Chemokine Receptor Expression on Mucosal Dendritic Cells from the Endocervix of Healthy Women

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Dendritic cells (DCs) may be an initial target of human immunodeficiency virus (HIV) during heterosexual transmission. An analysis of DCs in the intraepithelial layer of the endocervix of the female genital tract from healthy women showed that ~20% expressed CD1a, and 30% expressed cutaneous leukocyte antigen (CLA). Langerin, a molecule associated with Langerhans DCs, was on CD1a-positive and -negative DCs and on CLA-positive cells. CCR5 and CXCR4 were detected on CD1a-positive and -negative cervical DCs. These findings suggest that DCs in the genital tract are potential targets for macrophage-tropic and lymphotropic strains of HIV.

The mucosal-associated lymphoid tissue (MALT) of the female genital tract continually encounters commensal bacteria and pathogenic microorganisms. By contrast to the MALT of the respiratory and intestinal tracts, knowledge is scarce about the local immunity of the female genital mucosa. Important components of this local immune system are dendritic cells (DCs), which are responsible for presenting antigens and initiating an immune response [1]. DCs have been proposed to be targets of HIV during heterosexual transmission [2]. After infection or the attachment to DCs via DC-specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN), they migrate to the draining lymph node and are thought to transmit virus to helper T cells during antigen presentation [3]. The results of in vitro studies have shown that the highest level of virus replication occurs in these DC–T cell clusters [4]. In addition to a CD4 molecule, HIV requires a chemokine coreceptor for cellular entry. The 2 main chemokine coreceptors used by HIV are CCR5 and CXCR4, which are necessary for infection by macrophage (M)– and lympho (T)–tropic variants, respectively [5, 6].

After exposure to HIV, invariably it is M-tropic variants that become established, even when it is known that virus was transmitted from an individual carrying both M- and T-tropic strains of HIV [7]. Langerhans cells from skin have been reported to express CCR5 but not CXCR4; on the basis of this observation, it has been suggested that mucosal Langerhans cells might facilitate the preferential transmission of M-tropic HIV variants [8]. In the present report, we show that DCs in the endocervix express both CCR5 and CXCR4; therefore, preferential transmission by M-tropic variants cannot be explained solely by a lack of appropriate chemokine coreceptors.

Patients, materials, and methods. The study group consisted of 28 women attending the Genito-Urinary Medicine department for sexual health screening. Details of previous sexually transmitted infections, the time of last sexual intercourse, and type of contraception used were obtained from all women, including dates of the last menstrual period. Informed consent was obtained from all individuals who participated in the study. The study received approval from the Harrow ethics committee.

Participants were placed in the standard position for gynecological examination, and samples of endocervical secretions were obtained using sterile cotton-tipped swabs, for the screening of bacterial vaginosis, candida, Chlamydia trachomatis, Neisseria gonorrhoeae, and herpes simplex virus culture. Women with evidence of any of these infections were excluded from the study. A 10-μL plastic loop was used to collect endocervical samples, which were then smeared onto a glass slide and Gram stained for the quantification of polymorphonuclear leukocytes (PMNLs) per high-powered field (hpf). To minimize any cellular variability due to hormonal cycling, samples from women were obtained during the midcycle (median, 12 days; range, day 8–15 of the menstrual cycle). None of the women had evidence of vaginal warts, although specific tests for human papillomavirus to detect subclinical infection were not done.

The cervical os was wiped clean, to reduce genital secretions, before the insertion of a fine cervical cytobrush (Medscand). This was placed within the cervical canal and rotated, withdrawn, and held in a sterile centrifuge tube of sterile PBS that contained 100 U penicillin/mL, 100 μg streptomycin/mL, and 100 μg glutamine/mL. All cytobrush samples had negative results for blood contamination. We have previously presented
Figure 1.  

A, Percentage of cervical dendritic cells (DCs) expressing CD1a and cutaneous leukocyte antigen (CLA). B, Expression of CCR5 and CXCR4 on DC subpopulations. Left, White bar, CD1a+ DCs; shaded bar, CD1a/H11002 DCs. Right, White bar, CLA+ DCs; shaded bar, CLA/H11002 cells. Data are the mean no. of DCs in healthy women with minimal inflammation and no infection (n = 25). Vertical bars, SE; significance is denoted by **P < .0001 and *P < .05.

evidence that cells obtained by this procedure are derived predominantly from the intraepithelial compartment [9]. Samples were transported to the laboratory and processed within 2 h of collection, a process that involved minimal culturing. Cervical specimens were vortexed before the cytobrush was removed. They were then filtered through a 70-μm nylon cell strainer (Becton Dickinson) and centrifuged at 200 g for 10 min, and the resulting pellet was immunolabeled.

