

Secretory Leukocyte Protease Inhibitor Inhibits Infection of Monocytes and Lymphocytes with Human Immunodeficiency Virus Type 1 but Does Not Interfere with Transcytosis of Cell-Associated Virus across Tight Epithelial Barriers

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In the present study, we demonstrate that recombinant human secretory leukocyte protease inhibitor (rhSLPI) inhibits infection of lymphocyte- and monocyte-derived tumor cell lines and peripheral blood lymphocytes with laboratory-adapted isolates and with the primary isolate, NDK, of free human immunodeficiency virus type 1 (HIV-1). In contrast, rhSLPI did not exhibit inhibitory activity toward transcytosis of cell-associated HIV-1 through a tight monolayer of endometrial epithelial cells. These observations indicate that the inhibitory effect of SLPI is restricted to free HIV-1 in corporal fluids.

Human secretory leukocyte protease inhibitor (SLPI) is an 11.7-kDa single-chain polypeptide serine protease inhibitor, produced in epithelial cells (20), that concentrates in extravascular fluids, lining the mucosal surfaces. The physiological role of SLPI is primarily believed to ensure the protection of connective tissues from degradation by endogenous proteolytic enzymes of inflammatory leukocytes (15). SLPI may also exhibit additional functions as a host defense factor, consistent with its location in areas of the body routinely exposed to potential pathogens (e.g., the oral cavity, upper respiratory tract, and genital tract). In particular, SLPI is expressed at high concentrations in the lower female genital tract (6, 7), as shown in Fig. 1.

A specific role for SLPI as an inhibitor of human immunodeficiency virus type 1 (HIV-1) infection has been suggested (12). Thus, the high concentrations of salivary SLPI could contribute to the antiviral activity of saliva that is associated with the infrequent oral transmission of HIV-1 (12, 13, 22, 23). SLPI was reported to inhibit the infection of purified monocytes and T cells and of peripheral blood mononuclear cells (PBMCs) with monocytotropic and lymphocytotropic HIV-1 isolates *in vitro* (18, 22). The antiviral activity of SLPI was shown to reside in the disruption of the infection process soon after virus binding (13). Thus, SLPI could prevent the transmucosal penetration of HIV-1 by inhibiting infection of mononuclear cells that are target cells for HIV in the mucosa (11). However, attempts to document the anti-HIV activity of SLPI unexpectedly failed in the hands of another group (21). This discrepancy has not been explained. In the present study, we reevaluated the anti-HIV-1 activity of SLPI toward lymphocytes and monocytes and further investigated its function by exploring its ability to inhibit viral transcytosis through the mucosal barrier.

We have assessed the activity of recombinant human SLPI (rhSLPI) against free HIV-1 in cultures of the human lympho-

cyte-derived tumor cell line SupT1 and in peripheral blood lymphocytes (PBLs). A primary isolate of HIV-1 subtype A was obtained by culturing PBMCs of an African HIV-1-infected patient and further propagating the virus in the SupT1 cell line, as described previously (17). The strain was of the syncytium-inducing phenotype in a conventional assay with MT2 cells. Replication of HIV was quantitated by measuring p24 antigen released in culture supernatants with an immunocapture enzyme-linked immunosorbent assay (DuPont de Nemours, Wilmington, Del.). Purified rhSLPI was purchased from R & D Systems, Minneapolis, Minn. The biological activity of rhSLPI was measured by its ability to inhibit trypsin-catalyzed hydrolysis of tosyl-Gly-Pro-Lys-4-nitroanilide acetate at a 1:1 molar ratio of rhSLPI to activated trypsin. Free HIV-1 in an amount corresponding to 6,000 pg of p24 antigen was incubated with 20 μ g of rhSLPI in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), under a final volume of 500 μ l, or with culture medium alone, for 60 min at 37°C. The virus was then added to 2×10^6 SupT1 cells and incubated in RPMI 1640, in the presence of 10% FCS and antibiotics (penicillin and streptomycin) at 5% CO₂ for 2 h at 37°C. The cells were washed three times with RPMI 1640 and further cultured for 10 days. The cultures were fed twice a week by replacement of culture medium. The concentration of p24 antigen was measured in culture supernatants at 0, 4, 7, and 10 days. As shown in Fig. 2, the exposure of free virus to rhSLPI resulted in decreases in HIV replication to 28% at day 4 and 33% at day 10, as compared with replication in control SupT1 cells that had been incubated with untreated virus. Similar results were obtained when the human promonocyte-derived tumor cell line U937 infected by HIV-1_{LAI} was used (Fig. 2). In a control experiment, infectivity of free HIV collected in the basal chamber after transcytosis was conserved. We further assessed the inhibitory activity of rhSLPI on PBLs as an HIV-1 carrier. PBLs (8×10^5) were obtained by stimulating PBMCs of healthy donors with phytohemagglutinin (2 μ g/ml) for 3 days prior to interleukin 2 stimulation (10 IU/ml) for 24 h. The primary HIV-1 NDK isolate (19) (5 ng of p24 antigen per ml) was incubated for 1 h at 37°C with rhSLPI (20 μ g) or with RPMI 1640 before being used for infection of

