

Effect of Perinatal Zidovudine Prophylaxis on the Evolution of Cell-Free HIV-1 RNA in Breast Milk and on Postnatal Transmission

Olivier Manigart,¹ Montcho Crépin,² Valériane Leroy,³ Nicolas Meda,¹ Diane Valéa,¹ Edward N. Janoff,⁵ François Rouet,² Laurence Dequae-Merchadoux,³ François Dabis,³ Christine Rouzioux,⁴ and Philippe Van de Perre,^{1,a} for the Diminution de la Transmission Mere-Enfant Study Group^b

¹Centre MURAZ, 01BP390 Bobo-Dioulasso, Burkina Faso; ²PAC-CI Programme, Abidjan, Côte d'Ivoire; ³Institut National de la Santé et de la Recherche Médicale Unit 593, Université Victor Segalen Bordeaux 2, Bordeaux, and ⁴Laboratory of Virology, Necker-Enfants Malades Hospital, Paris, France; ⁵Mucosal and Vaccine Research Center, Veterans Affairs Medical Center, University of Minnesota School of Medicine, Minneapolis

Perinatal zidovudine (ZDV) prophylaxis decreases rates of perinatal transmission of human immunodeficiency virus type 1 (HIV-1). Its relationship with levels of HIV-1 RNA in breast milk and postnatal transmission in breast-fed African children is unknown. At day 8 after delivery, levels of HIV-1 RNA in breast milk from 28 women who transmitted HIV-1 (Ts) postnatally and from 130 women who did not transmit HIV-1 (NTs) were lower for women receiving ZDV than for women receiving placebo. Levels of HIV-1 RNA in breast milk remained low over time in NTs but increased by 8–16-fold in Ts treated with ZDV from baseline to days 45/90 after delivery. Levels of HIV-1 RNA in breast milk at day 8 after delivery and the increase in levels of HIV-1 RNA in breast milk from day 8 to days 45/90 after delivery were independently associated with postnatal transmission. An increase in the levels of HIV-1 RNA in breast milk from day 8 to 45 after delivery was associated with maternal ZDV prophylaxis. The rebound in levels of HIV-1 RNA in breast milk after discontinuation of maternal antiretrovirals needs to be further explored—it may justify prolonging antiretroviral prophylaxis during the entire breast-feeding period.

In 2001, >800,000 children were infected with HIV-1 worldwide, and nearly 580,000 died of HIV-1–related disease, particularly in resource-poor nations [1]. Breast-feeding accounts for one- to two-thirds of all mother-to-child transmission (MTCT) [2–5], although a significant part of MTCT occurs late in utero or during labor and delivery, with or without prophylaxis. De-

tection of cell-free HIV-1 RNA in breast milk has been associated with MTCT in some [6–9] but not all [10] studies. Breast-fed infant children of HIV-1–infected mothers have been estimated to ingest ~322,000 free viral particles [6] and ~25,000 infected cells [11] daily. Ingestion of 1 L of breast milk from an HIV-1–infected woman exposes infants to a risk of acquiring HIV-1 infection that has been proposed as the equivalent to an unprotected sex act for adults [9]. In 1998, short courses of maternal zidovudine (ZDV) were confirmed to reduce MTCT in non-breast-fed infants in Thailand [12]. Because alternatives to breast-feeding are difficult to apply in much of Africa, in 1999, the short-term efficacy of a similar ZDV regimen was demonstrated among breast-feeding mothers in Côte d'Ivoire and Burkina Faso [13–15]. However, the efficacy of such short-course regimens may diminish over time in breast-fed infants [16]. Whether such short-course antiretroviral regimens reduce levels of HIV-1 RNA in breast milk as well as in plasma and the duration of this reduction need to be determined [8]. Therefore, we compared

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^a Present affiliation: Laboratory of Bacteriology-Virology, CHR Arnaud de Villeneuve, Montpellier, France.

^b Study group members are listed after the text.

Reprints or correspondence: Dr. Philippe Van de Perre, Laboratory of Bacteriology-Virology, CHR Arnaud de Villeneuve, 371 Ave. du Doyen Gaston Giraud, 34395-Montpellier Cedex 5, France (p-van_de_perre@chu-montpellier.fr).

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Table 1. Definition of the timing of transmission of HIV-1.

Period ^a	D1/D8	D45	D90	D180
Perinatal	Positive	Positive	Positive	Positive
Early postnatal	Negative	Negative or positive	Positive	Positive
Late postnatal	Negative	Negative	Negative with positive result later	Positive or negative with positive result later

^a Infection determined by the presence of HIV-1 RNA in plasma from infants, by use of reverse-transcription polymerase chain reaction.

levels of HIV-1 RNA in breast milk over time in both women who transmitted HIV-1 (Ts) and women who did not transmit HIV-1 (NTs) to their infants by breast-feeding, after they had received either short-course perinatal ZDV prophylaxis or placebo [14].

