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HIV-Infected Langerhans Cells Preferentially Transmit Virus to Proliferating Autologous CD4⁺ Memory T Cells Located within Langerhans Cell-T Cell Clusters

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Langerhans cells (LC) are likely initial targets for HIV following sexual exposure to virus and provide an efficient means for HIV to gain access to lymph node T cells. The purpose of this study was to examine the nature of the CD4⁺ T cell that becomes infected by HIV-infected LC. We infected human LC within tissue explants ex vivo and then, 3 days later, cocultured HIV-infected LC with different subsets of autologous CD4⁺ T cells. Using multicolor flow cytometric analyses of LC-CD4⁺ T cell cocultures, we documented that HIV-infected LC preferentially infected memory (as compared with naive) CD4⁺ T cells. Proliferating and HIV-infected CD4⁺ memory T cells were more frequently detected in conjugates of LC and autologous CD4⁺ T cells, suggesting that T cells become activated and preferentially get infected through cluster formation with infected LC, rather than getting infected with free virus produced by single HIV-infected LC or T cells. p24⁺ Memory CD4⁺ T cells proliferated well in the absence of superantigens; by contrast, p24⁺ T cells did not divide or divided only once in the presence of staphylococcal enterotoxin B, suggesting that virus production was rapid and induced apoptosis in these cells before significant proliferation could occur. These results highlight that close interactions between dendritic cells, in this case epidermal LC, and T cells are important for optimal HIV replication within specific subsets of CD4⁺ T cells. Disrupting cluster formation between LC and memory CD4⁺ T cells may be a novel strategy to interfere with sexual transmission of HIV. The Journal of Immunology, 2004, 172: 2219–2224.

Understanding the biologic events that underlie sexual transmission of HIV is critical in designing interventions that interfere with transmission efficiency. Langerhans cells (LC), defined as dendritic cells (DC) that reside within stratified squamous epithelia, have been identified as the dominant cell type initially infected following intravaginal exposure to SIV in rhesus macaques (1). An earlier macaque study by Ho and colleagues (2) also implicated LC as initial targets for SIV. Importantly, Hu and colleagues (1) examined genital tissue at time points <24 h following intravaginal exposure and used flow cytometry to identify SIV within individual LC; these particular strategies were not used in earlier studies that did not detect intraepithelial LC infection (2–4).

It is now clear from numerous in vitro studies that there are at least two pathways by which HIV interacts with DC: 1) CD4- and CCR5-mediated infection by virus and 2) cell surface capture of virus by DC-specific ICAM-3 grabbing nonintegrin and other C-type lectins (5–11). Using a tissue explant model, however, we found no evidence that C-type lectins expressed by LC within epithelium are involved in transmission of virus from LC to CD4⁺ T cells (11). Unlike monocyte-derived DC that can capture HIV via DC-specific ICAM-3 grabbing nonintegrin and transmit virus in trans to T cells (6), LC-mediated transmission of virus to T cells requires LC infection in a CD4- and CCR5-dependent manner (11). Our work with LC, as well as the macaque findings by Hu et al. (1), suggests that infection of LC, rather than capture of virus by LC, is an early biologic event that occurs during sexual transmission of HIV.

Single mature LC and other types of mature DC possess the characteristic ability to cluster with numerous T cells during immune activation. In addition, the DC-T cell microenvironment has been described as an “explosive” site for HIV replication, with many studies now showing transmission of virus to T cells by LC and other types of DC (5, 8–13). This phenomenon is likely to be important in understanding several biologic aspects of HIV transmission. For instance, efficient LC-mediated transmission of HIV to T cells could sufficiently explain how virus could move from mucosal tissues and gain rapid access to draining lymph nodes (the normal sites of LC emigration following Ag exposure). This phenomenon also explains how minute amounts of infecting virus or how relatively few infected LC could lead to productive infection of large numbers of CD4⁺ T cells.

Previously, using a different model system, we showed that HIV preferentially infected HIV-specific CD4⁺ T cells (14). The precise nature of the type of T cells that become infected by HIV-infected LC, however, is unknown. In this study, we examined this issue by infecting human LC with HIV ex vivo and subsequently coculturing these cells with autologous memory and naive CD4⁺ T cells. Using multicolor flow cytometric analyses of LC-CD4⁺ T cell cocultures, we document that HIV-infected LC preferentially transmitted virus to proliferating, CD4⁺ memory T cells located within LC-T cell clusters. These results suggest that disrupting cluster formation between LC and CD4⁺ T cells may be a novel strategy to interfere with sexual transmission of HIV.

