Chapter 3

Chapter 3: Dendritic cell-to-T cell transmission of HIV is a drug-insensitive mode of infection

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ABSTRACT

Cell-to-cell transmission of HIV-1 allows for a high amount of concentrated virus to be directed to uninfected target T cells, which overwhelms the capacity of anti-retroviral drugs. During dendritic cell (DC)-to-T cell transmission, uninfected DCs can concentrate infectious virions and transmit them to T cell targets via cellular contacts. Here, we report DCs amplify the efficiency of T cell infection, which results in anti-retroviral drug insensitivity compared to T cell infection in the absence of DCs. The DC-mediated amplification and drug-insensitivity of T cell infection are both entirely dependent on physical cellular interactions. Further, we find the input of virus is important to the drug insensitivity of DC-to-T cell infection, but not DC-free T cell infection. DC-to-T cell transmission of HIV may have important implications for viral persistence *in vivo*.

INTRODUCTION

Cell-to-cell transmission of HIV is a robust mode of infection, whereby multiple virions infect a target cell (Sigal et al., 2011). During this process, a synapse forms between the infected donor and uninfected target $CD4^+$ T cells(Sattentau, 2011, Jolly et al., 2011, Sattentau, 2008, McDonald et al., 2003). This close physical proximity enables a high number of virions to be transmitted from the infected donor cell to the uninfected target cell, resulting in a reduced sensitivity to anti-retroviral drugs (Sigal et al., 2011, Sigal and Baltimore, 2012). Cell-to-cell transmission of HIV via a synapse can also occur between DCs and $CD4^+$ T cells (McDonald et al., 2003, Felts et al., 2010). Infection of the donor DC is not required because DCs can capture infectious virions and transmit them to uninfected target T cells via *trans* infection (Wu and KewalRamani, 2006, Cameron et al., 1992, Dong et al., 2007). It is unclear if DC-to-T cell transmission of HIV is a robust and drug-insensitive mode of infection.

In this study we found that human monocyte-derived DCs amplify HIV infection of autologous T cells in co-culture. This mode of transmission is relatively resistant to reverse transcriptase inhibitors compared to T cell infection in the absence of DCs. Further, it is dependent on physical contact between DCs and T cells. The drug insensitivity of DC-to-T cell infection is dependent on the virus input.

RESULTS

DCs amplify T cell infection

To determine the effect of DCs on HIV infection of T cells, we infected PBMCs with NL4-3 in the presence or absence of autologous human monocyte-derived DCs. DCs were matured with LPS prior to co-culture with T cells (**Supplementary Fig. 1**). Previous work indicates that LPS-matured DCs capture significantly more HIV-1 than immature

DCs (Dong et al., 2007, Izquierdo-Useros et al., 2014). To measure T cell infection, cells were analyzed for CD3 surface expression and intracellular p24 by flow cytometry. We found that T cell infection was on average 4-fold higher in DC-T cell coculture compared to DC-free culture across multiple donors (**Fig. 1**, **Supplementary Fig. 2**). As previously described, LPS-matured DCs showed little evidence of infection by p24 staining (Granelli-Piperno et al., 1999). The ability of DCs to amplify HIV infection was not specific to the viral strain. We observed that DC amplification of T cell infection occurred with various CXCR4-tropic, CCR5-tropic, dual-tropic, and transmitted founder strains (**Supplementary Fig. 3**).

DC-T cell transmission of HIV is drug-resistant

We next asked whether DC amplification of T cell infection leads to anti-retroviral drug insensitivity. We compared T cell infection in DC-free culture and DC-T cell coculture in the presence or absence of the reverse transcriptase inhibitor Tenofovir. We found that T cell infection decreased precipitously in the DC-free culture compared to DC-T cell coculture (**Fig. 2a**). We previously quantified T cell infection sensitivity to drugs using the transmission index (T_X), which is the fraction of T cells infected in the presence of drug divided by the fraction of T cells infected in the absence of drug (Sigal et al., 2011). Drug insensitivity (T_X) is dependent on the number of infectious particles transmitted to the target T cells. Thus, if the multiplicity of infection (m.o.i) of the target T cell is low, then infection is sensitive to the effect of drugs and T_X will be $<<1$. Alternatively, if the m.o.i. is high, then infection is insensitive to the effect of drugs and T_X will approach 1. We found the T_X in DC-T coculture was 4-fold higher compared to DC-free culture (**Fig. 2b**), suggesting that the ability of DCs to concentrate and transmit a high number of virions to a target T cells results in a relatively drug-insensitive mode of infection.

