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## Breast-feeding and Transmission of HIV-1

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### Abstract

Breast-feeding substantially increases the risk of HIV-1 transmission from mother to child, and although peripartum antiretroviral therapy prophylaxis significantly decreases the risk of mother-to-child transmission around the time of delivery, this approach does not affect breast-feeding transmission. Increased maternal RNA viral load in plasma and breast milk is strongly associated with increased risk of transmission through breast-feeding, as is breast health, and it has been suggested that exclusive breast-feeding could be associated with lower rates of breast-feeding transmission than mixed feeding of both breast- and other milk or feeds. Transmission through breast-feeding can take place at any point during lactation, and the cumulative probability of acquisition of infection increases with duration of breast-feeding. HIV-1 has been detected in breast milk in cell-free and cellular compartments; infant gut mucosal surfaces are the most likely site at which transmission occurs. Innate and acquired immune factors may act most effectively in combination to prevent primary HIV-1 infection by breast milk.

### Keywords

breastfeeding; mother-to-child transmission; postnatal transmission; risk factors; mechanisms

## TRANSMISSION OF HIV THROUGH BREAST-FEEDING

Breast-feeding substantially increases the risk of HIV-1 transmission from mother to child; the rate of breast-feeding transmission is estimated to be at least 16% and prolonged breast-feeding nearly doubles the overall infant HIV-1 infection rate.<sup>1</sup> Although peripartum antiretroviral therapy prophylaxis has been shown to significantly decrease the risk of mother-to-child transmission (MTCT) around the time of delivery, this intervention approach does not provide protection from breast-feeding transmission.<sup>2</sup> For example, short-course peripartum zidovudine resulted in a 50% reduction in infant HIV-1 infection rates in a nonbreast-feeding population in Bangkok,<sup>3</sup> but with a similar regimen in breast-feeding mothers in 2 trials in West Africa the efficacy assessed at 3 months of age was only about 37%.<sup>2,4,5</sup> In a pooled analysis of the West African studies using short-course zidovudine prophylaxis, the rate of MTCT at 24 months in the group randomized to peripartum zidovudine was about 8% lower than in the placebo group and the relative efficacy of the intervention was reduced to about 28% at 2 years from 37% at 3 months.<sup>2</sup> The decreased efficacy of the zidovudine peripartum regimen in preventing perinatal transmission in the West African cohorts at 2 years of age was primarily due to postnatal transmission through breast-feeding. However, although HIV-1 transmission continues after cessation of peripartum antiretroviral therapy, there is no evidence to suggest that administration of antiretroviral therapy in this early period is associated with an increased rate of breast-feeding transmission due to viral rebound after cessation of antiretrovirals.

## FACTORS ASSOCIATED WITH THE RISK OF TRANSMISSION THROUGH BREAST-FEEDING

The rate of postnatal transmission through breast-feeding is associated with clinical, immunologic, and virologic factors (Table 1). Increased maternal RNA viral load in plasma and breast milk is strongly associated with increased risk of transmission through breast-feeding. High levels of virus in plasma, and probably also in breast milk, are seen in primary HIV infection, when the rate of postnatal transmission has been estimated to be nearly 30%.<sup>6</sup> In a study in Kenya, the relative risk of MTCT was increased about 6-fold during primary infection of the mother.<sup>7</sup> Breast milk HIV-1 RNA levels correlate with systemic viral load<sup>8,9</sup> and are likely to be associated with risk of breast milk HIV-1 transmission.<sup>9,10</sup> Maternal immunosuppression defined by low CD4<sup>+</sup> cell count, although strongly correlated with plasma RNA viral load, is an independent risk factor for breast-feeding transmission risk. In the West African short-course zidovudine trials, the cumulative postnatal transmission risk of HIV at 2 years of age was higher among mothers with CD4<sup>+</sup> cell counts of <500 cells/mL than among those with CD4<sup>+</sup> cell counts of ≥500 cells/mL; within the zidovudine arm the rates were 22 and 2%, respectively.<sup>11</sup>

