ELSEVIER

Antiviral Research 29 (1996) 269–277

Antiviral Research

# Human immunodeficiency virus type-1 (HIV-1) replication is unaffected by human secretory leukocyte protease inhibitor

Jim A. Turpin[a], Catherine A. Schaeffer[a], Ming Bu[a], Lisa Graham[a], Robert W. Buckheit Jr.[c], David Clanton[b], William G. Rice[a,*]

[a]*Laboratory of Antiviral Drug Mechanisms, SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Building 431T-B, P.O. Box B, Frederick, MD 21702, USA*

[b]*Anti-AIDS Virus Drug Screening Laboratory, SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Building 431T, P.O. Box B, Frederick, MD 21702, USA*

[c]*Virology Research Group, Southern Research Institute-Frederick Research Center, 431 Aviation Way, Frederick, MD 21701, USA*

Received 31 August 1995; accepted 8 November 1995

**Abstract**

Human secretory leukocyte protease inhibitor (SLPI), a serine protease inhibitor found concentrated in secretory fluids, has been postulated to participate in the body's natural defense against infection by the human immunodeficiency virus type-1 (HIV-1) by affecting trypsin-like enzymes on the surface of target cells. SLPI was evaluated for potential antiviral activity against laboratory, clinical and monocytotropic strains of HIV-1 in human T-cell lines, peripheral blood lymphocytes and monocyte/macrophage cultures. SLPI was tested in a single cycle of infection assay and under conditions in which SLPI was preincubated both with target cells and with virus and then maintained during the virus-to-cell adsorption phase and throughout the entire culture period. However, SLPI did not exert anti-HIV activity under any experimental conditions, and mechanistic studies showed SLPI to have no inhibitory activity on HIV-1 binding, reverse transcriptase or protease. Thus, SLPI exhibited no suggestive anti-HIV-1 activity.

*Keywords:* Secretory leukocyte protease inhibitor (SLPI); Lymphocytes; Monocytes; Human immunodeficiency virus type-1 (HIV-1); Antivirals; Saliva

## 1. Introduction

Human secretory leukocyte protease inhibitor (SLPI) is an 11.7-kDa serine protease inhibitor that concentrates in extravascular mucous secretions and serves to protect connective tissues from degradation by endogenous proteolytic enzymes of inflammatory leukocytes (Rice and Weiss, 1990). However, a number of lines of evidence led us to first propose in 1990 that SLPI might also function as a natural barrier against human immunodeficiency virus type-1 (HIV-1) infection. First, human saliva, which is rich in SLPI, was

* Corresponding author: Tel: + 1 301 8465060; fax: + 1 301 8466846.


*SSDI* 0166-3542(95)00907-4

found to inhibit HIV-1 replication in human peripheral blood lymphocytes (Fox et al., 1988, 1989; Archibald and Cole, 1990). Secondly, the laboratories of Hattori (Hattori et al., 1989) and Kido (Kido et al., 1990, 1991) described a putative trypsin-like serine protease on the surface of T-cells that might proteolytically cleave the viral gp120 surface protein, thereby exposing the membrane fusion sequence of the viral gp41 transmembrane protein and allowing for fusion of the virus to target cells. From these observations we postulated that SLPI might inhibit the trypsin-like enzyme and prevent cleavage of the gp120 and subsequent fusion of the viral envelope with the cellular membrane.

Our earlier studies confirmed that human saliva inhibited HIV-1 infection, but a fully active recombinant form of SLPI was found neither to prevent replication of HIV-1 nor protect the cells from the cytopathic effects of the virus (Rice et al., 1991). Interestingly, a more recent report (McNeely et al., 1995) indicated that SLPI inhibited infection of human monocyte/macrophage (M/M) cultures by the monocytotropic Ba-L strain of HIV-1 and replication of HIV-$1_{IIIB}$ in proliferating T cell lines. To determine if we had failed to detect an antiviral effect of SLPI, we once again tested SLPI in various assays to assess in vitro anti-HIV efficacy and to determine any potential mechanism of antiviral action of the molecule. The data herein describe our finding that a recombinant form of human SLPI that possessed full antiprotease activity was incapable of exerting any notable anti-HIV activity.

## 2. Materials and methods

### 2.1. Virus replication and inactivation assays

Anti-HIV screening with a human T-cell line was performed with CEM-SS cells and HIV-$1_{RF}$ (MOI = 0.01) using the XTT cytoprotection assay as previously described (Weislow et al., 1989). Effective antiviral concentrations providing 50% cytoprotection ($EC_{50}$) and cellular growth inhibitory concentrations causing 50% cytotoxicity ($IC_{50}$) were calculated. 3′-Azido-2′,3′-dideoxythymidine (AZT) and dextran sulfate were utilized as positive control compounds for anti-HIV activity. HIV-1 isolates included common laboratory strains (RF, IIIB, LAV and MN), as well as the HIV-$1_{WEJO}$ clinical isolate (Rice et al., 1993a; Cushman et al., 1994) and the monocytotropic HIV-$1_{ADA}$ and HIV-$1_{Ba\text{-}L}$ strains.