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‡ Abbreviations used in this paper: LC, Langerhans cell; DC, dendritic cell; SEB, staphylococcal enterotoxin B

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Materials and Methods

Study participants

The Institutional Review Board of the National Cancer Institute approved all aspects of this study, and informed consent was obtained from all healthy individuals volunteering for the suction blister procedure and venipuncture.

Reagents

All of the murine mAbs were purchased from BD Pharmingen (San Diego, CA), except for PE-conjugated rat anti-p24 mAb (Beckman Coulter, Fullerton, CA). CFSE was purchased from Molecular Probes (Eugene, OR), and staphylococcal enterotoxin B (SEB) and brefeldin A were purchased from Sigma-Aldrich (St. Louis, MO). Purified, pelleted, and titered HIV-1inu (stock at 2.47 \times 10^4 virus particles/ml) was purchased from Advanced Biotechnologies (Columbia, MD).

HIV infection of LC ex vivo

Epithelial tissue explants were prepared from suction blister roofs of healthy volunteers, and LC within these explants were infected as previously described (9, 11). Briefly, 50 µl droplets containing HIV-1inu at 1/100 dilution in RPMI 1640 were placed on the inside surface of sterile plastic culture dish covers and explants were draped over droplets and incubated together at 37°C for 2 h. Explants were washed three times with sterile PBS, and then floated in 6-well plates containing 4 ml of complete medium.

Assessment of HIV infection in LC

LC that had emigrated from explants were collected 3 days following HIV exposure, preincubated in staining buffer for 10 min at room temperature to block nonspecific staining, and then incubated with 10 µg/ml PE-conjugated mouse anti-human HLA-DR mAb for 30 min at 4°C. Cells were then incubated with Dead Red (Molecular Probes) for 20 min at room temperature to label dead cells and fixed and permeabilized with Cytofix/Cytoperm reagents (BD Pharmingen) for 20 min at 4°C. Cells were then incubated with 10 µg/ml FITC-conjugated rat anti-p24 mAb diluted in Perm/Wash for 30 min at 4°C and examined by flow cytometry using a FACSscan (BD Biosciences, Mountain View, CA) equipped with Lysis II software (BD Biosciences). Cells were acquired and analyzed with FlowJo software (Tree Star, San Carlos, CA).

Statistical analyses

Statistical analyses were performed using Mann-Whitney U tests with STATVIEW 5.0.1 software (SAS Institute, Cary, NC).

Results

HIV-infected LC preferentially infect autologous memory CD4+ T cells in the presence or absence of SEB

Recently, we established an ex vivo model whereby we expose LC within epithelial tissue explants to HIV, and then allow emigration of LC from tissue (9). In this study, to model interaction of LC with CD4+ T cells in vivo, we cocultured HIV-infected LC with autologous naive or memory peripheral blood CD4+ T cells. Before coculture with T cells, HIV infection levels in LC were determined by flow cytometry; infection levels varied among different skin donors (range, 0.94 to 15.6%; mean, 5.78%), consistent with our past results (9, 11). We previously have shown that emigrated LC exhibited a mature DC phenotype, including high expression of the costimulatory molecules CD40, CD80, CD86, and MHC class II (9), which is a phenotype known to facilitate clustering of DC with T cells (15).

HIV-infected mature LC transferred infection to cocultured CD4+ T cells as determined by HIV p24 positivity in gated T cell populations. Gating was performed so that background HIV p24 staining in cultures containing only T cells and in those cocultures containing uninfected LC and T cells were <0.07%. We previously confirmed a 1:1 relationship between the number of p24+ cells and copies of gag DNA (14), thus validating our multicolor flow cytometric analyses as an appropriate means to detect single HIV+ cells. HIV p24+ CD4+ T cells were detected in both naive and memory CD4+ T cell populations, however, CD4+ memory T cells were more susceptible to infection (Fig. 1). Addition of SEB increased proliferation of CD4+ T cells, however, SEB did not increase the overall percentages of p24+ CD4+ T cells in cocultures. In other words, the overall percentage of infected T cells did not increase with the addition of SEB because SEB induced a significant increase in the number of uninfected T cells. There was no relationship between infection levels in LC and those in CD4+ T cells (data not shown). This result is not surprising because in LC-T cell cocultures, HIV infection levels in T cells are more likely to be related to T cell activation by LC rather than to HIV infection levels in LC (5). In additional experiments described below, only CD4+ memory T cells were cocultured with LC.

