Transmission of Cell-Free and Cell-Associated HIV-1 Through Breast-Feeding

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Background: Transmission through breast-feeding is an important cause of infant HIV-1 infections in developing countries; however, its mechanism remains largely unknown. We have explored the association between cell-free virus (CFV) and cell-associated virus (CAV) levels in breast milk (BM), as reflected by viral RNA and proviral DNA, respectively, and the risk of infant HIV-1 infection after 6 weeks postpartum.

Methods: Sixty-one HIV-positive mothers who transmitted HIV-1 by BM were matched to 61 HIV-positive nontransmitting mothers based on their infant's age at sample collection. CFV and CAV were quantified in a single milk specimen per mother preceding the infant's first HIV-positive result.

Results: After adjusting for maternal $CD4^+$ cell counts and disease stage, each 10-fold increase in CFV or CAV load was associated with an almost 3-fold increase in BM transmission. Whereas CAV load was predictive of transmission before and after 9 months postpartum, CFV was a significant predictor of transmission occurring only after 9 months. Phylogenetic analyses of the C2 to C5 env region showed that 85% of infants (11 of 13 infants) harboring viruses that clustered with CFV in their mother's milk were infected after 9 months postpartum.

Conclusion: A reduction in milk CAV and CFV loads might significantly decrease HIV-1 transmission by breast-feeding.

Key Words: HIV-1, breast-feeding, mother-to-child transmission, provirus

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HIV-1 infections through breast milk (BM) diminish the benefits of perinatal antiretroviral regimens in the prevention of mother-to-child transmission (MTCT).[1] Whereas complete avoidance of breast-feeding is best for minimizing postnatal HIV-1 transmission, this is not practical in many developing countries, given the health hazards and potential social stigma associated with alternative feeding practices.[2] A substantial risk for HIV-1 transmission through breast-feeding has been observed in Tanzania.[3] Transmission via BM in infants who were uninfected at 6 weeks of age increased from 3.9% at 4 months to 18.1% by 24 months of age.[3] In agreement with other studies, the incidence rate of postnatal infections remained constant over time in this Tanzanian cohort.[4] Although several clinical, demographic, and immunologic risk factors for BM transmission of HIV-1 have been identified, the mechanism of transmission remains poorly understood.[3,5,6]

During breast-feeding, the oral and/or buccal mucosa and the upper gastrointestinal tract are the principal portals of entry for cell-free virus (CFV) and/or cell-associated virus (CAV).[7–10] Primary intestinal epithelial cells could selectively transfer R5 HIV-1 to $CD4^+$ cells through transcytosis.[11] The amount of CAV was reportedly less than 1% of the amount of CFV necessary for viral translocation through a tight epithelial monolayer, however.[12,13] Conversely, gingival keratinocytes were susceptible to HIV-1 infection only via cell-free $CD4^-$ independent infection.[7] It is not known whether such differences in transmission efficiency exist in vivo and whether CFV or CAV is the predominant infectious component transferred to the infant. Even though HIV-1 RNA and DNA loads in milk have been associated with increased risk of MTCT,[14–20] no studies have specifically compared their effects on the risk of transmission through BM in the same population. Recently, it was shown that HIV-1 DNA load in blood as well as cervical and vaginal fluids was a better predictor of perinatal infant infection than HIV-1 blood RNA load.[21]

We examined the risk of postnatal HIV-1 infection associated with CFV and CAV loads in a single milk sample collected close to the time of transmission and before the infant's first HIV-positive result. Considering that the cell numbers in milk as well as the levels of maternal IgG in the infant decrease over time,[14,22,23] different mechanisms of BM HIV-1 transmission at early and late lactation stages could account for the relatively constant risk of postnatal HIV-1 infections in Tanzania.[3,4] Therefore, we also tested whether the transmission efficiency of CFV versus CAV may differ in early and late postpartum periods. Phylogenetic analysis was used to compare viral C2 to C5 env sequences from infant peripheral blood mononuclear cells (PBMCs) with C2 to C5 env clones from milk CAV and CFV of the respective mother according to time of transmission.

