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Significant Virus Replication in Langerhans Cells following Application of HIV to Abruaded Skin: Relevance to Occupational Transmission of HIV

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The cellular events that occur following occupational percutaneous exposure to HIV have not been defined. In this study, we studied relevant host cellular and molecular targets used for acquisition of HIV infection using split-thickness human skin explants. Blockade of CD4 or CCR5 before R5 HIV application to the epithelial surface of skin explants completely blocked subsequent HIV transmission from skin emigrants to allogeneic T cells, whereas preincubation with C-type lectin receptor inhibitors did not. Immunomagnetic bead depletion studies demonstrated that epithelial Langerhans cells (LC) accounted for > 95% of HIV dissemination. When skin explants were exposed to HIV variants engineered to express GFP during productive infection, GFP+ T cells were found adjacent to GFP+ LC. In three distinct dendritic cell (DC) subsets identified among skin emigrants (CD1a-langerin-DC-specific intercellular adhesion molecule grabbing non-integrin (SIGN)-, CD1a-langerin+ DC-SIGN+ dermal DC, and CD1a-langerin+ DC-SIGN+ dermal macrophages), HIV infection was detected only in LC. These results suggest that productive HIV infection of LC plays a critical role in virus dissemination from epithelium to cells located within subepithelial tissue. Thus, initiation of antiretroviral drugs soon after percutaneous HIV exposure may not prevent infection of LC, which is likely to occur rapidly, but may prevent or limit subsequent LC-mediated infection of T cells. The Journal of Immunology, 2008, 180: 3297–3304.

Occupational exposures place health-care personnel (HCP)¹ at risk for infection with blood-borne pathogens via sharps injuries, exposure of mucous membranes, or contact with nonintact skin (e.g., exposed skin that is chapped, abraded, or dermatitic) (1). In prospective studies of HCP, the average risk of HIV transmission after a single percutaneous exposure to HIV-infected blood has been estimated to be 0.3% (2). Although episodes of HIV transmission after exposure to nonintact skin have been documented (3), the average risk for transmission by this route has not been precisely quantified (1). Epidemiologic and laboratory studies suggest that several factors increase the risk of HIV transmission after an occupational exposure, including contact with a device visibly contaminated with the patient’s blood, contact with a needle that was in a vein or artery, exposure to hollow-bore needles, a deep injury, and exposure to R5 strains of HIV that use CCR5 for cell entry (1).

Genetic studies have shown that individuals homozygous for a 32-nt deletion in the chemokine receptor CCR5, CCR5A.32, are protected from primary HIV infection despite numerous exposures (4–7). The importance of CCR5 as a critical coreceptor involved in the sexual transmission of HIV is also supported by the observation that the majority of HIV strains isolated from patients shortly after primary infection are R5 viruses (8–10). In addition, topical application of high doses of the N terminus-modified chemokine Na-nonanoyl[thioproline2, cyclohexylglycine3]RANTES (PSC-RANTES) provided full protection against intravaginal chimeric SIV/HIV challenge in female rhesus macaques, suggesting a critical role for CCR5-mediated infection-dependent pathways in HIV entry (11).

In a primate model of SIV infection, there is controversy regarding which cells in the genital mucosa are initially infected with SIV. Studies have demonstrated that the primary infected cells present in the lamina propria of the cervicovaginal mucosa 48–72 h after intravaginal exposure to SIV are T cells or submucosal dendritic cells (DC), but not epithelial Langerhans cells (LC) (12, 13). When vaginal tissue was examined within 18 h following vaginal inoculation, however, up to 90% of the SIV-infected cells were LC (14). These conflicting observations may be the result of SIV-infected LC emigrating from epithelium relatively soon after viral exposure.

DC-specific intercellular adhesion molecule grabbing non-integrin (DC-SIGN), a C-type lectin receptor (CLR) expressed on dermal macrophages and monocyte-derived DC (MDDC) (15, 16), has been shown to bind HIV gp120 and to facilitate HIV infection of T cells in trans (16, 17). Although results from other studies indicate a minor contribution by DC-SIGN in the transmission of HIV from MDDC to T cells (18, 19), DC-SIGN may be involved in viral dissemination. In addition, langerin, an LC-specific CLR, and the mannose receptor, which is expressed on dermal DC, have

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3 Abbreviations used in this paper: HCP, health-care personnel; CLR, C-type lectin receptor; DC, dendritic cell; EGFP, enhanced GFP; IRES, internal ribosome entry site; LC, Langerhans cell; MDDC, monocyte-derived DC; PEP, postexposure prophylaxis; PSC-RANTES, Na-nonanoyl[thioproline2, cyclohexylglycine3]RANTES; DC-SIGN, DC-specific intercellular adhesion molecule grabbing non-integrin; TCID₅₀, 50% tissue culture infecting dose.

