BRIEF REPORT

High Concentrations of Interleukin 15 in Breast Milk Are Associated with Protection against Postnatal HIV Transmission

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Given the central role that interleukin 15 (IL-15) plays in human immunodeficiency virus (HIV) immunity, we hypothesized that IL-15 in breast milk may protect against postnatal HIV transmission. In a nested case-control study, we compared breast milk IL-15 levels in 22 HIV-infected women who transmitted HIV to their infants to those in 72 nontransmitters. Samples were collected in the first month of life, prior to HIV infection. IL-15 concentrations were associated with a decreased risk of HIV transmission in unadjusted analysis and after adjusting for milk viral load, CD4 cell count, and other cytokines in breast milk. IL-15–mediated immunity may protect against HIV transmission during breast-feeding.

Most infants breast-fed by human immunodeficiency virus (HIV)–infected women escape infection even in the absence of maternal or infant prophylactic treatment, suggesting that some immune protection of infants exists [1]. However, the underlying mechanisms are not well understood. Cytokines, which are key modulators of immunity, have been only partially investigated for their role in HIV transmission via breast milk [2–4].

Interleukin 15 (IL-15) plays a key role in anti-HIV responses by stimulating both CD8 T cells and natural killer cells [5]. Despite a wealth of in vitro data, a protective association between mucosal IL-15 concentrations and HIV transmission has, to our knowledge, not yet been observed in humans. In addition, in vitro data suggest that IL-15 might also permit infection of resting T cells by HIV [6]. IL-15 may therefore have a harmful effect.

Given the central role of IL-15 in HIV immunity [5], its potential clinical relevance as an adjuvant in vaccination studies [7], and its prior detection in breast milk [8], we hypothesized that higher levels of IL-15 in breast milk may protect against postnatal HIV transmission. To test this hypothesis, we conducted a case-control study nested within a clinical trial in Zambia. We measured 3 other cytokines—namely, CXCL12 (previously known as stromal cell–derived factor 1α [SDF-1α]), CCL5 (RANTES [regulated upon activation, normal T cell expressed and secreted]), and interleukin 8 (IL-8) (CXCL8), which has previously been found [2, 3] or proposed [9] to be associated with the risk of HIV transmission through breast-feeding—as well as maternal CD4 cell counts, breast milk sodium concentrations, and cell-free HIV RNA in breast milk to adjust for possible confounding.

Methods. This study was nested within the Zambia Exclusive Breast-feeding Study (ZEBS), a clinical trial of the prevention of mother-to-child HIV transmission and infant mortality in Zambia [10, 11]. In brief, 1435 HIV-infected pregnant women were enrolled at prenatal clinics in Lusaka, Zambia. Half were randomized to an intervention promoting abrupt weaning at 4 months postpartum; the remaining women were encouraged to breast-feed exclusively for 6 months and then continue breast-feeding as long as they wished. All women were counseled to exclusively breast-feed until at least 4 months postpartum. Infant dried blood spots were collected at delivery, at 1 week, and at 1, 2, 3, 4, 4.5, 5, 6, 9, 12, 15, 18, 21, and 24 months postpartum, and infant HIV status was tested by HIV polymerase chain reaction. Positive results were confirmed with a second sample. All women were given single-dose nevirapine as prophylaxis. None of the women included in this analysis received antiretroviral treatment because it was not available in Zambia at the time.

We selected as case patients 22 HIV-infected women who transmitted HIV to their infants later than 1 month postpartum, as indicated by a last negative HIV DNA test result for

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Table 1. Characteristics of Breast Milk Cytokine Concentrations in 94 Human Immunodeficiency Virus (HIV)–Infected and 18 HIV-Uninfected Women from Lusaka, Zambia