Phytohemagglutinin-stimulated human peripheral blood lymphocytes (PBLs) were prepared and utilized in antiviral assays as previously described (Rice et al., 1993a); $EC_{50}$ values for these cultures identify the drug concentration that provided a 50% reduction in viral p24 production. PBLs were infected with the lymphocytotropic HIV-$1_{WEJO}$ isolate. Human peripheral blood monocytes were isolated from buffy coats of normal HIV-1 negative donors after routine leukapheresis by centrifugal elutriation. Monocytes (>95% positive by non-specific esterase staining) were cultured for 7–10 days in AIM V medium (Gibco BRL, Rockville, MD), after which the M/M cultures were infected with either the monocytotropic HIV-$1_{ADA}$ or HIV-$1_{Ba\text{-}L}$ (MOI 0.1–0.01) viral strains. After a 2-h virus adsorption, the spent virus supernatant was removed and cultures were either washed three times with medium or fresh medium was merely added without washing. Cultures were continued for up to 30 days and fed every 3 days by half-volume replacement with or without replenishment of SLPI as indicated. The ability of compounds to directly inactivate viral infectivity was tested by treatment of an undiluted stock of HIV-$1_{LAV}$, followed by end-point titration in human PBL cultures, as previously described (Rice et al., 1993b).

Recombinant human SLPI was kindly provided by Amgen Inc. (Thousand Oaks, CA), and was determined to possess full antiprotease activity against human neutrophil elastase and chymotrypsin after the antiviral studies had been completed. The 3-nitrosobenzamide (NOBA) was kindly provided by Octamer, Inc. (Tiburon, CA).

### 2.2. Single cycle of infection assay

In order to identify the stage(s) of HIV replication that might be affected by a test compound, a single cycle of infection assay is performed in

which the compound is added to cultures at different times during the replication cycle and then XTT cytoprotection, p24 production and PCR-based detection of intracellular proviral DNA synthesis are measured as endpoints. This time course assay has been described previously in detail (Cushman et al., 1994; Rice and Bader, 1995; Clanton et al., 1995). In preparation for these studies we first experimentally determined the optimal concentration of the SLPI to be used in the time course assay by addition of a range of concentrations under conditions in which the SLPI was present during the preincubation of virus (HIV-$1_{IIIB}$) with cells (CEM-SS) and then added back immediately following removal of unbound virus.

### 2.3. Virus attachment and enzymatic assays

Binding of HIV-1 to target cells was measured by a p24-based assay (Rice et al., 1993a). Briefly, various concentrations of SLPI were preincubated with a concentrated stock of virus (HIV-$1_{RF}$) and separately with 2.5 × $10^5$ CEM-SS cells for 2 h at 37°C and the virus/SLPI and cells/SLPI samples were then mixed and incubated for 1 additional h at 37°C, after which the unbound virus was washed away and the cell-associated virus was solubilized in 1% Triton X-100, 1% BSA and analyzed by the p24 antigen capture assay. In addition, HeLa CD4$^+$ cells harboring a $\beta$-galactosidase gene under transcriptional control of the HIV-1 promoter (HeLa-CD4-LTR-$\beta$-gal cells) were used to quantitate the binding of infectious virus to cells, as well as to study HIV-1 specific membrane fusion interactions (Cushman et al., 1994).

The effects of SLPI on the in vitro activity of purified RT were determined by measurement of incorporation of [$^3$H]TTP onto the artificial poly(rA):oligo(dT) homopolymer template/primer system or [$^3$H]GTP onto the poly(rC):oligo(dG) system (Cushman et al., 1994). Samples (5 $\mu$l) were blotted onto DE81 paper, washed with 5% dibasic sodium phosphate, and then quantitated on a Packard Matrix 9600 direct beta counter. 3′-Azido-2′,3′-dideoxythymidine-5′-triphosphate (AZTTP) served as a positive control for inhibition of RT. HIV-1 protease activity was quantitated by an HPLC assay utilizing the Ala-Ser-Glu-Asn-Tyr-Pro-Ile-Val-Glu-Amide substrate (Multiple Peptide Systems, San Diego, CA) as previously described (Rice et al., 1993a).

## 3. Results

### 3.1. SLPI does not directly inactivate HIV-1 infectivity

To determine if SLPI could directly inactivate the infectivity of HIV-1, cell-free virions ($\sim 10^5$ $TCID_{50}$) were treated with various concentrations of the molecule and then serial dilutions of the mixtures were incubated with PBLs, after which the relative $TCID_{50}$ of each dilution was measured by quantitation of viral p24 in the cultures. The data in Table 1 clearly illustrate that SLPI was unable to directly inactivate the virus, whereas a control compound (3-nitrosobenzamide, NOBA) that attacks the HIV-1 nucleocapsid protein zinc fingers (Rice et al., 1993a,b) potently inactivated HIV-1 infectivity.