Conjugates of LC and autologous CD4+ T cells are more frequently detected in populations of proliferating CD4+ memory T cells

DC (e.g., LC) readily form clusters when cocultured with T cells (12, 16). Such clusters are composed of one DC surrounded by numerous T cells. During staining procedures, these clusters are either disrupted or excluded by the flow cytometry machine. Thus,
only conjugates of single DC with single T cells can be identified by expression of both DC and T cell markers when examined by flow cytometry. We used Abs directed against CD1a, a marker for LC, and CD3, pan-T cell marker, to identify conjugates. First, we gated on either proliferating or nonproliferating CD4⁺/H11001 memory T cells (i.e., CFSE diluted vs CFSE undiluted) and examined these two populations for evidence of LC-T cell conjugates. Populations of proliferating CD4⁺ T cells preferentially contained CD1a⁺/H11001 CD3⁺ conjugates, whereas nondividing CD4⁺ T cells rarely contained CD1a⁻CD3⁺ conjugates (Fig. 2A). We confirmed those conjugates were larger and more granular (higher forward light scatter and higher side light scatter) as expected (data not shown). Most proliferating CD4⁺ T cells cultured without SEB were located in LC-T cell clusters (Fig. 2B). This general pattern occurred whether T cells were cocultured with HIV-infected or uninfected LC, or either in the presence or the absence of SEB (Fig. 2B). Of note, SEB decreased the overall percentage of conjugates, suggesting either increased number of proliferating CD4⁺ T cells per one cluster or increased proliferating CD4⁺ T cells located outside the clusters.

Conjugates of LC and autologous CD4⁺ T cells are more frequently detected in populations of HIV-infected CD4⁺ memory T cells

Next, we gated on either p24⁺ or p24⁻ memory T cell populations and examined each group for evidence of LC-T cell conjugates. The HIV-infected p24⁺ CD4⁺ T cell population preferentially contained CD1a⁺CD3⁺ conjugates (Fig. 3A). In fact, CD1a⁺CD3⁺ conjugates were rarely observed in p24⁻ cell populations. This general pattern occurred either in the presence or the absence of SEB (Fig. 3B). This suggested that T cells became infected through cluster formation with infected LC, rather than getting infected by free virus produced by single HIV-infected LC or T cells.
More proliferating T cells are detected in populations of p24+ cells in the absence of SEB, whereas most HIV-infected T cells do not divide or divide only once in the presence of SEB.

The relationship between HIV infection and CD4+ T cell proliferation was examined in more detail by examining proliferation, i.e., CFSE dilution, either in CD4+ memory T cells cocultured with HIV-infected LC or uninfected LC, or in gated populations of HIV-infected or uninfected CD4+ memory T cells. SEB induced more proliferation of CD4+ memory T cells whether they were cocultured with HIV infected or uninfected LC. The percentage of proliferating cells tended to be smaller and the division index, the average number of divisions that a cell has undergone, was significantly smaller when cocultured with HIV-infected LC.

Next, we focused on gated populations of HIV-infected or uninfected CD4+ memory T cells. In cocultures grown in the absence of SEB, the p24+ CD4+ memory T cells were present in cell fractions that had divided 0–4 times and many showed ≥3 divisions (Fig. 4B). The percentage of proliferating cells and the division index were much higher compared with p24− CD4+ memory T cells (Table I).

**Discussion**

In this report, we describe a novel coculture and multiparameter flow cytometry model system that we have used to examine the nature of the CD4+ T cells that are infected by HIV-infected LC. We first found that HIV-infected LC preferentially infected memory (as opposed to naive) CD4+ T cells. Next, we showed that conjugates of LC and autologous CD4+ T cells were more frequently detected in populations of proliferating and HIV-infected CD4+ memory T cells. p24+ Memory CD4+ T cells proliferated well in the absence of superantigen; by contrast, p24− T cells did not divide or divided only once in the presence of SEB.
results could be important in designing strategies to block spread of HIV following sexual exposure to virus.