In a recent study from Nairobi, breast milk RNA levels were assessed in serial samples from 275 women up to 2 years after delivery.<sup>12</sup> Higher maternal plasma virus load and lower CD4<sup>+</sup> cell counts and rates of detection of HIV-1 DNA in genital secretions were significantly associated with higher breast milk HIV-1 RNA levels. Median RNA load in colostrum and early milk was higher than in mature milk collected >14 days after delivery. Breast milk RNA load was significantly associated with transmission through breast-feeding. In the same setting, the estimated probability of transmission was 0.00064 per liter of breast milk ingested and 0.00028 per day of breast-feeding.<sup>14</sup>

Breast health has also been associated with the risk of transmission through breast-feeding, with breast pathologies such as clinical and subclinical mastitis, nipple bleeding, abscess, or fissures relatively common in both the general and HIV-infected population. In Kenya, clinical mastitis was detected in 7–11% of HIV-1-infected mothers,<sup>7,12</sup> while the estimated

prevalence of subclinical mastitis, defined by elevated levels of sodium or potassium, in studies of HIV-infected mothers 6–14 weeks after delivery elsewhere ranged from 11–16%.<sup>9,10,13</sup> Nipple lesions have been detected in 10–13% of mothers in several cohort studies.<sup>7,12,15</sup> Breast abscess on clinical examination was detected at least once in 12% of breast-feeding mothers over a 2-year follow-up period in one study and in 3% of mothers in another study with shorter maternal follow-up.<sup>12,15</sup> Mastitis, abscess, and nipple lesions have all been associated with a relative increase in the risk of transmission through breast-feeding.<sup>7,9,10,12,15</sup> Oral candidiasis in the child has also been associated with MTCT, but the direction of causality is difficult to establish since early HIV-1 infection may be associated with thrush.<sup>7,15</sup>

Maternal nutritional status may influence risk of transmission overall, as well as breast-feeding transmission. In a recent paper, results were presented from an additional analysis of data from a randomized trial investigating the effect of multivitamins and vitamin A on the risk of transmission.<sup>16</sup> Multivitamins excluding A had no effect on the overall risk of transmission, but vitamin A alone was associated with a slight increase in transmission overall, and increased postnatal transmission. Multivitamins were associated with a nonsignificant reduction in breast-feeding transmission and mortality in the first 2 years of life. These results need to be confirmed.

The mode of infant feeding may be of particular relevance at a population level in determining rates of breast-feeding transmission. Although breast-feeding is recognized to be the optimum for maternal and child health, exclusive breast-feeding for up to 6 months is rare.<sup>17</sup> It has been suggested that exclusive breast-feeding could be associated with lower rates of breast-feeding transmission than mixed feeding of both breast- and other milk or feeds. In a study in Durban, South Africa, women self-selected to breast-feed or formula feed after being counseled.<sup>18</sup> Children who had received both breast milk and other feeds were more likely to be infected by 15 months of age (36%) than those who were exclusively breast-fed for at least 3 months (25%) or those who had been exclusively formula fed (19%), but the only statistically significant difference in transmission risk was between ever breast-fed versus never breast-fed children.<sup>18</sup> It has been hypothesized that an association between increased risk of transmission and mixed feeding early in life could be associated with increased gut permeability or levels of local inflammation, but neither hypothesis has been confirmed.<sup>19</sup> Another potential mechanism is that mixed feeding may be associated with suboptimal breast-feeding practice and with subclinical mastitis,<sup>13</sup> or the result could have been due to confounding between mixed feeding and susceptibility to infection.

Transmission of HIV through breast milk can take place at any point during lactation, and the cumulative probability of acquisition of infection increases with duration of breast-feeding.<sup>2,20–22</sup> The persistence of maternal antibodies and the presence of a “window period” during which infection is undetectable using currently available technology make it difficult to determine whether an infant has been infected during delivery (intrapartum) or through early breast-feeding in the period immediately following birth. To avoid this problem, the risk of MTCT associated with breast-feeding has been estimated based on the group of young infants born to infected mothers with negative HIV-1 diagnostic assay results after 1 month of age. These children are then followed until after they cease breast-feeding to determine their rate of acquisition of HIV infection through breast-feeding (defined as late postnatal transmission or LPT). Among 139 Tanzanian infants born to HIV-1-infected mothers and a negative HIV-1 DNA polymerase chain reaction (PCR) test at 6 months of age, 8 infants (5.8%) became infected at 1 year or later, yielding an estimated incidence of 6.2 per 100 child-years of breast-feeding between 6–27 months.<sup>23</sup> In a Malawian study 47 (8.2%) of 572 infants at risk became infected after 6 weeks of age, with a cumulative risk of 10.3% at 24 months and an incidence of 6.9 per 100 child-years.<sup>20</sup> In a