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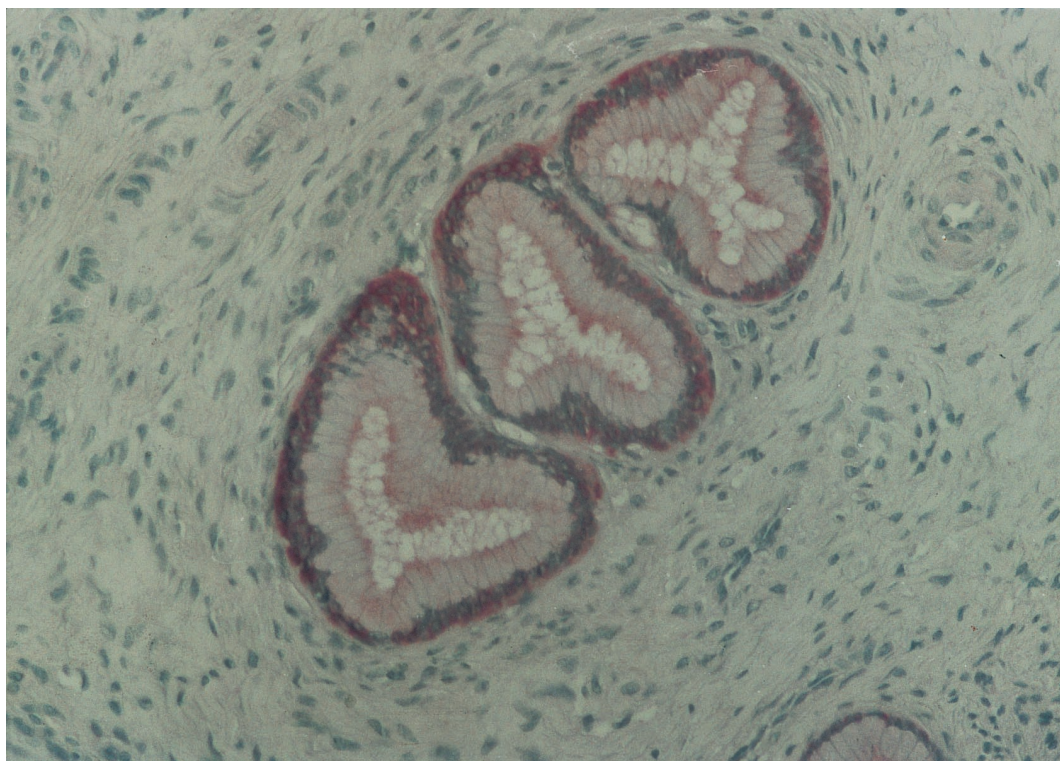