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MATERIALS AND METHODS

Study Population

The BM samples were collected at delivery and at 3-month intervals thereafter from 1078 HIV-1–positive mothers enrolled in a randomized, double-blind, placebo-controlled trial that examined the effect of vitamin supplements on MTCT of HIV-1 in Tanzania.[24,25] Information on sociodemographic, clinical, and laboratory characteristics as well as on duration of breast-feeding and potential risk factors was recorded during the trial. Blood $CD4^+$ lymphocyte counts were measured at enrollment with the FACScount system (Becton-Dickinson, San Jose, CA), and maternal disease stage was determined according to the World Health Organization (WHO) HIV stage disease classification.[26]

The HIV-1 status of the infants was determined using the Amplicor HIV-1 detection kit (Roche Diagnostic Systems, Branchburg, NJ) on blood samples collected at birth, 6 weeks, and every 3 months thereafter or by enzyme-linked immunosorbent assay (ELISA) and/or Western blot analysis in children older than 18 months of age.[27] Transmission times for all HIV-1–infected infants from this cohort have been published elsewhere.[28] Children were considered infected through BM if they had a negative HIV-1 polymerase chain reaction (PCR) assay at 6 weeks of age and a positive PCR assay at any time thereafter. Antiretroviral treatment was not available in Tanzania at the time of this study. After counseling, mothers were free to choose to breast-feed their children. The project was approved by the Human Subjects Committee of the Harvard School of Public Health and the Research and Publications Committee of Muhimbili University College of Health Sciences.

Study Design

A case-control study was nested within the vitamin supplementation trial in Dar es Salaam. Cases were 61 mothers who transmitted HIV-1 to their infants through BM. All cases were asymptomatic at WHO HIV-1 disease stage 1 (65.6%) or stage 2 (34.4%). Transmission was estimated to have occurred around the midpoint of the interval between an infant's last negative test result and first positive PCR result. The average length of this interval was 20 weeks. One BM sample, collected close to the estimated time of transmission (ETT), was used for each case. Seven mothers were excluded from the study because they had not provided a BM sample before their infant's first positive PCR result. The average time of sample collection was 5.56 weeks before the ETT (median = 2.5 weeks). In 51 (84%) of 61 cases, a sample was available within 12 weeks before or after the ETT, whereas an earlier sample was used in the remaining 10 cases (mean = 27 weeks before the ETT, range: 12.7–49.7 weeks). In 11 of 61 cases, the only BM sample available was at the time of the child's first positive test result (n = 8) or within the prior 4 weeks (n = 3). Unfortunately, samples collected at the ETT were not available from the blood compartment in most of the mothers enrolled in this study.

For each case, 1 control was randomly chosen from among breast-feeding HIV-1–positive nontransmitting mothers who had provided a BM sample within 1 week of the sample date for the case. This matching on time of sample collection was intended to minimize the potential bias of varying concentrations of CFV and CAV as well as total cell numbers in milk over time. The length of follow-up for controls averaged 162 weeks (range: 98–234 weeks). All milk samples were collected while the mother was still breast-feeding.

Clinical mastitis in the 6 months preceding the ETT was reported in only 3 mothers.

Isolation of HIV-1 Proviral DNA and Free Virus RNA

Milk samples were collected by manual expression and immediately centrifuged at 1500 g for 12 minutes at 4°C. The top cream layer, aqueous interface, and milk cell pellet were cryopreserved separately and transported to the laboratory in Boston. All samples were kept at −70°C until further analysis. Cell pellets were washed with phosphate-buffered saline (PBS), and genomic DNA extraction was performed using the QIAamp Blood Kit (Qiagen, Valencia, CA).

Viral RNA was isolated from the cell-free aqueous milk fraction using the High Pure Viral RNA Kit (Roche Diagnostics, Indianapolis, IN).