Leukocytes were preincubated for 15 min at room temperature with 20 μL of normal rabbit serum. After incubation on ice for 20 min, cells were washed once with 6 mL of FACS buffer (10% fetal calf serum, 1 mmol EDTA/L, and 10 mmol NaN₃/L), fixed in 1% paraformaldehyde (buffered with 0.1 mol phosphate buffer/L [pH 7.2]), and stored overnight at 4°C.

An analysis of chemokine receptor expression was performed on the first 25 samples, and a further 3 samples were used for studies of langerin expression. Monoclonal antibodies used were fluorescein isothiocyanate conjugated to anti-CD1a and to cutaneous leukocyte antigen (CLA); phycoerythrin conjugated to anti-CD3, anti-CD14, anti-CD16, anti-CD19, anti-CD56, and anti-langerin; peridinin chlorophyll protein conjugated to anti-HLA-DR; allophycocyanin conjugated to anti–chemokine coreceptors (anti-CCR5 and anti-CXCR4); and anti-CD1a. DCs were identified as those cells that expressed major histocompatibility class II DR but did not label with anti-CD3, anti-CD14, anti-CD16, anti-CD19, and anti-CD56 (antigens that are expressed by T and B lymphocytes, macrophages, and NK cells, respectively). Cell preparations were labeled in parallel with the appropriate isotype control antibodies (Becton Dickinson). Events were acquired by use of an acquisition gate, to reduce non–mononuclear cell components. A minimum of 50,000 events was acquired within a leukocyte acquisition gate. Data were recorded and counted using FACSCalibur software (Becton Dickinson).

These data are presented as arithmetic mean ± SE. The mean values were compared by use of Student’s t test for unpaired means, and P < .05 was considered to be significant.

Results. There were 28 women in the study, all of whom showed minimal inflammation of the endocervix (<5 PMNLs/hpf). On culture, all specimens had negative results for C. trachomatis, herpes simplex virus, and N. gonorrhoeae. All participants were HIV antibody negative according to the results of standard ELISA.

As was previously described, cervical specimens exhibited a high level of granularity and autofluorescence that could be attributed to a combination of factors, including dislodged epithelial debris and dead cells expelled from the uterus. Background fluorescence was minimized by the introduction of an acquisition gate on the forward-scatter (FSC) versus side-scatter (SSC) profile, which excluded most of the nonleukocyte fraction. This allowed labeled cells to be differentiated and reliably distinguished from cellular debris and epithelial cells [9]. Cervical DCs were identified by the absence of labeling with a mixture of lineage antibodies (anti-CD3, anti-CD14, anti-CD16, anti-CD19, and anti-CD56) and the expression of HLA-DR. In a typical cervical specimen, ~1% of cells were mononuclear, of which 10% were DCs.

Cervical DCs could be divided into 2 populations on the basis of their expression of CD1a, with the CD1a-positive cells constituting ~20% (range, 8%-39%) of the population (figure 1A). Two major populations of DCs are found in mucosal
tissue, the more superficially located Langerhans cells and the deeper dermal DC population. Langerhans cells express CLA and, uniquely, langerin. We therefore analyzed cervical DCs for the expression of both of these molecules. CLA was detected on ∼30% (range, 12%–48%) of DCs (figure 1A), and >90% of the CLA-positive DCs expressed langerin (figure 2C). CCR5 was present at similar levels on CD1a-positive (range, 0%–10%) and CD1a-negative (range, 1%–15%) DCs. CXCR4 was expressed at a higher level than CCR5 on both CD1a-positive (range, 5%–40%) and CD1a-negative (range, 4%–30%) DCs (figure 1B). Both chemokine coreceptors were detected on CLA-positive DCs. Higher numbers of CLA-negative (range, 0%–8%) than CLA-positive (range, 6%–13%) DCs expressed CCR5 (P < .05), as shown in figure 1B. The expression of CXCR4 on CLA-negative (range, 1%–15%) and CLA-positive (range, 1%–20%) DCs was similar.