DC-T cell transmission requires cell-to-cell contact

We next determined if amplification of T cell infection and drug insensitivity was dependent on cell-to-cell contact between DCs and T cells. We found amplification (**Fig. 3a**) and drug insensitivity (**Fig. 3a**,**b**) of T cell infection were abolished if DCs and T cells were physically separated by a transwell membrane. These data suggest drug-insensitivity associated with DC-to-T cell infection is due to the DC's ability to concentrate and transmit virions to T cell through physical interactions.

Virus input is important to DC-T cell transmission

We next sought to determine the effect of virus input on drug insensitivity. Despite increasing the amount of NL4-3 added to DC-free culture, T_X was unaffected (**Fig. 4**). However, as the virus input was increased in DC-T cell cocultures, the T_X also increased, indicating DC-to-T cell transmission is dependent on the amount of virus in the system. These results suggest that the drug insensitivity of DC-to-T cell transmission is directly related to the amount of virions available for capture and transmission by DCs.

DISCUSSION

In our previous work we found that cell-to-cell transmission between an infected donor T cell and an uninfected T cell target was a high multiplicity of infection, which allowed for ongoing replication in the presence of anti-retroviral drug (Sigal et al., 2011). Others have also found cell-to-cell transmission to be dependent on high local concentrations of virus particles at sites of cellular contacts (Monel et al., 2012). In this report, we examined cell-to-cell transmission of HIV between DCs and T cells and asked whether this mode of transmission would allow replication in the presence of anti-retroviral drug. We found that DCs were essential to two processes. First, consistent with previous work, we found DCs were difficult to infect and their infection was not required for robust

amplification of T cell infection (Granelli-Piperno et al., 1999). This DC-mediated enhancement of T cell infection was entirely dependent on cell-to-cell contact. Second, DC-to-T cell infection displayed reduced sensitivity to anti-retroviral drug and also required physical contact between the donor DCs and target T cells. We further found that drug insensitivity of DC-to-T cell transmission increased as the virus input was increased. In contrast, the amount of virus in the system had no effect on drug insensitivity of DC-free T cell infection. These results suggest that DCs can efficiently capture virions and direct them to T cell targets, which leads to amplification of T cell infection and anti-retroviral drug insensitivity. Without DCs, primary T cell infection is a very inefficient process.

Our findings raise important questions regarding the importance of DCs in the cell-to-cell spread of HIV. During initial infection, DCs are among the first immune cells to encounter HIV at mucosal surfaces (Wu and KewalRamani, 2006). It is possible that DCs carry virions back to lymphoid organs where DC-to-T cell transmission plays an important mode of spreading the virus. The contribution of DCs to the viral reservoir is under investigation. Others have found follicular DCs capture infectious virus for days and transmit them to T cells, suggesting a possible cellular virus reservoir (Keele et al., 2008).

We suspect the mode of viral transmission may also be an important factor contributing to the viral reservoir. The high multiplicity of infection associated with cell-to-cell infection allows for anti-retroviral drug insensitivity, which leads to active replication (Sigal et al., 2011). This may be especially problematic in certain anatomical niches such as lymphoid tissue, which are conducive to cell-to-cell interactions and have been found to also have reduced anti-retroviral drug level (Fletcher et al., 2014). Thus, DC-to-

T cell transmission may have real implications for persistent virus replication and reservoir replenishment *in vivo*. It is interesting to speculate whether cell-to-cell transmission of HIV contributes to low-level viremia in HIV-infected patients on anti-retroviral therapy. Altogether, if the viral reservoir is to be eradicated, then a deeper understanding of factors contributing to its formation and maintenance is required.

EXPERIMENTAL PROCEDURES

Antibodies and Flow cytometry. Single-cell suspensions were stained with the following antibodies from Biolegend: PE-anti-human CD11c (301606), PerCP-anti-human CD14 (325621), APC-anti-human CD86 (305411), and APC/Cy7-anti-human HLA-DR (307617). For dead cell staining, 1ug ml^{-1} of propidium iodine was added. Cells were analyzed on a MACSQuant analyzer (Miltenyi), and data were analyzed with FlowJo software (TreeStar).

