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

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Occupational exposures place health-care personnel (HCP) 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, CCR5A32, 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
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/CCR5-mediated infection-dependent pathway, and not by CLR-mediated capture pathways (23, 24). LC infection levels in this model correlated with host CCR5 genotype (e.g., CCR5Δ32), and the genetic susceptibility of LC to HIV infection paralleled genetic susceptibility to HIV in cohorts of HIV-infected individuals (23). These results, along with the finding that immature resident LC express surface CCR5, but not surface CXCR4 (25), suggest that selective R5 HIV transmission observed in epidemiologic studies most likely occurs at the level of the LC. This has been referred to as the primary gatekeeper model.

In our current study, we have modified our previous explant model to focus more on the cellular mechanisms that may be involved following occupational HIV exposure to nonintact skin. HIV was applied to the abraded epithelial surfaces of split-thickness 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-laminin 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-RANTES (a newer, more potent analog of RANTES) (26). N. Yamamoto (Tokyo Medical and Dental University, Tokyo, Japan) provided KRH-1636, a small molecule CXCR4 antagonist that blocks X4 HIV-1 entry into target cells (27).

Viruses

Purified, pelleted, and titered HIVBa-L, an R5 HIV laboratory isolate, stock content by ELISA (Beckman Coulter). Supernatant were measured by ELISA (ZeptoMetrix). The p24Gag insert was rescued from insertion of the IRES sequence. The ATG codon of EGFP was placed 2 bp downstream of gp41 termination codon, and nef expression was rescued from insertion of the IRES sequence. The R5 virus-expressing GFP (NLCSF-V3EGFP) was constructed by replacing the V3 sequence in the NL-EGFP with the V3 sequence from JRCSF. Another R5 HIV-1 expressing GFP (JRLF-GFP) was generated through insertion of the EGFP/IRES fragment in pNL4-3 by inserting an enhanced GFP (EGFP) gene and an internal ribosome entry site (IRES) sequence between gp41 and the nef sequence by PCR-based subcloning. The ATG codon of EGFP was placed 2 bp downstream of gp41 termination codon, and nef expression was rescued from insertion of the IRES sequence. The nef sequence was previously described (28, 29). Briefly, the X4 HIV-1 expressing GFP (NL-EGFP) was constructed from pNL4-3 by inserting an enhanced GFP (EGFP) gene and an internal ribosome entry site (IRES) sequence between gp41 and the nef sequence by PCR-based subcloning. The ATG codon of EGFP was placed 2 bp downstream of gp41 termination codon, and nef expression was rescued from insertion of the IRES sequence. The nef sequence was previously described (28, 29). Briefly, the X4 HIV-1 expressing GFP (NL-EGFP) was constructed from pNL4-3 by inserting an enhanced GFP (EGFP) gene and an internal ribosome entry site (IRES) sequence between gp41 and the nef sequence by PCR-based subcloning. The ATG codon of EGFP was placed 2 bp downstream of gp41 termination codon, and nef expression was rescued from insertion of the IRES sequence. The nef sequence was previously described (28, 29).

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 HIVBa-L 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 t-glutamine, 10 mM nonessential amino acids, 1X penicillin/streptomycin, 10 mM sodium pyruvate, 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 NLCSF-V3EGFP 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 (Lympo-Kwik; One Lambda), according to manufacturer’s guidelines. The emigrating cells from explants were collected 3–4 days after HIV exposure, and then cocultured 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 Ig-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).

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 described above, and analyzed by flow cytometry, as previously described (23). Briefly, skin emigrants were preincubated with mouse anti-CD16 mAb and anti-CD52 mAb (Eagle Tech), according to the manufacturer’s instructions. The emigrating cells from uninfection were incubated with FITC-conjugated rat anti-p24 mAb or isotype control Ab diluted in Perm/Wash (BD Biosciences) containing 2% rat serum for 30 min at 4°C. Cells were then examined by flow cytometry using a FACSCan (Becton Dickinson) and a Vantage VH Analyzer (H1A5, KEYENCE). In some experiments, the emigrating cells were labeled with PKH67 Red (Sigma-Aldrich), according to manufacturer’s instructions, before adding to CD4+ T cells in coculture.
that obtained by others (31, 32). We next characterized DC/macrophages from the skin explants was similar to the mean cell yield of cells emigrating from individual skin explants were determined, and the expression levels in each population are shown (bold line) along with isotype control staining (thin line) (lower panels). A. Entire skin explants were preincubated with mannan (200 μg/ml) or indicated Abs (40 μg/ml) before exposure to HIV-1Ba-L, and emigrant cells were cocultured with allogeneic CD4+ T cells. * p < 0.05 compared with the control IgG-preincubated explants. B. Cells migrating from HIV-exposed skin explants were collected, 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 T cells. HIV p24 levels in coculture supernatants (SN) were assessed by ELISA. Data shown represent at least two separate experiments derived from separate donors.

**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**

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 numbers 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 allogeneic 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 CD3+ 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.

**After epidermal exposure to HIV, HIV-infected LC 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 mannan or KRN1-1636, a small molecule CXC4R 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 coculture 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 emigrant cells from HIV-exposed skin explants were stained for the surface Ags shown or intracellular HIV p24. HIV p24 levels in coculture supernatants (SN) were assessed by ELISA. Data shown represent at least two separate experiments derived from separate donors.

**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, 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).
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\(^+\)/H11001 T cells, NLCSF\(_{\text{R}}\)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 being cocultured with T cells. Images derived with FITC (green: GFP) and rhodamine filters (red: langerin) were combined (yellow). Representative HIV-infected GFP PKH\(^+\) LC (arrows, A and B) and HIV-infected GFP PKH\(^+\) T cells (arrows, C) are shown. Insets (A): higher magnifications. B, GFP\(^+\) cells in PKH\(^+\) cells were counted in the cocultures of emigrating cells from NLCSF\(_{\text{R}}\)EGFP (○)- or NL43EGFP (△)-exposed skin. In PKH\(^+\) cells, GFP\(^+\) cells were not detected during the first week. Scale bar: A, 50 μm; C, 10 μm. D, The cocultures were processed for flow cytometry following langerin and CD3 staining. GFP\(^+\) cells of each gated population in emigrating cells are shown. Data shown represent at least two separate experiments derived from separate donors.

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 observed from NLCSFV3EGFP or JRFLEGFP infections, but not from NL43EGFP infections (data not shown). To quantify numbers of HIV-transmitted T cells at the single-cell level, emigrating cells were labeled with PKH67 Red before coculture with T cells and then analyzed by flow cytometry. PKH^+ (R1) or PKH^- (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.

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^+ (R1) or PKH^- (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 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-dependent 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 infection of dermal DC. The conflicting findings regarding infection of dermal DC subsets, T cells within migratory cells also contributed partially. This suggests 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).

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Furthermore, HIV replication in cocultures predominantly 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, whereas PEP probably may prevent systemic infection and larger viral replication in these 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 test the effects of PEP administered at various times following HIV exposure.

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Disclosures

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