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Salivary Secretory Leukocyte Protease Inhibitor Is Associated with Reduced Transmission of Human Immunodeficiency Virus Type 1 through Breast Milk

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Abstract

Secretory leukocyte protease inhibitor (SLPI), a protein found in saliva, breast milk, and genital secretions, is capable of inhibiting human immunodeficiency virus (HIV) type 1 in vitro. The aim of this study was to determine whether SLPI in infant saliva provides protection against mother-to-child HIV-1 transmission. In total, 602 saliva specimens were collected from 188 infants at birth and at ages 1, 3, and 6 months. Infants' median salivary SLPI concentrations were higher at birth than at 6 months (341 vs. 219 ng/mL; $P = .001$). There was no association between SLPI concentration and HIV-1 transmission overall. However, among 122 breast-fed infants who were HIV-1 uninfected at 1 month, higher salivary SLPI levels were associated with a decreased risk of HIV-1 transmission through breast milk (hazard ratio, 0.5; 95% confidence interval, 0.3–0.9; $P = .03$). These results suggest that SLPI plays an important role in reducing HIV-1 transmission through breast milk.

Approximately 2 million infants are born to human immunodeficiency virus (HIV) type 1–infected mothers each year [1]. Despite exposure to HIV-1 in utero, during delivery, and through breast-feeding, only an estimated 30% of these infants (~600,000) will become HIV-1 infected [1]. Escape from HIV-1 infection may be due to viral factors and maternal or infant immunity against HIV-1 [2, 3].

Endogenous immune mechanisms present at the site of exposure, predominantly the oral and gastrointestinal mucosa in the fetus and infant, may also contribute to an infant's resistance to HIV-1 infection. Mucins and thombospondins present in infant saliva can inactivate HIV-1 by physically entrapping and sequestering virus particles [4]. In addition, several soluble components of saliva have activity against HIV-1, including lysozyme, cystatins, lactoferrin, and secretory leukocyte protease inhibitor (SLPI) [5]. Among these, only SLPI, a 12-kDa nonglycosylated protein secreted in serous secretions by acinar cells of submucosal glands, is capable of inhibiting HIV-1 replication in vitro at physiologic concentrations [4, 5].

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Written informed consent was obtained from all study participants. This study received ethical approval by the institutional review boards of the University of Washington and the University of Nairobi and was conducted according to the guidelines set forth by the US Department of Health and Human Services.

Although SLPI is found in highest concentrations in saliva, it is also found in semen, cervical secretions, breast milk, tears, synovial fluid, and cerebral spinal fluid [4]. To date, 2 studies have evaluated the role of nonsalivary SLPI in mother-to-child HIV-1 transmission. A study in the Central African Republic [6] measured SLPI levels in breast milk of HIV-1–seropositive women and found no association between subsequent infant HIV-1 infection and SLPI levels in either colostrum or breast milk. A second study in South Africa determined that there was a significantly lower rate of mother-to-infant HIV-1 transmission among pregnant HIV-1–seropositive women who had elevated SLPI levels in cervicovaginal secretions at week 32 of gestation [7].

In both of these studies, maternal SLPI levels were evaluated to determine whether elevated SLPI within virus-containing secretions might affect the risk of HIV-1 infection in the exposed infant. Neither study evaluated whether elevated levels in infant saliva may inhibit HIV-1 within the infant oropharynx. In this study, we evaluated infant salivary SLPI levels in a cohort of HIV-1–exposed infants to characterize SLPI production and to determine whether infant salivary SLPI levels affect the risk of mother-to-child HIV-1 transmission.

Subjects and Methods

Study population

From July 1999 through December 2001, HIV-1–seropositive pregnant women participating in an ongoing perinatal HIV-1 cohort study in Nairobi were monitored antenatally, during delivery, and postpartum with their infants. Women received zidovudine from week 36 of gestation through delivery and were counseled regarding the risks of HIV-1 transmission through breast milk. Infants were fed according to maternal preference. Mother-infant pairs were evaluated by study physicians after delivery and monthly thereafter, for 12 months of follow-up.