Quantification of HIV-1 Cell-Associated Virus and Cell-Free Virus

The proportion of HIV-1 proviral copies per total cell numbers was quantified by real-time PCR using the FastStart DNA Master SYBR Green I mix and the LightCycler instrument (Roche Diagnostics). The double-stranded DNA binding dye SYBR Green I was used for sequence-independent fluorescent detection, followed by melting curve analysis of the amplification products to ensure their specificity. Samples were tested in duplicate using HIV-1–specific primers designed to amplify a conserved 150–base pair (bp) region of gag. Ten-fold serially diluted genomic DNA from 8E5 cells (ATCC number: CRL 8993), known to carry a single HIV provirus per cell, was used as a positive control and for the construction of standard curves. Proviral copies were normalized according to copies of beta-globin, reflecting cell numbers present in each sample. The quantification results of this procedure were expressed as proportion of infected BM cells, and the lower limit of detection was determined to be 5 proviral copies.

Quantification of CVF in the cell-free aqueous milk fraction was done using the ultrasensitive protocol of the Amplicor HIV-1 Monitor Test, v 1.5 (Roche Diagnostics). We evaluated the performance of this procedure in BM specimens by spiking uninfected human milk samples with known amounts of virus obtained from a subtype C infectious molecular clone,[29] followed by centrifugation under the same conditions used for obtaining the aqueous milk layer from infected specimens. The results reflected the free viral load concentration accurately and within the expected variability range inherent to the assay. The lower limit of detection was 100 viral copies/mL.

Amplification of HIV-1 Cell-Associated Virus DNA and Cell-Free Virus RNA for Phylogenetic Analysis

The first positive blood sample from each infant was used for DNA sequencing and phylogenetic analyses. As

previously described, a nested PCR assay was used to amplify the C2 to C5 env fragment from genomic DNA purified from milk cells and infant PBMCs.[27] The same region was also amplified from purified HIV-1 RNA using the C. therm. Polymerase One-Step RT-PCR System (Roche Diagnostics). Cloning of gel-purified PCR and reverse transcriptase (RT) PCR products was done with the TOPO 2.1 TA Cloning Kit (Invitrogen, Carlsbad, CA). An average of 10 clones from each milk sample and 5 clones from PBMCs was sequenced with dye terminators using an automated sequencer.

The Clustal X software package[30] was used to align milk viral and proviral sequences to clones from the respective infant and to HIV-1 subtype references recommended by the Los Alamos HIV Sequence Database.[31] All alignments were gap-stripped for the generation of trees and subjected to phylogenetic analysis using the neighbor-joining method and the Kimura 2-parameter model for calculation of genetic distances.

Reliability of phylogenetic trees was estimated from 100 bootstrap replicates. To rule out mislabeling or potential cross-contamination, all sequences obtained from milk were aligned to sequences previously amplified from the subject's PBMCs and to viral sequences isolated from the respective infant in cases of transmitting mothers. As expected, tighter clusters were observed for viruses from different maternal compartments and from the respective infants than for any other viral sequences amplified from the study population.

TABLE 1. Baseline Characteristics of HIV-1–Positive Mothers Transmitting by Breast-Feeding and Their Matched Controls

Maternal Characteristic	Controls* (n = 61)	Cases* (n = 61)	P†
Age (y)	26.0 (4.9)	25.6 (4.9)	0.98
Primiparous (%)	24.6 (15)	34.4 (21)	0.26
Money spent on food per person-day	594 (320)	483 (265)	0.18
Lack primary schooling (%)	13.1 (8)	13.1 (8)	1.00
Height (cm)	155.5 (4.7)	156.9 (5.8)	0.27
Weight (kg)	56.8 (9.2)	58.7 (8.8)	0.13
Arm circumference (cm)	25.6 (3.2)	25.9 (3.1)	0.33
Duration of breast-feeding (mo)	20.5 (4.7)	20.1 (4.0)	0.51
$CD4^+$ cell counts/mm^3	467 (208)	405 (295)	0.03
$CD8^+$ cell counts/mm^3	703 (306)	817 (377)	0.15
Hemoglobin (g/L)	9.8 (2.0)	9.4 (1.7)	0.39
Malaria (%)	9.8 (6)	13.1 (8)	0.77
Symptomatic HIV disease (%)	16.4 (10)	34.4 (21)	0.03
Any STDs	17 (28)	13 (21)	0.23
Birth weight (g)	3037 (573)	3035 (517)	0.65
Gestational age at delivery (wk)	38.9 (2.9)	38.4 (3.2)	0.48

*Mean values reported; standard deviation is shown in brackets.
†Wilcoxon matched pairs rank-sum test for continuous variables and McNemar test for categoric variables.
STDs indicates sexually transmitted diseases.

