SUPPLEMENT ARTICLE

The Role of Cell-Associated Virus in Mother-to-Child HIV Transmission

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Mother-to-child transmission (MTCT) of human immunodeficiency virus (HIV) continues to contribute to the global burden of disease despite great advances in antiretroviral (ARV) treatment and prophylaxis. In this review, we discuss the proposed mechanisms of MTCT, evidence for cell-free and cell-associated transmission in different routes of MTCT, and the impact of ARVs on virus levels and transmission. Many population-based studies support a role for cell-associated virus in transmission and in vitro studies also provide some support for this mode of transmission. However, animal model studies provide proof-of-principle that cell-free virus can establish infection in infants, and studies of ARVs in HIV-infected pregnant women show a strong correlation with reduction in cell-free virus levels and protection. ARV treatment in MTCT potentially provides opportunities to better define the infectious form of virus, but these studies will require better tools to measure the infectious cell reservoir.

Keywords. breast milk; cell-associated virus; cell-free virus; genital secretions; HIV-1; infected leukocytes; mother-to-child transmission.

Worldwide, over 260 000 children were infected with human immunodeficiency virus (HIV) in 2012—almost 30 children per hour [1]. The majority of these infections were via mother-to-child transmission (MTCT), which can occur while the child is in utero, during labor and delivery, or via breastfeeding. In the absence of any interventions, the risk of MTCT is approximately 30%–40% [2]. This risk of transmission depends on a number of facets, but high levels of maternal virus have consistently been shown to be a major risk factor [2–5]. Antiretroviral (ARV) therapy can lower maternal viral loads and provide prophylaxis to the infant to significantly reduce this risk. In fact, single-dose nevirapine provided to the mother and infant near birth can decrease transmission by half, presumably by reducing both intrapartum (during labor/delivery) and early breast milk infections [6]. Furthermore, provision of combination ARVs during pregnancy and breastfeeding can reduce transmission risk to less than 5% [7–9]. Despite these great advances, a significant number of infants are still infected every year and a number of questions remain regarding the biologic mechanisms of transmission.

One question that remains to be elucidated is the molecular mechanism of virus transmission in MTCT and whether the most infectious form is free virus or an infected cell. Insights into this question have been gleaned from in vitro cell culture models, experimental infections in animals, and via clinical correlates of MTCT identified through population-based studies. In this article, we review proposed mechanisms of MTCT and the evidence for cell-free and cell-associated virus transmission in different routes of MTCT. We also discuss the dynamic between antiretroviral treatment, virus levels, and transmission and what such data suggest about the likely source of transmitted virus.

Molecular Mechanisms of MTCT Across Epithelial Barriers

While a number of MTCT mechanisms have been described, a majority of transmission events are believed to occur across infant mucosal surfaces, such as the

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The Journal of Infectious Diseases® 2014;210(S3):S631–40

DOI: 10.1093/infdis/jiu344

gastrointestinal tract and nasopharyngeal surfaces. These mucosal barriers are in contact with HIV-infected maternal fluids throughout gestation, delivery, and the breastfeeding period, providing ample time and opportunity for transmission to occur. However, transmission does not occur in the majority of cases, and the polarized epithelial barrier that overlies mucosal surfaces certainly contributes to the infant's protection. For systemic infection to occur, maternal HIV must infect susceptible cells within or underneath the epithelial barrier and then traffic to underlying layers to disseminate the virus to lymphatic and blood vessels.

Despite the protection afforded by epithelial barriers, there is evidence that exposure of these surfaces to virus does result in infection. Firstly, limiting infant exposure to infected maternal fluids (including blood, cervicovaginal fluid, and breast milk [10–13]) has been shown to reduce the risk of MTCT. This reduction in transmission has been most clearly shown in cases where breastfeeding is replaced with formula and infant infection is reduced by almost half [14]. Similarly, elective Cesarean sections, conducted prior to the onset of labor and membrane rupture, avoid infant exposure in the birth canal and reduce risk of transmission [7, 15]. Data from nonhuman primates also provide proof-of-concept that infection can occur at these mucosal sites. Following oral challenge of cell-free simian immunodeficiency virus (SIV), viral replication has been observed in oral, esophageal, and gastrointestinal mucosa of infant macaques [16, 17].