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been shown to bind HIV gp120 (20), suggesting their participation in virus transmission from DC to T cells. In addition, CLR may also enhance de novo CD4/coreceptor-dependent infection of DC (21). The cooperation of CLR and CD4/HIV coreceptors in facilitating de novo infection of DC has been termed cis infection (19–21).

To understand how HIV traverses the skin and genital mucosa, we recently developed an ex vivo model in which epithelial tissue explants obtained from suction blister roofs were exposed to HIV (22). By contrast to the studies using MDDC, results from this model indicated that resident LC transmit HIV to T cells via a CD4/CCL5-mediated infection-dependent pathway, and not by CLR-mediated capture pathways (23, 24). LC infection levels in this model correlated with host skin explants, and infection of all the possible cell types present in skin was studied in detail.

Materials and Methods

Reagents and Abs

All mAbs were purchased from BD Biosciences, except for anti-p24 mAb and anti-langerin mAb (Beckman Coulter), and anti-DC-SIGN mAb (R&D Systems). Mannan and mannose were purchased from Sigma-Aldrich. R. Offord (University of Geneva, Geneva, Switzerland) provided PSC-Systems). Mannan and mannose were purchased from Sigma-Aldrich.

Assessment of HIV infection

To quantify numbers of infected cells, cells that spontaneously emigrated from skin explants were collected 3–4 days following HIV exposure, as indicated in the Materials and Methods, the emigrant cells from uninfected skin showed occasional low positive staining, while those from HIV-exposed skin explants were incubated with Dispase II (2.5 mg/ml) in RPMI 1640 at 4°C. After 4–6 h, the skin was washed to remove dispase, and using fine forceps, the epidermis was separated from the dermis. Epidermal sheets were then exposed to 100-μl droplets containing NLCSFv3EGFP at 10,000 TCID50/ml for 2 h at 37°C, washed to remove unbound virus, and then floated on culture medium to allow migration of LC from the explants. The emigrating cells from epidermal sheets were collected 3 days following HIV exposure.

Assessment of HIV transmission to CD4+ T cells

PBMC were isolated by density centrifugation and enriched for CD4+ T cells by negative selection using a commercially prepared mAb mixture/ complement reagent (Lympho-Kwik; One Lambda), according to manufacturer’s guidelines. The emigrating cells from infected explants were collected 3–4 days after HIV exposure, and then co-cultured with 2.5 × 106 resting allogeneic CD4+ T cells in an approximate skin cell emigrant/T cell ratio of 1:100. In some experiments, HIV-exposed skin explants were incubated with Dispase II (2.5 mg/ml) in RPMI 1640 at 4°C. After 6 h, the epidermis was separated from the dermis, and both layers were washed in PBS. Epidermal and dermal sheets were floated on culture medium for 3–4 days to allow migration of cells from the separated sheets. Cells emigrating from three epidermal or dermal sheets were collected and washed before adding to CD4+ T cells in coculture. In some experiments, the emigrant cells from HIV-exposed skin explants were incubated with control IgG or mAbs against CD3, HLA-DR, or langerin, followed by sheep anti-mouse IgG-coated magnetic beads (Dynal Biotech), according to the manufacturer’s protocol. Negative populations were cocultured with CD4+ T cells, respectively. For detection of secreted HIV p24 protein, supernatants were examined for p24 protein content by ELISA (Beckman Coulter).