Parameter	HIV-infected mothers			HIV-uninfected mothers (n = 18)
	Transmitter (n = 21)	Nontransmitter (n = 72)	P	
Maternal characteristics at enrollment				
$CD4^+$ cell count, cells/μL	218 ± 112	403 ± 211	<.001	867 ± 258
Plasma HIV load, $\log_{10}$ HIV RNA copies/mL	5.0 ± 0.4	4.5 ± 0.9	<.001	…
Maternal age	29.1 ± 5.4	25.9 ± 4.7	.01	25.7 ± 7.2
Previous live births, median (IQR)	3 (2 to 4)	2 (1 to 3)	.04	2 (0 to 4)
Infant variables				
Birth weight, g	2653 ± 642	3055 ± 507	.004	3168 ± 641
Low birth weight, no. (%)	7 (33)	7 (10)	.02	2 (12)
Breast milk				
1-week samples, no. (%)	15 (68)	50 (69)	.91	13 (72)
HIV load, median (IQR), copies/mL	346 (198 to 4043)	<50 (<50 to 289)	<.001	…
HIV load ⩾50 copies/ml, no. (%)	19 (86)	32 (44)	<.001	…
Sodium level, median (IQR), mmol/L	<13 (<13 to 13)	<13 (<13 to 15)	.64	<13 (<13 to 16)
Sodium level, ⩾13 mmol/L, no. (%)	6 (27)	23 (32)	.68	7 (39)
IL-8 level, $\log_{10}$ pg/mL	2.4 ± 0.5	2.6 ± 0.6	.17	2.3 ± 0.7
IL-15 level, $\log_{10}$ pg/mL	1.3 ± 0.4	1.5 ± 0.4	.02	1.4 ± 0.4
CXCL12 level, median (IQR), pg/mL	<156 (<156 to <156)	<156 (<156 to 386)	.02	<156 (<156 to 223)
CXCL12 level, ⩾156 pg/mL, no. (%)	3 (14)	33 (46)	.01	8 (44)
CCL5 level, median (IQR), pg/mL	76 (38 to 165)	84 (<31 to 169)	.93	<31.2 (<31 to 71)
CCL5 level, ⩾31 pg/mL, no. (%)	17 (77)	52 (72)	.64	7 (39)

NOTE. Data are means ± standard deviations, unless otherwise indicated. Numbers may be smaller than the total due to missing data. IL-8, interleukin 8; IL-15, interleukin 15; IQR, interquartile range.

their infant at the 1-month visit or later. The infants of the 22 transmitters first tested positive between 2 and 6 months (n = 14), 9 and 12 months (n = 4), and 15 and 24 months (n = 4). Breast milk cytokine concentrations of the 22 transmitting women were compared with those of 72 nontransmitting HIV-infected women, who were randomly selected from among all those who had not transmitted HIV to their infants through 24 months or through the time of collection of their last available sample if they were lost to follow-up or had died. Eighteen HIV-uninfected women recruited from the same community over the same period were included as control subjects. Breast milk samples were frequency-matched by time of sample collection at 1 week (70%) or 1 month (30%) postpartum. All samples were collected between 2001 and 2004.

All women signed informed-consent forms for participation in the study, which was approved by the institutional review boards at the respective institutions of the investigators.

Breast milk was collected and processed as described elsewhere [4]. Concentrations of HIV-1 RNA were determined by an ultrasensitive assay with a lower limit of detection of 50 copies/mL (version 1.5; Roche Amplicor). Sodium was quantitated using an anion selective electrode (Synchron LX20; Beckman Coulter). Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to determine concentrations of IL-8, IL-15, CCL5 (R&D Systems), and CXCL12 (BD Biosciences), according to the manufacturers' instructions. Concentrations below the standard range of the assay were imputed with a value just below the lowest detectable standard concentration (for IL-8, 2 pg/mL; for IL-15, 3.89 pg/mL; for CXCL12, 155 pg/mL; for CCL5, 31.1 pg/mL).

Maternal CD4 cell counts (FacsCount; BD Immunocytometry Systems) and plasma HIV RNA (Roche Amplicor) levels were measured in samples collected at enrollment during pregnancy.

We used the χ^2 test to compare categorical variables, the Fisher exact test if the expected cell size was <5, the Wilcoxon rank sum test to compare non–normally distributed continuous variables, and the t test for normally distributed variables. Pearson correlation coefficients were calculated to describe univariate associations between 2 normally distributed variables, and Spearman rank order coefficients were calculated for non–normally distributed variables. Paired plasma and breast milk cytokine concentrations were compared using the Wilcoxon signed rank test. Logistic regression modeling was conducted to adjust for possible confounding. We did not observe evidence of variance inflation. All statistical analyses were performed using SAS software (version 9.1; SAS).

Results. The 22 transmitters in this study had more advanced HIV disease than did the control group of 72 nontransmitters, as reflected by lower CD4 cell counts and higher plasma and breast milk HIV RNA concentrations. In addition, transmitters were older, had higher parity, and had infants of lower birth weight than did the nontransmitters (Table 1).