In human infants, virus may infect susceptible lymphocytes within the epithelial layer and/or traverse the barrier to reach other target cells. Recently, fetal and infant gut epithelia were shown to contain large numbers of activated target cells ($CD4^+CCR5^+$ T cells) that are susceptible to HIV infection, suggesting that infection may occur at these surfaces if they come into contact with HIV-infected fluids [18]. Alternatively, viruses may penetrate this epithelium via breaks in the mucosal barrier or via transcytosis [19, 20]. Transcytosis is the vesicular transport of materials, such as HIV, across a cell. In the infant, this mechanism may permit HIV to be transferred from the gastrointestinal tract lumen through the cell to the basolateral face of the epithelium where it is released. This transfer brings the virus into contact with susceptible target cells, seeding infection. In vitro studies of fetal oral and intestinal tissue have shown that cell-free and cell-associated virus can transmigrate across these barriers, and virions that pass through these cells remain highly infectious [21, 22].

Studies in cell culture models, while not specifically designed to recapitulate MTCT, do provide insight into cell-free and cell-associated transmission across epithelial barriers. HIV-infected cells may transmigrate through epithelial cell layers or transmit virus through these layers by forming virologic synapses with epithelial cells and releasing virus at the apical surface of these cells [19, 20, 22, 23]. Virologic synapses, which aid in transcytosis, are similar to immune synapses and may also protect the virus from recognition (and subsequent elimination) by the host's immune system [24]. In vitro studies of transcytosis across epithelial layers suggest that cell-associated virus is more infectious than cell-free virus [19, 25]. Similarly, kinetic studies suggest that cell-to-cell spread of infection in culture is more efficient than that of cell-free virus [26–28]. Thus, these in vitro studies support a potential role of cell-associated virus in cell-to-cell spread of infection. The studies, however, utilized a variety of epithelial cell lines, not all relevant to MTCT, and as with all in vitro models, they do not fully recapitulate the complexity of infection in exposed infants.

Defining the Infectious Form of Virus in HIV-Infected Women and Their Infants

The study of cell-free and cell-associated virus in cohorts of HIV-infected women and their infants is the only way to determine the relative role of these 2 viral forms in the presence of the complex host-pathogen interactions that occur during MTCT. Population-based studies present their own challenges and limitations, however, because it is difficult to capture events at the precise moment of transmission. Nonetheless, the window period of infection is perhaps best defined for MTCT compared to other modes of HIV transmission, as infants born to HIV-positive mothers are monitored regularly to determine if and when transmission occurs.

Cell-free and cell-associated virus have both been detected in maternal blood (plasma), breast milk, and genital secretions, and virus levels in these fluids have all been correlated with MTCT (reviewed in [2] and discussed further below). However, examining the potential role of these different viral forms in MTCT has relied on the use of surrogate measures rather than a specific measure of infectious virus. Cell-free virus is typically measured by HIV RNA levels. These levels, while easily quantifiable, do not directly measure the number of infectious particles, as most virions are noninfectious [29]. Nevertheless, a majority of studies examining correlates of MTCT have observed that maternal HIV RNA levels do correlate with transmission risk (reviewed in [2]). However, there is also considerable evidence in the setting of MTCT that infected cells (cell-associated virus) are also involved in HIV transmission (reviewed in [2]). Infected cells have typically been quantified by levels of HIV proviral DNA. As with cell-free virus, DNA levels do not directly quantify the infection potential of the cells as many integrated proviruses are not capable of producing infectious virions [30], but they do correlate with MTCT risk. Because DNA and RNA viral loads are often correlated, it has made it more difficult to clarify the specific contribution of cell-associated versus cell-free virus to transmission risk. Thus, studies that measure both forms of the virus in the same cohort are essential to determine the relative contribution of each form.

Evidence for Cell-Associated and Cell-Free Virus Transmission During in Utero Transmission

In utero transmission is the least common route of MTCT. While HIV has been detected in fetuses as early as 8 weeks, the majority of in utero transmission occurs during the third trimester and only 5%–10% of infants born to HIV-infected mothers become infected via this route [2, 31].

