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Cell-Free Human Immunodeficiency Virus Type 1 in Breast Milk

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Abstract

Breast-feeding may be an important route of human immunodeficiency virus type 1 (HIV-1) vertical transmission in settings where it is routinely practiced. To define the prevalence and quantity of HIV-1 in cell-free breast milk, samples from HIV-1-seropositive women were analyzed by quantitative competitive reverse transcription-polymerase chain reaction (QC-RT-PCR). HIV-1 RNA was detected in 29 (39%) of 75 specimens tested. Of these 29 specimens, 16 (55%) had levels that were near the detection limit of the assay (240 copies/mL), while 6 (21%) had >900 copies/mL. The maximum concentration of HIV-1 RNA detected was 8100 copies/mL. The prevalence of cell-free HIV-1 was higher in mature milk (47%) than in colostrum (27%, $P = 0.1$). Because mature milk is consumed in large quantities, these data suggest that cell-free HIV-1 in breast milk may contribute to vertical transmission of HIV-1.

Breast-feeding is associated with lower infant morbidity and mortality than is formula-feeding and is the recommended form of infant feeding worldwide [1]. After the onset of the human immunodeficiency virus type 1 (HIV-1) pandemic, the possibility of vertical transmission through breast-feeding raised concerns about this universal recommendation. Postpartum transmission of HIV-1, probably through breast-feeding, has been described in both case reports and prospective studies [2]. As a result, advice on breast-feeding for HIV-1–infected women has been modified on a regional basis. For example, in the United States, where formula and potable water are readily available, mothers infected with HIV-1 are counseled to formula-feed their infants [3]. The World Health Organization initially recommended breast-feeding by HIV-1–seropositive mothers in settings where infectious diseases are a major cause of infant mortality, and the risk of formula-feeding may be considerable [4]. These recommendations were adapted in 1996 in an interim statement by the Joint United Nations Programme on HIV/AIDS, which considered the growing evidence that breast milk HIV-1 transmission was appreciable [5]. The new recommendations advise counseling of HIV-1-infected women on the risks of HIV-1 transmission through breast milk and the risks and possible benefits of other methods of infant feeding. The risk of HIV-1 vertical transmission in breast-feeding compared with formula-feeding mother-infant pairs has been difficult to determine because in most populations a single form of feeding predominates. A metanalysis of six studies involving women with established HIV-1 infection estimated that breast-feeding was associated with a 14% risk of transmission. Similar analysis of four studies among women who acquired HIV-1 infection in the

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Informed consent was obtained from all patients. This study was reviewed and approved by ethical review committees of the University of Washington and the University of Nairobi. Human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of research.

postpartum period found that the risk of transmission to infants through breast-feeding was 29% [6].

Breast milk has been demonstrated to be the major route of perinatal transmission for human T cell leukemia virus and to be a route of transmission for animal retroviruses in experimental and natural settings [2, 7, 8]. For example, simian immunodeficiency virus (SIV) can be transmitted to neonatal macaques by oral feeding of cell-free virus [9]. Experimental transmission of feline immunodeficiency virus (FIV) has been demonstrated by oral administration of either cell-free FIV, FIV-infected peripheral blood mononuclear cells, or milk from a queen infected with FIV after delivery [10–12]. Neither the relative importance of cell-free versus cell-associated virus nor the impact of inoculum size was specifically evaluated in these models.

Breast milk contains 10^4–10^6 cells/mL, including macrophages, lymphocytes, and ductal epithelial cells. Although all of these cell types are susceptible to HIV-1 infection in vitro [13], there are few convincing reports that HIV-1 can be cultured from breast milk [14,15]. However, HIV-1 proviral DNA has frequently been detected in breast milk cells using the polymerase chain reaction (PCR). The prevalence of HIV-1 DNA detection ranged from 44% to 58% in four published studies [16–19]. In a study of women in Kenya, the frequency of infected cells ranged from as few as 1 in 10^4 cells to as many as 1 in 3 cells. In the same cohort, the detection of HIV-1–infected cells was more likely in women with low $CD4^+$ lymphocyte counts and low vitamin A levels. There are no published data on the prevalence or quantity of cell-free HIV-1 in breast milk or on its relationship to the number of infected breast milk cells. The following study was undertaken to complement and extend the data on breast milk cells by describing the prevalence and quantity of cell-free HIV-1 in the breast milk of women with established HIV-1 infection.