Table 1
Viral inactivation studies

| Virus treatment | $TCID^a_{50}$ | Percent of control |
|---|---|---|
| None | 79 423 | 100 |
| SLPI | | |
| 10 $\mu$g/ml | 87 152 | 109.7 |
| 50 $\mu$g/ml | 82 202 | 103.5 |
| 100 $\mu$g/ml | 70 256 | 88.5 |
| 1000 $\mu$g/ml | 76 069 | 95.8 |
| NOBA 100 $\mu$M | 11 | <0.001 |

[a]HIV-$1_{LAV}$ (~$10^5$ $TCID_{50}$) was incubated without or with the indicated concentration of reagent for 1 h at 37°C, serial 10-fold dilutions were prepared and added to $10^5$ PBL (ten cultures per dilution), and cultures analyzed for viral antigens after 9 days of culture. Cultures were scored positive if their absorbance in the antigen capture ELISA scored >3 S.D. above the mean absorbance of ten uninfected cultures. Resultant $TCID_{50}$ (50% tissue culture infective dose) values were then calculated.

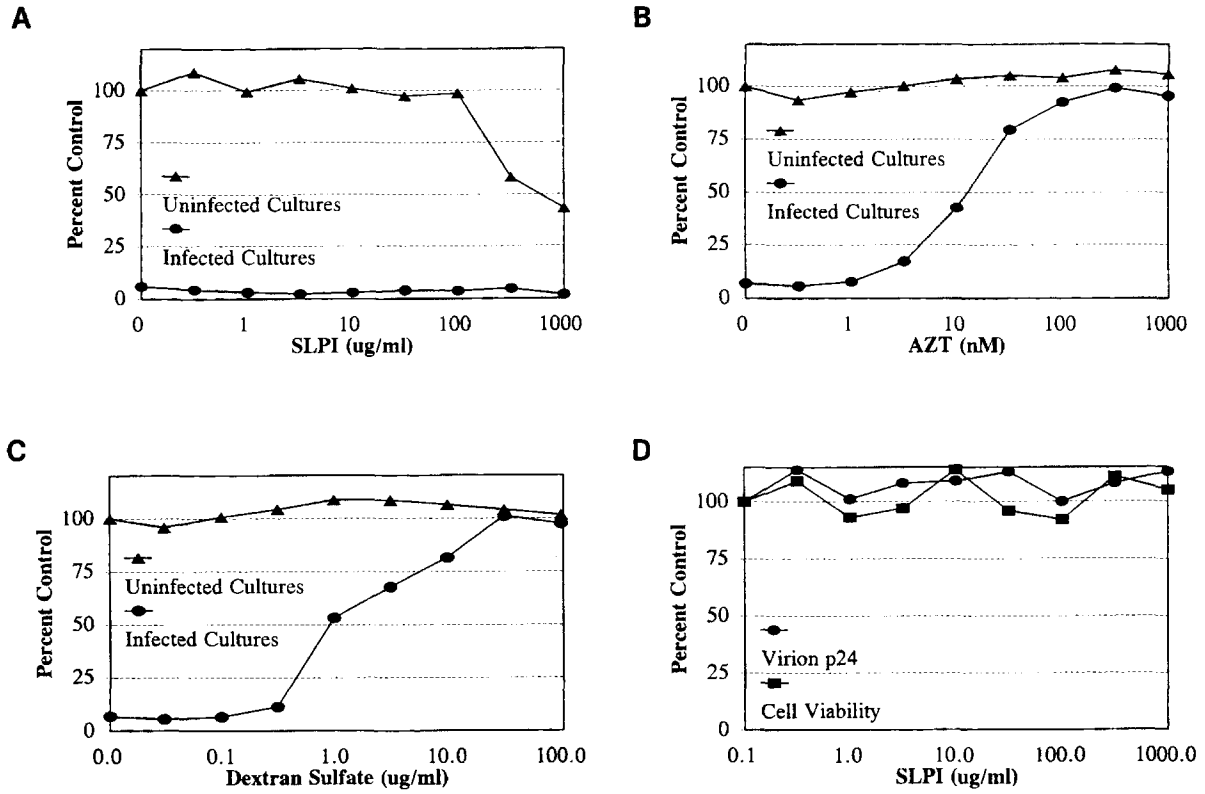


Fig. 1. Effects of SLPI on infection by lymphocytotropic strains of HIV-1. (A-C) Growth and metabolic activity (XTT) of uninfected (▲) and HIV-$1_{RF}$-infected (●) CEM-SS cells were assessed as a function of increasing concentrations of SLPI (A), AZT (B) or dextran sulfate (C). Note that SLPI did not protect the infected cultures from the cytopathic effects of HIV-1. (D) Human PBL ($10^5$/well in 96-well plates) were exposed to 250 $TCID_{50}$ of the HIV-$1_{WEJO}$ clinical isolate, incubated 7 days, and then virus production monitored by p24 antigen capture (●) and cell viability determined by the biscarboxyethyl-5(6)-carboxyfluorescein acetoxymethyl ester dye (■). Activities are expressed as a percentage of the drug-free control. SLPI did not reduce supernatant p24 levels.