HIV infection during pregnancy is hypothesized to occur across the placenta. Placental trophoblasts (syncytiotrophoblasts and cytotrophoblasts) form a polarized epithelial barrier between maternal and fetal blood supplies. A number of early in vitro studies suggested that cell-free HIV can infect placental trophoblasts [32–35]. Newer studies, however, suggest that while cell-associated virus can cross the placenta and cause productive infection in target cells, cell-free virus cannot productively infect placental cells [25, 36, 37]. These newer studies utilized trophoblastic cells organized as a polarized monolayer, similar to the barrier encountered in vivo, while older studies often used unorganized trophoblast target cells [32, 34, 35]. Other studies have also supported a role for cell-associated virus transmission across placental trophoblasts by transcytosis [38, 39]. If the data from these in vitro studies reflect events in vivo, they suggest that cell-associated virus may significantly contribute to in utero infections.

Alternatively, in animal models, there is evidence for cell-free HIV transmission through amniotic fluid. As a proof-of-concept, direct injection of SIV into the amniotic fluid of pregnant macaques resulted in infant infection in 2 different studies [40, 41]. The role of cell-free virus transmission through amniotic fluid in humans, however, is unclear and controversial. While there have been cases where HIV has been detected in amniotic fluid [42, 43], others have shown no evidence of HIV in amniotic fluid [44, 45]. These studies suggest that if HIV is present in amniotic fluid, it is likely rare or present at low levels. Furthermore, even in the study where HIV was reported in amniotic fluid, the presence or level of virus was not correlated with infant infection risk in utero [43]. Studies of virus in amniotic fluid are limited, partially over concerns that such an invasive procedure may increase the transmission risk [46–48]. Given the difficulty of sampling viral reservoirs and infected tissues during pregnancy, as well as the challenge of accurately estimating the time of infection in the fetus, the roles of cell-associated and cell-free virus in in utero MTCT are still largely undefined.

Evidence for Cell-Associated and Cell-Free Virus Transmission During Labor and Delivery

A large proportion of HIV-positive infants are infected intrapartum. MTCT around the time of delivery accounts for approximately one-third to half of infant infections in breastfeeding populations [2].

HIV may be transmitted during pregnancy or labor and delivery if a breach in the maternal-infant blood barrier, a placental microtransfusion, occurs. While the exact cause of placental microtransfusions is unknown, they have been associated with contractions during the early stages of labor when membranes rupture, and they ultimately result in the exchange of small amounts of maternal and fetal blood [49, 50]. This exchange may result in the transfer of HIV-infected cells and free virus from the mother to the infant, increasing infant infection risk. Two studies of HIV-infected Malawian women found placental microtransfusions to be strongly associated with the risk of intrapartum MTCT [51, 52]. These studies and others, however, have not attempted to clarify the impact of cell-free and cell-associated HIV on transmission [51–54].

During labor and delivery, infection may also occur as the infant is exposed to and swallows cervical and vaginal fluids infected with HIV. HIV has been isolated from gastric/oropharyngeal aspirates at birth, and firstborn twins who spend a longer time in the birth canal in contact with HIV-infected fluids are more likely than their second born siblings to become infected [55–59]. Epidemiologic studies examining levels of HIV RNA and DNA and their correlation with infant infection have attempted to clarify the relative impact of cell-free versus cell-associated virus on this route of transmission (Table 1). While HIV RNA in the genital tract has been associated with intrapartum transmission risk in a few studies [60, 61, 63], somewhat more compelling evidence exists for a role of cell-associated virus. Several studies have found a significant correlation between levels of genital tract HIV DNA and risk of intrapartum transmission [5, 61–63]. Although relatively few studies have examined both DNA and RNA virus levels, results from at least 2 studies support a role for cell-associated virus over that of cell-free virus in transmission. Tuomala et al observed that every 1-log increase of HIV DNA in cervicovaginal lavage specimens was associated with a significantly higher risk of transmission. This association was not seen when examining HIV RNA levels in the genital samples, supporting a role for cell-associated virus transmission [63]. Similarly, in a study of 279 HIV-exposed infants, maternal cervical and vaginal DNA were significantly associated with transmission, independent of plasma HIV RNA levels [5]. Overall, these cohort studies suggest a significant association between cell-associated HIV and intrapartum transmission risk. While the number of intrapartum studies is limited at this time, other studies of oral infection (such as via breastfeeding) may provide additional insight into mechanisms of intrapartum transmission.