Collection and processing of specimens

Maternal blood specimens were obtained at week 32 of gestation for CD4 T cell subsets. Infant saliva specimens were collected at birth and at ages 1, 3, and 6 months, using 6 dacron swabs saturated with pooled saliva under the tongue and in the buccal mucosa. Each of the 6 swabs was estimated to absorb ~0.17 mL of saliva, resulting in the collection of a total saliva volume of ~1 mL [8]. Saliva specimens were obtained at least 30 min after an infant had completed breast-feeding and were kept on ice until they were processed. The sample then was diluted 4-fold with the addition of 3 mL of PBS. After centrifugation through a 0.22-μm filter, the protease inhibitor 4-(2-aminoethyl)-benzenesulfonylflouride was added to the samples, which then were frozen at −70°C. SLPI levels in saliva were quantified using a commercial ELISA assay (R & D Systems) and reported without adjustment for the dilution factor, as in previous reports [7].

To determine infant HIV-1 infection status, infant blood specimens were collected on filter paper at birth and at ages 1, 3, 6, 9, and 12 months. HIV-1 DNA *gag* sequences were detected in filter paper specimens using polymerase chain reaction (PCR) assays [9]. Infants were considered to be HIV-1 infected if they had either positive PCR assay results on 2 consecutive dates or a single positive PCR assay result at the last clinic visit.

Statistical methods

To characterize factors associated with elevated SLPI, SLPI levels were dichotomized at the median. Infants with SLPI levels above the median were compared with remaining infants, using the Mann-Whitney U test for continuous variables and Pearson's χ^2 for categorical variables. Salivary SLPI levels at birth and at ages 1, 3, and 6 months were compared using

the Wilcoxon signed rank test for paired samples. To determine whether SLPI levels at birth were associated with subsequent HIV-1 infection, Cox proportional hazards regression with HIV-1 infection as the outcome was done.

Results

In total, 602 saliva specimens were collected from 188 infants for analysis. Specimens were collected from 165 (88%) infants at birth, from 168 (89%) infants at 1 month, from 146 (78%) infants at 3 months, and from 123 (65%) infants at 6 months. Specimens were collected at all 4 time points from 87 (46%) infants. Overall, SLPI was detected in 600 infant saliva samples (99%), and the median SLPI concentration was 255 ng/mL. Salivary SLPI levels at different infant ages decreased from birth to 6 months, with the median SLPI concentration at birth (341 ng/mL) being significantly higher than the median SLPI concentrations at ages 1 month (275 ng/mL; $P = .035$), 3 months (199 ng/mL; $P = .001$), and 6 months (219 ng/mL; $P = .001$) (table 1). These results did not change when the analysis was restricted to the 87 infants with all 4 samples.

For mother-infant pairs in the cohort, the median maternal CD4 cell count at week 32 of gestation was 470 cells/mL. The median gestational age at delivery was 40 weeks (range, 32–49 weeks), and the median birth weight was 3.1 kg (range, 1.5–4.6 kg). Eighty-six percent of women chose to breast-feed their infants. Elevated infant SLPI levels were not associated with any maternal or infant characteristics, including maternal CD4 T cell count, mode of delivery, duration of labor, duration of ruptured membranes, maturity by Dubowitz scoring, birth weight, or the presence of infant oral ulcers, oral candidiasis, or other oral abnormalities (data not shown). In addition, salivary SLPI levels for HIV-1–infected infants were no different from those in HIV-1–uninfected infants at any age (table 1).

HIV-1 PCR data from at least one filter paper blood specimen were available for 184 (98%) of the 188 infants with salivary SLPI results. Among these 184 infants, 38 (21%) were HIV-1 infected during follow-up, of whom 31 (82%) were infected before age 1 month. An additional 7 (4%) infants were infected after the first postpartum month. HIV-1 transmission rates at month 1 and month 12 were 18% and 25%, respectively. During a median follow-up period of 12 months, 28 (15%) infants died, 17 (61%) of whom were HIV-1 infected. Seventeen (9%) infants were lost to follow-up.