### *3.2. SLPI does not demonstrate in vitro efficacy against HIV-1*

SLPI was evaluated for its ability to inhibit replication of HIV-1 in cultures requiring multiple rounds of infection. The microtiter XTT cytoprotection assay was used to determine if SLPI could inhibit the productive, cytopathic infection of HIV-$1_{RF}$ in CEM-SS cells. As shown in Fig. 1A, SLPI at concentrations as high as 1000 $\mu$g/ml could not inhibit the HIV-1-induced cytopathicity, while AZT (nucleoside RT inhibitor, Fig. 1B) and dextran sulfate (inhibitor of virus attachment to target cells, Fig. 1C) achieved 50% cellular protection ($EC_{50}$) with approximately 14 nM and 0.96 $\mu$g/ml, respectively. Likewise, infection by the lymphotropic HIV-$1_{WEJO}$ clinical isolate in fresh human PBL cultures was unaffected by SLPI (Fig. 1D). Utilizing two other clinical isolates (HIV-$1_{BAKI}$ and HIV-$1_{VIHU}$ ) we observed that the high test concentration of SLPI (1000 $\mu$g/ml, many fold above the physiological levels) resulted in only 58% and 28% inhibition of RT activity in the cell-free supernatants, respectively. The WEJO and BAKI isolates are syncytia-inducing (SI), while the VIHU isolate is a non-syncytia inducing (NSI) virus, and AZT inhibited these strains with $EC_{50}$ values of 126 nM, 45 nM and 23 nM, respectively.