Evidence for Cell-Associated and Cell-Free Virus Transmission in Breastfeeding

Breastfeeding also contributes substantially to MTCT, accounting for up to 40% of infant infections [2]. Both cell-free and

Table 1. Cohort Studies of Cell-Associated and Cell-Free Virus in Maternal Genital Fluids and Risk of MTCT

Study	Mother-Infant Pairs (n)	Maternal Genital Virus Levels Measured[a]	Summary of Findings
Chuachoowong (2000) [60]	310	CVL RNA	High plasma VL (>10 000 copies/mL) and quantifiable CVL RNA were associated with transmission ($P < .001$) CVL RNA levels were associated with risk of transmission after adjusting for plasma RNA
Panther (2000) [61]	24	CVL DNA CVL RNA CVL CA RNA	CVL DNA ($P = .04$), and RNA ($P = .01$) levels were associated with perinatal transmission CA CVL RNA levels did not correlate with transmission
John (2001) [5]	279	Cervical DNA Vaginal DNA	Cervical ($P = .004$) and vaginal ($P = .03$) DNA levels were associated with perinatal transmission, independent of plasma VL
Montano (2003) [62]	84	CVL DNA	CVL DNA was associated with increased risk of transmission, independent of plasma RNA VL ($P = .034$)
Tuomala (2003) [63]	78	CVL DNA CVL RNA CVL CA RNA	CVL DNA ($P = .01$) and RNA ($P = .04$) titers were associated with transmission risk, while CVL CA RNA was not ($P = .08$) CVL DNA titers were associated with transmission risk ($P = .03$) in vaginal and nonelective cesarean deliveries after controlling for plasma VL. CVL RNA and CA RNA titers were not associated with transmission after controlling for plasma RNA

Abbreviations: CA, cell-associated; CVL, cervicovaginal lavage; MTCT, mother-to-child transmission; VL, viral load.

[a] CVL DNA represents cell-associated virus; CVL RNA represents cell-free virus; CVL CA RNA represents RNA present in the cellular fraction (cell-associated).

cell-associated viruses have been detected in breast milk. HIV RNA levels in breast milk typically correlate with those of plasma but are approximately 100-fold lower [11, 64–66]. HIV DNA has also been detected in breast milk $CD4^+$ T cells and macrophages [12]. It has been difficult to isolate infectious HIV from breast milk, perhaps because levels are low, and past efforts in our lab were unsuccessful; however, there have been at least 2 reports of HIV being cultured from breast milk [65, 67]. These studies isolated the virus from cellular and cell-free fractions of breast milk, suggesting that infectious virus may exist in either form.

Studies in animal models have provided limited evidence that transmission may occur by cell-free or cell-associated virus. First, studies using oral inoculations of cell-free virus have been shown to cause infection in infant macaques [68]. While these studies typically use high doses of virus in the challenge inoculum, which may not be directly relevant to human exposure, they do provide proof-of-concept that cell-free virus can cause infection via the oral route. More recently, a study in humanized mice suggested that either cell-free or cell-associated HIV can result in oral HIV transmission [69].

Similar to studies of genital fluids, cohort studies of breastfeeding populations have tried to clarify the impact of cell-free versus cell-associated virus in MTCT (Table 2). In humans, HIV RNA levels in breast milk have been associated with transmission risk [64, 70–72, 74–79]. Multiple studies have also suggested a role for cell-associated virus transmission during breastfeeding [5, 66, 73–75, 78, 79]. Of the studies that examined both DNA and RNA, the majority suggest an increased role for cell-associated virus transmission during the breastfeeding period. In a study of 291 HIV-infected women from Kenya, cell-associated HIV in breast milk was significantly associated with transmission risk after adjusting for cell-free virus [73]. In this study, each log10 increase in infected cells tripled the risk of infant infection. Koulinska et al also found that cell-associated virus levels in breast milk were associated with transmission throughout the breastfeeding period, while cell-free virus levels were only predictive of transmission risk after 9 months of age [74]. Additionally, Kantarci et al observed that in a multivariate analysis, only cell-associated virus levels were directly associated with mastitis (inflammation of the breast tissue), a known risk factor for breastfeeding transmission [75].