Virus infection of skin explants ex vivo

Split skin was obtained from HIV-negative healthy donors undergoing plastic or corrective surgery (written consent was obtained from all tissue donors, according to the Local Research Ethics Committee). The epidermal surface of skin was abraded with a wire brush to remove the corneal layer. The skin was stored at 4°C and used within 2 h of collection. Skin explants were prepared by cutting abraded skin into 8.0-mm circular pieces. For infection, skin explants were placed in wells of 24-well plastic plates, and, as previously described (30), 3% agarose was added to confine the inoculates to the epidermis by sealing the surrounding area. Virus was added to the epidermal surface, and the plates were incubated at 37°C for 2 h. In other experiments, virus was added directly to culture medium, and entire skin plants were floated on the culture medium. For some experiments, skin explants were preincubated for 20 min at 37°C with various inhibitors, and then HIV was added at 1/100 final dilution was added before incubation for an additional 2 h at 37°C. After incubation, skin explants were extensively washed to remove unbound virus and inhibitors. After a wash step, three to five infected skin explants were floated on culture medium, RPMI 1640 (Invitrogen Life Technologies) supplemented with heat-inactivated 10% FCS (Sigma-Aldrich), 2 mM l-glutamine, 10 mM nonessential amino acids, 1X penicillin/streptomycin, and 25 mM HEPES, in 6-well plates, with each experimental condition performed in duplicate. In some experiments, skin explants were incubated with Dispase II (2.5 mg/ml, Roche Diagnostics) in RPMI 1640 at 4°C. After 4–6 h, the skin was washed to remove dispase, and using fine forceps, the epidermis was separated from the dermis. Epidermal sheets were then exposed to 100-μl droplets containing NLCSFv3EGFP at 10,000 TCID50/ml for 2 h at 37°C, washed to remove unbound virus, and then floated on culture medium to allow migration of LC from the explants. The emigrating cells from epidermal sheets were collected 3 days following HIV exposure.

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After epidermal and dermal exposure to HIV, emigrant cells from HIV-exposed skin transmit infection to T cells. During ex vivo culture of skin explants, resident LC, dermal DC, and T cells spontaneously emigrated from explants into surrounding medium in 1–3 days. In experiments in which the numbers of cells emigrating from individual skin explants were determined, the mean cell yield ± SD was 8.4 ± 1.7 × 10^3 (n = 5). The number of cells recovered from the skin explants was similar to that obtained by others (31, 32). We next characterized DC/macrophage subsets migrating from skin explants. HLA-DR^+ migratory cells contained three distinct subsets, as follows: CD1a^+/langerin^+ DC-SIGN^+ LC, CD1a^+/langerin^+ DC-SIGN^+ dermal DC, and CD1a^+/langerin^+ DC-SIGN^+ dermal macrophages, and each subset exhibited comparable surface expression levels of CD4 and CCR5 (Fig. 1A).

In initial experiments, HIV_{1Ba.L} (an R5 virus) was added to the entire skin explants, and the emigrating cells from the explants were collected 3–4 days following HIV exposure. As shown in Fig. 1, emigrating cells from HIV-exposed skin explants induced high levels of HIV infection when cocultured with resting allo- geneic CD4^+ T cells. We could not detect p24 protein in culture supernatants of emigrating cells cultured alone (data not shown), suggesting that the main source of secreted p24 protein in the cocultures was T cells. When anti-CD4 mAb was preincubated with skin explants before HIV exposure, HIV p24 production in the supernatants was substantially inhibited (Fig. 1B). By contrast, mannan, a known inhibitor of CLR binding, partially, but significantly inhibited HIV p24 production in the supernatants, and when combined with CD4 mAb did not increase its inhibition provided by CD4 mAb alone (Fig. 1B). These data suggest that, after spontaneous epidermal and dermal exposure, transmission of R5 HIV from skin emigrants to T cells is dependent upon a CD4- and CLR-dependent infection process. We then investigated the cell type or types responsible for transmission of virus to T cells. As shown in Fig. 1C, HLA-DR^+ cells accounted for as much as 95% of HIV-1 dissemination, whereas DC^3^ cells contributed partially. These results suggest that HIV-1 dissemination by migratory cells is largely mediated by DC subsets, and DC-T cell conjugates also contribute to the dissemination.