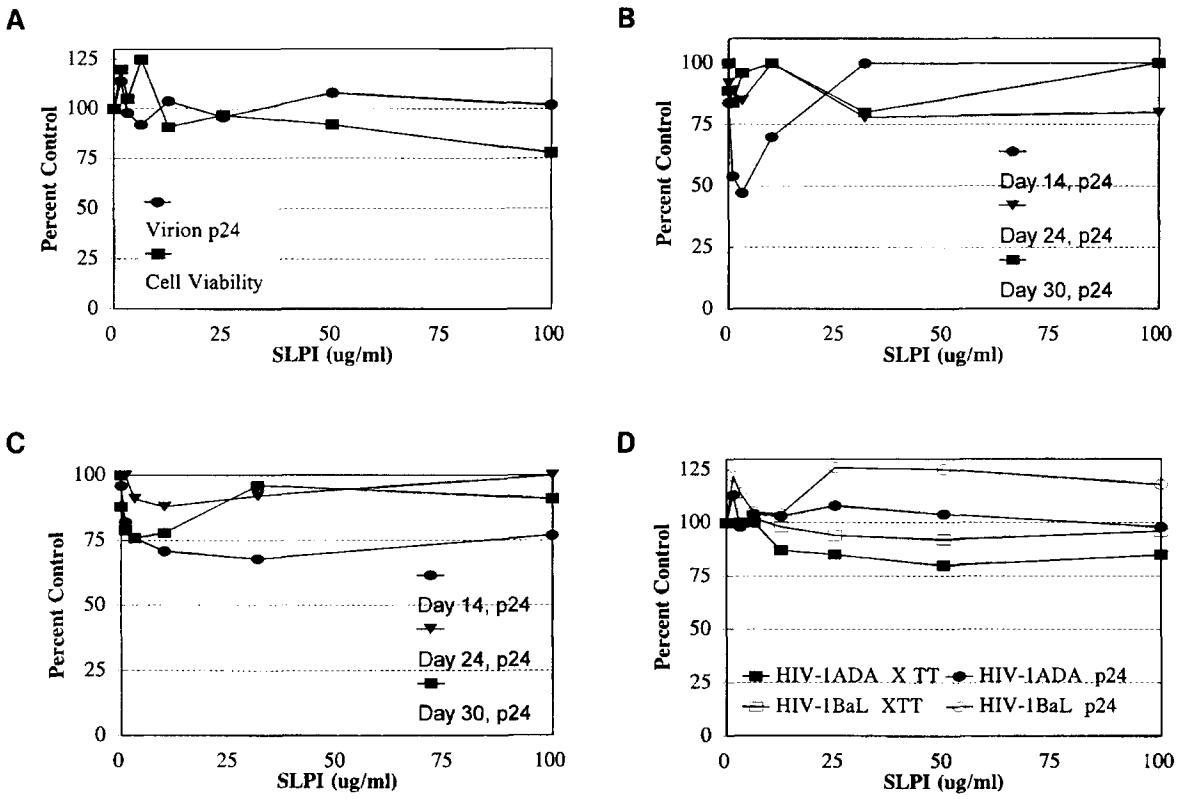


Fig. 2. Lack of inhibition of HIV-$1_{ADA}$ or HIV-$1_{Ba\text{-}L}$ replication in monocyte/macrophage cultures by SLPI. Monocytes were cultured for 10 days prior to infection with HIV-1. SLPI was added at indicated times, and infection was determined by p24 ELISA assays. (A) M/M cultures were infected with HIV-$1_{ADA}$ at an MOI of 0.1. After a 2 h virus adsorption the unbound virus was removed (without washing). SLPI was added at the indicated concentrations and then maintained at those concentrations throughout the subsequent 14 days in culture. At 14 days of infection cell-free virion p24 production (●) and cell viability by XTT (■) were determined. (B) M/M cultures and virus were treated as described by McNeely et al. (1995). HIV-$1_{ADA}$ was preincubated with indicated doses of SLPI for 30 min at 20°C followed by 30 min at 37°C. The virus-SLPI mixtures were then allowed to adsorb to M/M for 1 h at 37°C, after which cultures were washed three times with medium to remove unbound virus. Cultures were continued for 30 days with half-volume replenishment every 3 days with readdition of SLPI. Cell-free virion p24 was determined at 14 (○), 24 (▽) and 30 days (□). (C) Infection and pretreatment of virus was performed as in (B), but cultures were not washed after virus adsorption. (D) Either HIV-$1_{ADA}$ (closed symbols) or HIV-$1_{Ba\text{-}L}$ (open symbols) were preincubated with the indicated doses of SLPI for 1 h at 37°C. The virus-SLPI mixtures were then placed on M/M cultures for a 2 h adsorption. Virus was removed (without washing) and medium with SLPI added back. Cultures were continued for 15 days with resupplementation of SLPI. Cell-free virion production (circles) and XTT-based cell viability (squares) were determined.

To determine if SLPI might demonstrate a specific antiviral effect on monocytotropic strains of HIV-1, M/M cultures were exposed to a 2-h adsorption of HIV-$1_{ADA}$ to cells (pre-cultured for 10 days prior to infection), followed by removal of unbound virus and addition of various concentrations of SLPI. The initial SLPI concentration was then maintained throughout the culture period by re-addition of SLPI during the feeding of cultures every 3 days. Cultures were monitored at 14 days of infection for cell-free virion p24 production and for cell viability. Fig. 2A illustrates that SLPI was not toxic to the M/M cultures at the high test concentration of 100 $\mu$g/ml by XTT and visual determinations, yet there was no inhibitory effect on HIV-1 replication.

It was possible that SLPI may have blocked infection if present with the virus prior to virion

attachment to the cells and if the cultures were washed thoroughly to remove unbound virus. To address these issues, virus was preincubated with SLPI for 30 min at room temperature and then 30 min at 37°C and then placed on M/M. The mixtures (virus ± SLPI) were allowed to incubate with the adherent M/M for 1 h at 37°C, after which the cultures were washed three times to remove unbound virus (Fig. 2B). There was a transient decrease in extracellular p24 production after 14 days in culture in the 1–10 μg/ml SLPI concentration range, but the p24 rebounded to the level of untreated infected controls by days 24 and 30. Also, no inhibition of infection was observed when cultures were not washed after virus adsorption (Fig. 2C)

Finally, experiments were performed to compare the effects of SLPI on two different monocytotropic strains of HIV-1 (Ba-L and ADA) under conditions in which the SLPI was preincubated with the cells and with the virus separately for 1 h at 37°C and during the following 2-h virus adsorption. Afterwards, the virus was removed and SLPI was added back to the cultures. Even under these pre-exposure conditions, SLPI demonstrated no antiviral activity against either viral strain (Fig. 2D). We also observed no inhibitory effect of SLPI on virus replication under these pre-exposure conditions when tested against two further dilutions of input titers of each virus (not shown), indicating that our virus titers were not so high as to negate an antiviral effect of SLPI.

### *3.3. Time course and mechanism of action studies*

Although SLPI had been inactive in all assays requiring multiple rounds of infection, it was possible that we might detect a subtle inhibitory effect on some particular aspect of the HIV-1 replication cycle. To test this possibility, SLPI was to be evaluated in a single-cycle acute phase assay that identifies the phase(s) of the viral replication cycle in which an antiviral compound acts. However, preliminary studies to determine the optimal amount of SLPI to use in the assay indicated that SLPI was completely inactive in the single cycle assay (Fig. 3). During control experiments (see also Fig. 3), dextran sulfate and DDC successfully prevented infection. In addition, analogous studies with other antiviral compounds, such as cosalane (Cushman et al., 1994) and the Farmitalia sulfonates and phosphonates (Clanton et al., 1995), readily identified compounds that could inhibit attachment or fusion of the virus to target cells.

The lack of an inhibitory effect of SLPI in the single cycle assay was consistent with mechanistic studies that measured the effect of SLPI on individual stages in the virus replication cycle. Two independent methods revealed no interference by SLPI on the binding of HIV-1 to target cells (Fig. 4A). Likewise, SLPI did not inhibit the fusion of cells-to-cells (Fig. 4A) or the enzymatic activities of HIV-1 p66/p51 RT or protease (Fig. 4B).

## 4. Discussion

In the current study, we extensively evaluated the ability of SLPI to exert antiviral activity against laboratory, clinical lymphocytotropic and monocytotropic isolates of HIV-1 in cultures of CD4(+) T-cell lines, fresh human peripheral blood lymphocytes or monocyte/macrophage cultures. In addition, we sought to identify any potential mode of anti-HIV action of SLPI. Our findings demonstrate that a fully active form of recombinant SLPI was unable to mount any appreciable inhibitory effect on HIV-1 replication, and that the molecule was without effect on virion attachment and fusion to target cells or on the in vitro activities of the HIV-1 reverse transcriptase and protease enzymes.