FIG. 1. Immunohistochemical localization of SLPI in the endocervix. A sample of cervix tissue was obtained from a 35-year-old HIV-seronegative woman. Serial sections were saturated by treatment with 3% bovine serum albumin in phosphate-buffered saline (PBS) and further incubated with rabbit IgG anti-human SLPI (3 $\mu\text{g}/\text{ml}$) in PBS-0.1% bovine serum albumin. The sections were washed with PBS and revealed with biotinylated mouse Ig to rabbit Fc γ . After the sections had been washed, streptavidin-biotin-phosphatase alkaline complex was added for 30 min, and the slides were incubated with a chromogenic solution containing fast red. After the slides had been washed, they were counterstained with hematoxylin. Lung tissues were used as a positive control (not shown). The negative control included normal rabbit serum IgG instead of the anti-SLPI antibody. The secretory epithelium and glandular structures of the endocervix, but not the exocervix (not shown), showed marked immunoreactivities for SLPI (in red). Magnification, $\times 200$.

PBLs. The cells were further processed as described above. As shown in Fig. 2, exposure of free virus to rhSLPI resulted in decreases in HIV replication of 64% at day 4 and 47% at day 10, as compared with replication in control PBLs that had been incubated with untreated virus. The possible toxic effect of rhSLPI on cell line SupT1, promonocyte-derived tumor cell line U937, and PBLs was evaluated according to Trypan blue coloration at 4, 7, and 10 days of incubation. The percentages of dead cells were strictly similar in cells incubated with medium alone or with medium plus rhSLPI. These observations demonstrate that rhSLPI inhibits infection of both lymphocyte- and monocyte-derived tumor cell lines and PBLs with laboratory-adapted and primary isolates of HIV-1. Our observations are in accordance with previously reported findings of anti-HIV activity of rhSLPI in cultures of human monocytes (12) and lymphocytes (22).

Several mechanisms are involved in the penetration of HIV-1 through the mucosal barrier. Both free and cell-associated HIV (11, 16) could reach submucosal CD4⁺ cells (i.e., T cells, monocytes/macrophages, and Langerhans' cells), provided that the integrity of the mucosa is compromised. In the presence of an intact mucosal surface, free HIV could enter the mucosa by infecting a susceptible cell harboring the CD4 receptor, such as Langerhans' cells, or by penetrating the mucosal epithelium through a cyclic AMP-dependent transport mechanism involving the interaction of the lectin-like domain of gp120 and mannose residues on glycoproteins on the mucosal surface (9). In addition, mononuclear cell-associated HIV may cross the genital mucosa, as shown in appropriate in

vitro models of transcytosis (4). In order to test whether SLPI interferes with the latter mechanism of transmucosal penetration of HIV, in addition to its inhibitory effect on viral infection of lymphocytes and monocytes, we assessed the effect of rhSLPI on transcytosis of cell-associated HIV-1 in vitro. We used cells of the previously described model of the endometrial epithelial cell line HEC1 (3, 4) grown as a monolayer on a permeable support (4, 5, 8, 14). HEC1 cells do not express CD4, but express galactosylceramide (5) and CXCR4 coreceptor, as assessed by immunofluorescence staining with anti-human CXCR4 monoclonal antibody (R & D Systems) and reverse transcription-PCR (RT-PCR) for mRNA of CXCR4 by using previously described primers (2; data not shown). We verified that HEC1 cells do not produce SLPI, as assessed by negativity of immunofluorescence staining with polyclonal antibody to SLPI, as previously described (10), and lack of detection of SLPI mRNA in HEC1 cells by RT-PCR, as previously described (1). In brief, HEC1 cells were grown as a tight polarized monolayer on a permeable support of 0.4- μm -pore-diameter polycarbonate (Transwell; Costar, Cambridge, Mass.). The tightness of the monolayer of HEC-1 cells was monitored by measuring resistivity at the apical and basolateral poles of the cells, which was above 200 Ω/cm^2 . SupT1 cells were infected with HIV-1 subtype A, as described above. A total of 2×10^6 infected SupT1 cells were preincubated with RPMI 1640 medium supplemented with 10% FCS alone or in the presence of 1, 10, or 20 μg of rhSLPI, under a final 300- μl volume, prior to being deposited on the apical pole of the HEC1 monolayer. The cells were allowed to interact for 180

