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Recruitment of HIV and Its Receptors to Dendritic Cell–T Cell Junctions

David McDonald,[1] Li Wu,[2] Stacy M. Bohks,[3] Vineet N. KewalRamani,[2] Derya Unutmaz,[3] Thomas J. Hope[1*]

[1]Department of Microbiology and Immunology, University of Illinois, Chicago, IL 60612, USA. [2]HIV Drug Resistance Program, National Cancer Institute at Frederick, Frederick, MD 21702, USA. [3]Department of Microbiology and Immunology, Vanderbilt University, Nashville, TN 37232, USA.

*To whom correspondence should be addressed at thope@uic.edu

Monocyte-derived dendritic cells (MDDCs) can efficiently bind and transfer HIV infectivity without themselves becoming infected. Using live-cell microscopy, we found that HIV was recruited to sites of cell contact in MDDCs. Analysis of conjugates between MDDCs and T cells revealed that, in the absence of antigen-specific signaling, the HIV receptors CD4, CCR5, and CXCR4 on the T cell were recruited to the interface while the MDDCs concentrated HIV to the same region. We propose that contact between dendritic cells and T cells facilitates transmission of HIV by locally concentrating virus, receptor, and coreceptor during the formation of an infectious synapse.

Dendritic cells (DCs) comprise a multivariate family of cell types whose principal function is in the primary initiation of immune responses. Myeloid-derived dendritic cells (MDDCs) patrol areas of the body that are susceptible to invasion by pathogens and engulf antigens for later processing and presentation to T lymphocytes. The human immunodeficiency virus (HIV) has apparently appropriated this feature of the immune system to better establish and maintain infection of its primary target, CD4-positive T cells. HIV is taken up by MDDCs through interaction between its envelope glycoprotein, gp120, and mannose C-type lectin receptors (MCLRs) expressed on the DC surface (*1*). The best characterized of these is DC-SIGN, a DC-specific C-type lectin (*2*). Rather than having the DC-HIV interaction lead to infection of the DCs, the bound HIV is efficiently transferred to target cells (*2*, *3*). Further, HIV is internalized into a trypsin-resistant compartment, where its infectivity can be retained for an extended period of time before transfer (*4*).

Dendritic cells are remarkably efficient at enhancing infection of targets. When we exposed cultured MDDCs to a CCR5-tropic HIV-1 vector encoding the firefly luciferase marker and then added appropriate targets, infectivity was enhanced significantly over a large range of input virus (Fig. 1A). We detected no luciferase activity when the Hut/CCR5 targets were not included, which indicated that the DCs did not become detectably infected under these conditions. To verify that the infectivity of CXCR4-tropic HIV that was tagged with green fluorescent protein (GFP) joined with HIV viral protein R (Vpr) (*5*) was also enhanced by MDDCs, we performed a similar experiment using β-galactosidase indicator cells (Fig. 1B). In addition to the enhancement seen under the coculture conditions, significant capture and transmission of infectious HIV was revealed by washing away unbound virus before challenging the target cells. All subsequent experiments used GFP-Vpr–labeled HIV, which allowed direct fluorescent imaging.

Cultured MDDCs are highly motile cells that tend to grow in suspension in vitro (*6*). After exposure to HIV, MDDCs bound varying amounts of virion particles, from a few to a few hundred per cell. Images of different focal planes of a representative MDDC showed that most of the HIV was found at or near the cell surface (Fig. 1, C and D; movie S1) (*7*).

We next asked whether the localization of HIV particles was altered when DCs contacted target cells. In a time-lapse experiment, two HIV-pulsed DCs were observed shortly after contact with adherent, CD4-positive cells (Fig. 2; movie S2). At the first time frame, the HIV was evenly distributed throughout both DCs. Within 6 min, the DC in the top of the frame began to spread out on the target, and the majority of the HIV relocated to the initial site of contact. Similarly, movement was observed in the other DC at 18 min. Although the HIV in the two DCs moved at different times, the majority of the particles moved within one 3-min time frame, which suggested rapid relocalization after cell-cell recognition.