Although SLPI-rich human salivas express antiviral activity, we cannot attribute the anti-HIV activity of saliva to SLPI. Recent reports have associated the bulk of the anti-HIV activity of saliva to the mucin-rich fraction (Yeh et al., 1992; Bergey et al., 1994). In addition, a smaller and non-filterable antiviral factor in saliva has been noted for several years, and as early as 1990 we proposed that SLPI might represent the non-filterable component. SLPI was postulated to block HIV-1 infectivity by inhibiting a putative trypsin-like serine protease activity associated with the surface of CD4(+) T-cells. The protease was

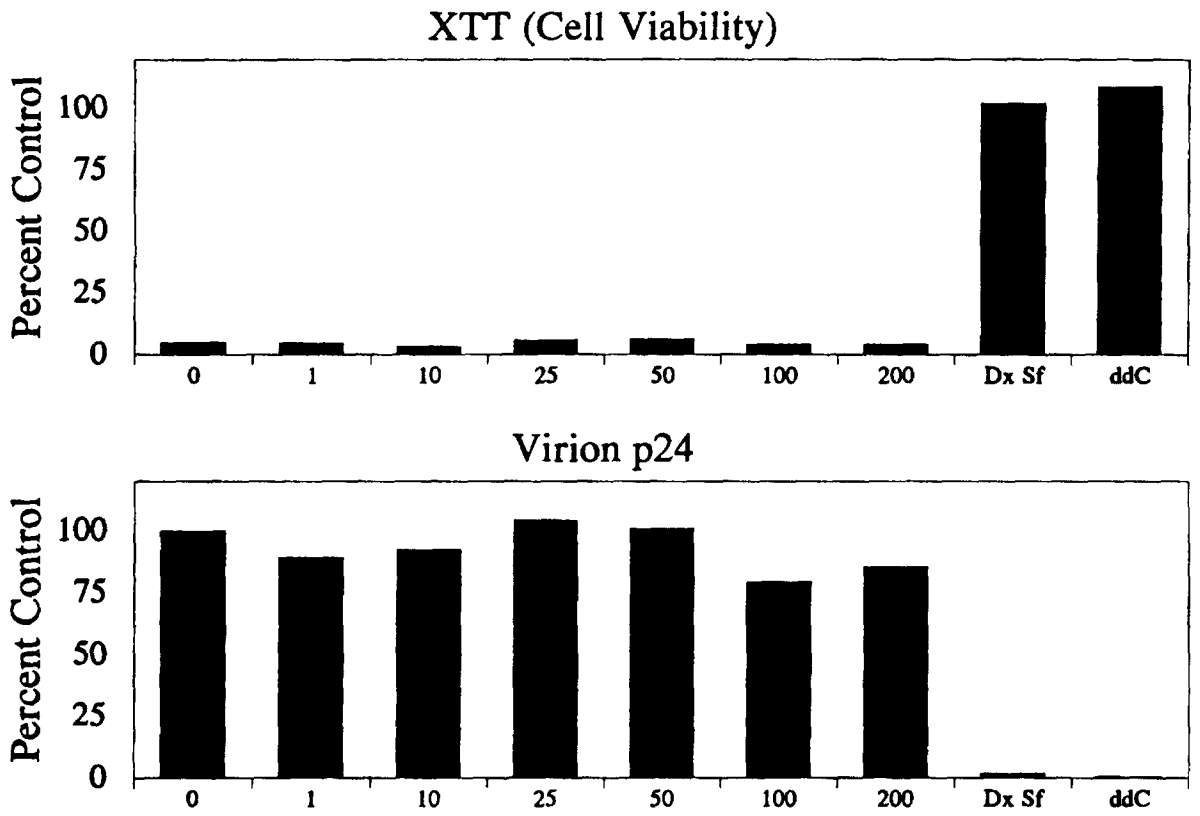


Fig. 3. Effects of SLPI in an acute, high-MOI, single cycle of infection of HIV-1. Various concentrations of SLPI (0, 1, 10, 25, 50, 100 or 200 $\mu$g/ml), 50 $\mu$g/ml dextran sulfate or 1 $\mu$M DDC were exposed to $10^5$ CEM-SS cells and 50 $\mu$l of a concentrated stock of HIV-$1_{IIIB}$ during a 1 h preincubation that allows for adsorption of virus to cells. Cells were then washed free of unbound virus and test reagent was added back to the cells. The cells were then cultured for 3 additional days, at which time quantitative measurements were taken for cell viability by XTT and for viral production by supernatant p24 levels. Note that dextran sulfate and DDC completely blocked p24 production and protected the cells from HIV-induced cytopathicity, while SLPI was ineffective.

thought to proteolytically cleave the viral gp120 surface protein following attachment of the viral gp120 to the CD4 receptor on the T-cell surface. The cleavage of the gp120 would result in exposure of the membrane fusion sequence of the viral gp41 transmembrane protein, thereby allowing the virus to fuse with the T-cell membrane. Unfortunately, our earlier studies found that SLPI did not prevent HIV-1 replication or the cytopathic effects of the virus (Rice et al., 1991). However, the recent report by McNeely et al. (1995) suggested that our initial assessment of the anti-HIV activity of SLPI may have been in error; therefore, we once again surveyed the effectiveness of SLPI as an anti-HIV agent.