Generation of DCs. Human moDCs were generated by culture for 7 d of $CD14^+$ peripheral blood monocytes (UCLA Center for AIDS Research (CFAR) Virology Core Lab) in media containing human GM-CSF and IL-4 (Peprotech). DCs were cultured in RPMI-1640 medium supplemented with 10% (vol/vol) fetal bovine serum (FBS) (Sigma), 1% (vol/vol) non-essential amino acids (HyClone), 1mM sodium pyruvate (Gibco), 10mM HEPES (Gibco), and 0.05mM 2-mercaptoethanol (Gibco).

Virus Production. The pNL4-3 plasmid used to generate infectious HIV-1 was obtained from the AIDS Reagent Program, Division of AIDS, NIAID, NIH. Virus was prepared by culturing HEK293T/17 cells in 10-cm tissue culture dishes and transfecting with BioT (Bioland Scientific, Paramount CA) according to manufacturer's instructions using a total of 10 µg DNA pMDLg/pRRE and pRSV-Rev. All viral supernatants were harvested at 36,

48, and 60 h post-transfection and filtered through a 0.45-μm filter. The concentration of gag was measured by p24 capture ELISA Kit (ImmunoDiagnostics).

Statistical Analyses. GraphPad Prism 6.0 software was used for data analysis. Statistical significance was determined by unpaired, two-sided Student's *t*-tests for two groups or one-way ANOVA (with post-hoc Tukeys' multiple comparison test) for three or more groups.

FIGURE LEGENDS

Figure 1. DCs significantly amplify T cell infection. (**a**) Infection of PBMCs with (*center*) or without DCs (*left*) in co-culture was analyzed by flow cytometry. Uninfected DC-T coculture (*right*) was included as a negative control. Number in the top right gate indicates percentage of infected T cells. (**b**) Percentages of $p24^+$ T cells were measured in infected DC-free culture or DC-T cell co-culture. Each symbol represents one donor. Mean ± s.e.m (η = 6 donors). Data is representative of six independent experiments (**a**) or pooled from six independent experiments (**b**).

Figure 2. DC-T cell infection is drug-resistant. (**a**) Infection of DC-free PBMCs (left two plots) or DC-T cell co-culture (right two plots) in the absence of presence of 10μm of TFV. (**b**) Transmission index when infection occurs in DC-free culture (blue bars or squares) or DC-T cell coculture. TFV at 0 μM, 5 μM, 10 μM (wedges). Mean ± s.e.m (η = 3 donors). Data is from one experiment (**a**,**b**).

Figure 3. DC-T cell drug resistance depends on physical contact between cells. (**a**) Infection of DC-free PBMCs or DC-T cell co-culture with (*bottom*) or without (*top*) a transwell system, in which DCs are physically separated from PBMCs by a transwell membrane. Infection occurs in the absence of presence of 10μm of TFV. (**b**) Transmission index when infection occurs in DC-free culture or DC-T cell coculture with or without 10 μM of TFV in the absence of presence of a transwell system. Mean ± s.e.m (η = 3 donors). Data is from one experiment (**a**,**b**).

Figure 4. DC-to-T cell transmission is dependent on the amount of virus in the system. DC-free culture (*left*) or DC-T cell co-culture (*right*) were infected with varying amounts of NL4-3 in the absence of presence of 10μm of TFV. *x*-axis is ng of p24 of

NL4-3. *y*-axis is the transmission index. Each symbol represents a donor. Data is representative of one experiment.

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. LPS activation of monocyte-derived DCs. DCs were treated with LPS and analyzed by flow cyotometry. (**a**) Number in the top left gate indicates percentage of cells with the DC phenotype $CD14^{-}DC\text{-}SIGN^{+}$. (**b**) The surface expression of activation markers CD86 and HLA-DR were analyzed. Data is representative of two independent experiments.

Supplementary Figure 2. DC amplification of HIV infection of T cells in six donors. Infection of PBMCs in the presence or absence of DCs was analyzed by flow cytometry. Percentages of infected $p24^{+}$ T cells were measured in six donors. Each symbol represents a technical replicate. Horizontal bar indicates the mean. Data is representative of six independent experiments.

Supplementary Figure 3. DC amplification of T cell infection with different HIV-1 strains. Infection of PBMCs with or without DCs by CXCR4-tropic (LAI), CCR5 tropic (JRCSF, NFNSX), dual-tropic (89.6) and CCR5-tropic founder strains (11745, 11739) were analyzed by flow cytometry. Number in the top right gate indicates percentage of infected T cells.