Methods

Study participants and procedures

Breast milk and blood samples were obtained from HIV-1–seropositive women participating in a randomized clinical trial of breast- versus formula-feeding in Nairobi, Kenya [16]. At 32 weeks of gestation, women underwent a standardized evaluation of demographic, social, sexual, and obstetrical history and a physical examination. At that time, heparinized blood was obtained for white blood cell and differential counts and CD4 and CD8 lymphocyte counts using monoclonal antibodies (Becton Dickinson, San Jose, CA) and flow cytometry (FACscan; Becton Dickinson). Women provided samples of manually expressed breast milk within a few days of delivery and at 6- to 12-week intervals thereafter.

Processing of breast milk

The aqueous supernatant fraction of breast milk was prepared by centrifugation of freshly expressed milk at 710 *g* for 20 min. The lipid layer was removed and discarded. The remaining supernatant was stored at −70°C or in liquid nitrogen until used. Prior to RNA extraction, the breast milk supernatant was centrifiiged at 10,000 *g* for 5 min to remove any remaining cells.

Nucleic acid isolation

RNA was extracted from the breast milk supernatant using RNAzol LS (TelTest, Friendswood, TX) according to the manufacturer's instructions. Twenty micrograms of glycogen (Boeringer Mannheim, Indianapolis) were added prior to isopropanol precipitation to maximize recovery of RNA. RNA samples were resuspended in diethylpyrocarbonate-treated water and used immediately for cDNA synthesis.

Quantitative competitive reverse transcription-PCR (QC-RT-PCR)

Primers and protocols for QC-RT-PCR were identical to those described by Piatak et al. [20], except that a different competitor RNA was used. The competitor plasmid, pGGD, was constructed by first inserting the 622-bp Hindill fragment of HIV-1_{Lai} (nt 632–1259) into the *Hind*lll site of pGEM-1 (Promega, Madison, WI). Subsequently, the 71-bp *Pst*I-*Sty*I fragment (nt 966–1027) within the HIV-1 insert was deleted to produce pGGD. Competitor RNA was generated by in vitro transcription of pGGD with the Ribomax Kit (Promega), following the manufacturer's instructions. The competitor RNA was quantitated by spectrophotometry, diluted, and stored in individual PCR tubes at −70°C until used.

For each quantitative assay, RNA extracted from 250 to 425 μL of breast milk supernatant was resuspended in 60 μL of diethyl-pyrocarbonate-treated water. Ten microliters of this RNA solution was added to each of 5 tubes containing 0, 0, 10, 33, and 100 copies of competitor RNA, respectively. For cDNA synthesis, 15 μL of a master cDNA reaction mix was added to each tube to produce a 30-μL final volume with the following concentrations: 10 m*M* Tris (pH 8.3), 50 m*M* KCl, 5 m*M* $MgCl_2$,5 m*M* dithiothrei-tol, 1 m*M* each dNTP, 5 ng/μL random hexamers, 0.5 U/μL RNAse Inhibitor (US Biochemical, Cleveland, OH), and 1 U/μL Superscript II reverse transcriptase (Life Technologies Gibco BRL, Grand Island, NY). One of the two tubes with 0 copies of competitor RNA, which was designated as a control to detect HIV-1 DNA, received all of the components of the cocktail except for reverse transcriptase. After a 30-min incubation at 42°C, cDNA synthesis was halted by heating to 95°C for 5 min. PCR amplification was initiated after adding 30 μL of a solution containing 10 m*M* Tris (pH 8.3), 50 m*M* KCl, 2.5 units of Taq polymerase (Perkin-Elmer, Foster City, CA), and 0.5 μg of primers GL4 (5′-CATICTATT-TGTTCITGAAGGGTACTAG-3′) and GL6 (5′-GCITTIAGC-CCIGAAGTIATACCCATG-3′; I = inosine). After a 5-min dena-turation at 94°C, PCR amplification was carried out for 45 cycles of 94°C for 1 min, 50°C for 2 min, and 72°C for 1 min. PCR products were analyzed by gel electrophoresis through 3% agarose followed by ethidium bromide staining. Quantitative RNA levels were determined by comparing the intensity of wild type and competitor product bands [20] using NIH Image software (NIH, Bethesda, MD). The quantity of competitor in the reaction that produces wild type and competitor bands of equal intensity approximates the amount of wild type RNA in the reaction. After correcting for the volume of milk extracted and the fraction of total RNA used per reaction, the concentration of HIV-1 RNA in milk was calculated. When wild type RNA was present in very small amounts, it could be detected in the reaction lacking competitor but not in the reaction with the lowest concentration of competitor. These samples were scored as "detected/not quantified."