To prevent bias, SLPI was evaluated for anti-HIV efficacy in three separate laboratories. Each lab independently found SLPI to be inactive against HIV-1, and the data are presented in the body of this article. Although we did observe minimal antiviral activity against the BAKI and VIHU clinical HIV-1 isolates in the concentration range of 1000 $\mu$g/ml, this concentration is 40- to 250-fold higher than the reported physiological concentrations of SLPI in human saliva (4–24 $\mu$g/ml) (McNeely et al., 1995). We therefore consider the significance of this finding to be of little or no merit.

McNeely et al. (1995) reported that SLPI inhibited infection in M/M cultures by HIV-$1_{Ba\text{-}L}$ un-

der a set of defined conditions that included washing of cultures after virus adsorption. In an attempt to confirm their findings we repeated their conditions. It should be noted that extensive washing of M/M monolayers after virus adsorption can result in loss of cells and conversion of virus spread from a cell-to-cell mode to that of a supernatant transmission mode, resulting in transient delays in infection that can be misconstrued as inhibition. Indeed, we did observe a transient decrease at 14 days of culture that later rebounded to untreated levels of infection. Thus, we were unable to detect a significant and sustained inhibition of infection under the conditions described.

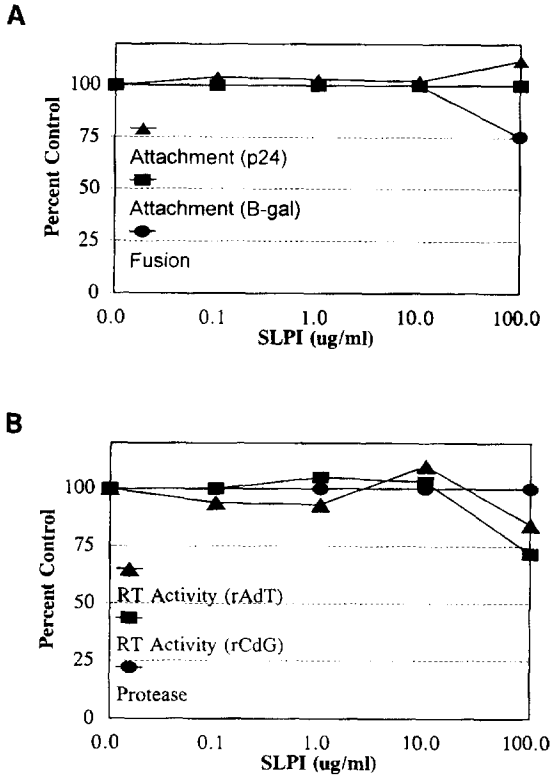


Fig. 4. Effects of SLPI on binding interactions and on HIV-1 enzymatic activities. (A) Binding of HIV-$1_{RF}$ to CEM-SS cells was quantitated by the association of virion p24 with the cells (▲), and by the binding of HIV-$1_{IIIB}$ to HeLa CD4+/LTR/$\beta$-gal cells (■). Effects of SLPI on fusion interactions were measured by co-cultivation of HeLa CD4+/LTR/$\beta$-gal cells with HL2/3 cells expressing env and tat proteins (●). (B) Effects of SLPI on the enzymatic activity of p66/p51 RT were analyzed against the rAdT (▲) and rCdG (■) template/primer systems. Actions of SLPI on the enzymatic activity of HIV-1 protease were evaluated (●). All points on the curves represent the mean of at least three replicates and are depicted as the percent of the drug-free control.

In addition to these efforts to detect an anti-HIV activity of SLPI, M/M were utilized that were derived from multiple donors and infections were initiated with various HIV-1 strains and virus input titers. Moreover, both cells and virus were pretreated with SLPI to provide for an optimal opportunity for the molecule to exert an antiviral effect; yet, no anti-HIV effect was observed. The identical batch of SLPI from Amgen Inc. was utilized in our study and in the study by McNeely et al. (1995); therefore, this cannot be the source of disparity in our findings. It is possible that culturing the M/M on glass surfaces, as was done by the other investigators (McNeely et al., 1995), rather than on tissue culture plastic (as we did), may account for our differences in results. Culture conditions are known to influence the expression of cell surface receptors as the cells mature from monocytes to the macrophage-like phenotype (Kaplan and Gaudernack, 1982; Shepherd et al., 1982). It may be that SLPI coats the cell surface by interaction with one or more of these factors that are upregulated differentially by the different substrata.

In summary, our findings clearly do not support the contention that SLPI exerts any overt anti-HIV activity. It is possible that SLPI might participate in the prevention of HIV-1 infection in the oral cavity by some multi-component process, but any notion that SLPI might be developed as a therapeutic agent for the treatment of HIV infection should be questioned.

## Acknowledgements

This work was supported by the National Cancer Institute under contract with SAIC Frederick and Southern Research Institute. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization im-

ply endorsement by the US Government. We thank Ms. Judy Duears for preparation of the manuscript.

## References


Archibald, D.W. and Cole, G.A. (1990) In vitro inhibition of HIV-1 infectivity by human salivas. AIDS Res. Hum. Retrovir. 6, 1425–1432.