To determine whether intracellular viral particles can also traffic to sites of cell contact, we performed time-lapse experiments on DCs that had been treated with protease after exposure to HIV. Much of the HIV signal remained after protease treatment, which suggested that these particles reside in an internal compartment not accessible to protease (*4*). When the HIV membranes were stained with a lipophilic dye during viral production (*5*), most of the GFP-labeled particles remained associated with the membrane dye even after trypsinization, which suggested that they were internalized as intact virions (*8*, *9*). When the protease-treated DCs were immediately placed onto target cells, their adhesion to other cells was impaired. However, when the DCs were allowed to recover for an hour, adhesion was restored, and recruitment of the internalized viral particles occurred in a manner similar to that of DCs which had not been treated with protease (fig. S1, movie S2 to S5).

The recruitment of virus to sites of cell contact suggested a mechanism by which DCs might enhance viral infectivity. Antigen-presenting B cells interact with T cells to form a highly ordered, antigen-dependent junction known as the immunological synapse (*10*). Interactions between DCs and T cells are less well characterized. One apparent difference is that DCs are known to form antigen-independent structures with T cells. This interaction results in the recruitment of T cell receptor, CD4, and other molecules seen in the immunological synapse and induces low levels of signaling in the T cell but does not lead to activation (*11*). To determine whether HIV was recruited to the site of contact during antigen-independent interactions between DCs and T cells, we incubated HIV-pulsed MDDCs in solution with

autologous CD4-positive T cells. Under these conditions about 10 to 30% of the DCs were found closely associated with T cells. When we immunostained such conjugates for different surface molecules (Fig. 3, fig. S2), we observed recruitment of CD4 in the majority of conjugates. Recruitment of the adhesion molecule LFA-1 was also apparent in nearly all conjugates examined (Fig. 3B). Likewise, the HIV coreceptors CXCR4 and CCR5 often accumulated at the interface (Fig. 3, C and D). Staining for the DC-specific markers human leukocyte antigen (HLA) or DC-SIGN (Fig. 3, E and F) yielded more ambiguous results. Although all conjugates had significant amounts of HLA or DC-SIGN at the interface, neither was significantly depleted in distal regions of the cell, so the signal at the interface was not obviously higher than in other regions.

In some conjugates, HIV was concentrated in the area of the interface (Fig. 3E). In addition, HIV was often detected in the conjugated T cells, which suggested viral transfer during these short-term experiments (Fig. 3, A, B, and E). However, in the majority of conjugates, much of the virus remained spread throughout the DC with no apparent concentration at the interface. To quantify the number of DCs that had recruited some or all of the HIV to the T cell junction, we randomly selected conjugates and determined the fraction of the total GFP signal in the half of the DC in which T cell interaction occurred (Fig. 4A). A random distribution of HIV would result in a DC with 50% of the signal in each half of the cell, whereas recruitment of all the virus to the site of contact would result in 100% of the signal in the T cell half. Most of the cells did not fall into these two categories, but there were significantly more cells that contained greater than 90% of the signal in the T cell half. On average, about 8% of the cells reside in each of the first nine categories (Fig. 5A). If this is taken as the baseline of recruitment to sites other than the contact region, about 16% of the DCs recruit nearly all of the virus to the side of the DC in contact with the T cell in these experiments.

Maturation of MDDCs by a variety of inflammatory signals improves their ability to transmit HIV (*3*). Consistent with this, lipopolysaccharide (LPS)–activated MDDCs enhanced infection better than immature MDDCs (Fig. 4B). As did immature DCs, the mature MDDC formed antigen-independent interactions with T cells (Fig. 4C). Quantification of viral recruitment showed that virtually all of the HIV was either recruited to the side of the DC in contact with the T cell or to the opposite side of the cell. More than 90% of the HIV resided in the T cell half in 42% of mature MDDCs (Fig. 4D) compared with 24% of the immature DCs (Fig. 4A). Conversely, 27% of mature DC conjugates had essentially all the HIV on the opposite side, compared with 6% in the immature cells. Recruitment away from the site of contact was surprising but was consistent with reports that molecules in the immunological synapse are sometimes recruited to distal regions (*11*, *12*). The increased ability of mature DCs to enhance HIV infectivity and to recruit HIV relative to immature DCs establishes a correlation between HIV recruitment and enhancement of infectivity.