SUBJECTS, MATERIALS, AND METHODS

Study population. The Diminution de la Transmission Mere-Enfant (DITRAME) Agence Nationale de Recherches sur le SIDA (ANRS) 049a trial was conducted in 2 large cities in west Africa: Abidjan, Côte d'Ivoire, and Bobo-Dioulasso, Burkina Faso. The trial protocol was approved by the ANRS ethical review boards, the Ethical Committee of the Côte d'Ivoire National Ministry of Health, the Centre MURAZ Ethical Committee, and the Ministry of Health of Burkina Faso. Written, informed consent was obtained from all participants. The methodology of this randomized, double-blind, placebo-controlled trial has been described elsewhere [14]. In brief, eligible HIV-1-infected pregnant women were randomized at 36–38 weeks of gestation to receive either ZDV (250–300 mg twice daily) or a matching placebo until the beginning of labor, then a single oral dose of 500/600 mg until delivery, and a 7-day postnatal prophylactic treatment of 500/600 mg once daily. No prophylaxis was given to neonates. Adherence to prophylaxis was estimated by regular pill counts and repeated measurements of maternal red cell corpuscular volume. Prophylaxis adherence >80% of maximum drug use was 75% during the prenatal period, 81% during labor, and 83% during the postnatal period [14]. At enrollment, only 2 women fulfilled the clinical definition of AIDS, and 7.7% had CD4⁺ cell counts <200 CD4⁺ cells/mm³. Five women died by 6 months after delivery [14]. The median duration of breast-feeding was 8.1 months in women from Abidjan (interquartile range [IQR], 7–10 months) and 19.4 months in women from Bobo-Dioulasso (IQR, 18–22 months). The postnatal transmission rate estimated at 24 months of age was similar in the 2 populations (10.5 [95% confidence interval {CI}, 4.8–16.2]/100 child-years of breast-feeding in Abidjan and 7.4 [95% CI, 3.2–11.6]/100 child-years of breast-feeding in Bobo-Dioulasso) [17]. The present study is a nested case-control study of Ts and NTs within the DITRAME prospective cohort of HIV-1-infected pregnant women and their live-born children. All women from whom breast milk samples were available at day 8 after delivery (>1 mL) were included. HIV-1 RNA was measured in breast-

milk samples from Ts and randomly selected NTs who were comparable for study site (Abidjan vs. Bobo-Dioulasso) and treatment allocation (ZDV vs. placebo).

Laboratory analysis and definition of timing of transmission. Blood samples from infants were collected at days 1–8, 45, 90, 180, and every 3 months until at least 18 months of age or 3 months after complete cessation of breast-feeding if the child was still breast-feeding at 18 months of age. Samples were stored at –80°C. Cells and plasma samples collected at day 180 after delivery, or an earlier sample when samples from day 180 were not available, were systematically processed by use of polymerase chain reaction (PCR) or by use of reverse-transcription (RT) PCR if the serologic test at 15 months of age was positive (after the loss of HIV-1 maternal antibodies). If the first sample tested was found to be positive, the same test was then applied to all the preceding available samples.

Noncommercial DNA PCR was used in Abidjan, as described elsewhere [14]. In Bobo-Dioulasso, blood samples were analyzed first by noncommercial DNA PCR of cells and commercial quantitative plasma RNA RT-PCR (Amplicor HIV Monitor, version 1.5; Roche Diagnostics Systems), which gave concordant results, and then by plasma RNA RT-PCR only. Serum samples collected between 9 and 15 months of age were screened for HIV-1 and HIV-2 antibodies by use of a commercial ELISA (Genelavia Mixt [Diagnostics Pasteur] or Murex ICE 1-O-2 [Murex Biotech]). A synthetic peptide ELISA (Peptilav 1–2; Diagnostics Pasteur) was used to confirm results on the same sample. A positive antibody test at 15 months of age or later was also considered to be diagnostic for HIV-1 infection. Children who had no sample available for PCR and could not be followed beyond 6 months of age were considered to be of indeterminate HIV-1 status. The diagnosis of pediatric HIV-1 infection was considered on the basis of 1 positive PCR result or by a positive RT-PCR result [18]. Postnatal transmission of HIV-1 (early/late) was diagnosed on the basis of the sequence of results on ≥2 PCRs using blood mononuclear cells from infants, RT-PCR using plasma, or serologic testing at 18 months of age and later (table 1).

Breast-milk samples were obtained by manual expression of either the left or the right breast at the end of breast-feeding at days 8, 45, and 90 after delivery. Samples were centrifuged at 2000 g for 10 min within 5 h of collection, to separate 3 layers: lipids, aqueous supernatant (lactosera or whey), and

cells. The lipid layer was discarded. Whey was stored at -80°C . Cells were washed in PBS 3 times and then were stored as dry pellets at -80°C .