Bergey, E.J., Cho, M.-I. Blumberg, B.M., Hammarskjold, M.-L., Rekosh, D., Epstein, L.G. and Levine, M.J. (1994) Interaction of HIV-1 and human salivary mucins. J. Acquir. Immune. Defic. Syndr. 7, 995–1002.

Clanton, D.J., Buckheit, R.W., Jr., Terpening, S.J., Kiser, R., Mongelli, N., Borgia, A.L., Schultz, R., Narayanan, V., Bader, J.P. and Rice, W.G. (1995) Novel sulfonated and phosphonated analogs of distamycin which inhibit the replication of HIV. Antiviral Res. 27, 335–354.

Cushman, M., Golebiewski, W.M., McMahon, J.B., Buckheit, R.W., Jr., Clanton, D.J., Weislow, O., Haugwitz, R.D., Bader, J.P., Graham, L. and Rice, W.G. (1994) Design, synthesis, and biological evaluation of cosalane, a novel anti-HIV agent which inhibits multiple features of virus production. J. Med. Chem. 37, 3040–3050.

Fox, P.C., Wolff, A., Yeh, C.-K., Atkinson, J.C. and Baum, B.J. (1988) Saliva inhibits HIV-1 infectivity. J. Am. Dent. Assoc. 116, 635–637.

Fox, P.C., Wolff, A., Yeh, C.-K., Atkinson, J.C. and Baum, B.J. (1989) Salivary inhibition of HIV-1 infectivity: functional properties and distribution in men, women, and children. J. Am. Dent. Assoc. 118, 709–711.

Hattori, T., Koito, A., Takatsuki, K., Kido, H. and Katanuma, N. (1989) Involvement of tryptase-related cellular protease(s) in human immunodeficiency virus type 1 infection. Fed. Eur. Biochem. Soc. Lett. 248, 48–52.

Kaplan, G. and Gaudernack, G. (1982) In vitro differentiation of human monocytes. J. Exp. Med. 156, 1101–1114.

Kido, H., Fukutomi, A. and Katunuma, N. (1990) A novel membrane-bound serine esterase in human T4+ lymphocytes immunologically reactive with antibody inhibiting syncytia induced by HIV-1. Purification and characterization. J. Biol. Chem. 265, 21979–21985.

Kido, H., Fukutomi, A. and Katunuma, N. (1991) Tryptase TL2 in the membrane of human T4+ lymphocytes is a novel binding protein of the V3 domain of HIV-1 envelope glycoprotein gp120. Fed. Eur. Biochem. Soc. Lett. 286, 233–236.

McNeely, T.B., Dealy, M., Dripps, D.J., Orenstein, J.M., Eisenberg, S.P. and Wahl, S.M. (1995) Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. J. Clin. Invest. 96, 456–464.

Rice, W.G. and Bader, J.P. (1995) Discovery and in vitro development of AIDS antiviral drugs as biopharmaceuticals. In: J.T. August, M.W. Anders, F. Murad and J.T. Coyle (Eds), Advances in Pharmacology, Vol. 33, pp. 389–438. Academic Press, Inc., San Diego, CA.

Rice, W.G. and Weiss, S.J. (1990) Regulation of proteolysis at the neutrophil-substrate interface by secretory leukoprotease inhibitor. Science 249, 178–181.

Rice, W.G., Schaeffer, C.A., McDougal, J.S. and Orloff, S.L. (1991) Nature of inhibitors of HIV in human secretory fluids. Abstract, Contemporary Developments in Salivary Research, Buffalo, NY.

Rice, W.G., Schaeffer, C.A., Graham, L., Bu, M., McDougal, J.S., Orloff, S.L., Villinger, F., Young, M., Oroszlan, S., Fesen, M., Pommier, Y., Mendeleyev, J. and Kun, E. (1993a) The site of antiviral action of 3-nitrosobenzamide on the infectivity process of human immunodeficiency virus in human lymphocytes. Proc. Natl. Acad. Sci. USA 90, 9721–9724.

Rice, W.G., Schaeffer, C.A., Harten B., Villinger, F., South, T.L., Summers, M.F., Henderson, L.E., Bess, J.W., Jr., Arthur, L.O., McDougal, J.S., Orloff, S.L., Mendeleyev, J. and Kun, E. (1993b) Inhibition of HIV infectivity by zinc-ejecting *C*-nitroso compounds. Nature 361, 473–475.

Shepherd, V.L., Campbell, E.J., Senior, R.M. and Stahl, P.D. (1982) Characterization of the mannose/fucose receptor on human mononuclear phagocytes. RES: J. Ret. Soc. 32, 423–431.

Weislow, O.S., Kiser, R., Fine, D.L., Bader, J., Shoemaker, R.H. and Boyd, M.R. (1989) New soluble-formazan assay for HIV-1 cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. J. Natl. Cancer Inst. 81, 577–586.

Yeh, C.-K., Handelman, B., Fox, P.C. and Baum, B.J. (1992) Further studies of salivary inhibition of HIV-1 infectivity. J. Acquir. Immune. Defic. Syndr. 5, 898–903.