We propose a model whereby the DC binds HIV over its large cell surface area and then effectively concentrates virions by specific recruitment to an infectious synapse structure into which the T cell concentrates the HIV entry receptors CD4, CCR5, and CXCR4. The recruitment of virus and receptors to the same region enhances the likelihood of a productive infectious event, consistent with our observation that even under conditions where virus concentration is too low to initiate infection, addition of MDDCs results in successful infection. The recruitment of trypsin-insensitive viral particles to intercellular junctions suggests that this process involves intracellular compartments. It seems likely that HIV has co-opted antigen capture functions of the DCs to facilitate infection of CD4-positive T cells in vivo (*13*). Recent reports that C-type lectins on dendritic cells play a role in a number of viral (*14*–*16*), bacterial (*17*, *18*) and parasitic (*19*) infections suggest that this antigen capture pathway of DCs may be a weak point that is exploited by many pathogens.

References and Notes

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Supporting Online Material
www.sciencemag.org/cgi/content/full/1084238/DC1
Materials and Methods
Fig. S1, S2
Movie S1 to S5

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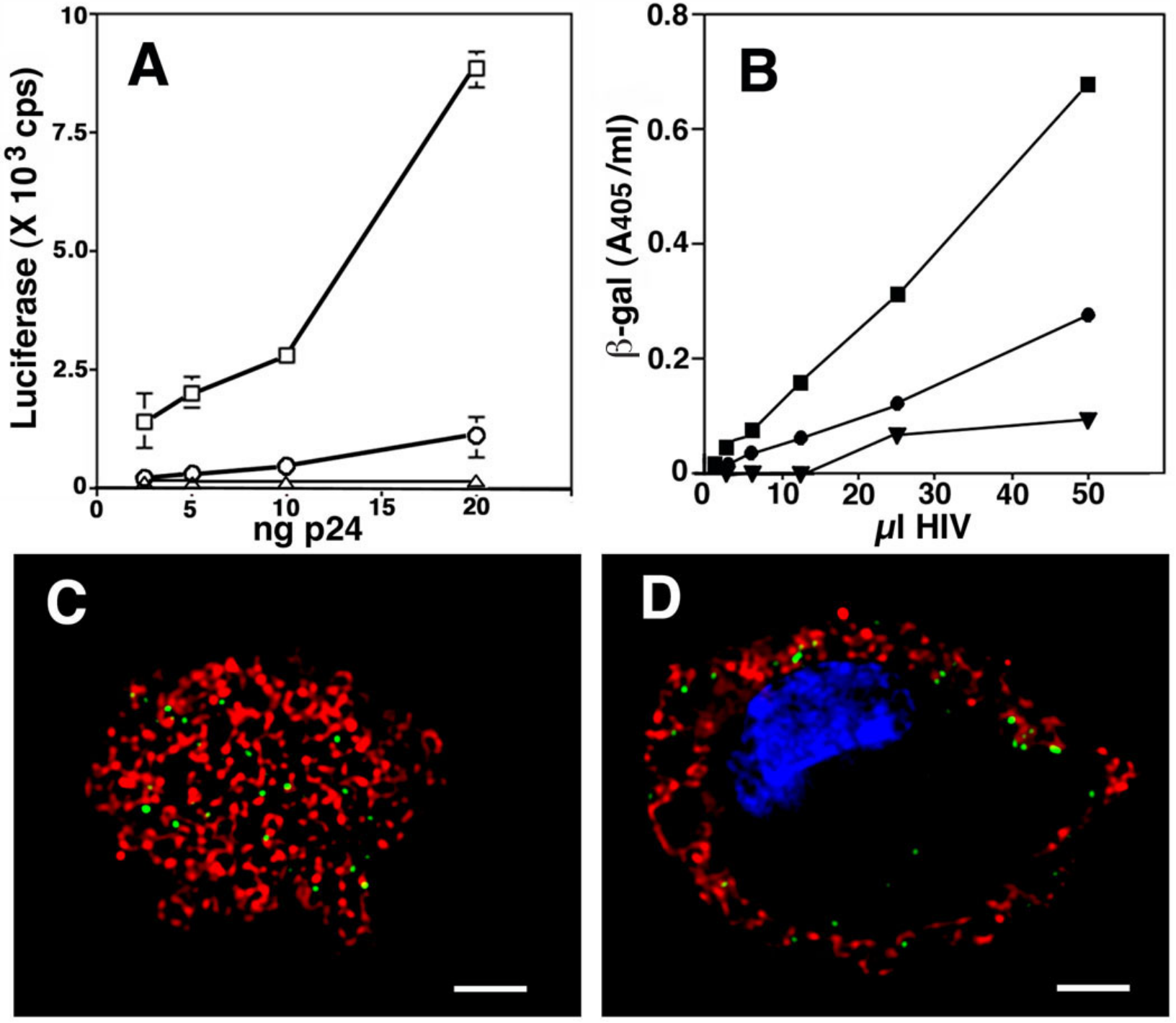
Fig. 1. Dendritic cells enhance HIV infection. (**A**) MDDCs (10^4) (□) were incubated with HIV-Luciferase^{ADA} and then cocultured with Hut/CCR5 cells (10^4). MDDCs (△) or Hut/CCR5 cells (❍) were pulsed with the HIV-Luciferase^{ADA} and cultured alone. Cells were assayed for luciferase activity 48 hours later. (**B**) GFP-Vpr–labeled HIV^{LAI} was added to MAGI indicator cells (10^4) (*20*) without (▼) or with (■) MDDCs (10^4). MDDCs (10^6/ml) (●) were incubated in the same viral supernatant for 1 hour at 37ºC, washed, and plated