We quantified cell-free HIV-1 in breast-milk whey after a Boom extraction of RNA, which eliminates breast-milk inhibitors of PCR [19, 20] and allows the use of a greater volume of breast milk. In brief, RNA was purified from $800\ \mu\text{L}$ of breast milk in the presence of an internal RNA standard. In conditions of high-salt concentration, nucleic acids bind to silica particles and, after washing, can be eluted. An aliquot was amplified by use of PCR, after RT, with *Thermus thermophilus* HB8 (Tth) DNA polymerase in the presence of dUTP and Uracil-N-glycosylase (AmpErase). The quantitation of HIV-1 RNA copies and the threshold of the reaction is a function of the amplification of a known number of copies of the internal standard that is revealed by ELISA.

We used quantitative competitive RT-PCR (Amplificor 1.5 kit; Roche Molecular Systems), in accordance with the manufacturer's recommendations, and the Nuclisens Kit (Organon Teknika). Maternal levels of HIV-1 RNA in plasma have been reported elsewhere [21].

Statistical analysis. Comparisons of levels of HIV-1 RNA in breast milk between Ts and NTs and between treatment groups were made by use of the Wilcoxon nonparametric test. Median CD4^+ cell counts and maternal age were described and compared by use of the Wilcoxon nonparametric test. Univariate logistic regression analysis estimated the odds ratios (ORs) for postnatal transmission of HIV-1 according to (1) the levels of HIV-1 RNA in breast milk at days 8 and 45 after delivery, (2) the difference between these 2 time points, and (3) the maternal plasma viral load at day 8 after delivery and the median CD4^+ cell count at study entry. In univariate analysis, variables associated with MTCT of HIV-1 with $P < .25$, as well as variables similarly associated with study site and treatment allocation, were included in a multivariate logistic regression stepwise-descendant model. An additional multivariate logistic regression analysis was performed to measure the determinants of an increase in \log_{10} HIV-1 RNA in breast milk between days 8 and 45 after delivery.

RESULTS

Rates of MTCT over time. From September 1995 to February 1998, 421 women were enrolled in the study and were delivered of 401 live children (200 ZDV; 201 placebo). Ten children with indeterminate HIV-1 status and 7 mothers with indeterminate CD4^+ cell counts (including 1 T) were excluded from the DITRAME ANRS 049a analysis. Among the remaining 384 mother-child pairs, 98 mothers (25.5%) transmitted and 286 mothers did not transmit HIV-1 within the first 24 months after delivery. According to our definition (table 1), 61 transmissions (62.2%) occurred pre- or perinatally, and 37 (37.8%)

occurred postnatally. Thus, 9.6% of all women in this cohort (37/384) transmitted HIV-1 postnatally, of which 23 transmissions (62.2%) occurred during the early postnatal period and 14 (37.8%) occurred during the late postnatal period.

Of the 98 Ts, 68 (69.4%; 40 [65.6%] of 61 perinatal Ts and 28 [75.7%] of 37 postnatal Ts) had available breast-milk samples collected at day 8 after delivery. From the 286 NTs, we randomly selected 130 women to be comparable to the 68 Ts, in terms of study site (Abidjan vs. Bobo-Dioulasso; $P = .13$) and treatment allocation (ZDV vs. placebo; $P = .74$), for the substudy of virologic outcomes. The present study focused on postnatal transmission; therefore, we analyzed a selection of 158 women that included 28 postnatal Ts and 130 NTs.

Effect of ZDV prophylaxis on levels of free HIV-1 RNA in breast milk over time. From Ts and NTs selected for our breast-milk studies, 314 breast-milk samples were available for measurements of HIV-1 RNA: 158 collected at day 8 after delivery (28 from Ts and 130 from NTs), 104 collected at day 45 after delivery (22 from Ts and 82 from NTs), and 52 collected at day 90 after delivery (6 from Ts and 46 from NTs). The reasons for missing breast-milk measurements were as follows: 110 insufficient amounts, 37 missing samples, 3 children weaned by day 45 after delivery, 1 woman lost to follow-up at day 45 after delivery, and 5 women lost to follow-up at day 90 after delivery. Levels of HIV-1 RNA in breast milk ranged from below the limit of detection (5 copies/mL) to 21,161 copies/mL. Levels of HIV-1 RNA in breast milk were routinely quite low, with only 8.9% of samples (14/158) at day 8 after delivery, 6.7% of samples (7/104) at day 45 after delivery, and 5.8% of samples (3/52) at day 90 after delivery having >1000 copies/mL.