study in Côte d'Ivoire, LPT was defined as having occurred if HIV infection was detected after a negative HIV-1 PCR at 3–6 months of age. Among 45 children at risk, 4 became infected, with an estimated LPT risk of 12% (95% CI, 3–23%) and an estimated incidence of 9.2 per 100 child-years.<sup>21</sup> Results from a meta-analysis of individual patient data from 4 cohorts from industrialized and 4 from developing countries indicate an overall risk of acquisition of infection after 3 months of age of about 3 per 100 child-years of breast-feeding.<sup>21</sup> In another meta-analysis of published data, the risk of postnatal transmission after 3–6 months of age was in the order of 4%.<sup>12</sup> Thus, the rate of LPT by breast-feeding is substantial and persists for the duration of breast-feeding.

Results from these studies are difficult to compare because of differences in defining LPT and variable follow-up schedules. To overcome these difficulties a comprehensive meta-analysis of LPT was initiated using data from 9 randomized placebo-controlled clinical trials in sub-Saharan Africa (South Africa, Kenya, Côte d'Ivoire, Burkina Faso, Tanzania, and Uganda) with stringent case definitions and inclusion/exclusion criteria.<sup>24</sup> Early transmission was defined by a positive HIV test before 4 weeks, and LPT by a negative diagnostic test at or after 4 weeks of age, followed by a positive test result. A child was presumed to be no longer at risk for becoming infected once breast-feeding had ceased, and the methodology used adequately dealt with such censoring. A total of 4085 children were breast-fed and had HIV testing performed. The overall rate of transmission was 23%, and of the 993 infected children, the timing of acquisition was early in 314 (31%), late in 231 (23%), and unknown in 454 (45%). Therefore, the contribution of LPT to overall transmission risk was estimated to be at least 23% but could be as high as 42%. Results show that children are at continued risk throughout the breast-feeding period and that the risk is approximately constant as long as breast-feeding continues.<sup>24</sup> The cumulative probability of acquiring HIV infection after age 4 weeks of age was about 1.5% at 3 months, 4% at 6 months, 7% at 12 months, and 9.5% at 18 months. The risk of LPT was lower for girls than for boys (relative risk [RR] = 0.6; 95% CI, 0.4–0.9) and for children whose mothers had higher CD4 counts at the time of delivery (CD4 < 200, RR = 8.0; CD4 299–499, RR = 3.7). The gender difference was not explained by differences in duration of breast-feeding.

## DRUG RESISTANCE

Worldwide, short-course antiretroviral regimens, typically including zidovudine, nevirapine, or lamivudine, are rapidly being introduced for prevention of infant HIV-1. This widespread use of short-term dual or monotherapy has the potential to select antiretroviral resistance mutations in populations with programs for the prevention of MTCT. Common mutations that are seen for zidovudine include M41L, D67N, K70R, L210W, and T215W/F; for lamivudine M184V; and for nevirapine K103N, K101N, Y181C/I, Y188C, and G190A. Several mutations can cause cross-resistance. T215W/F confers resistance to zidovudine and stavudine; the M184V mutation confers resistance to didanosine, zalcitabine, abacavir, lamivudine, zidovudine, stavudine, and tenofovir<sup>25–28</sup>; and the K103N, Y181C/I, Y188C, and G190A mutations confer resistance to nevirapine and other nonnucleoside reverse transcriptase inhibitors. The rates of maternal genotypic mutation (M184V/L) when lamivudine is given in combination with zidovudine are about 40% after 8 weeks of treatment.<sup>29</sup>