HIV-1 stability in breast milk

To demonstrate the efficiency of extraction of HIV-1 from breast milk, serial dilutions of a cell-free HIV-1_{LAI} stock were made in PBS, plasma, and cell-free breast milk. Plasma and cell-free breast milk were from HIV-1–seronegative donors. RNA extraction and QC-RT-PCR were then performed on samples of each diluent. The stability of HIV-1 virions in breast milk was studied by incubating breast milk containing HIV-1 for 0–16 h at room temperature prior to RNA extraction and QC-RT-PCR.

Southern hybridization

In a minority (18/90) of the breast milk samples, RT-PCR in the absence of competitor produced DNA products of an ambiguous size, smaller than the expected wild type product but larger than the competitor. Southern hybrid-ization/autoradiography of these PCR products following standard methods was used to clarify their origin [21]. For these analyses, a ^{32}P-labeled probe was synthesized by random priming of an HIV-1 *gag* fragment

(nt 905–1044, HIV-1_{LAI}) using the Megaprime kit (Amersham, Arlington Heights, IL), following the manufacturer's instructions. This fragment is internal to the PCR primers used for this assay. Hybridization to the HIV-1–specific probe was used to determine whether the ambiguously sized products were derived from HIV-1.

Analysis of cell-associated HIV-1 in breast milk

In a previous study, HIV-1 DNA in breast milk cells was analyzed using nested PCR with primers in the *gag* region of HIV-1 [16]. HIV-1 DNA data were available for the cellular component in 73 (97%) of the 75 breast milk supernatant specimens analyzed in the current study.

Data analysis

Data were analyzed using the SPSS-PC (SPSS, Chicago) statistical program. The Mann-Whitney test was used to compare continuous variables; Yates's corrected χ^2 and Fisher's exact tests were used for categorical variables.

Results

Characteristics of the study population

The study population from which this sample was drawn has been previously described [16]. It consists of HIV-1–seropositive women enrolled in an ongoing clinical trial of breast- versus formula-feeding in Nairobi, Kenya. For this study, samples from 90 women were analyzed. Fifteen samples were excluded from further analysis because HIV-1 PCR product was detected in the absence of reverse transcription, suggesting that HIV-1 DNA from infected cells was present in the samples. The remaining 75 women had a median age of 23 years (range, 17–34); 69% were married, and the median lifetime number of sex partners was 3. HIV-related immunosuppression was assessed using clinical criteria and absolute CD4 lymphocyte counts. Using the modified World Health Organization clinical case definition [22], none of the women had AIDS. Of the 74 women with clinical data available, 27 (36%) had HIV-related symptoms (fever or cough for > 1 month, >10% loss of body weight, or history of skin rash or shingles); 5 (7%) of 68 women had clinical signs of HIV (rash, herpes zoster, thrush or oral ulcers). Of 64 women with available CD4 lymphocyte counts, 13% had <200 cells/mm^3, 59% had 200–499 cells/mm^3, and 28% had ≥500 cells/mm^3.