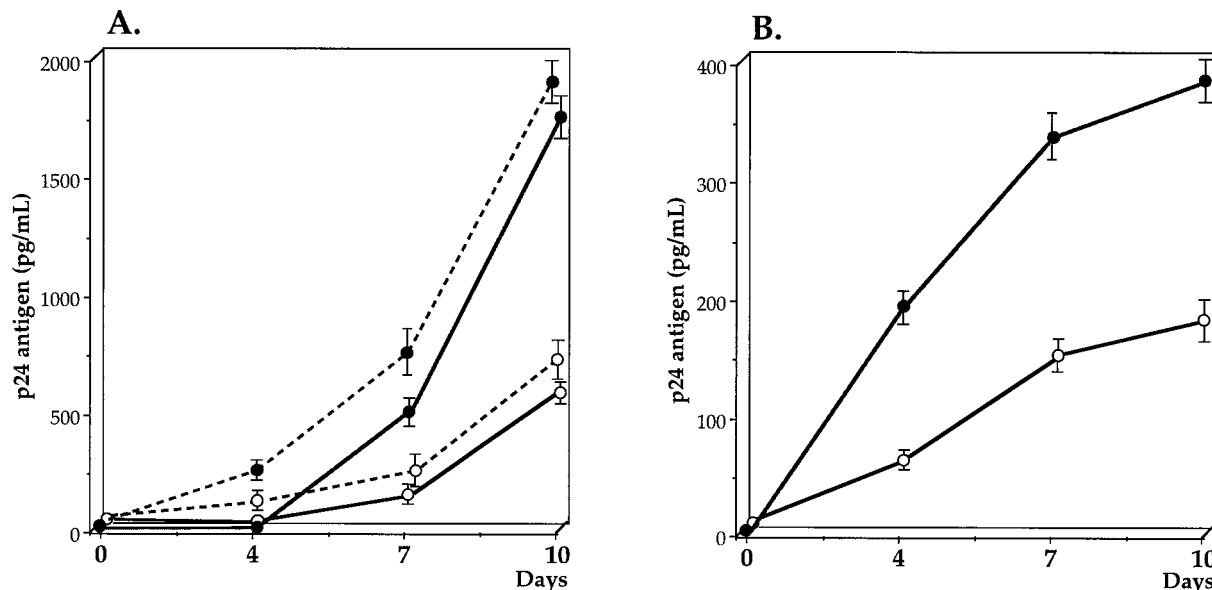


FIG. 2. Effect of rhSLPI on HIV-1 infection of lymphocyte tumor cell line SupT1, promonocyte-derived tumor cell line U937, and PBLs. (A) Free laboratory-adapted HIV-1 subtype A was incubated with rhSLPI (20 μ g) (open circles) or RPMI 1640 (solid circles) for 1 h prior to addition to SupT1 cells (solid line) and further incubation for 2 h. The cells were then washed and cultured for 10 days. Cell supernatants were removed at 3- to 4-day intervals, and p24 antigen was quantitated in supernatants. Free HIV-1_{LAI} was incubated with rhSLPI (open circles) or RPMI 1640 (closed circles) prior to addition to U937 cells (dotted line), as described above in the case of SupT1 cells. (B) Free primary isolate HIV-1 NDK was incubated with rhSLPI (20 μ g) (open circles) or RPMI 1640 (solid circles) for 1 h at 37°C before infection of PBLs, as described above. Data are expressed as the mean \pm standard error from the same experiment performed in duplicate.

min at 37°C. A positive control for inhibition of HIV transcytosis consisted of specific anti-HIV immunoglobulin G (IgG) antibodies purified from pooled HIV-seropositive sera. HIV transcytosis was monitored in the basolateral chamber by measuring the concentration of p24 antigen. As shown in Fig. 3, at all concentrations of rhSLPI that we used, preincubation of infected cells with rhSLPI did not result in inhibition of trans-