Previous experience with antiretrovirals is associated with a higher risk of zidovudine mutations. Among treatment-naïve women in the US, West African, and Thai cohorts, baseline resistance to T215Y was minimal, whereas among antiretroviral-experienced women, T215Y mutation rates were 12–25%.<sup>30</sup> No conclusive data support the conclusion that the presence of such resistance is associated with increased transmission risk among

women receiving zidovudine for prevention of transmission.<sup>31</sup> Some but not all studies have noted an increased risk among drug-experienced women with this mutation. At baseline, 2.3% of treatment-naïve women have nevirapine resistance. Single-dose nevirapine is associated with induction of nevirapine resistance in women with unsuppressed virus. For mothers receiving single-dose nevirapine, the risk of developing transient detectable genotypic nevirapine-resistant virus (usually K103N or Y181C) among women with replicating virus was between 15–19% based on data from several trials.<sup>30,32</sup> After nevirapine is stopped, resistance mutations decrease to undetectable levels in the absence of drug selection pressure. In the HIVNET 012,<sup>32</sup> this mutation was no longer detectable when reassessed at 12 months; and in the Thai Centers for Disease Control (CDC) study<sup>30</sup> it was not detectable by 4 months postpartum. Results from the first of these studies also suggest that women with subtype D are more likely to develop nevirapine resistance.<sup>32</sup> The long-term implications of nevirapine-related mutant selection following single-dose therapy are as yet undefined, but concerns arise about the drug's efficacy during subsequent pregnancies and future treatment options.<sup>31</sup>

It is anticipated that with use of highly active antiretroviral therapy (HAART) regimens capable of achieving nondetectable viral loads, the risk of development of resistance for either lamivudine or nevirapine should be much lower. Genotypic resistance to zidovudine usually develops more slowly and is dependent on a series of mutations occurring; high-level resistance is usually not detected until at least several months after initiation of therapy. With the short-course ZDV regimens, no maternal genotypic resistance has been reportable from the CDC Abidjan short-course trial<sup>33</sup> or in the CDC Thai zidovudine plus single-dose nevirapine study.<sup>30</sup>

Among infected infants, resistance to zidovudine has been reported in 25% of infants in PACTG 239 who received 6 weeks of zidovudine prophylaxis and whose mothers also received zidovudine prenatally<sup>31</sup> and in 10% of infants in the CDC Thai study<sup>30</sup> with short-course maternal zidovudine, 4 weeks of infant zidovudine, and single-dose nevirapine. However, in the latter study, 20% of infected infants had evidence of a nevirapine-resistant virus; and in the HIVNET 012 study, 40% had detectable resistant virus.<sup>32</sup>

To further understand and to prevent HIV transmission through breast-feeding, it is important to determine whether there is compartmentalization of resistance in breast milk either due to separate viral origin or inadequate drug levels. Pharmacokinetic studies on antiretroviral diffusion in breast milk are also needed. As regimens are developed for antiretroviral use during lactation, viral resistance in breast milk will become increasingly important. In the planned maternal HAART studies (in which HIV-infected mothers receive HAART while breast-feeding their children), it is likely that children will be exposed to subtherapeutic levels of antiretrovirals through breast milk and some infants will become HIV infected. It is not known whether this will lead to the development of HIV resistance to antiretroviral drugs among children who become infected and whether this would have any impact on their future HIV treatment. HIV-infected children will therefore require long-term follow-up to assess this issue.

## VIROLOGY

HIV-1 has been detected in breast milk in cell-free and cellular compartments. Most studies to date have used DNA or RNA PCR assays to evaluate breast milk for HIV-1. In an early study from Kenya, breast milk HIV-1 RNA was detected in 39% of 75 specimens.<sup>8</sup> In this study, viral levels in breast milk were lower (~1 log lower) than in plasma. However, there were some cases that suggested compartmentalization of virus to breast milk with higher levels in breast milk than plasma. Most studies of breast milk HIV-1 have focused on the

cell-free supernatant and cellular compartment and have excluded the lipid fraction. These data may thus underestimate the amount of HIV-1 in breast milk; in an unpublished study by Becquart et al. (personal communication, 2002), there is evidence HIV-1 RNA may be entrapped in the lipid compartment.