QC-RT-PCR assay of cell-free breast milk

The QC-RT-PCR assay described here was sensitive for the detection of HIV-1 in breast milk. Ten copies of HIV-1 RNA target produced by in vitro transcription were routinely amplified at levels that could be visualized on an ethidium bromide–stained gel. Equal efficiency of extraction of viral RNA from PBS, plasma, and breast milk was demonstrated by spiking samples with identical amounts of HIV-1 and performing the standard QC-RT-PCR assay. The concentration of HIV-1 determined by QC-RT-PCR was identical in all three diluents (data not shown). The stability of HIV-1 in breast milk was demonstrated by incubating identical samples of HIV-1-spiked breast milk for 0 or 16 h at room temperature. As illustrated in figure 1 A, no loss in quantitative signal was noted.

Results from a typical assay with patient specimens is illustrated in figure 1B. The specimen from subject MM 97 had no detectable HIV-1 RNA, and that from subject MM 06 contained ~ 10 copies/reaction, corresponding to 240 copies/mL. Because QC-RT-PCR incorporates an internal control in each reaction, the lower limit of detection of the assay was determined for each specimen. For the 46 samples in which no HIV-1 was detected, the lower limit

ranged from <240 copies/mL to <1500 copies/mL. In 38 (82%) of these 46 samples, the lower limit of detection was <500 copies/mL, and in 16 (35%) it was <240 copies/mL.

Prevalence and correlates of cell-free HIV-1 in breast milk

Samples from the 75 women included in this study consisted of 30 obtained during the first postpartum week (colostrum), 23 from days 8 to 90, and 22 from 3 and 12 months. HIV-1 RNA was detected in 29 (39%) of the 75 specimens. The association between the detection of HIV-1 RNA and the time after delivery is illustrated in figure 2. The prevalence of HIV-1 RNA was lower in the first postpartum week than at later times (27% vs. 47%, $P = .1$). During the first week, the prevalence of HIV-1 RNA in breast milk was similar on days 0–3 (29%) and days 4–7 (23%). In 13 (45%) of the 29 samples with detectable HIV-1 RNA, the concentration was high enough that a quantitative level could be determined. Among these 13 specimens, the range of HIV-1 levels was 240–8100 copies/mL with a mean of 1687 copies/mL (SD = 2087) and a median of 946 copies/mL.

Detection of HIV-1 RNA in breast milk supernatant was not related to absolute CD4 or CD8 lymphocyte count or CD4:CD8 ratio (table 1). There was a trend for women with detectable HIV-1 RNA in breast milk supernatant to have a higher concentration of HIV-1–infected cells in the cellular specimen from the same sample (313 infected cells/104 cells vs. 100 infected cells/10^4 cells, $P = .1$). There was no significant association between the detection of cell-free HIV-1 in breast milk and age, HIV-1–related signs or symptoms, or vitamin A levels. Small sample size, however, limited the power of this study to evaluate such associations.

Discussion

This is the first study to describe the quantity of cell-free HIV-1 in breast milk using a highly sensitive PCR assay for viral RNA. The concentration of cell-free HIV-1 has been extensively studied in blood. If no antiretroviral drugs are used, the plasma of most HIV-1-infected persons contains 10,000– to 1,000,000 copies of HIV-l/mL [23], while the breast milk samples tested here contained <240 to 8100 copies/mL. This difference is not surprising for a number of reasons: Breast milk is produced in large volumes each day, it contains a much lower concentration of cells than does blood, and it has less contact with the lymphoid organs, where most HIV-1 production probably occurs.