Statistical Analysis

Conditional logistic regression analysis was used to assess the association between CFV and CAV loads in milk and the risk of BM transmission. Multivariate models included maternal blood $CD4^+$ cell counts and disease stage at enrollment. Adjustment for vitamin A supplementation status was also done initially because an adverse effect on MTCT had been previously reported[32]; however, it was subsequently dropped from all models because it did not change the point or interval estimates of the associations of interest. CFV and CAV loads were analyzed on a decimal logarithmic scale and as binary outcomes using the median value among cases as a cutoff. A value equal to the assay cutoff was assigned to samples below the detectable level for the purpose of logarithmic transformation.

Differences in categoric variables were assessed by the McNemar test in matched analyses or by the Fisher exact test or 2-sample proportion test. The Wilcoxon matched pairs rank-sum and Mann-Whitney *U* tests were used for nonparametric analysis of continuous variables. Spearman rank correlation was used for estimating the correlation between continuous variables.

RESULTS

Cell-Free Virus and Cell-Associated Virus Loads in Milk and Transmission by Breast-Feeding

Information on several potential risk factors for MTCT transmission recorded for each mother at baseline is shown in Table 1. Cases were more likely to have lower $CD4^+$ cell counts ($P = 0.03$) and a more advanced disease stage ($P = 0.03$) than controls.

CFV was detected in 57% of BM samples (70 of 122 samples) from all mothers and in 74% of cases (45 of 61 cases), with values ranging from 101 to 27,347 copies/mL. CAV was detected in the BM of 74% of all mothers (90 of 122 mothers) and in 87% of those (53 of 61 mothers) transmitting the virus. Up to 14.7% of cells in milk were found to carry HIV-1 proviruses. In nonparametric analyses, CFV and CAV loads were significantly higher among cases than controls ($P = 0.006$ and $P = 0.0011$, respectively, by Wilcoxon matched pairs rank-sum test).

In univariate regression analyses, each 10-fold increase in CAV or CFV load was associated with approximately a 3-fold increase in transmission (odds ratio [OR] = 2.83 and OR = 3.24, respectively; Table 2). Mothers with high CAV and CFV loads were almost 9 times more likely to transmit HIV-1 by breast-feeding than mothers with low CAV and CFV loads (OR = 8.51, 95% confidence interval [CI]: 2.22 to 32.69; $P = 0.002$). After adjustment for maternal $CD4^+$ counts and disease stage at recruitment, there was still a significant association between CAV or CFV load in milk and transmission through breast-feeding (OR = 2.69, 95% CI: 1.36 to 5.32 and OR = 3.07, 95% CI: 1.44 to 6.58, respectively).

The risk for infant infection was 5-fold higher if the proportion of infected milk cells or cell-free virions was equal to or greater than the median observed among transmitters (7.48 copies per 104 cells and 666 copies/mL, respectively; see Table 2). CAV and CFV loads in BM were positively correlated (Spearman $\rho = 0.46$; $P < 0.00001$). There was no threshold concentration below which transmission did not

TABLE 2. HIV-1 CAV and CFV Load in BM of Mothers Transmitting the Infection by Breast-Feeding and Their Nontransmitting Controls

	Cases	Controls	Regression Analysis of Continuous CAV/CFV‡§	Regression Analysis of Binary CAV/CFV‡‖
CAV load* Mean (SD)	5.91 (19.94)	1.25 (4.23)	OR = 2.83, P = 0.003 95% CI: 1.43 to 5.58	OR = 5.00, P = 0.001 95% CI: 1.59 to 11.85
CFV load† Mean (SD)	2561 (5138)	618 (1780)	OR = 3.24, P = 0.002 95% CI: 1.55 to 6.76	OR = 5.50, P = 0.002 95% CI: 1.90 to 15.96

*HIV-1 CAV: proviral copies per 10,000 cells.
†HIV-1 CFV: copies/mL.
‡Univariate conditional logistic regression.
§CAV and CFV loads analyzed after a $\log_{10}$ transformation.
‖CAV and CFV loads analyzed as high versus low using the median load among cases as a cutoff.
SD indicates standard deviation.

occur: 7 mothers with CAV and CFV loads in BM below the level of detection transmitted HIV-1 to their infants.