**Results**

**After epidermal and dermal exposure to HIV, DC and T cells that have emigrated from HIV-exposed skin explants transmit virus to CD4^+ T cells**

We next tested whether CLR were involved in HIV transmission from skin emigrants to CD4^+ T cells after epidermal exposure to HIV. HIV_{1Ba.L} was applied to the surface of abraded skin explants, and the emigrating cells from the explants were collected 3–4 days following HIV exposure. Consistent with previous report (31), abrasion of the epidermal surface had no detectable effect on the phenotype of the emigrant cells (data not shown). As shown in Fig. 2, emigrating cells from HIV-exposed skin explants induced high levels of HIV infection when cocultured with allogeneic CD4^+ T cells. We could not detect p24 protein in culture supernatants of emigrating cells cultured alone (data not shown). Interestingly, when PSC-RANTES, a chemically modified RANTES analog and potent CCR5 inhibitor, was preincubated with skin explants before HIV exposure, HIV p24 production in the supernatants was clearly inhibited (Fig. 2, A and B). By contrast, mannan did not affect HIV p24 production in the supernatants (Fig. 2, A and B). No cellular toxicity was observed for PSC-RANTES at the doses used in these experiments (24). HIV transmission mediated by skin emigrants was also not affected by preincubation of skin explants with mannos or KRH-1636, a small molecule CXCR4 antagonist (Fig. 2B and data not shown). Preincubation of skin explants with CD4 mAb blocked subsequent transmission of HIV_{1Ba.L} to cocultured CD4^+ T cells, whereas preincubation with either DC-SIGN mAb or langerin mAb did not affect HIV p24 production in culture supernatants (Fig. 2A). These data suggest that, after epidermal exposure, transmission of R5 HIV from skin emigrants to T cells is totally dependent upon a CD4- and CCR5-dependent and CLR-independent infection process.

We then investigated the cell type or types responsible for transmission of virus to T cells after epidermal exposure to HIV. We first examined the relative contributions of emigrating cells from the epidermis and from the dermis to HIV dissemination. The surface of abraded skin explants was exposed to HIV_{1Ba.L} for 2 h, and the epidermis was then separated from the dermis using dispase. The emigrating cells from the epidermis or dermis were examined for virus carriage to cocultured allogeneic CD4^+ T cells. Interestingly, emigrating cells from epidermal, but not dermal, sheets carry HIV (Fig. 2C). Consistent with this finding, LC-depleted emigrating cells from HIV-exposed skin explants failed to transmit infection to cocultured T cells (Fig. 2D). These results indicate that...
LC play a critical role in virus dissemination from skin emigrants to T cells.

**HIV-infected cells were detected in LC, but not in dermal DC or macrophages, emigrating from R5 HIV-exposed skin explants**

Others have shown that emigrating cells from skin explants contain three main populations of cells, as follows: HLA-DR^+^CD3^−^LC/DC, CD3^+^HLA-DR^−^T cells, and HLA-DR^−^CD3^+^LC/DC-T cell conjugates (31, 33). To determine which populations are infected by R5 HIV using our new model, HIVBa-L was applied to the surface of abraded skin explants. The number of LC/DC and T cells emigrating from the explants was variable, depending on the donor. In a series of five experiments, analysis of the emigrant cells showed a mean ± SD of 22.9 ± 8.3% T cells, 17.7 ± 9.5% LC/DC-T cell conjugates, and 35.1 ± 14.5% LC/DC. HIV p24^+^ cells were detected in HLA-DR^−^ populations: LC/DC and LC/DC-T cell conjugates, but not in HLA-DR-negative populations (Fig. 3A). To determine which DC/macrophage subsets (i.e., CD1a^+^langerin^+^DC-SIGN^−^LC, CD1a^+^langerin^+^DC-SIGN^−^dermal DC, and CD1a^−^langerin^+^DC-SIGN^+^dermal macrophages observed in Fig. 1A) are infected by HIV, HLA-DR^−^ cells migrating from HIV-exposed skin were further analyzed for infection. Interestingly, we could detect HIV p24^+^ cells in langerin^+^LC (R2), whereas langerin^−^DC/macrophages (R1) demonstrated <1% HIV-infected cells (Fig. 3B). In three experiments in which HIV-infected emigrant cells were characterized, the results for percentage of HIV p24^+^ cells in LC vs dermal DC/macrophages were 9.4 vs 0.0%, 5.6 vs 0.8%, and 12.2 vs 0.1%, respectively. These results suggest that LC are the major target for HIV infection when the epidermal surface of abraded skin is exposed to virus. Variability in LC infection levels may be due to CCR5 heterogeneity in skin donors, as documented by previous findings (23). In this model, disruption of the corneal layer of the epidermis was necessary for infection, because no HIV-infected cells were detected in the migrating cells when virus was applied to the surface of nonabraded skin (data not shown).