It is possible that cell-associated virus is less susceptible than cell-free virus to inhibitory factors found in breast milk. In vitro studies have suggested that many innate factors in milk can inhibit cell-free HIV; however, a similar effect has not been observed with cell-to-cell viral spread [80–87]. One interpretation of this finding is that cell-associated virus may contribute to more breastfeeding infections because cell-free virus is neutralized by innate factors. The impact of such inhibitors on MTCT, however, is largely unclear when considering population-based studies. For example, secretory leukocyte protease inhibitor (SLPI) levels in breast milk from HIV-infected women did not correlate with transmission risk, but other studies have suggested that SLPI levels in infant saliva and maternal cervicovaginal fluid correlate with decreased risk of transmission [88–90].

Contrary to many in vitro studies, however, oral challenge studies of humanized mice have observed that human breast milk strongly inhibits transmission of both cell-free and cell-associated HIV [69]. Along these same lines, evidence from

Table 2. Cohort Studies of Cell-Associated and Cell-Free Virus in Maternal Breast Milk and Risk of MTCT

Study	Mother-Infant Pairs (N)	Maternal BM Virus Levels Measured[a]	Summary of Findings
Van de Perre (1993) [66]	129	BM DNA	The presence of infected BM cells was predictive of transmission ($P < .05$)
Semba (1999) [70]	134	BM RNA	BM RNA levels were associated with transmission ($P < .0001$)
Pillay (2000) [71]	79	BM RNA	BM RNA levels were associated with transmission ($P = .04$)
John (2001) [5]	141	BM DNA	Trend for higher concentration of HIV-infected cells among transmitting mothers compared to nontransmitting mothers ($P = .09$)
Rousseau (2003) [72]	275	BM RNA	BM RNA levels were significantly associated with transmission ($P = .002$)
Rousseau (2004) [73]	134	BM DNA BM RNA	BM DNA associated with increased risk of transmission after adjusting for cell-free virus in plasma ($P = .03$) and BM RNA ($P = .1$) In multivariate analyses, BM RNA was not associated with transmission
Koulinska (2006) [74]	122	BM DNA BM RNA	BM DNA ($P = .001$) and RNA ($P = .006$) were associated with transmission. BM DNA was predictive of transmission before ($P = .04$) and after 9 mo postpartum ($P = .05$) BM RNA was only predictive of transmission after 9 mo ($P = .02$)
Kantarci (2007) [75]	118	BM DNA BM RNA	BM DNA ($P = .001$) and BM RNA ($P = .002$) were associated with transmission Positive association between BM DNA and mastitis, which was associated with transmission
Semrau (2008) [76]	138	BM RNA	Detection of BM RNA was significantly associated with transmission ($P < .01$)
Lunney (2010) [77]	559	BM RNA	BM RNA viral load was associated with transmission ($P = .02$)
Neveu (2011) [64]	72	BM RNA	BM RNA shedding was associated with transmission ($P < .001$)
Ndirangu (2012) [78]	72	BM DNA BM RNA	BM RNA ($P < .001$) and DNA ($P < .001$) levels were associated with transmission. Prior to 6 mo, this association was stronger for BM DNA. After 6 mo, this association was stronger for BM RNA
Kuhn (2013) [79]	839	BM DNA BM RNA	HIV RNA and DNA concentrations were strongly associated with postnatal transmission HIV RNA concentrations remained associated with postnatal transmission after adjusting for maternal CD4 count and plasma RNA VL.

Abbreviations: BM, breast milk; HIV, human immunodeficiency virus; MTCT, mother-to-child transmission; VL, viral load.