Association of Cell-Associated Virus and Cell-Free Virus Loads for HIV-1 Transmission in Different Stages of Lactation

Separate conditional logistic regression models were fit for case-control pairs in which transmission was estimated to have occurred before and after 9 months postpartum. The 9 months cutoff was chosen because it represents the time when a significant drop has been reported in the numbers of HIV-1–infected milk cells[14] and the titers of passively transferred maternal IgG antibodies.[22,33]

In univariate and multivariate models, high CAV loads in BM were associated with a 5- to 6-fold increase in the risk of BM transmission before and after 9 months postpartum (Table 3); however, the 95% CIs for the crude and adjusted ORs were quite wide. In contrast, detectable CFV loads seemed to be a stronger predictor of transmission after 9 months of lactation than before (see Table 3).

Comparison of C2 to C5 env Sequences in Breast Milk With Sequences Isolated From the Infant

An average of 10 clones from the viral C2 to C5 env region was sequenced from CAV and CFV in milk and compared with clones obtained from the infant at the time of the first positive PCR assay. Phylogenetic analysis was used to examine the presence of any preferential clustering of the transmitted infant viral sequences with CAV or CFV sequences in maternal milk. Amplification of CAV and CFV was possible in 41 (67.2%) of 61 cases. In 13 (31.7%) of 41 cases, the infant sequences showed closer genetic relatedness to CFV, and in 16 (39.0%) of 41 cases, the infant sequences clustered with CAV. No clear clustering pattern was observed in 12 (29.2%) of 41 cases, of which 6 transmitted before 9 months and 6 transmitted after 9 months. Those pairs were excluded from the subsequent statistical analysis. Phylogenetic trees typical of CAV, CFV, and indeterminate transmission clusters are shown in Figure 1. A significantly smaller proportion (2 [15.3%] of 13 clusters) of CFV clusters than CAV clusters (8 [50%] of 16 clusters) occurred in samples from mothers transmitting before 9 months of breast-feeding (1-sided 2-sample proportion test, P = 0.03). The ETTs of the 2 infants infected before 9 months who harbored viruses clustering with BM CFV were 20.4 and 31.4 weeks. Phylogenetic clustering was not predetermined by high CAV or CFV load; 2 mothers with a low CAV load still seemed to transmit cell-associated variants before 9 months in the presence of high CFV loads. Seventy-one percent of cases (10 of 14 cases) in which the transmitted sequences clustered with CFV had a high CAV load.

Although the average CFV loads tended to be higher in RNA versus DNA phylogenetic clusters (3.28 vs. 2.81 $\log_{10}$, respectively), this difference was not significant (Mann-Whitney U test, P = 0.17).

DISCUSSION

We quantified CFV and the proportion of HIV-1–infected cells in milk samples collected from transmitting and nontransmitting mothers. In agreement with previous studies, RNA viral loads in BM were lower than those generally detected in plasma,[15–17] whereas HIV-1 DNA levels were

TABLE 3. Association of HIV-1 CAV and CFV Levels in Milk With HIV-1 Transmission by Breast-Feeding Occurring Before and After 9 Months Postpartum

	Transmission ≤9 Months		Transmission >9 Months	
	Crude OR	Adjusted OR†	Crude OR	Adjusted OR†
CAV*	5.0, P = 0.038, 95% CI: 1.10 to 22.82	6.09, P = 0.04, 95% CI: 1.06 to 34.94	5.0, P = 0.011, 95% CI: 1.45 to 17.27	5.29, P = 0.05, 95% CI: 0.98 to 28.61
CFV*	4.5, P = 0.054, 95% CI: 0.97 to 20.82	3.97, P = 0.1, 95% CI: 0.78 to 20.26	6.5, P = 0.014, 95% CI: 1.47 to 28.80	6.73, P = 0.018, 95% CI: 1.39 to 32.52

*HIV-1 milk CAV and CFV levels analyzed as high versus low using the median among cases as the cutoff.
†OR adjusted for CD4 cell counts at delivery and HIV disease stage at baseline.