The amount of viral RNA in breast milk would be underestimated if HIV-1 were unstable in breast milk or if viral RNA were degraded prior to nucleic acid extraction. Neither of these possibilities seems likely on the basis of control experiments in which we processed breast milk, plasma, and PBS spiked with identical quantities of HIV-1. We found that the virus was as stable in breast milk as it was in either PBS or plasma. In addition, room temperature incubation of HIV-1 in breast milk for 16 h prior to RNA extraction did not lead to a quantitative loss of signal.

We found a trend for a lower prevalence of cell-free HIV-1 in the first week after birth than at later times. The prevalence of cell-free HIV-1 in colostrum may have been underestimated because 11 (73%) of the 15 samples excluded from the analysis because of cellular DNA contamination were colostrum. Nonetheless, nearly half (47%) of the mature milk samples contained detectable cell-free HIV-1. Furthermore, 4 of the 6 specimens with the highest levels of cell-free HIV-1 were from mature milk. This implies that the risk of cell-free HIV-1 shedding in milk persists after the milk supply has matured. This finding does not support the concept of withholding colostrum from infants of HIV-1–seropositive women, an intervention that has been proposed to prevent postpartum transmission of HIV-1.

In 61% of breast milk samples tested, no viral RNA could be detected using this QC-RT-PCR assay, which has a lower limit of detection of <500 copies/mL. In addition, 16 (55%) of the 29 samples that contained cell-free HIV-1 had levels that were near the detection limit of the assay. Six samples (21%), however, had >900 copies/mL. Although the methods of virus quantitation were different than those used here, two studies of SIV infection in rhesus monkeys suggest that oral exposure to small amounts of cell-free retrovirus may lead to infection. In the one study, 4 of 4 neonatal rhesus monkeys were infected after nontraumatic oral feeding of 100 animal infectious doses of SIVmac251 [9]. More recently, the same group showed that adult rhesus monkeys could also be infected by the oral route with 6000 times less virus than was needed for infection by the rectal route [24]. Although exposure to HIV-1 probably involves both cell-free and cell-associated virus, the SIV oral infection model suggests that cell-free virus, even in small amounts, may be sufficient for transmission.

Despite the low concentrations of HIV-1 RNA detected in cell-free breast milk versus plasma, it is important to consider that mature milk is consumed in large volumes, averaging >700 mL/day during the first 4 months of lactation [1]. For the infants consuming milk with >900 copies/mL, the oral exposure is >630,000 copies of HIV-1 RNA/day. Even if a minority of virus particles are infectious [25], this is a substantial exposure.

In summary, a significant fraction (39%) of cell-free breast milk specimens from HIV-1–infected women contained HIV-1 RNA, and the prevalence of HIV-1 RNA did not decrease as the milk supply matured. The overall risk of transmission of HIV-1 through breast milk is likely to be related to the quantity of HIV-1 in cell-free breast milk in addition to other factors, such as the number of HIV-1–infected cells in breast milk, the presence of antiviral substances in breast milk, and factors determining infant susceptibility.

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References

1. Lawrence, R. Breastfeeding: a guide for the medical profession. 4th ed. Mosby; St. Louis: 1994. p. 28-33.
2. Van de Perre P, Lepage P, Homsy J, Dabis F. Mother-to-infant transmission of human immunodeficiency virus by breast milk: presumed innocent or presumed guilty? Clin Infect Dis. 1992; 15:502–7. [PubMed: 1445596]
3. Centers for Disease Control. Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotropic virus type III/ lymphadenopathy-associated virus and acquired immunodeficiency syndrome. MMWR. 1985; 34:721–32. [PubMed: 2999576]
4. World Health Organization. Consensus statement from WHO/UNICEF consultation on HIV transmission and breast feeding. Wkly Epidemiol Rec. 1992; 7:177–84.
5. Joint United Nations Programme on HIV/AIDS (UNAIDS). Wkly Epidemiol Rec. 1996; 71:289–91. [PubMed: 8885700]
6. Dunn DT, Newell ML, Ades AE, Peckham CS. Risk of human immunodeficiency virus type 1 transmission through breast feeding. Lancet. 1992; 340:585–8. [PubMed: 1355163]
7. Oxtoby MJ. Human immunodeficiency virus and other viruses in human-milk: placing the issues in broader perspective. Pediatr Infect Dis J. 1988; 7:825–35. [PubMed: 2850525]

