory T cells; T_N, naïve T cells.

FIG. 4. Depletion of endogenous myeloid DCs from HLACs decreases memory T-cell infection. CD1c⁺ myeloid DCs were depleted from HLACs by magnetic bead selection. The myeloid DC-depleted cultures (10^6 /well) were infected with NL43-GFP-Nef and analyzed by flow cytometry 2 days postinfection. **(A)** Depletion of myeloid DCs decreased HIV-1 infection, 1.6 ± 0.2 -fold. Addition of MDDCs (5×10^4 /well) restored overall infection to near the level of predepleted cultures with MDDCs (right plots). **(B)** Depletion of myeloid DCs decreased T_{CM} infection, 1.78 ± 0.4 -fold, and T_{EM} infection, 1.4 ± 0.1 -fold. Addition of MDDCs restored infection to near the level of predepleted cultures in T_{CM} and T_{EM} ($p = .3$ and $.5$, respectively). Data are compiled from multiple experiments (**A**, $n = 6$; **B**, $n = 4$); ns, not significant ($p > 0.05$).

fusion into naïve T cells was significantly lower than fusion into central and effector memory T cells (Fig. 5C). Taken together, these data indicate that MDDCs facilitate HIV delivery to both naïve and memory T cells, whereas increased infection is only observed in memory T-cell populations.

Depletion of myDCs from HLACs reduces HIV fusion into memory T cells

To test whether resident myDCs influence target cell fusion, we performed depletion and readdition experiments in the HLACs as above. Depletion of myDCs from the cultures significantly reduced HIV fusion and readdition of MDDCs to the depleted cultures restored it nearly to predepletion levels (Fig. 6A, B), suggesting that tonsillar DCs can mediate

HIV fusion in HLACs. In the experiment shown in Supplementary Figure S1, CD1c⁺ DCs represented 0.35% of tonsillar lymphoid cells (Supplementary Fig. S1). A comparable number of MDDCs (0.5%) was used in readdition experiments to better capture the range of DCs among donors compared with the addition of excess (5%) MDDCs. Readdition of 0.5% MDDCs to the cultures restored fusion to levels similar to nondepleted HLACs, and excess (5%) MDDCs increased fusion to levels similar to HLACs plus MDDCs (Fig. 6C). Subset analysis revealed that DC depletion resulted in a decrease in fusion into central and effector memory cells, but did not significantly impact fusion into naïve cells. (Fig. 6C, D). Addition of MDDCs to DC-depleted HLACs resulted in fusion levels comparable with HLACs with MDDCs and restored fusion into the effector memory T cells. Taken together, these experiments suggest that myDCs enhance HIV infection in lymphoid tissues by increasing HIV delivery to all CD4⁺ T cells and facilitating the infection of memory cell populations.

Discussion

Chronic immune activation is a hallmark of HIV/AIDS that is thought to drive replication and pathogenesis of HIV in infected individuals throughout the course of untreated infection.⁴⁷ One consequence of systemic activation is the accumulation of activated myDCs in secondary lymphoid tissues,⁴⁸ which may contribute to HIV dissemination through suboptimal induction of T-cell responses and transfer of infectious HIV into the very cells designed to contain the infection. In this study, we investigated whether DCs impact HIV infection in the complex cellular milieu of lymphoid tissues using human tonsil tissue cultures. When cultured as intact tissues, we found that exogenously added MDDCs increased overall HIV infection in the blocks compared with cell-free HIV; CD4⁺ T-cell infection increased three to fourfold when DCs were added. In dissociated cell cultures (HLACs), addition of MDDCs increased infection of CD4⁺ T cells that expressed decreased levels of HLA-DR, suggesting that the DCs can mediate HIV infection of T cells with lowered activation status. Analysis of T-cell subsets demonstrated that MDDCs increased infection of central and effector memory cell populations, but had no impact on infection of naïve cells, which was nearly undetectable in these experiments in either condition.