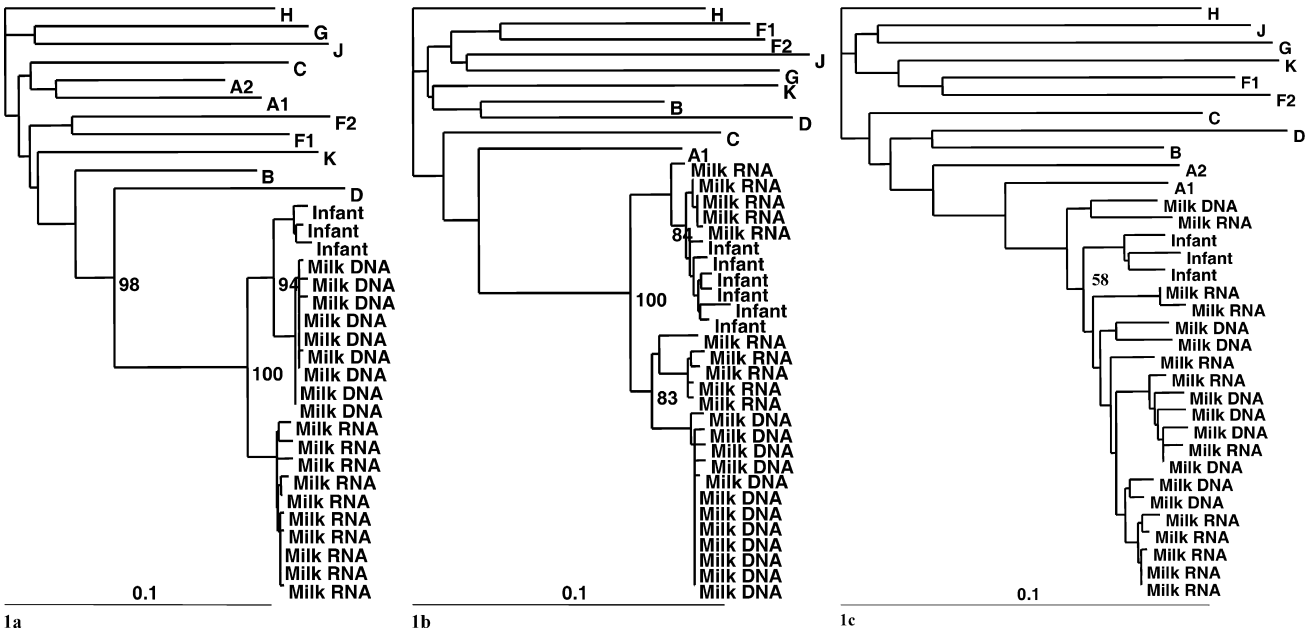

FIGURE 1. Phylogenetic clustering of infant HIV-1 env C2 to C5 sequences with maternal milk provirus or free virus clones from the same genetic region. a, Mother-infant pattern with phylogenetic pattern suggestive of HIV-1 proviral transmission. b, Mother-infant pattern with phylogenetic pattern suggestive of HIV-1 free virus transmission. c, Mother-infant pattern with indeterminate phylogenetic pattern.

higher than those reported in the blood of asymptomatic individuals.[34–37] Although the origin of CFV and CAV in milk is not known, it is possible that they enter the mammary compartment separately from blood or other peripheral mucosal sites.[38] The inability to detect HIV-1 p24 antigen in milk samples even after immune complex dissociation[39,40] suggests low levels of local replication and indicates that the correlation between viral RNA and DNA levels in milk may simply reflect a similar correlation in blood or other body sites. Alternatively, local viral expression might occur only at certain stages of lactation or maternal disease and might itself constitute a risk factor for transmission.[41]

Our ability to detect HIV-1 RNA and DNA in milk samples was comparable to that cited in other reports.[15–19,39,40] CVF was detected in 70 (57%) of 122 milk samples and in 45 (74%) of 61 samples from transmitting mothers. CAV was detected in 74% of all mothers (90 of 122 mothers) and in 87% of those (53 of 61 mothers) whose infants became postnatally infected. A report from Uganda in which CAV was found in BM cell pellets in 80% of HIV-1–transmitting women (16 of 20 women) at 6 weeks after delivery[40] found no correlation between the presence of CAV in BM and transmission; however, no quantification was performed.