Among breast-milk samples collected at day 8 after delivery, just as maternal ZDV prophylaxis was interrupted, HIV-1 RNA was more often detectable in breast-milk samples from women who received placebo (54.1% [53/98]; 95% CI, 43.7%–64.2%) than in those from ZDV-treated women (33.3% [20/60]; 95% CI, 21.7%–46.7%; $P = .011$). Levels of HIV-1 RNA in breast milk were lower for ZDV-treated women (median, 25 copies/mL; range, 6–1982 copies/mL) than for women who received placebo (median, 53 copies/mL; range, 5–21,161 copies/mL; $P = .012$). Among breast-milk samples collected at day 45 after delivery, levels of HIV-1 RNA were similar for ZDV-treated women (median, 27 copies/mL; range, 10–6567 copies/mL) and for women who received placebo (median, 25 copies/mL; range, 5–12,537 copies/mL).

Relationship of postnatal transmission of HIV-1 with levels of HIV-1 RNA in breast milk over time. At days 8, 45, and 90 after delivery, HIV-1 RNA was more frequently detected in breast milk from Ts (78.6% [22/28], 81.8% [18/22], and 100% [6/6], respectively) than in breast milk from NTs (39.2% [51/130] [$P \leq .0002$], 46.3% [38/82] [$P = .003$], and 41.3% [19/

Table 2. Univariate analysis of determinants of postnatal mother-to-child transmission of HIV-1.

Variable	Postnatal transmitting mothers (n = 20)	Nontransmitting mothers (n = 60)	OR (95% CI)	P
Abidjan site, %	70.0	70.0	1.0 (0.33–3.02)	1.0
Maternal ZDV prophylaxis, %	50.0	38.3	1.6 (0.58–4.46)	.36
CD4 ⁺ cell count at study entry, median [range], × 10 ⁶ /L	335 [123–1355]	504 [93–1176]	1.30 ^a (1.03–1.64)	.03
Maternal log ₁₀ HIV-1 RNA in plasma at day 8 after delivery, mean [SE]	4.61 [0.57]	3.77 [0.90]	4.51 ^b (1.85–11.00)	.001
Log ₁₀ HIV-1 RNA in breast milk at day 8 after delivery, mean [SE]	2.37 [0.99]	1.75 [0.65]	2.71 ^b (1.37–5.37)	.0042
Difference in log ₁₀ HIV-1 RNA in breast milk between days 8 and 45 after delivery, mean [SE]	+0.11 [1.11]	−0.21 [0.74]	4.87 ^c (2.05–11.58)	.0003

NOTE. CI, confidence interval; OR, odds ratio; ZDV, zidovudine.

^a Per 100 cell decrement in CD4⁺ T cells.

^b Per log₁₀ increment in level of HIV-1 RNA in breast milk.

^c For 1 log increase, adjusted on initial value.

46] [$P = .0087$], respectively). At each time point (days 8, 45, and 90 after delivery), median levels of HIV-1 RNA were significantly higher in breast milk from Ts than in breast milk from NTs: 197 copies/mL (IQR, 49–1684 copies/mL), 413 copies/mL (IQR, 72–796 copies/mL), and 191 copies/mL (IQR, 55–1620 copies/mL), respectively, for Ts, versus 32 copies/mL (IQR, 13–83 copies/mL) ($P < .0001$), 20 copies/mL (IQR, 12–54 copies/mL) ($P < .0001$), and 15 copies/mL (IQR, 9–37 copies/mL) ($P = .0019$), respectively, for NTs.

Among 80 women (20 Ts and 60 NTs [matched controls], the 2 groups being strictly comparable for variables unrelated to selection criteria) for whom complete data sets on relevant potential determinants were available, univariate analysis revealed 4 immunologic and virologic factors associated with postnatal transmission of HIV-1: (1) median maternal CD4⁺ cell count, (2) mean maternal plasma viral load at day 8 after delivery, (3) mean copies of HIV-1 RNA in breast milk at day 8 after delivery, and (4) the mean difference in log₁₀ HIV-1 RNA in breast milk between days 8 and 45 after delivery (table 2). In multivariate analysis, both the mean log₁₀ HIV-1 RNA in breast milk at day 8 after delivery and the mean difference in log₁₀ HIV-1 RNA in breast milk between days 8 and 45 after delivery remained significantly and independently associated

with postnatal transmission of HIV-1 (table 3). Levels of HIV-1 RNA in plasma also showed a strong trend toward significance. However, because only breast milk, and not blood, was transferred from mother to child after birth, this association with plasma levels likely relates to the correlation between levels of virus in plasma and breast milk. Previous maternal ZDV prophylaxis (interrupted at day 7 after delivery) was the only factor associated with an increase of 0.5 log₁₀ HIV-1 RNA in breast milk between days 8 and 45 after delivery, with ORs of 4.67 by univariate analysis ($P = .017$) and 5.66 by multivariate analysis (95% CI, 1.52–21.1; $P = .0098$).