Selective gating onto lineage-negative HLA-DR-positive DCs enabled the analysis of CD1a and CLA expression. Coexpression of these markers was seen on ∼10% of DCs (figure 2A), and 25% of DCs expressed only CLA. Further analysis showed that approximately one-half of the CD1a-positive DCs and one-half of the CD1a-negative DCs stained positive for langerin (figure 2B). By contrast, virtually all of the CLA-positive DCs stained positive for langerin (figure 2C). Less than 20% of CLA-negative DCs expressed langerin, and then only at a very low level (figure 2C). These observations suggest that CLA could be used as a marker to identify Langerhans cells in cervical-brush cell preparations.

**Discussion.** DCs within the female genital tract are essential for the initiation of mucosal immunity against invading pathogens. However, DCs are also thought to be initial targets for HIV after transmission. The coexpression of CCR5 and...
CXCR4 was observed on different DC subpopulations in the endocervical mucosa. Zaitseva et al. [8] detected the expression of CCR5, but not CXCR4, on skin Langerhans cells. It was presumed that these findings mirrored the situation in the genital tract; thus, this was hypothesized to be a potential mechanism for the preferential transmission of M-tropic variants across genital mucosal tissue. We have now shown that chemokine coreceptor expression on DCs differs in the genital mucosa. CXCR4 is normally up-regulated, because DCs are stimulated to migrate to the draining lymphoid tissue [10]. The antigenic stimulation of DCs in mucosal tissue is likely to be significantly higher than that of DCs in skin, which are protected from the external environment by the stratum corneum—this may explain the observed differences in CXCR4 expression. The entry of T-tropic variants may be blocked by the local production of SDF-1, the ligand for CXCR4 [11].

The expression of CD1a on DCs was evident in 20% of DCs within the intraepithelial layer of the cervix. DCs and Langerhans cells derived in vitro from CD34 stem cells express CD1a [12]. The reason for the low proportion of DCs expressing CD1a in the cervix is unclear. The acquisition gate on the FSC versus SSC profile was based on the location of cells labeling for CD45, and one would not expect a non-DC bone marrow–derived population to express HLA-DR and to be negative for a mixture of lineage markers. DCs do lose CD1a during maturation and migration to lymph nodes, although it seems unlikely that such a high proportion of DCs would be in the process of migrating.

The CLA molecule functions as part of the tissue-specific homing pathway interacting with E-selectin [13]. The majority of cells expressing CLA were positive for langerin, an antigen that is specifically expressed by Langerhans cells. Thus, CLA could be used as an additional marker, although it is marginally less sensitive than langerin, for mucosal Langerhans cells. The cytobrush technique collects superficial leukocytes that predominantly lie in the intraepithelial compartment; thus, one might expect to acquire mainly Langerhans-type DCs rather than the deeper dermal-type DCs. The CLA-positive DCs constitute only 30% of the total DC population, and whether the CLA-negative DCs are destined to become Langerhans cells or constitute another DC population will require further study.

Cytobrushing predominantly samples the intraepithelial layer of the endocervix. However, it is possible that some of the DCs detected in the present study may have migrated out of or been expelled from the uterus. To minimize this potential source of contamination, genital secretions were wiped away before cervical sampling.

The role of DCs after HIV exposure remains controversial, and there are 2 main hypotheses. The first proposes transmission by the initial infection of mucosal DCs. The second hypothesis is that virus binds to DCs via DC-SIGN but does not infect the DCs. In both proposals, it is postulated that there is transmission to CD4 T cells after the migration of DCs to the draining lymph node. The observation made in the present study that mucosal DCs express CCR5 and CXCR4 suggests that they could be potential targets for HIV infection. However, this does not rule out the possibility of transmission also occurring via the DC-SIGN route.

Little is known about where in the female genital tract the transmission of HIV occurs. Although the cervix is considered to be a potential site of HIV entry, because the mucosa is overlaid by a thin columnar epithelial layer, infection may also occur in other regions of the genital tract and may be influenced by hormones [14]. The vagina and ectocervix have a thick, stratified, squamous epithelial layer overlying the mucosa, which may render these regions less susceptible to infection. The protective effect of the stratified epithelial layers is illustrated by experiments in which monkeys given progesterone were found to be more susceptible to simian immunodeficiency infection after vaginal challenge, compared with placebo-treated control monkeys [15]. These findings were proposed to reflect thinning of the vagina mediated by progesterone. In summary, the expression of both CCR5 and CXCR4 by cervical DCs suggests that the favored transmission of a HIV variant after sexual intercourse is not dependent on chemokine coreceptor expression alone, and other mechanisms should be investigated.

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References