In a previous report by our group (14), using a different experimental approach, we found that HIV preferentially infected HIV Ag-specific T cells. In that paper, naive and memory T cells were stimulated with SEB 3 days before HIV infection and then analyzed for p24 expression. We found that more p24 was expressed in naive T cells (or more correctly, in the cells that had been naive before they were exposed to SEB). In this current report, SEB was added at the time that LC and T cell cocultures were initiated. We also did not attempt to correlate HIV infection levels with Ag specificity using our LC-T cell system described in this study. In another paper by our group (17), we showed that memory T cells were more susceptible to SEB activation when compared with naive T cells. Thus, because memory T cells respond more readily to SEB activation, we believe this may be the reason why we saw higher p24 expression in memory T cells when compared with naive T cells in our current short-term culture experiments. Alternatively, memory T cells may have exhibited greater p24 expression because they interact with and respond to LC more readily than naive T cells (with or without SEB).

Our results suggest that most LC-mediated HIV infection of CD4+ T cells does not occur randomly or in the absence of some T cell selection. Recent reports have shown that HIV infection of DC induces expression of chemokines in these cells that differentially attract certain T cell subsets. HIV Tat or Nef protein expression within monocyte-derived DC increased production of IFN-inducible protein-10 and monokine induced by IFN-γ (18, 19) or macrophage inflammatory protein-1α and -β, respectively (20, 21). These chemokines are ligands for either CXCR3 or CCR5, which are more highly expressed on memory CD4+ T cells as compared with naive T cells (22, 23). Thus, although we did not specifically study this in our system, it is possible that induction of specific chemokines within HIV-infected LC led to preferential chemotraction and infection of memory CD4+ T cells (Fig. 1). Alternatively or additionally, because CCR5 is also important as a coreceptor for R5 HIV, differences in susceptibilities between naive and memory CD4+ T cells in our system could be attributed to higher expression levels of CCR5 in the latter cell population (data not shown).

Cellular activation is critical for replication of HIV (24). Not surprisingly, we preferentially detected conjugates of LC and autologous CD4+ T cells in populations of proliferating and HIV-infected CD4+ memory T cells, suggesting that T cells become activated and infected through cluster formation with infected LC rather than getting infected from free virus produced by single HIV-infected LC or T cells (Figs. 2 and 3). This is consistent with data from numerous groups who have identified the DC-T cell milieu as an explosive site for HIV replication. As emigrated LC exhibited a mature DC phenotype, it is not surprising that HIV-exposed LC cultured in the absence of exogenous Ag were able to induce cluster formation, activation, and infection in autologous T cells. CD4+ memory T cells cocultured with HIV-infected LC proliferated less compared with those cocultured with uninfected LC (Fig. 4A and Table I). The difference, however, was very small probably because of small percentages of HIV-infected LC in this system. This phenomenon was consistent with our previous report in which we showed that HIV-infected DC were poor stimulators of allogeneic CD4+ T cell proliferation and IL-2 production (10). Interestingly, in the presence of SEB, HIV-infected CD4+ T cells did not divide or divided only once (Fig. 4B). We hypothesize that virion production by CD4+ T cells is efficient and rapid in the presence of HIV-infected LC and SEB, inducing apoptosis of infected T cells before significant proliferation can occur. HIV-infected LC may have produced pro-apoptotic factors in the presence of SEB, because HIV-infected APCs have previously been reported to produce pro-apoptotic factors, including TNF-α (20, 21, 25), Fas ligand (25), and TNF-related apoptosis-inducing ligand (26, 27).

In summary, these results highlight that close interactions between DC, in this case epidermal LC, and T cells are important for optimal HIV replication within specific subsets of CD4+ T cells. Strategies designed to decrease conjugate formation between DC and T cells, and thus decrease T cell activation and proliferation,
may be particularly effective in blocking the spread of HIV following sexual exposure to virus. One strategy may be to block chemotaxis of T cells toward HIV-infected DC using chemokine or chemokine receptor antagonists. A second strategy may involve blocking full activation of T cells by HIV-infected DC by addition of Abs that interfere with costimulatory molecule function.

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References