onto MAGI cells. Plates were washed at 16 hours and incubated an additional 24 hours before assaying for β-galactosidase activity. (**C** and **D**) Localization of HIV in DCs in suspension. MDDCs were incubated with GFP-Vpr–labeled HIV^{LAI} (green) for 1 hour at 37ºC, washed and stained for surface HLA class II (HLA-DR, -DP, -DQ) by indirect immunofluorescence (red). Cells were fixed, stained with Hoechst dye (blue), and resuspended in Gel Mount. Cells were imaged in z-section and deconvolved. (C) Single plane z-section at the surface of the cell. (D) Single-plane z-section through the interior. Scale bars, 5 μm. See also online movie S1.

Fig. 2. Recruitment of HIV to sites of cell contact. MDDCs were incubated with GFP-Vpr–labeled HIV^{LAI} (green) for 1 hour at 37ºC, washed and stained with a mitochondrial dye (red). The DCs were added to Hos/CD4 cells growing on a coverslip chamber premounted on a microscope and warmed to 37ºC. Hos/CD4 are the reticular structures under the bright DCs. Starting at 1 min after the DCs contacted the target cells, images were collected in z-series every 2 min, deconvolved and reconstructed as three-dimensional volume projections. Scale bar, 10 μm. See also online movie S2.

Fig. 3. Localization of HIV and cell surface molecules in antigen-independent DC–T cell conjugates. (**A** to **F**) MDDCs were incubated with GFP-Vpr–labeled HIV^{LAI} (green) for 1 hour at 37ºC, washed and resuspended (2×10^7/ml). An equal volume of purified $CD4^+$ T cells (8×10^7/ml) was added and incubated at 37ºC for 15 min. Cells were plated onto poly-L-lysine–coated coverslips, allowed to adhere for 2 min at room temperature, and then fixed and stained by indirect immunofluorescence with monoclonal antibodies (mAbs) specific for (A) CD4, (B) LFA-1, (C) CXCR4, (D) CCR5, (E) HLA (-DR, -DP, -DQ), (F) DC-SIGN. Images on the left are actin (red) and Hoechst (blue) staining patterns. Images to right are mAb-specific stains (red). All images are deconvolved volume renderings of four sequential 0.2-μm optical sections through the interior of the cell and rotated so that the T cell is at the top of the frame. Scale bars, 5 μm. See also online fig. S2.