Survival analysis was conducted to determine whether elevated salivary SLPI was associated with decreased HIV-1 transmission from mother to child. The overall HIV-1 transmission risk was evaluated for the 165 infants with saliva specimens collected at birth. There was no association between SLPI levels at birth and risk for HIV-1 infection overall. To define the role of SLPI in breast milk HIV-1 transmission, analysis was conducted to determine the association of SLPI levels with risk of HIV-1 transmission occurring after month 1, when late in utero and intrapartum transmission can definitely be excluded. In survival analysis, infants with elevated SLPI levels at 1 month of age had significantly decreased late breast milk HIV-1 transmission (table 2). Among 122 breast-fed infants who were HIV-1 uninfected at age 1 month and had month 1 saliva specimens available, 7 (6%) were HIV-1 infected by month 12. There was ~50% decreased risk of transmission for every 100 ng/mL increase in salivary SLPI concentration at month 1 (hazard ratio, 0.5; 95% confidence interval, 0.3–0.9; $P = .03$).

Discussion

In adults, HIV-1 acquisition across oral mucosal surfaces is infrequent, compared with other modes of transmission, and accounts for <10% of primary HIV-1 infections [10, 11]. In

infants, HIV-1 infection after oral exposure is more common; HIV-1 transmission through breast milk is responsible for ~50% of HIV-1 infections among breast-fed infants [12]. Salivary antiviral factors and, specifically, the SLPI protein may contribute to protecting oropharyngeal mucosa against invasion by HIV-1 [4]. Establishing a role for SLPI in preventing oral HIV-1 infection could lead to the development of novel interventions, such as using recombinant SLPI for HIV-1 prevention.

In the present study, the first to evaluate the protective effect of salivary SLPI, we determined salivary SLPI concentrations in a cohort of 188 breast-fed, HIV-1–exposed infants. Salivary SLPI levels among these infants were inversely correlated with infant age and not significantly associated with any maternal or infant characteristics. SLPI concentrations at birth were not associated with the overall risk of HIV-1 infection. However, SLPI did play a role in protection from late HIV-1 transmission through breast milk. Higher salivary SLPI levels at month 1 were associated with a decreased risk of HIV-1 infection among infants who were HIV-1 exposed via breast-feeding after month 1. These findings are consistent with results from a study of SLPI in cervicovaginal secretions from HIV-1–infected pregnant women [7] but contrast with the results of a study on colostrum and breast-milk SLPI concentrations in a cohort of HIV-1–infected, breast-feeding mothers [6]. Elevated cervicovaginal SLPI was found to protect against infant HIV-1 infection in the former study [7], but, in the latter study, neither colostrum nor breast milk SLPI was associated with protection against HIV-1 transmission [6].

Differences in results are unlikely to be explained by variations in the mechanism of action of SLPI, because this would be the same in saliva, breast milk, and cervicovaginal secretions. SLPI interferes with HIV-1 entry into monocytes and lymphocytes by binding to a host cell transmembrane protein rather than by interfering directly with viral replication or binding to HIV-1 [13]. In studies in which virus was pretreated with SLPI, there was no inhibition of viral entry into cells; pretreatment of host cells with SLPI in the absence of virus also did not inhibit viral entry into cells [14]. Thus, both SLPI and virus need to be present simultaneously at the site of viral invasion for SLPI to exert an inhibitory effect. Cervicovaginal, breast milk, and salivary SLPI, therefore, would be expected to act in a similar manner to prevent HIV-1 infection of infant mucosal cells.