The origin of HIV-1 in the breast milk is poorly defined. Studies have evaluated cell-free virions and latent nonproductively infected cells, but not productive HIV-1 infective cells. It will be important to study the latter to determine how much viral replication occurs in the breast milk compartment. Cells, including macrophages and lymphocytes, and cell-free virus may migrate from the systemic compartment to breast milk. It is not known whether ductal and alveolar cells within the mammary compartment contribute to local viral replication. In a recently published study, viral variants in blood and breast milk were found to be distinct, with some major variants in breast milk not detected in blood. This suggests that some virus in breast milk originates, or replicates independently, in the mammary compartment.<sup>34</sup>

Following ingestion of HIV-1-infected breast milk, infant gut mucosal surfaces are the most likely site at which transmission occurs. Cell-free or cellular HIV-1 may penetrate to the submucosa in the setting of mucosal breaches or lesions, via transcytosis through M cells or enterocytes expressing galactosyl ceramide (Gal Cer) or Fc receptors. In vitro models suggest that secretory IgA or IgM may inhibit transcytosis of HIV-1 across enterocytes.<sup>35,36</sup> Thus, breast milk HIV-1 immunoglobulins may play a role in protection from transmission. Primary human enterocytes express CCR5, but not CXCR4, on their cell surface and thus may play a role in selection of viruses that can be transmitted to the infant.<sup>37</sup> This transfer is not inhibited by fusion inhibitors such as T20. Tonsils may also be a portal of entry for HIV-1 in breast milk transmission. Tonsils include M cells in proximity to lymphocytes and dendritic cells, and tonsillar M cells are capable of HIV-1 replication.<sup>38,39</sup> Oral transmission has been demonstrated in a macaque simian immunodeficiency model, in which nontraumatic inoculation with cell-free virus resulted in infection of lymphocytes close to M cells in the tonsillar crypts, without infection of epithelial cells.<sup>40</sup>

Many questions remain regarding breast milk HIV-1 virology. It will be important to determine the origin of HIV-1 in breast milk and whether local viral production in breast milk influences transmission, whether viral selection or resistance in breast milk has an impact on transmission, and what the portal of entry to the infants is. Moreover, it remains unclear which cell types are infected with HIV-1 in vivo and whether cell-free or cell-associated virus is more closely related to rates of postnatal transmission. Answers to these questions may promote rational design of interventions to minimize the risk of HIV-1 transmission through breast-feeding.

## IMMUNOLOGY

In general, mucosal immunity exists to protect the host from infections and harmful foreign antigens. The transmission site in the infant is likely to be either the upper intestine or the tonsils, or even the oral or buccal mucosae. Properties of these sites may influence the likelihood of transmission at the site as well as the type of virus transmitted. The characteristics of enterocytes have been better characterized than those of tonsillar cells. In the upper intestine, there is a single-cell epithelial layer with columnar cells. As noted, intestinal epithelial cells express galactosyl ceramide as HIV receptors, rather than CD4, as well as CCR5 coreceptors.<sup>41,42</sup> Tonsils have a multi-cell layer of stratified squamous epithelium. Although the receptors and coreceptors of the cells in tonsils have not been defined, uptake of a CXCR4-tropic (X4) HIV-1 isolate, IIIB, by primary gingival epithelium has been shown in vitro.<sup>43</sup> CD4<sup>+</sup> T lymphocytes in tonsils may be less consistently activated than those in the gastrointestinal tract.

Many innate mechanisms may contribute to prevention of HIV-1 transmission. Perhaps the most well-characterized innate factor that has been considered for protection against mucosal transmission of HIV-1, including by breast milk, is secretory leukocyte protease inhibitor (SLPI). This low-molecular-weight protein secreted by polymorphonuclear and epithelial cells has HIV-1 inhibitory activity in vitro. SLPI acts on the target cell rather than the virus and in vitro inhibits internalization of HIV-1, but not binding, at concentrations >100 ng/mL.<sup>44</sup> In a study of uninfected control mothers, saliva collected from mothers postpartum had the ability to neutralize HIV-1 during the postpartum period, as did breast milk in a time-dependent manner (Janoff, et al., manuscript submitted). Clinically, increased SLPI levels in maternal cervicovaginal fluids have been associated with protection from MTCT of HIV-1 in South Africa.<sup>45</sup> Infant salivary SLPI has also been associated with decreased risk of late breast milk HIV-1 transmission.<sup>46</sup> However, levels of SLPI in breast milk were not associated with decreased transmission in a study from Cameroon.<sup>41</sup> Further controlled studies are needed to confirm the role of maternal and infant SLPI in transmission, either alone or in combination with other innate and specific immune factors. Indeed, many other innate factors are present at mucosal sites and each factor may act in isolation, synergistically, or even antagonistically with others. Thus, detailed studies of the mucosal milieu will be useful to define the interplay between innate protective factors and the relationship between innate and adaptive immunity.