FIGURES

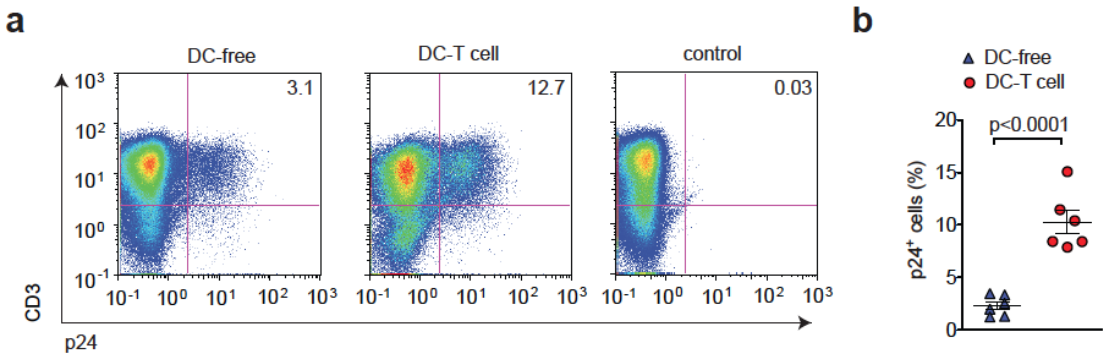


Figure 1. DCs significantly amplify T cell infection.

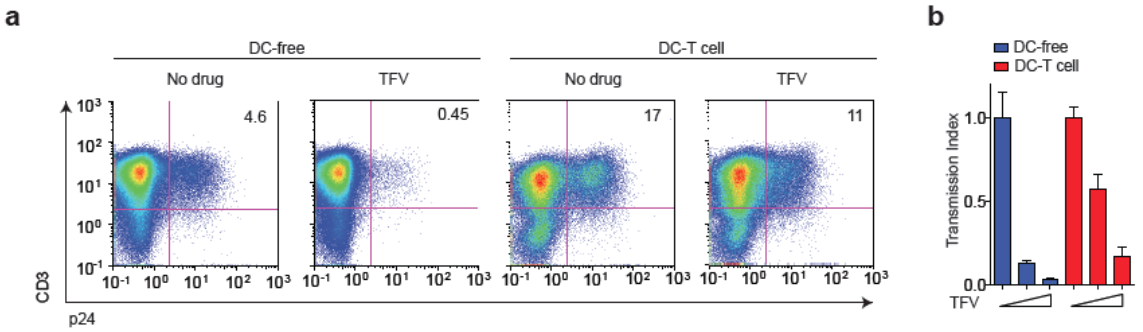


Figure 2. DC-T cell infection is drug-resistant.