Comparison of levels of HIV-1 RNA in Ts and NTs, according to treatment group. ZDV prophylaxis was associated with significant differences in the levels and kinetics of HIV-1 RNA in breast milk. At day 8 after delivery, the virus was detected more frequently in breast milk from women who received placebo than in breast milk from ZDV-treated women. This difference was no longer observed later during follow-up (table 4). Among Ts at day 8 after delivery, the median level of HIV-1 RNA in breast milk was 56 copies/mL for ZDV-treated women and was significantly higher (1608 copies/mL; $P = .0015$) for women who received placebo. Among Ts at day 45 after delivery, the median level of HIV-1 RNA in breast milk

Table 3. Multivariate analysis of determinants of postnatal mother-to-child transmission of HIV-1.

Variable	OR (95% CI)	P
Abidjan site	0.56 (0.12–2.61)	.46
Maternal ZDV prophylaxis	3.92 (0.79–19.5)	.095
CD4 ⁺ cell count at study entry	1.04 ^a (0.81–1.33)	.77
Maternal log ₁₀ HIV-1 RNA in plasma at day 8 after delivery	2.78 ^b (0.98–7.93)	.056
Log ₁₀ HIV-1 RNA in breast milk at day 8 after delivery	6.24 ^b (1.57–24.84)	.0093
Difference in log ₁₀ HIV-1 RNA in breast milk between days 8 and 45 after delivery	3.77 ^b (1.49–9.56)	.0052

NOTE. CI, confidence interval; OR, odds ratio; ZDV, zidovudine.

^a Per 100 cell decrement in CD4⁺ T cells.

^b Per log₁₀ increment in level of HIV-1 RNA in breast milk.

increased to 471 copies/mL for ZDV-treated women and decreased to 346 copies/mL for women who received placebo ($P = .72$). Among NTs at day 45 after delivery, the median level of HIV-1 RNA in breast milk was ~20 copies/mL for both treatment groups.

DISCUSSION

Postnatal transmission by breast-feeding accounts for up to one-third of all MTCT, but the determinants of infection and the effect of perinatal antiretroviral therapy or prophylaxis on these rates and determinants have not been well characterized. Using clear definitions of postnatal infection and prospectively collected specimens, we generated data that reveal 3 distinct and novel observations: (1) maternal ZDV prophylaxis has a significant effect on levels of HIV-1 RNA in breast milk; (2) ZDV prophylaxis and withdrawal have a significant effect on the temporal evolution of expression of HIV-1 RNA in breast milk, with a viral burst on interruption of treatment; and (3) both baseline levels of HIV-1 RNA in breast milk and the increment in viral RNA in breast milk are significantly associated with postnatal MTCT of HIV-1.

In contrast to previous reports [6–9], with the notable exception of a recent inconclusive report on 6 postnatally infected children [10], our study clearly has separated the independent role that levels of HIV-1 RNA in breast milk play in postnatal transmission from the role that they play in overall MTCT. This distinction is important, since levels in breast milk would have little effect on transmission that occurs before breast-feeding has been initiated. The present study is unique in that it has represented the largest reported cohort of mother-child pairs with confirmed postnatal transmission and has provided direct comparisons of cell-free HIV-1 RNA in breast milk from postnatal Ts and NTs and the relationship of breast-milk HIV-1 RNA to transmission.

Cell-free HIV-1 RNA is a critical viral compartment in breast milk [6–9]. In the present study, levels of HIV-1 RNA in breast milk were measured after a Boom extraction [19]. This extraction procedure increased the sensitivity of viral detection in milk to ~10 copies/mL by eliminating PCR inhibitors and increasing the volume analyzed (in our experience, up to 800 μ L). This increased sensitivity allowed us to recognize that 4 women in our cohort, including 2 who had received perinatal ZDV, transmitted the virus postnatally despite the presence of ≤ 50 HIV-1 copies/mL in their breast milk around the time of transmission (data not shown). That transmission of HIV-1 by breast-feeding can occur despite very low HIV-1 RNA copy numbers in breast milk suggests that the ability of the virus to cross the epithelium and its replicative capacity, rather than just its concentration in breast milk, may be important determinants of transmission. Other factors that may also effect successful transmission in the presence of only very low levels

Table 4. Frequency of detection of HIV-1 RNA in breast milk, by time, treatment group, and transmission.