HIV-1-specific antibodies are present in breast milk, with higher levels of HIV-1-specific IgG than IgA.<sup>47</sup> These antibodies are directed to gp120, the V3 loop, and gp41. In vitro studies have shown that these HIV-1-specific antibodies present in blood and mucosal fluids can inhibit transcytosis of the virus.<sup>48,49</sup> Moreover, CD8<sup>+</sup> cytotoxic T lymphocytes that recognize a range of HIV-1 antigens have recently been identified in milk from infected women in the United States and Zambia.<sup>50</sup> However, results from clinical studies have not yet confirmed that postnatal transmission is inhibited by antibodies to HIV-1 or related receptors in breast milk. Current research is directed to determine whether mucosal vaccines may stimulate a protective humoral response. This approach may be particularly promising in light of recent animal studies demonstrating protection against mucosal challenge with HIV-1 by passive immunization with monoclonal antibodies given parenterally and locally.<sup>51</sup> Innate and acquired immune factors may act most effectively in combination to prevent primary HIV-1 infection by breast milk.

Overall, the majority of breast-feeding infants of HIV-1-infected mothers do not acquire HIV infection. Levels of HIV-1 in breast milk are low compared with those in plasma<sup>10,13</sup>; HIV-1 is difficult to culture from breast milk, perhaps because of these low viral levels or due to the effects of innate or specific anti-HIV-1 factors in milk. Vaccine strategies for prevention of postnatal transmission of HIV should take into account these unique features of breast milk HIV-1 transmission. A combined approach that integrates innate, adaptive humoral, and cellular immunity, and that enhances existing maternal and infant defenses, would be ideal. A mucosal vaccine for both mother and infant may most effectively accomplish this goal.

## SUMMARY

Given the need to avoid HIV transmission to infants through breast-feeding while at the same time avoiding the increased risk of other morbidity and mortality associated with formula feeding, current United Nations recommendations state that “When replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breast-feeding by HIV-infected mothers is recommended. Otherwise, exclusive breast-feeding is recommended during the first months of life and should then be discontinued as soon as it is feasible.” HIV-1-infected women should thus make a decision regarding formula feeding on

the basis of safety, feasibility, and affordability of formula.<sup>52</sup> For women choosing to breast-feed, exclusive breast-feeding is recommended for 4 months, with cessation as rapidly as possible thereafter. The United Nations targets as a priority to decrease by 20% the number of newly HIV infected children worldwide by 2005 and 50% by 2010.<sup>53</sup> To accomplish these ambitious and important goals, further interventions to decrease HIV-1 transmission through breast-feeding remain a high priority. In addition to avoiding breast-feeding where possible or exclusive breast-feeding with shortened duration, it is also important to optimize breast-feeding technique and to prevent breast pathology. Decreasing breast milk viral load with antiretrovirals throughout lactation is likely to decrease infectivity during breast-feeding. Because of the increased likelihood of breastmilk transmission among immunosuppressed women, this group may be a particularly important target for antiretroviral therapy during lactation.

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**TABLE 1****Factors Possibly Associated with Transmission Through Breastfeeding**

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Maternal
RNA viral load in milk
RNA viral load in plasma
Clinical or immunologic (CD4 count) disease progression
Breast health (subclinical or clinical mastitis, abscess, cracked nipples) (indirect)
Local immune factors in breast milk
Infant
Duration of breast-feeding
Mode of breast-feeding
Morbidity leading to less vigorous suckling, milk stasis, and increased leakage of virus across milk ducts

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