Table 2. Correlates of Cytokine Concentrations in Breast Milk Collected from 72 Breast-feeding Human Immunodeficiency Virus (HIV)–Infected Zambian Women Who Did Not Transmit HIV to Their Infants

Parameter	IL-8[a]	IL-15[a]	CXCL12[a]	CCL5[a]
1-week sample	2.7 ± 0.6	1.6 ± 0.4	<156 (<156 to 357)	80 (<31 to 144)
1-month sample	2.5 ± 0.5	1.4 ± 0.4	<156 (<156 to 474)	115 (<31 to 198)
P	.18	.06	.90	.53
HIV RNA level	R_S = 0.28	R_S = 0.28	R_S = 0.18	R_S = 0.25
P	.02	.02	.14	.03
⩾50 HIV RNA copies/mL	2.8 ± 0.6	1.6 ± 0.4	189 (<156 to 475)	127 (54 to 234)
<50 HIV RNA copies/mL	2.5 ± 0.5	1.4 ± 0.3	<156 (<156 to 336)	73 (<31 to 128)
P	.13	.03	.31	.06
Sodium level	R_S = 0.70	R_S = 0.63	R_S = 0.46	R_S = 0.47
P	<.001	<.001	<.001	<.001
⩾13 mmol/L sodium	3.2 ± 0.4	1.9 ± 0.4	458 (<156 to 712)	167 (79 to 420)
<13 mmol/L sodium	2.4 ± 0.4	1.4 ± 0.3	<156 (<156 to 228)	54 (<31 to 124)
P	<.001	<.001	.004	.001
IL-8 level	…	R_P = 0.47	R_S = 0.39	R_S = 0.37
P		<.001	.001	.001
IL-15 level	R_P = 0.47	…	R_S = 0.59	R_S = 0.63
P	<.001		<.001	<.001
CXCL12 level	R_S = 0.39	R_S = 0.59	…	R_S = 0.60
P	.001	<.001		<.001
CCL5 level	R_S = 0.37	R_S = 0.63	R_S = 0.60	…
P	.001	<.001	<.001	

NOTE. Numbers may differ slightly from the total due to missing values. IL-8, interleukin 8; IL-15, interleukin 15; R_P, Pearson correlation coefficient; R_S, Spearman correlation coefficient; SD, standard deviation.

[a] Means and standard deviations (IL-8 and IL-15 in $\log_{10}$ pg/mL) or medians and interquartile ranges (CXCL12 and CCL5 in pg/mL) are shown for dichotomous variables, and correlation coefficients (Pearson or Spearman as indicated) are shown for continuous variables.

Breast milk IL-8 and IL-15 concentrations were detectable in virtually all (111/112) of the tested samples. In contrast, CXCL12 was detectable in only 44 (39%) of 112 tested samples, and CCL5 was detected in 76 (68%) of 112 tested samples.

Nontransmitters had significantly higher concentrations of IL-15 in their breast milk than did transmitters (mean ± standard deviation, 1.5 ± 0.4 vs 1.3 ± 0.4 $\log_{10}$ pg/mL [34 vs 20 pg/mL]; $P = .02$). Nontransmitters were more likely to have detectable CXCL12 concentrations than transmitters (33 women [46%] vs 3 women [14%]; $P = .01$). Nontransmitters and transmitters did not differ in their IL-8 or CCL5 concentrations in breast milk (Table 1).

HIV-uninfected women differed from nontransmitters in that they were less likely to have breast milk samples with detectable CCL5 concentrations (7 HIV-uninfected women [39%] versus 52 nontransmitting infected women [72%]; $P = .01$). They differed from transmitters in that they had detectable CXCL12 concentrations more often (8 uninfected women [44%] versus 3 transmitting women [14%], $P = .04$) but detectable CCL5 concentrations less often (7 uninfected women [39%] vs 17 transmitting women [77%], $P = .01$). There was no difference in IL-8, IL-15, and CXCL12 concentrations in breast milk between HIV-uninfected women and nontransmitters or in IL-8 and IL-15 concentrations between uninfected women and transmitters (Table 1).

Among the nontransmitters, breast milk IL-8, IL-15, and CCL5 (but not CXCL12) concentrations correlated significantly with breast milk viral load. All 4 cytokines were also moderately to strongly correlated with breast milk sodium concentration and with each other (Table 2). There were, or tended to be, additional associations between some of the cytokines and plasma viral load at enrollment (for $\log_{10}$ IL-15 level, $R_P = 0.24$ and $P = .04$), maternal age (for $\log_{10}$ IL-8 level, $R_P = -0.25$ and $P = .04$), parity (for all 4 cytokines, $R_S = -0.35$ to -0.20 and $P \leqslant .1$), and infant birth weight (for IL-15, $R_P = -0.46$ and $P < .001$; for CXCL12, $R_S = -0.24$ and $P = .05$). None of the cytokines correlated with maternal CD4 cell counts at enrollment.