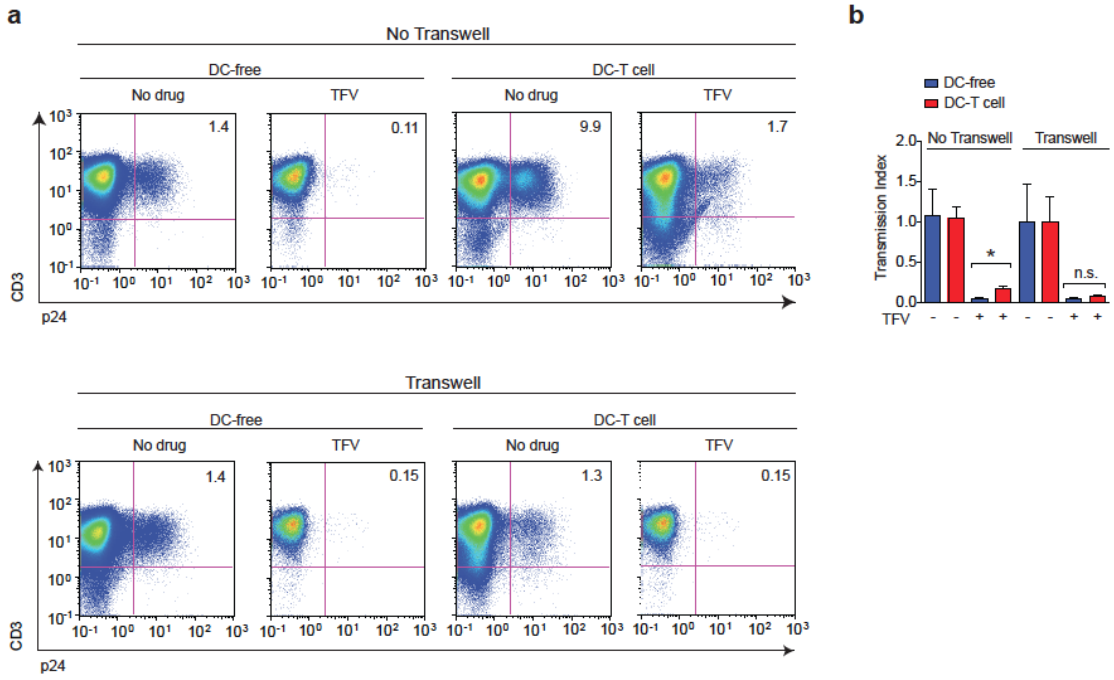


Figure 3. DC-T cell drug resistance depends on physical contact between cells.