Treatment group, transmitter status	Day 8	Day 45	Day 90
Placebo			
Yes	14/15 (93.3 ^a)	10/12 (83.3)	4/4 (100.0)
No	39/83 (47.0 ^b)	29/58 (50.0)	16/36 (44.4)
ZDV			
Yes	8/13 (61.5 ^a)	8/10 (80.0)	2/2 (100.0)
No	12/45 (25.5 ^b)	9/24 (37.5)	3/10 (30.0)

NOTE. Data are no. of detections/total no. tested (%). ZDV, zidovudine.

^a $P = .069$, for placebo group vs. ZDV group.

^b $P = .016$, for placebo group versus ZDV group.

of cell-free HIV-1 RNA are the contribution of cell-associated HIV-1 [11, 22], the ability of infected cells to cross the epithelium, and the contribution of HIV-1 RNA and infected cells in the lipid layer of breast milk [23]. These considerations suggest that HIV-1 may be sequestered in different strata of the breast-milk compartment. Considering this compartmentalization, in some women, maternal plasma viral load may well reflect the total breast-milk viral exposure, rather than just the cell-free whey viral exposure.

The present study has demonstrated that the use of maternal ZDV prophylaxis is associated with decreased levels of HIV-1 RNA, not only in plasma, but also in the breast-milk compartment. This observation suggests that maternal antiretroviral treatment or prophylaxis that is prolonged during the breast-feeding period could durably mitigate viral replication in breast milk and therefore substantially reduce the risk of postnatal transmission to infants. An apparently low rate of early postnatal transmission of HIV-1, by the use of the relatively long-acting drug nevirapine, in HIVNET 012 [24] was suggestive of such an effect of antiretrovirals after delivery, and several clinical trials to test this hypothesis are ongoing.

The present study has shown that levels of cell-free HIV-1 RNA in breast milk are closely and independently associated with postnatal MTCT of HIV-1. Such transmission accounted for at least 37.8% of all transmissions in the present study. We did not identify a consistent threshold level of HIV-1 RNA in breast milk below which no postnatal transmission occurred (data not shown), as noted in the 4 cases above. Although higher maternal plasma HIV-1 levels are well recognized as risks for perinatal transmission, we have confirmed this association for postnatal transmission.

Among the most-striking findings of the present study is the effect of ZDV on the evolution of HIV-1 RNA in breast milk over time. Among postnatal Ts who received placebo, the median level of HIV-1 RNA in breast milk decreased steadily over time. This progressive decrease in levels of HIV-1 RNA in breast milk, noted in a previous report [8], may relate to the release of increased amounts of virus by the increased cellular content

observed in human breast milk during earlier phases of lactation [25]. Changes in maternal immune response during the postnatal period and alleviation of gestational Th2-driven immune suppression [26] could also explain this decrease over time, as has been shown for HIV-1 in cervicovaginal secretions [27] and for other infectious agents, such as malaria parasitemia [28]. By sharp contrast, among ZDV-treated postnatal Ts, median levels of HIV-1 RNA in breast milk increased from days 8 to 45/90 after delivery. Multivariate analysis revealed an association between the magnitude of the increase in log₁₀ HIV-1 RNA in breast milk (day 45 minus day 8) and postnatal transmission. Previous maternal ZDV prophylaxis (interrupted at day 7 after delivery) was the only factor significantly and independently associated with this increase in levels of HIV-1 RNA in breast milk between days 8 and 45 after delivery. These data demonstrate the existence of viral rebound in breast milk in some women after ZDV withdrawal, as has been frequently observed in plasma a few weeks after various antiretroviral regimens were stopped [29–31]. The high viremic burst observed in these patients with profound pretreatment immunosuppression and experience with highly active antiretroviral regimens contrasts with the low-level increase in levels of HIV-1 RNA in breast milk observed in the present study. This difference is most probably due to a better immune status of our study participants and to their ZDV monoprophylaxis regimen. In our clinical trial protocol, maternal ZDV prophylaxis was interrupted at day 7 after delivery. Consistent with the temporal evolution of viral rebound observed in levels of plasma by other investigators [29–31], the increase in HIV-1 RNA was observed in breast-milk samples collected at day 45 after delivery from ZDV-treated Ts, after they had discontinued therapy. In the present study, the observed minimal rebound of viral load in breast milk contrasts with the high rebound observed in patients with profound pretreatment immunosuppression.