Fig. 4. (**A**) Distribution of HIV in MDDC–T cell conjugates. DC–T cell conjugates generated as in Fig. 3 were randomly selected by scanning coverslips in the actin channel, imaged in z-section, and deconvolved. The entire conjugate was displayed in volume projection. The intensity of the GFP signal was determined in each half of the DC and expressed as the percent of signal in the T cell half (x axis) then divided into 10 groups. The fraction of cells in each group (y axis) was determined from five independent experiments (n = 118). Striped column is the average value of the first nine groups. (**B**) Maturation of MDDCs increases enhancement of HIV infection. MDDCs (△) or LPS-matured MDDCs (10^4) (❍) were incubated with HIV-$Luciferase^{HXB2}$ and then cocultured with Hos/CD4 cells (10^4). (□) Hos/CD4 alone. Cells were assayed for luciferase activity 48 hours later. MDDCs without Hos/CD4 targets did not become detectably infected (not shown). (**C** and **D**) Distribution of HIV in mature MDDC–T cell conjugates. (C) Conjugates between HIV-pulsed (green), LPS-activated MDDCs, and autologous T cells were prepared as described in Fig. 3, fixed and stained for CD4 (right, red). Left shows actin (red) and nuclear (blue) staining pattern. Scale bar, 5μm. (D) Mature MDDC–T conjugates were prepared and analyzed as in (A). Data are from three independent experiments (n = 66).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5.0
2.5
0
0
5
10
15
20
ng p24
B
β-gal (A405 /ml)
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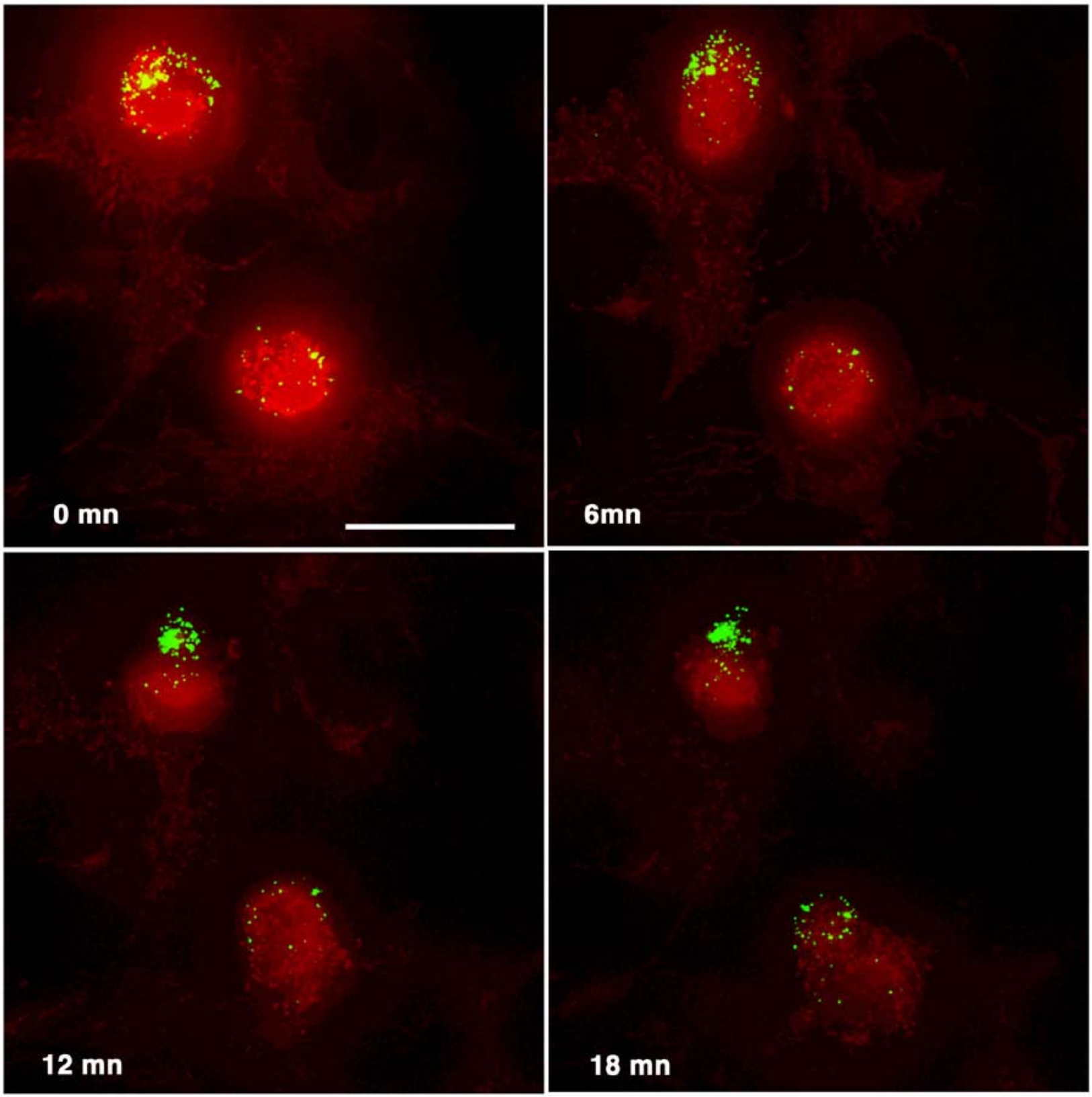
0.6
0.4
0.2
0
0
10
20
30
40
50
μl HIV
C