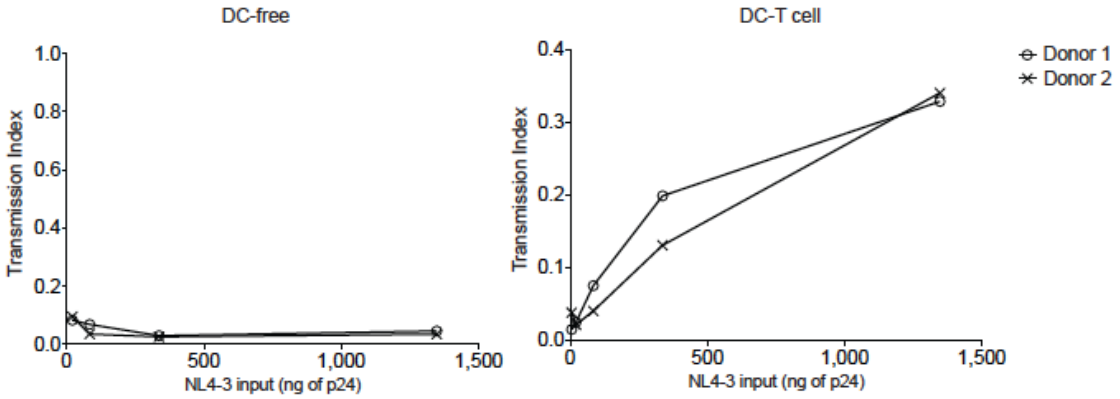
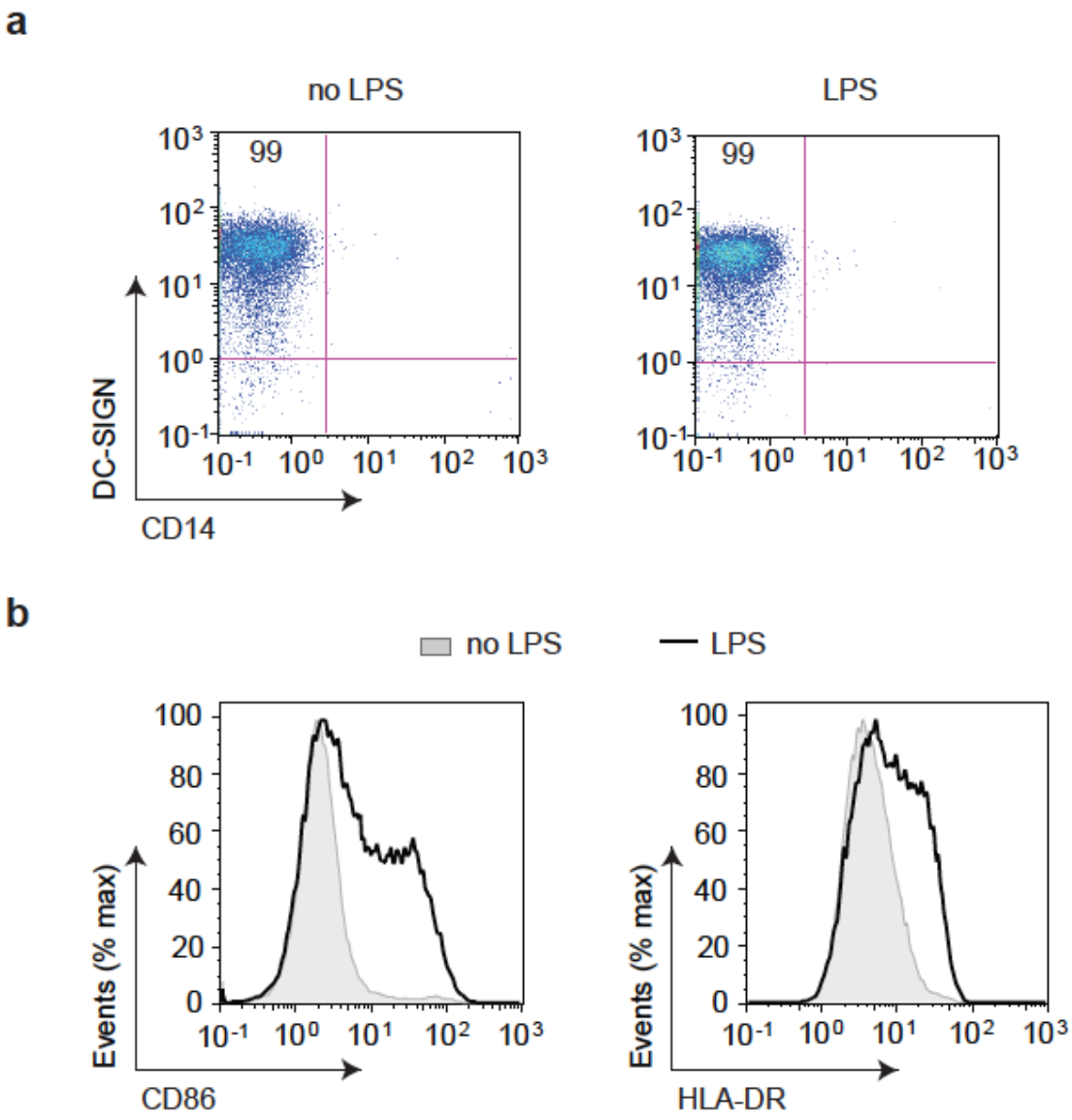
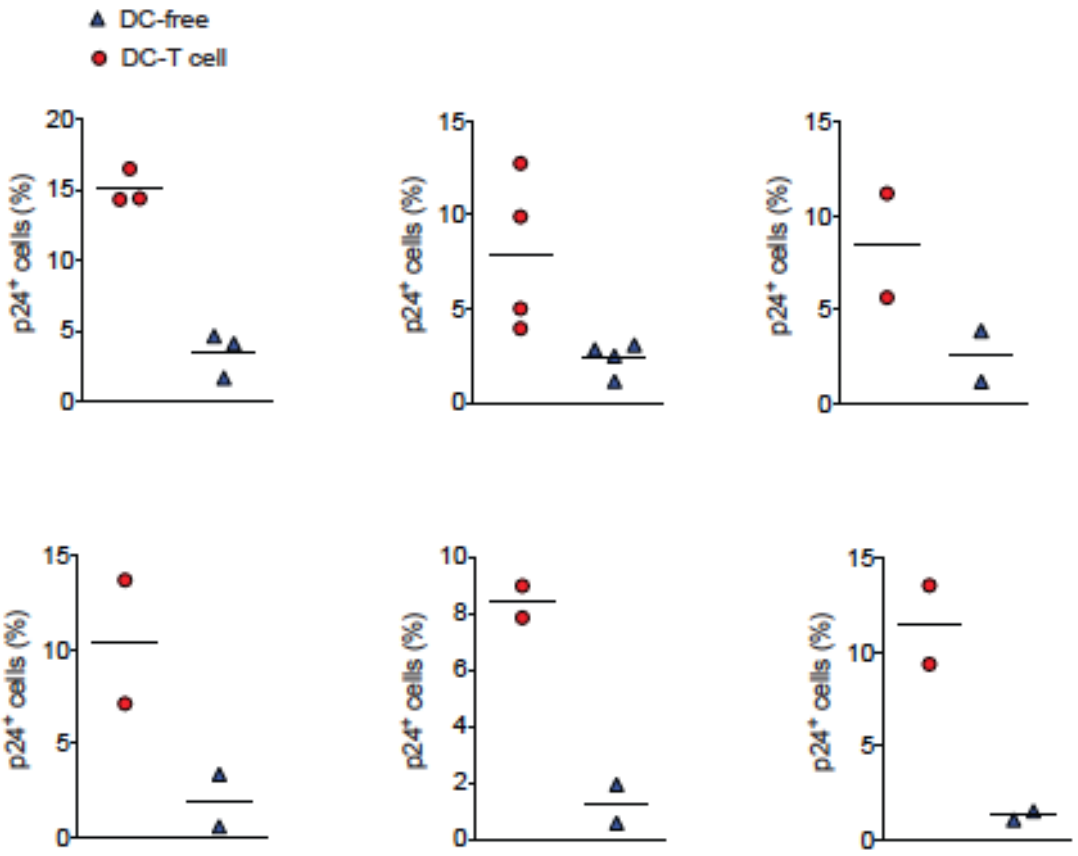