Recent data have shown that, by 24 months of age, some of the benefits of a short-course perinatal ZDV regimen have been lost due to transmission by breast-feeding [16]. No increase in postnatal transmission was observed when comparing infant children of ZDV-treated women with those of untreated women, all of whom were from the DITRAME cohort [15, 16, 17]. In the present study, which followed mother-child pairs from the DITRAME cohort, although an increased level of HIV-1 RNA in breast milk was independently associated with postnatal transmission and although this viral rebound was associated with previous ZDV prophylaxis, maternal ZDV prophylaxis was not associated with an additional risk of postnatal transmission [17]. However, it can be anticipated that interruption of more-suppressive prophylactic drug regimens or of highly active antiretroviral therapy during lactation may substantially increase the risk of transmission by breast-feeding [32]. Thus, strategies that limit postnatal breast-feeding or maintain effective anti-

retroviral therapy in the mother and/or infant for the duration of breast-feeding may have a significant effect on postnatal transmission and the overall rate of MTCT of HIV-1. The results of the present study, which has shown an effect of postnatal maternal ZDV prophylaxis on early levels of HIV-1 RNA in breast milk, suggest that longer-term maternal antiretroviral prophylaxis may be an option to prevent transmission by breast-feeding during the early postnatal period, when levels of HIV-1 RNA in breast milk are the highest.

DIMINUTION DE LA TRANSMISSION MERE-ENFANT (DITRAME) STUDY GROUP

Coordination: Institut National de la Santé et de la Recherche Médicale (INSERM) Unit 330, Université Victor Segalen Bordeaux 2, Bordeaux, France (F. Dabis).

Principal investigators: Centre Hospitalier Universitaire de Yopougon, Abidjan, Côte d'Ivoire (C. Welfens-Ekra); and Maternité Cochin Port-Royal, Paris, France (L. Mandelbrot).

Abidjan Center (Côte d'Ivoire): Centre de Diagnostic et de Recherche sur le Sida et les Maladies Opportunistes, Centre Hospitalier Universitaire de Treichville (D. Bonard, P. Combe, N. Elenga, R. Likikouet, C. Montcho, V. Noba, F. Sylla-Koko, I. Viho, and B. You); Centre Hospitalier Universitaire de Yopougon (R. Camara, M. Dosso, and M. Timité); DITRAME Project (G. Gourvellec and R. Ramon); Office de la Recherche Scientifique et Technique Outre-Mer Petit Bassam (P. Msellati [local coordinator]); and the Health Centers of Anonkouakoute, Ouassakara, Yopougon-Attie, and Yopougon.

Bobo-Dioulasso Center (Burkina Faso): Centre MURAZ/Organisation de Coordination et de Coopération pour la lutte contre les Grandes Endémies (OCCGE) (M. Cartoux, A. M. Cassel-Beraud, L. Gautier-Charpentier, O. Ky-Zerbo, O. Mangart, N. Meda [local coordinator], A. Ouangré, O. Sanou, A. Simonon, I. Sombié, S. Tiendrebeogo, and S. Yaro); Centre Hospitalier National Sourô Sanou (A. Bazié, B. Dao, B. Nacro, and F. Tall); the Health Centers of Accart-Ville, Farakan; and Social Security.

Data management: INSERM Unit 330, Bordeaux (L. Dequae-Merchadou).

Methodology: INSERM Unit 330, Bordeaux (V. Leroy and R. Salamon); Centre MURAZ/OCCGE (P. Van de Perre); and Laboratoire de Virologie, Hôpital Necker-Enfants Malades, Paris, France (C. Rouzioux).