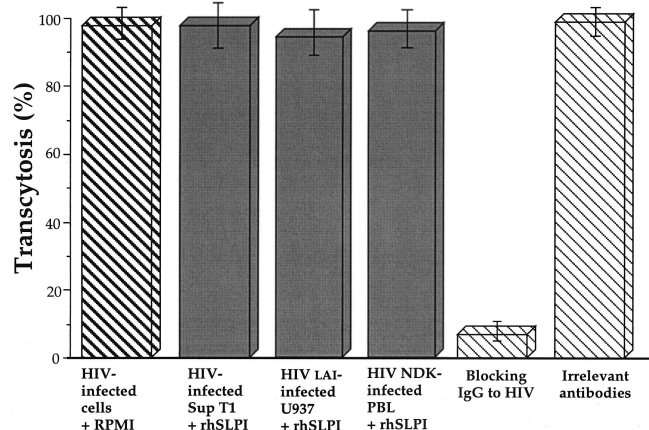


FIG. 3. Lack of inhibitory activity of rhSLPI on transcytosis through a tight epithelial monolayer of SupT1 cell-associated HIV-1 subtype A, of U937 cell-associated HIV_{LAI}, and of the PBL-associated primary HIV-1 NDK isolate. Transcytosis was expressed as the percentage of p24 antigen recovered in the basolateral chamber in the presence of rhSLPI compared with the amount of p24 antigen recovered in the presence of irrelevant IgG. HIV transcytosis was markedly inhibited by immunopurified human polyclonal anti-gp160 IgG antibodies (positive control). No inhibition of transcytosis was observed after preincubation of HIV-infected cells with HIV-seronegative IgG, RPMI 1640 alone, or rhSLPI. Data are expressed as the mean percentage \pm standard error in the same experiment performed in duplicate. Only data obtained with SLPI (20 μ g) are shown.

cytosis. HIV transcytosis was markedly inhibited, however, by anti-HIV IgG antibodies purified from pooled HIV-seropositive serum (1 μ g/ml), but not by purified HIV-seronegative IgG. rhSLPI did not inhibit transcytosis when preincubated with epithelial cells before the addition of HIV-infected cells. Similar results were obtained with PBLs infected with the primary isolate HIV-1 NDK (Fig. 3). The lack of inhibitory activity of rhSLPI on transcytosis was also observed with U937 monocytes infected with the HIV-1_{LAI} isolate (Fig. 3).

Our findings confirm that rhSLPI expresses antiviral activity against free HIV in a model using monocyte- and lymphocyte-derived tumor cell lines and PBLs and document the lack of inhibitory activity of rhSLPI on transcytosis of cell-associated HIV through a monolayer of epithelial cells. We have previously evaluated the cervicovaginal production of SLPI under normal conditions (unpublished data). Cervicovaginal secretion lavage samples from 15 healthy HIV-negative women without genital symptoms were evaluated for SLPI by enzyme-linked immunosorbent assay (R & D Systems). The cervicovaginal SLPI concentration (mean \pm standard error) in healthy HIV-negative women was 8.76 \pm 0.85 μ g/ml. Thus, the concentration of rhSLPI we used in the present study was close to the physiological cervicovaginal concentrations of SLPI.

Taken together with the results of previously published studies (4, 5, 18, 22), these observations suggest that the inhibitory effect of SLPI is restricted to free HIV-1 in corporal fluids, such as saliva, cervicovaginal secretions, or breast milk. The latter effect may be particularly relevant to hamper infection of both intraepithelial Langerhans' cells and submucosal CD4-expressing cells reached by the virus through lesions in the epithelium layer. The lack of inhibition of HIV transcytosis by rhSLPI suggests that SLPI has no effect on cell-mediated infection of mucosae. These observations may be of significance, given the fact that SLPI is largely expressed in the endocervix and the endometrium (6, 7). Our data indicate that the rele-

vance of SLPI in decreasing the infectiousness of the index partner or the susceptibility to HIV of an exposed individual should be considered in the context of the multiple pathways that may be used by HIV to cross the mucosae.

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