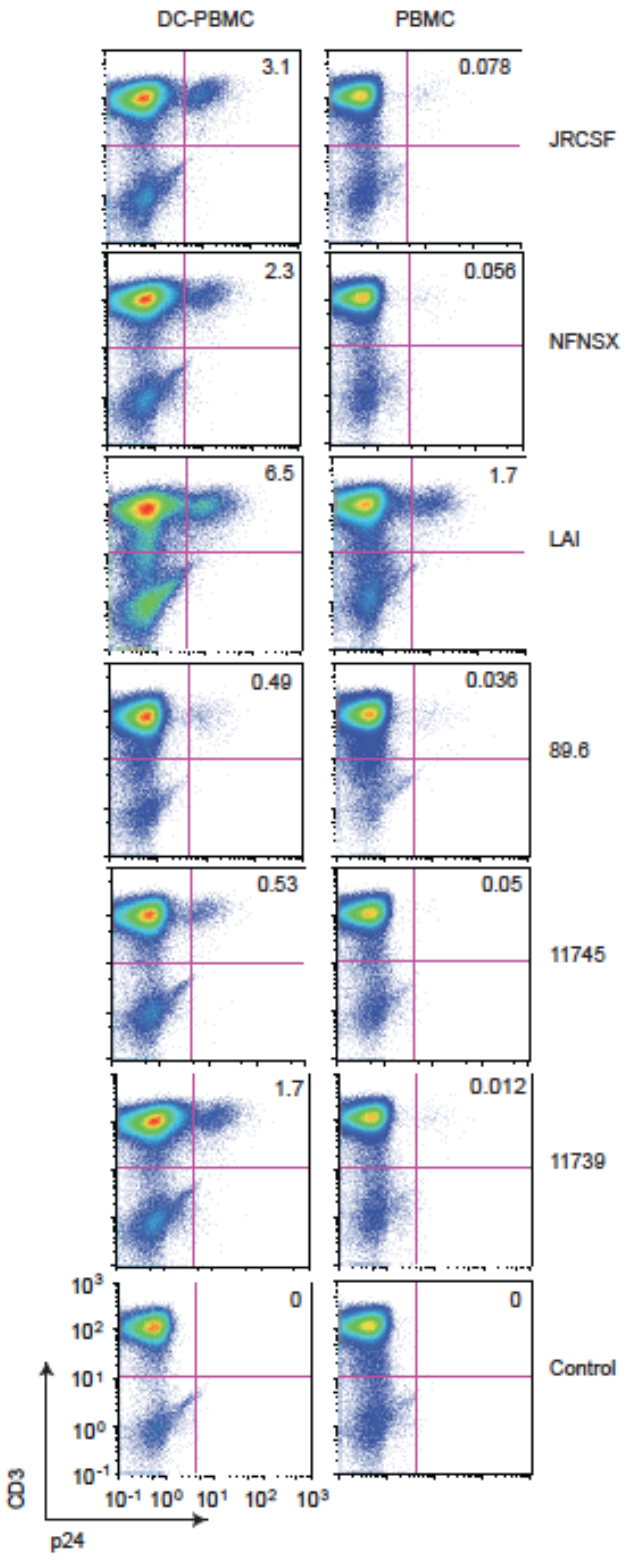
Figure 4. DC-to-T cell transmission is dependent on the amount of virus in the system.



Supplementary Figure 1. LPS activation of monocyte-derived DCs.



Supplementary Figure 2. DC amplification of HIV infection of T cells in six donors.



Supplementary Figure 3. DC amplification of T cell infection with different HIV-1 strains.