**HIV replicates within LC emigrating from R5 HIV-exposed skin explants**

Recently, we established an ex vivo model whereby resident LC within epithelial tissue explants are exposed to R5 HIV and found productive virus infection of LC, as evidenced by the following observations: 1) positive staining for HIV p24, 2) virions budding from cell surfaces, and 3) detection of HIV transcripts (22–24, 34–36). To test directly whether HIV can replicate within LC, skin explants from control skin explants unexposed to HIV were used as a representative of three separate experiments derived from separate donors.
virus exposure, GFP weakly positive cells with dendritic morphology were observed in emigrating cells from epidermal sheets (Fig. 4C). Because CD3$^+$ H11001 T cells were never detected in the emigrating cell populations (data not shown), this result indicates that low levels of productive R5 HIV infection occur in LC without interaction with T cells.

**Visualization of viral transmission from HIV-infected LC to T cells**

To visualize HIV transmission from LC to CD4$^+$ T cells, NLCSF$\nu_4$EGFP (an R5 virus) or NL43EGFP (an X4 virus) was applied to the surface of abraded skin explants, and emigrating cells from HIV-exposed skin were labeled with PKH67 Red before coculture with allogeneic T cells. In the cocultures of emigrants from NLCSF$\nu_4$EGFP-exposed skin and T cells, we could detect GFP expression within PKH$^+$ large cells with dendritic morphology (i.e., HIV-replicating LC), and the number of GFP$^+$ LC progressively increased over the first week (Fig. 5, A and B). By contrast, we could not detect GFP$^+$ cells within the coculture of emigrants from NL43EGFP-exposed skin and T cells (Fig. 5B). Between day 10 and 12 following coculture of emigrants from NLCSF$\nu_4$EGFP-exposed skin and T cells, a number of GFP$^+$ PKH$^+$ small T cells was visible in cocultures (Fig. 5C). PKH$^+$ T cells expressing high levels of GFP were found adjacent to PKH$^+$ GFP$^+$ LC, suggesting that HIV-infected LC directly transmitted HIV to T cells (Fig. 5C).

R5 HIV, but not X4 HIV, applied to skin explants induces HIV infection in T cells cocultured with skin emigrants

To compare the efficiencies of R5 HIV and X4 HIV dissemination using our new model, viral inoculates containing 10,000 TCID$_{50}$.
of NLCSFV3EGFP (R5 HIV), JRFLEGFP (R5 HIV), or NL43EGFP (X4 HIV) were applied to the surface of abraded skin explants, and emigrant cells were cocultured with allogeneic T cells. Twelve days following coculture, GFP cells were gated and further examined for GFP expression in each population. Data shown represent at least two separate experiments derived from separate donors.

FIGURE 6. Selective R5 HIV dissemination. Emigrating cells from skin explants exposed to the indicated HIV strains were labeled with PKH67 Red before coculture with T cells. Cultured cells were stained with anti-CD3 mAb and analyzed by flow cytometry. PKH<sup>+</sup> (R1) or PKH<sup>-</sup> (R2) cells were gated and further examined for GFP expression in each population. Data shown represent at least two separate experiments derived from separate donors.

Discussion

Percutaneous injury, usually inflicted by a hollow-bore needle, is the most common route of occupational HIV transmission. HIV may be transmitted to an accident victim by direct inoculation of exogenous virus into recipient blood vessels of the dermis. In addition, our results suggest that DC subsets that are resident within skin may also play a role in initial infection and dissemination of virus. There could be several possible pathways that HIV is transmitted from resident cutaneous DC to T cells, as follows: de novo or cis infection-dependent pathway or infection-independent pathways via CLR (37–39). Following spontaneous epidermal and dermal exposure, we found that transmission of HIV from skin emigrants to T cells occurs through a CD4- and a CLR-dependent manner. Blockade of CD4 substantially inhibited subsequent HIV dissemination, whereas blockade of CLR partially inhibited virus dissemination (Fig. 1). In addition, combination of CD4 and CLR blockade did not increase inhibition provided by CD4 mAb alone, suggesting that de novo infection is predominantly involved in the uptake of virus by resident skin DC. Of note is that, although HIV dissemination by migratory cells is largely mediated by DC subsets, T cells within migratory cells also contributed partially. This suggests that DC-T cell conjugates contribute greatly to viral dissemination, as suggested by previous findings that HIV infection is highest in DC-T cell conjugates when emigrated skin cells were directly exposed to HIV (33, 40).