Viral fusion assays demonstrated that DCs increased HIV delivery into all three subsets analyzed to a similar degree; however, naïve cells were infrequently targeted by the virus, with or without added DCs. In these experiments, HIV entered less than 8% of naïve T cells compared with 30% to 40% of memory T cells when MDDCs were added. Naïve T cell resistance to HIV infection is well documented and has been linked to their low metabolic rate⁴⁹ and the activity of restriction factors such as SAMHD1.⁵⁰ The studies presented here suggest that HIV fusion into naïve T cells is also relatively infrequent in histocultures even when excess MDDCs are added. Moreover, the fusion studies demonstrated that even in fusion-positive subsets, the vast majority of targeted T cells did not progress to productive infection. In both central and effector memory T-cell populations, ~10% of fusion-positive T cells became productively infected, consistent with other reports.⁵¹ While a subset of

FIG. 5. MDDCs increase HIV-1 fusion into naïve and memory T cells in histocultures. HLACs were infected with NL43-GFP-BlaM without or with MDDCs (5×10^4 /well) for 6 h (37°C , 5% CO_2), washed, and loaded with CCF2-AM for 1 h at room temperature. The cultures were washed and incubated for 18 h at room temperature in HLAC medium supplemented with 2.5 mM probenecid and 25 mM HEPES. The cells were then washed, stained, and analyzed by flow cytometry. Dead cells were gated out by viability dye uptake, and live cells were gated on forward and side scatter to identify the combined lymphocyte and lymphoblast populations. $\text{CD}3^+/\text{CD}4^+$ T cells were analyzed for cleaved CCF2, indicating HIV-1 fusion and cytosolic delivery of BlaM-vpr. **(A)** A representative experiment demonstrating increased fusion in MDDC-containing HLACs. Values refer to the % cleaved CCF2-positive $\text{CD}4^+$ T cells. **(B)** Addition of MDDCs increased HIV-1 fusion in total $\text{CD}4^+$ T cells (1.8 ± 0.2 -fold). **(C)** MDDCs increased fusion equally into T_N (1.7 ± 0.2 -fold), T_CM (1.9 ± 0.2 -fold), and T_EM (1.8 ± 0.2 -fold). Data in **(B, C)** are compiled from seven independent experiments.

FIG. 6. Tonsillar myDCs support HIV-1 fusion into memory T cells in histocultures. Myeloid DCs were depleted from HLACs by magnetic bead selection, infected with NL43-GFP-BlaM for 6 h, and analyzed for fusion into $\text{CD}4^+$ T cells as above. **(A)** Dot plots of a representative experiment. **(B)** Total HIV-1 fusion decreased 1.8 ± 0.2 -fold in myDC-depleted cultures. Addition of MDDCs (5×10^4 /well) restored fusion to levels similar to predepletion cultures with MDDCs ($p = .6$). **(C)** myDC-depleted cultures were supplemented with MDDCs (5×10^3 , 5×10^4 /well). Bars represent the average of three samples $\pm$ SEM. **(D)** myDC depletion results in decreased HIV fusion into T_CM (1.5 ± 0.1 -fold, $p = .04$) and T_EM cells (1.9 ± 0.2 -fold, $p = .02$). Decreased fusion into T_N was not statistically significant (1.4 ± 0.2 -fold, $p = .14$). Addition of MDDCs restored fusion to near predepletion levels into all subsets (T_N , T_CM , and T_EM $p > .05$). Data in **(B, D)** were compiled from five independent experiments. myDC, myeloid DC.

fused, incompletely transcribed virion particles may exist in a preintegration latent state,^{52,53} the majority of fusion events are thought to result in abortive infection, potentially fueling T-cell loss in uninfected T cells.^{36,37} Notably, addition of MDDCs did not impact the rate of conversion to productive infection, suggesting that the DCs act primarily to increase HIV delivery to the T cells and may not impact postfusion events. Importantly, depletion of myDCs from the histocultures resulted in decreased fusion and infection of memory T cells that was restored by addition of MDDCs, indicating that resident DCs in the tissues support HIV-1 infection.

DCs are key mediators of innate and adaptive immunity to HIV; however, the experiments presented here demonstrate that DCs may also be important in initiating productive infections in lymphoid tissues. In the course of a single day, one DC may interact with thousands of T cells in a lymph node,⁵⁴ providing ample opportunity to transmit HIV to antigen-activated or quiescent CD4⁺ T cells. Therefore, DCs can act as a replication-independent viral reservoir that is protected from attack both by current antiviral drug regimens and by host defense systems. Our studies implicate tissue-resident myDCs in presenting increased HIV delivery to memory T-cell populations, potentially providing the fuel to drive infection and depletion in these key sites of replication *in vivo*. Many current therapeutic strategies aimed at eradicating latent HIV infections rely on immune recognition of reactivated HIV-infected cells^{55,56}; however, these reactivation strategies run the risk of increasing DC sequestration and dissemination of infectious HIV. Understanding the underlying molecular and cellular mechanisms of DC transinfection within the complex milieu of lymphoid tissues will be essential to achieve an HIV cure.

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Author Disclosure Statement

No competing financial interests exist.

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