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References

1. Joint United Nations Programme on HIV/AIDS (UNAIDS). Report on the global HIV/AIDS epidemic [UNAIDS/02.26E]. Geneva: UNAIDS, 2002.
2. Dunn DT, Newell ML, Ades AE, et al. Risk of human immunodeficiency virus type 1 transmission through breastfeeding. *Lancet* 1992; 340:585–8.
3. Simonon A, Lepage P, Karita E, et al. An assessment of the timing of mother-to-child transmission of human immunodeficiency virus type 1 by means of polymerase chain reaction. *J Acquir Immune Defic Syndr* 1994; 7:952–7.
4. Ekpini ER, Wiktor SZ, Satten GA, et al. Late postnatal mother-to-child transmission of HIV-1 in Abidjan, Côte d'Ivoire. *Lancet* 1997; 349:1054–9.
5. Nduati R, John G, Mbori-Ngacha D, et al. Effect of breastfeeding and formula feeding on transmission of HIV-1: a randomized clinical trial. *JAMA* 2000; 283:1167–74.
6. Semba RD, Kumwenda N, Hoover DR, et al. Human immunodeficiency virus load in breast milk, mastitis, and mother-to-child transmission of human immunodeficiency virus type 1. *J Infect Dis* 1999; 180:93–8.
7. Pillay K, Coutoudis A, York D, et al. Cell-free virus in breast milk of HIV-1 seropositive women. *J Acquir Immune Defic Syndr* 2000; 24:330–6.
8. Rousseau CM, Nduati RW, Richardson BA, et al. Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and its relationship to infant infection and maternal disease. *J Infect Dis* 2003; 187:741–7.
9. Richardson BA, John-Stewart GC, Hughues JP, et al. Breast-milk infectivity in human immunodeficiency virus type 1-infected mothers. *J Infect Dis* 2003; 187:736–40.
10. Willumsen JF, Filteau SM, Coutoudis A, et al. Breastmilk RNA viral load in HIV-infected South African women: effects of subclinical mastitis and infant feeding. *AIDS* 2003; 17:407–14.
11. 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.
12. Shaffer N, Chaoowong R, Mock PA, et al. Randomized placebo-controlled trial of short-course antenatal zidovudine to reduce perinatal HIV transmission, Bangkok, Thailand. *Lancet* 1999; 353:773–80.
13. Wiktor SZ, Ekpini ER, Karon JM, et al. Randomized clinical trial of a short course of oral zidovudine to prevent mother-to-child transmission of HIV-1 in Abidjan, Côte d'Ivoire. *Lancet* 1999; 353:781–5.
14. Dabis F, Mselatti P, Meda N, et al. Six-month efficacy, tolerance and acceptability of a short regimen of oral zidovudine to reduce vertical transmission of HIV in breastfed children in Côte d'Ivoire and Burkina Faso: a double-blind placebo-controlled multicenter trial. *Lancet* 1999; 353:786–92.
15. DITRAME Study Group. 15-month efficacy of a maternal short regimen of oral zidovudine to reduce vertical transmission of HIV in African breastfed children. *Lancet* 1999; 354:2050–1.
16. Leroy V, Karon JM, Alioum A, et al. Twenty-four month efficacy of a maternal short-course zidovudine regimen to prevent mother-to-child transmission of HIV-1 in West Africa. *AIDS* 2002; 16:631–41.
17. Leroy V, Karon JM, Alioum A, et al. Postnatal transmission of HIV-1 after a maternal short-course zidovudine peripartum regimen in West Africa. *AIDS* 2003; 17:1493–1501.
18. Dabis F, Mselatti P, Newell ML, et al. Methodology of intervention trials to reduce mother-to-child transmission of HIV-1 with special reference to developing countries. *AIDS* 1995; 9 (Suppl A):S67–74.
19. Sheppard RN, Schock J, Robertson K, et al. Quantitation of human immunodeficiency virus type 1 RNA in different biological compartments. *J Clin Microbiol* 2000; 38:1414–8.
20. Boom R, Sol CJA, Saliman MM, et al. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990; 28:495–503.
21. Leroy V, Montcho C, Manigart O, et al. Maternal plasma viral load, zidovudine and mother-to-child transmission of HIV-1 in Africa: DITRAME ANRS 049a trial. *AIDS* 2001; 15:517–22.
22. Van de Perre P, Simonon A, Hitimana DG, et al. Infective and anti-infective properties of breast milk from HIV-1 infected mothers. *Lancet* 1993; 341:914–8.
23. Hoffman IF, Martinson FE, Stewart PW, et al. Human immunodeficiency virus type 1 RNA in breast-milk components. *J Infect Dis* 2003; 188:1209–12.
24. Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomized trial. *Lancet* 1999; 354:795–802.
25. Goldman AS, Garza CG, Nichols BL, Goldman RM. Immunologic factors in human milk during the first year of lactation. *J Pediatr* 1982; 100:563–7.
26. Luppi P, Haluszczak C, Betters D, Richard CA, Trucco M, DeLoia JA. Monocytes are progressively activated in the circulation of pregnant women. *J Leukoc Biol* 2002; 72:874–84.
27. Henin Y, Mandelbrot L, Henrion R, Pradinaud R, Coulaud JP, Montagnier L. Virus excretion in the cervicovaginal secretions of pregnant and nonpregnant HIV-infected women. *J Acquir Immune Defic Syndr* 1993; 6:72–5.
28. Mvondo JL, James MA, Campbell CC. Malaria and pregnancy in Cameroonian women: effect of pregnancy on *Plasmodium falciparum* parasitemia and response to chloroquine. *Trop Med Parasitol* 1992; 43:1–5.
29. de Jong MD, de Boer RJ, de Wolf F, et al. Transient overshoot of HIV-1 viraemia after early discontinuation of antiretroviral treatment: role of target cell availability. *AIDS* 1997; 11:F79–84.
30. Colven R, Harrington RD, Spach DH, et al. Retroviral rebound syndrome after cessation of suppressive antiretroviral therapy in three patients with chronic HIV infection. *Ann Intern Med* 2000; 133:430–4.
31. Ioannidis JP, Havlir DV, Tebas P, et al. Dynamics of HIV-1 viral load rebound among patients with previous suppression of viral replication. *AIDS* 2000; 14:1481–8.
32. Van de Perre P, Manigart O, Meda N. Long-term reduction of HIV transmission from mother to breastfed child by antiretrovirals: are more drugs better than less? *AIDS* 2001; 15:658–9.