The molecular and cellular events that occur following occupational exposure of nonintact skin to HIV have not been previously defined. Our data indicate that CD4- and CCR5-mediated productive HIV infection of LC, and not C-type lectin-mediated capture of virus by LC or DC, play a major role in virus dissemination when the epidermal surface of abraded skin is exposed to virus. Selective infection of epidermal LC within skin resident DC populations observed in our model may be due to restricted access to subepithelial cells conferred by desmosomes and tight junctions within epithelial tissue (38).

Reece et al. (31) also used skin explants to model early events of HIV transmission. These investigators exposed HIV to skin specimens overnight, and, using a PCR-based assay, demonstrated that R5 HIV was found in both epidermal and dermal emigrant DC. The conflicting findings regarding infection of dermal DC may be a result of the duration of virus exposure to the epidermal surface of abraded skin (overnight vs 2 h). Because similar conflicting results were observed in the rhesus macaques studies (12–14), it is probable that virus-infected LC emigrate from epithelial surfaces into subepithelial tissues during overnight virus exposure. Thus, our data suggest that epithelial LC play a critical early role in transmitting R5 HIV to cells within underlying subepithelial tissue.

Unlike effective R5 HIV dissemination by LC, LC emigrating from X4 HIV-exposed skin explants failed to transmit infection to cocultured T cells (Fig. 6). In cocultures, R5 viral infection in LC was much more efficient than X4 virus infection (Figs. 5 and 6), suggesting LC are preferentially infected with R5 HIV probably due to differential HIV coreceptor expression on resident LC (25). In this regard, it has been reported that DC-SIGN, and probably other CLR (including langerin), bind R5 and X4 viruses equally well (16), suggesting that these molecules may not be responsible for the preferential selection of R5 viruses observed in our model. By contrast, a recent study revealed that langerin on LC prevents LC infection of HIV and viral dissemination (41). This study showed that HIV captured by langerin was internalized into Birbeck granules and degraded. Nevertheless, our results indicate that when LC were exposed to HIV at high virus concentrations (10,000 TCID), significant LC infection of R5 virus and viral transmission to T cells were observed, suggesting that langerin is saturated at these concentrations and is not able to protect against infection. Because CCR5-dependent and CLR-independent virus dissemination was predominantly observed in our model using high concentrations of R5 HIV (Fig. 2), we believe that direct HIV infection of resident LC most likely plays a pivotal role in occupational transmission of HIV following exposure of nonintact skin. The disruption of the corneal layer of the epidermis before virus application to the surface of skin was necessary for LC infection of HIV, indicating that the corneal layer functions as a protective barrier for intact skin. Alternatively, it is possible the abrasion of skin might induce LC activation and subsequent down-regulation of langerin, leading to the enhanced infection of LC in our model.
Furthermore, HIV replication in cocultures predominately occurred in LC-T cell conjugates (Fig. 5). Because activated CD34-derived LC have been recently shown to facilitate the trans infection of cocultured T cells (42), LC-T cell conjugates may allow for T cell-mediated activation of LC and subsequent trans infection from HIV-infected LC to responding T cells.

In the retrospective case-control study of HCP, use of zidovudine as postexposure prophylaxis (PEP) was associated with a reduction in the risk of HIV infection (43). Animal studies have demonstrated the importance of starting PEP soon after an exposure (44, 45), and PEP probably is substantially less effective when delayed (46, 47). Our data support the importance of starting PEP soon after an exposure, because PEP may prevent systemic infection and larger viral production of T cells. Because LC most likely become infected soon after exposure, PEP is probably not acting upon HIV replication in these cells, but rather the later step of LC-mediated infection of T cells. In addition to the timing of starting PEP, our findings suggest that other factors (e.g., presence of R5 HIV strains in the source person) may influence the efficacy of PEP. Further studies are now underway using our model to determine the effects of PEP administered at various times following HIV exposure.

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Disclosures
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