Discrepant results are more likely to be due to differences in SLPI concentrations in infant saliva, cervicovaginal secretions, and breast milk. However, comparing SLPI levels in different body fluids is problematic, because some sample types were diluted before SLPI testing (i.e., infant saliva and cervicovaginal secretions) [7], whereas others were not diluted (i.e., colostrum and breast milk) [6]. To compare salivary SLPI collected in this study with colostrum and breast milk, a crude adjustment using the dilution factor must be made. This results in an ~4-fold increase in mean salivary SLPI concentration, from 255 to 1020 ng/mL. This higher value is comparable to adult levels (10^3–10^4 ng/mL) [4] and to SLPI concentrations that have been reported to maximally inhibit HIV-1 in vitro (10^3 ng/mL) [5, 15]. Mean SLPI concentrations in breast milk (~250 ng/mL) were lower than this inhibitory threshold, providing one explanation for the absence of protection associated with breast milk SLPI concentrations at 1 and 6 months.

Low SLPI concentrations do not explain why colostrum, with a mean SLPI concentration ~10-fold higher than that in breast milk, did not influence HIV-1 infection. The transient nature of colostrum may contribute to an inability to alter HIV-1 transmission rates. Alternatively, the small sample size (43 mother-infant pairs) in the study of breast milk SLPI may have limited the ability to define a protective effect on HIV-1 transmission through breast milk, making additional studies in larger populations important to confirm these findings.

In conclusion, this study is the first to report SLPI levels in infants and to evaluate the role of this important innate immune mechanism in mother-to-child HIV-1 transmission. The observation that higher salivary SLPI levels protect against HIV-1 infection after exposure to HIV-1 through breast milk supports further research into the development of pharmacological agents that would mimic the actions of SLPI in vivo. In populations where HIV-1–seropositive women often breast-feed their infants, such agents may contribute to the prevention of mother-to-child HIV-1 transmission. Further investigation of SLPI and other endogenous proteins will be important to develop new HIV-1 prevention strategies, strengthen our understanding of mucosal immunity, and clarify the role of SLPI in reducing mother-to-child HIV-1 transmission via breast milk.

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Table 1

Salivary secretory leukocyte protease inhibitor (SLPI) levels at different infant ages for human immunodeficiency virus (HIV) type 1–infected and HIV-1–uninfected infants.

	All infants		HIV-1 uninfected infants[a,b]		HIV-1 infected infants[a,b]	
Infant age	No.	SLPI concentration, median ng/mL (IQR)[c]	No.	SLPI concentration, median ng/mL (IQR)	No.	SLPI concentration, median ng/mL (IQR)
<48 h	165	341 (153–594)	156	337 (145–534)	7	385 (195–944)
1 month	168	275 (130–434)	139	278 (136–434)	28	227 (109–503)
3 months	146	199 (105–417)	121	191 (105–393)	23	232 (78–153)
6 months	123	219 (96–354)	104	204 (90–347)	18	279 (174–396)

NOTE. IQR, interquartile range.

[a] HIV-1 infection status at time of specimen collection. HIV-1 infection status was not available for all infants.

[b] Median SLPI levels were not significantly different between HIV-1–infected and HIV-1–uninfected infants at birth and at ages 1, 3, and 6 months.

[c] Median SLPI level at <48 h was significantly different from median SLPI levels at 1 month ($P = .035$), 3 months ($P = .001$), and 6 months ($P = .001$).

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Table 2

Median salivary secretory leukocyte protease inhibitor (SLPI) concentrations and survival analysis results for breast-fed infants who were human immunodeficiency virus (HIV) type 1-uninfected at age 1 month.

	HIV-1 infected at month 12		HIV-1 uninfected at month 12				
Infant age[a]	**No.**	**SLPI concentration, median ng/mL (IQR)**	**No.**	**SLPI concentration, median ng/mL (IQR)**	**HR, risk/100 ng/mL SLPI**	**95% CI**	***P***
<48 h	6	341 (136–534)	103	128 (58–245)	0.6	0.3–1.0	.07
1 month	7	114 (51–282)	115	291 (145–462)	0.5	0.3–0.9	.03

NOTE. CI, confidence interval; HR, hazard ratio; IQR, interquartile range.

[a]Age at the time of specimen collection for breast-fed infants who were HIV-1 uninfected at 1 month. Infants without specimens were excluded.

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