8. Hino S, Katamine S, Kawase K, et al. Intervention of maternal transmission of HTLV-1 in Nagasaki. Leukemia. 1994; 8(suppl l):S68–70. [PubMed: 8152307]
9. Baba TW, Koch J, Mittler ES, Greene M, Wyand M, Pennick D, Ruprecht RM. Mucosal infection of neonatal rhesus monkeys with cell-free SIV. AIDS Res Hum Retroviruses. 1994; 10:351–7. [PubMed: 8068415]
10. O'Neil LL, Burkhard MJ, Diehl LJ, Hoover EA. Vertical transmission of feline immunodeficiency virus. AIDS Res Hum Retroviruses. 1995; 11:171–82. [PubMed: 7734190]
11. Moench TR, Whaley KI, Mandrell TD, Bishop BD, Witt CJ, Cone RA. The cat/feline immunodeficiency virus model for transmucosal transmission of AIDS: nonoxynol-9 contraceptive jelly blocks transmission by an infected cell inoculum. AIDS. 1993; 7:797–802. [PubMed: 8395855]
12. Sellon RK, Jordan HL, Kennedy-Stoskopf S, Tompkins MB, Tompkins WAF. Feline immunodeficiency virus can be experimentally transmitted via milk during acute maternal infection. J Virol. 1994; 68:3380–5. [PubMed: 8151797]
13. Toniolo A, Serra C, Conaldi PG, Basolo F, Falcone V, Dolei A. Productive HIV-1 infection of normal human mammary epithelial cells. AIDS. 1995; 9:859–66. [PubMed: 7576319]
14. Vogt MW, Witt DJ, Craven DE, et al. Isolation of HTLV-III/LAV from cervical secretions of women at risk for AIDS. Lancet. 1986; 1:525–7. [PubMed: 2869261]
15. Thiry L, Sprecher-Goldberger S, Jonckheer T, et al. Isolation of AIDS virus from cell-free breast milk of three healthy virus carriers. Lancet. 1985; 2:891–2. [PubMed: 2864603]
16. Nduati RW, John GC, Richardson BA, et al. Human immunodeficiency virus type-1 - infected cells in breast milk: association with immunosuppression and vitamin A deficiency. J Infect Dis. 1995; 172:1461–8. [PubMed: 7594703]
17. Ruff AJ, Cobery J, Halsey NA, et al. Prevalence of HIV-1 DNA and p24 antigen in breast milk and correlation with maternal factors. J Acquir Immune Defic Syndr. 1994; 7:68–73. [PubMed: 7903381]
18. Van de Perre P, Simonon A, Hitimana D, et al. Infective and anti-infective properties of breast milk from HIV-1 infected women. Lancet. 1993; 341:914–8. [PubMed: 8096264]
19. Buranasin P, Kunakorn M, Petchclai B, et al. Detection of human immunodeficiency virus type 1 (HIV-1) proviral DNA in breast milk and colostrum of seropositive mothers. J Med Assoc Thai. 1993; 76:41–4. [PubMed: 8228693]
20. Piatak M, Luk K, Williams B, Lifson JD. Quantitative competitive polymerase chain reaction for accurate quantitation of HIV DNA and RNA species. Biotechniques. 1993; 14:70–80. [PubMed: 8424881]
21. Sambrook, J.; Fritsch, EF.; Maniatis, T. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1989.
22. De Cock KM, Selik RM, Soro B, Gayle H, Colebunders RL. AIDS surveillance in Africa: a reappraisal of case definitions. Br Med J. 1991; 303:1185–8. [PubMed: 1747620]
23. Piatak M Jr, Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science. 1993; 259:1749–54. [PubMed: 8096089]
24. Baba TW, Trichel AM, Li A, Liska V, Martin LN, Murphey-Corb M, Ruprecht RM. Infection and AIDS in adult macaques after nontraumatic oral exposure to cell-free SIV. Science. 1996; 272:1486–9. [PubMed: 8633242]
25. Boulerice F, Bour S, Geleziunas R, Lvovich A, Wainberg A. High frequency of defective human immunodeficiency virus type 1 and heterogeneity of viral expression in clone of infected U937 cells. J Virol. 1990; 64:1745–55. [PubMed: 1690823]