To determine whether breast milk concentrations of IL-15 and CCL5 were a result of local production or reflected blood levels, we compared plasma and breast milk concentrations in a subset of nontransmitting women. While all breast milk samples had IL-15 levels above the detection limit of the assay, plasma IL-15 concentrations were above the assay cutoff for only 2 (10%) of the 20 samples tested. The median difference

between breast milk and plasma IL-15 concentrations was 45 pg/mL (range 9–425 pg/mL; $P < .001$). The median CCL5 concentrations ($n = 53$) were >2000-fold higher in plasma than in breast milk (median CCL5 level in plasma, 184 ng/mL, interquartile range [IQR], 110–200 ng/mL; $P < .001$ for the comparison with breast milk). Plasma CCL5 concentrations did not correlate with breast milk CCL5 concentrations ($R_S = 0.15$; $P = .27$).

When adjusted for breast milk viral load and blood CD4 cell counts (both well-established risk factors for HIV transmission via breast-feeding), IL-15 remained strongly associated with protection against mother-to-child HIV transmission (adjusted odds ratio [AOR], 0.05 per $\log_{10}$ increase [95% confidence interval {CI}, 0.01–0.3]). These associations strengthened further when adjusted for the other 3 measured cytokines. In the final model, a $\log_{10}$ increase in IL-15 level was associated with a 200-fold lower risk of HIV transmission (AOR, 0.005 [95% CI, <0.001–0.09]) when adjusted for detectable breast milk CCL5 concentrations (≥31.1 pg/mL) (AOR, 13.8 [95% CI, 1.8–109.5]), breast milk viral load (AOR, 6.6 per $\log_{10}$ increase [95% CI, 2.2–19.3]), and blood CD4 cell counts (AOR, 0.5 per 100 cells increase [95% CI, 0.3–0.7]). None of the other variables listed in Table 1 remained significantly associated with the transmission risk or changed the effect estimates substantially when added to the model.

Discussion. In this cohort of HIV-infected women in Lusaka, Zambia, we found a protective association between breast milk IL-15 concentrations and postnatal HIV transmission that strengthened in multivariate analysis. This association was not explained by the correlation between IL-15 and other proinflammatory or chemotactic cytokines in breast milk, including IL-8, CCL5, and CXCL12, nor was it explained by maternal CD4 cell count or the concentration of cell-free virus in breast milk. These data therefore suggest that IL-15 in breast milk or an associated factor protects against HIV transmission via breast-feeding.

That IL-15 has a protective effect is corroborated by previous studies that demonstrated a high frequency of CD8 T cells in breast milk, including those with HIV specificity among HIV-infected women [12]. The ability of IL-15 to enhance CD8 T cell immunity may directly lower the risk of HIV transmission by lysis of HIV-infected breast milk cells. It is also possible that IL-15 has an effect on infant immunity. IL-15 receptors are expressed on infant intestinal intraepithelial lymphocytes, and paracrine IL-15 expression by epithelia cells is thought to activate these cells [13]. Although these processes have been implicated in the pathogenesis of inflammatory bowel disease [14], they may also be beneficial by enhancing infant immune responses against HIV at the intestinal epithelium. Furthermore, it was recently suggested that HIV-intrinsic antisense anti–IL-15 micro-RNA is used by HIV to overcome IL-15–mediated immunity [15], further corroborating the protective role played by IL-15 against HIV.

Our data are also in concordance those of with previous studies that found a detrimental effect of breast milk CCL5 concentrations on postnatal HIV transmission in adjusted analysis [2, 3]. We did not, however, observe a protective association with CXCL12 when adjustments were made for IL-15, CCL5, breast milk viral load, and blood CD4 cell counts, suggesting that the closely correlated cytokine IL-15 may be a more important factor for protection against HIV transmission than CXCL12. We also did not observe a detrimental effect of IL-8 as has previously been proposed [9]. It would be interesting to investigate if this association differs for women with clinical mastitis, who presumably have higher concentrations of IL-8.

The strengths of this study include the collection of samples prior to infant HIV infection and the measurement of proinflammatory cytokines other than IL-15. As in all observational studies, we cannot exclude the possibility of confounding by unmeasured variables. An additional limitation of this study is the inability of our assays to detect low levels of CCL5 and CXCL12 in a large proportion of breast milk; it may be possible that part of their effects was missed.

In summary, our data are concordant with the concept that IL-15–mediated immunity plays a role in protection against HIV transmission in humans. These results are encouraging for vaccine studies that explore the use of IL-15 as an adjuvant [7].

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