Using a matched case-control design, we found that CFV and CAV loads in milk were significantly associated with BM HIV-1 transmission. Each 10-fold increase in CFV or CAV concentration in BM was associated with approximately a 3-fold increase in the risk of postnatal infant infection. A 2-fold increased risk of transmission with every 10-fold increase in BM virus load has been previously reported when multiple milk samples per mother were examined; however, a distinction between intrapartum and BM transmission was not possible.[16] Despite the high value of CFV and CAV loads in milk for predicting the risk of postnatal infection, no lower threshold for transmission could be determined.

Detection of HIV-1 DNA in BM reportedly decreases 9 months after delivery,[14] and infection in the infant has only been associated with HIV-1 DNA detection before 6 months[39,42]; however, these studies were done before real-time PCR technology for proviral quantification was available. We found that whereas the association between the proportion of infected milk cells and postnatal HIV-1 transmission occurring before and after 9 months was similar, the association of CFV load in milk with postnatal HIV-1 transmission was stronger after 9 months postpartum. We performed phylogenetic analysis to support these findings. Most infant sequences (11 of 14 sequences) that were phylogenetically closer to maternal milk HIV-1 RNA than DNA belonged to infants infected after 9 months of age. In contrast, half of the infant sequences clustering with CAV were transmitted before 9 months postpartum. These results suggest that early postnatal transmission of HIV-1 CFV in human milk is less common than early transmission via CAV. Accordingly, after adjustment for CFV in plasma, CAV but not CFV load in BM was significantly associated with HIV-1 transmission occurring peripartum and through early breast-feeding in Nairobi, Kenya.[43] Unfortunately, analysis of the excess transmission attributed to BM CAV or CFV in comparison with plasma viral load was not possible in our study because plasma samples at the ETT were not available.

Although we did not measure virus loads at different time points, others have not found an increase of HIV-1 RNA

in milk over time.[17] Possible causes for enhanced infection by CFV at later stages of lactation could include a decline in neutralizing HIV-specific IgM in milk,[42] passively transferred IgG in blood,[22] soluble antiviral factors in milk,[44–48] introduction of mixed feeding, intestinal maturation, and higher receptor expression. Alternatively, the changing immune environment in maternal milk and infant blood could facilitate infection of oral epithelial cells with CFV.[7,8]

The phylogenetic clustering of infant sequences with CFV or CAV sequences from BM might not always indicate transmission of CFV versus CAV, respectively. Viral quasispecies dominant in the proviral fraction in milk might become the predominant population of CFV at a later time point and vice versa. Because we can only estimate the time of transmission, any rapid exchange of quasispecies between the 2 viral fractions in milk might not be detected. An average of 10 C2 to C5 env clones from CFV and CAV isolated from 10 mothers at 2 different time points of lactation did not suggest that such exchange patterns are common, however.

Cloning of multiple PCR amplicons from each specimen might have better represented the viral diversity in BM; however, this was not possible because of the low CFV levels and sample volume limitations. Nevertheless, because CFV and CAV sequences were generally amplified with the same primer set in each mother-infant pair, it would be unlikely that primer-specific differential amplification had significantly affected the phylogenetic clustering of viral C2 to C5 env clones between mothers and infants.

Although a neonatal anti-HIV vaccine is still not available, current intervention strategies considered in the prevention of MTCT by breast-feeding include antiretroviral therapy given to the mother to reduce viral load in plasma and BM, antiretroviral regimens providing prophylaxis to uninfected infants during the period of breast-feeding, formula feeding, and passive immunization of infants. Potential problems with the implementation of such approaches are related to drug toxicity in the infant; the inability of current drugs to clear the virus from long-lived and memory cells, which comprise most cells in BM milk[38,49,50]; and the need for frequent postnatal administration of anti-HIV antibodies. If the findings from this study are confirmed on a larger scale, further knowledge of the mechanism responsible for protection from infection with free HIV-1 virions at early lactation stages may help to develop safer and more effective preventive strategies.

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