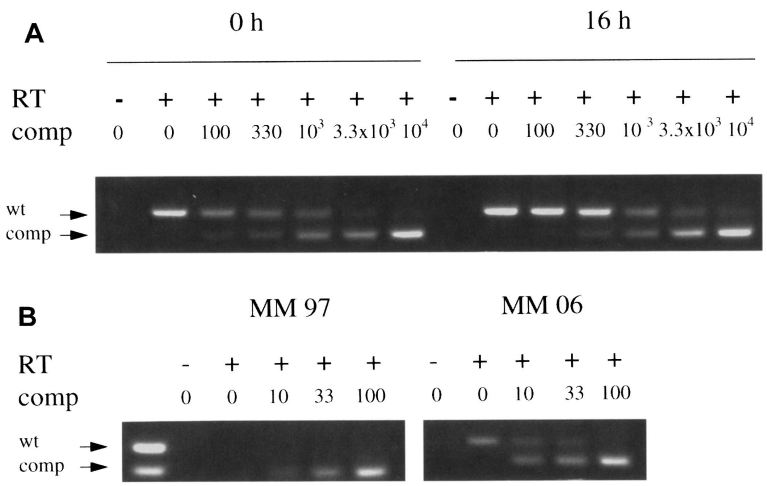

Figure 1.

Quantitative competitive reverse transcription-polymerase chain reaction (QC-RT-PCR) analysis of HIV-1 in breast milk. **A**, Comparison of QC-RT-PCR performed on HIV-1–spiked breast milk incubated for 0 or 16 h at room temperature. Presence of reverse transcriptase and quantity of competitor in each reaction is indicated above lanes. Sizes of wild type and competitor products are indicated by arrows on right. **B**, Representative patient samples MM 97 and MM 06, analyzed by QC-RT-PCR. Lane 1, wild type HIV-1 and competitor size markers.

Figure 2.
Prevalence of HIV-1 RNA in breast milk. Ratios inside bars are no. detected/no. of specimens tested.

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Table 1

Correlates of HIV-1 RNA in breast milk.

	HIV-1 RNA$^+$ ***n*** **= 29**	**HIV-1 RNA$^-$** ***n*** **= 46**	**OR (95% CI)**	**P**
Age (years, median)	23	23		1.0
HIV-related signs *	4% (1/25)	9% (4/43)	0.4 (0.04, 3.9)	.6
HIV-related symptoms †	41% (12/29)	33% (15/45)	1.4 (0.5, 3.7)	.6
CD4 cell count/mm^3 (median)	337	439		.3
CD4 cell count <400/mm^3	56% (13/23)	37% (15/41)	2.3 (0.8, 6.4)	.2
% CD4 cells (median)	22%	24%		.4
CD8 cell count/mm^3 (median)	680	820		.8
% CD8 cells (median)	51%	45%		.4
CD4:CD8 ratio (median)	0.4	0.5		.4
Breast milk cell HIV-1 DNA detected	69% (20/29)	57% (25/44)	1.7 (0.6, 4.5)	.3
Breast milk cell DNA viral load (infected cells/10^4) (median)	313	100		.1
Vitamin A (μ/dL) (median)	29.5	27.5		1.0

NOTE. OR, ratio; CI, confidence interval; +, positive; − negative.

* Rash, zoster, thrush, or oral ulcers.

† Fever; cough >1 month, >10% weight loss, or history of rash or shingles.