D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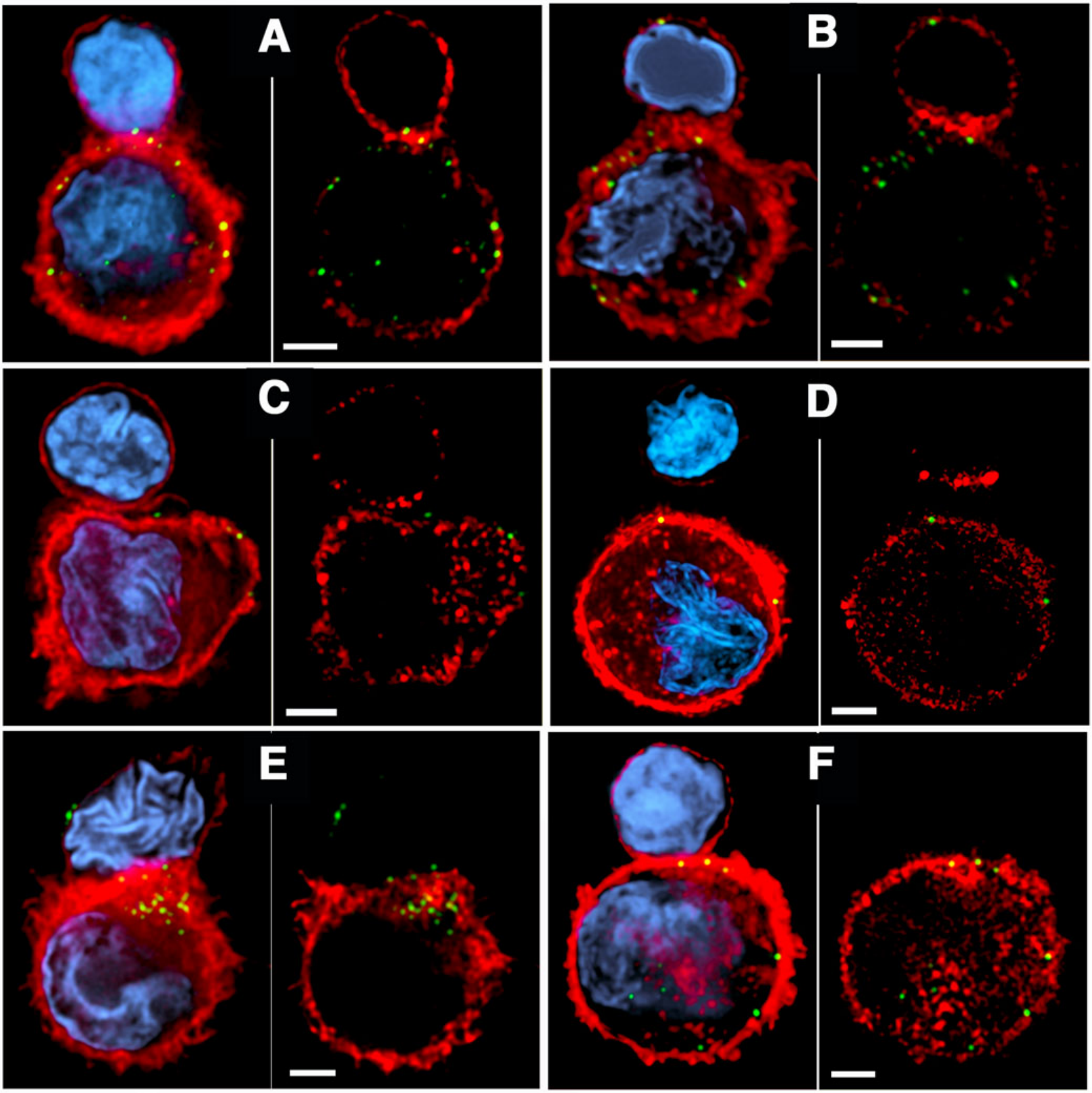
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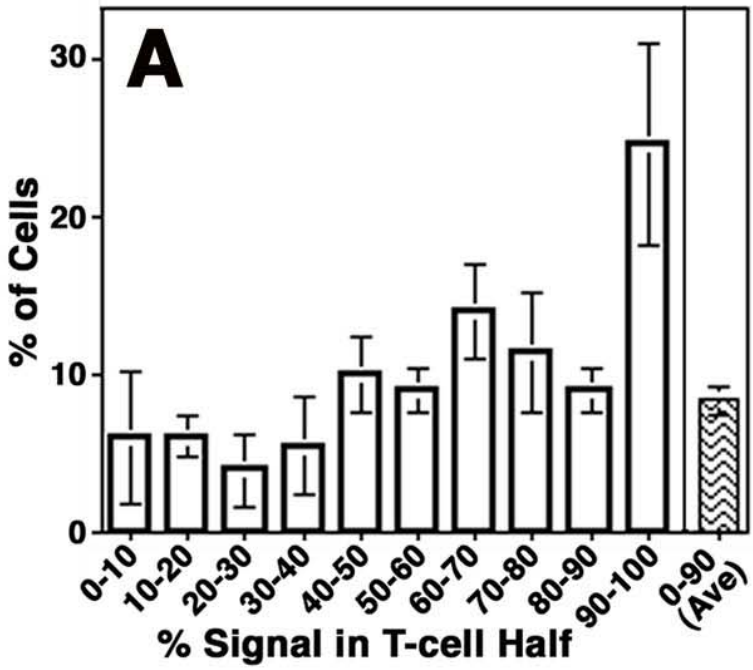
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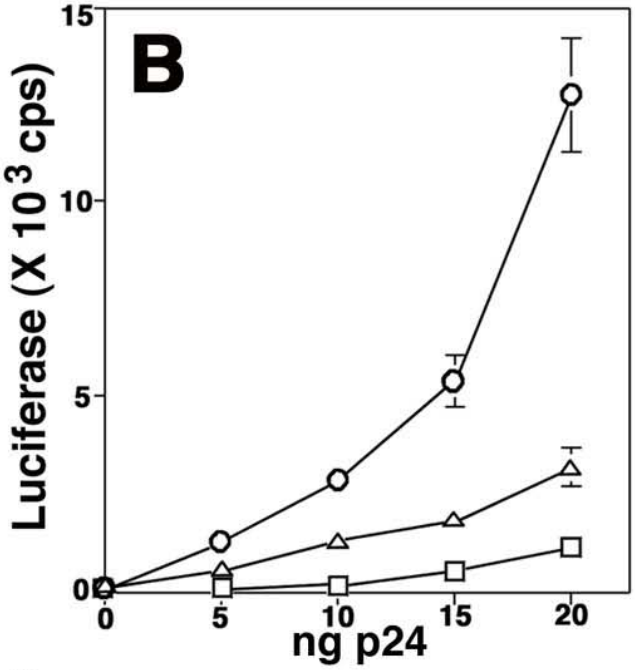
20
10
0
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
0-90 (Ave)
% Signal in T-cell Half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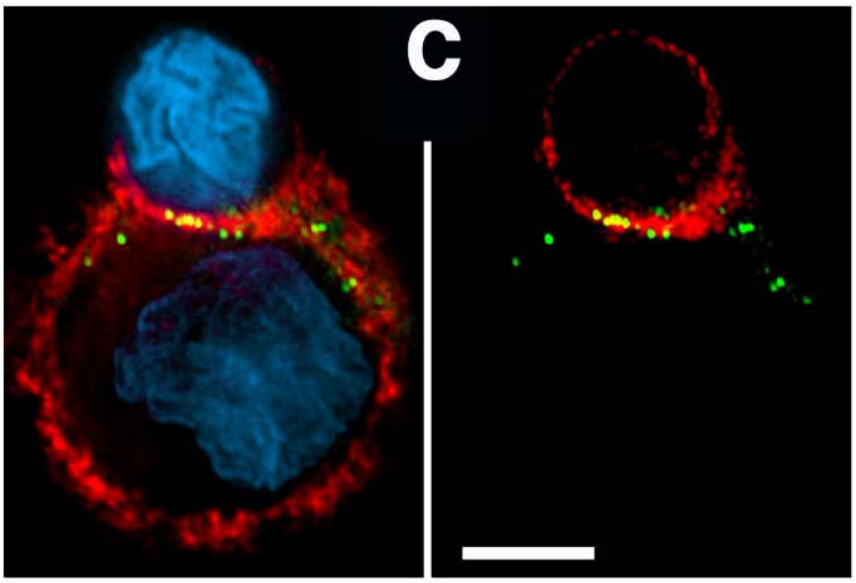
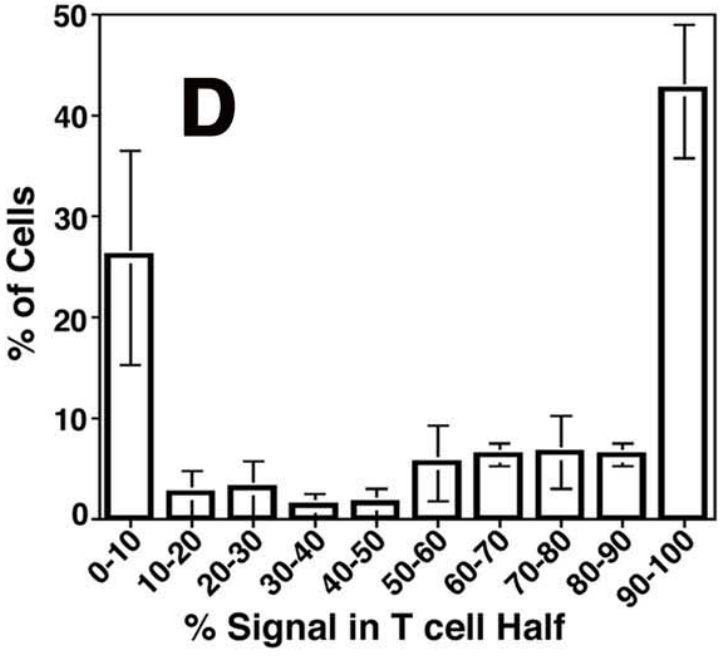

C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% Signal in T cell Half