[a] BM DNA represents cell-associated virus; BM RNA represents cell-free virus.

population-based studies suggest that other immune responses in breast milk may act to lower cell-associated virus levels. For example, in a cohort study of 170 women, HIV-*gag*-specific cytotoxic T lymphocyte (CTL) activity in breast milk correlated with reduced infant infection during the breastfeeding period [91]. HIV-specific maternal antibodies in breast milk may also reduce levels of cell-associated virus by mediating activities such as antibody-dependent cellular cytotoxicity (ADCC). ADCC activity results in the death of infected cells, thus preventing further spread of infection and reducing levels of cell-associated virus. In a small study of HIV-infected women, ADCC levels in breast milk were associated with reduced risk of infant infection during the breastfeeding period [92]. One interpretation of the findings that breast milk CTL and ADCC are associated with reduced transmission is that these responses act by lowering maternal cell-associated virus levels and reducing infant infection risk, although this hypothesis remains to be tested.

Overall, the most data regarding cell-free versus cell-associated virus transmission in MTCT are from the breastfeeding period, probably largely due to the fact that timing of infant infection can be more accurately estimated with regular follow-up, and collection of breast milk is easy and noninvasive. While cohort studies suggest that cell-associated virus may contribute to more infections, animal studies have shown that both cell-free and cell-associated virus can cause oral transmission. In vitro experiments have also suggested that factors in breast milk may inhibit cell-free or cell-associated virus and thus more studies need to be conducted to understand the relative impacts of such factors on virus levels and transmission in breastfeeding populations. Such studies can provide information on virus transmission during breastfeeding and may also provide insights into potential therapeutic interventions that could work by mimicking natural inhibitory processes.

The Role of Preexisting HIV-Specific Antibodies and Virus Transmission

MTCT is also a unique setting in which to study whether antibodies play a role in protection from infection. Specifically, maternal antibodies are transferred to the infant through the placenta, and infection occurs in the face of HIV-specific

antibodies present in the infant. HIV-specific antibodies may act through multiple mechanisms to prevent cell-free and/or cell-associated virus transmission. First, antibodies may bind to and neutralize cell-free virions, thus preventing initial infection. Alternatively, antibodies can block cell-associated virus through mechanisms such as ADCC, which target infected cells for destruction. The relative efficacy of these antibody functions could thus shift the relative contribution of cell-free or cell-associated virus transmission from one form to the other.

Passive immunization studies in macaques have shown that both neutralization and other antibody functions are important in protection from infection [93–95], but the relative contribution of different antibody functions in human infant protection is less clear. A number of population-based studies from MTCT cohorts have been conducted, and while there is some evidence to support a role for neutralizing and nonneutralizing antibodies in protection, there is inconsistency among studies, with a number of other studies suggesting no protective effect (reviewed in [96, 97]). Future studies are warranted to clarify how these antibody functions impact the transmission of cell-free and cell-associated virus transmission.

The Impact of Antiretroviral Therapy on Cell-Free and Cell-Associated Transmission

Treatment of women and their children with antiretrovirals during the course of pregnancy and breastfeeding has dramatically lowered the risk of MTCT, by reducing maternal viral burden and by providing prophylaxis to the infant. In fact, prevention of MTCT was one of the first settings to show that ARV prophylaxis can prevent infection [6, 98]. Multiple studies have observed that ARVs provided to the infant can prevent infection, even in the absence of maternal treatment [99, 100]. Thus, infant prophylaxis works independent of maternal viral loads, and the ARVs may be acting to prevent cell-free or cell-associated virus.

The reduction in maternal viral load associated with ARV treatment, however, also greatly contributes to reduction in transmission risk. Data by Chung et al suggest that the decrease in maternal RNA viral load associated with treatment is an independent protective factor against transmission [101]. The correlation between a reduction in maternal RNA viral load and infant infection risk has been shown in a number of compartments (blood, breast milk, and genital secretions) and thus suggests that cell-free virus significantly contributes to MTCT [4, 60, 102–106]. Similarly, disruption of antiretrovirals in the mother has been associated with an immediate increase in RNA viral load in breast milk and a subsequent increase in risk of breastfeeding transmission [107, 108].

While ARVs have been shown to reduce RNA viral load in maternal fluids, there is a limited effect on cell-associated virus (as measured by HIV DNA) in breast milk [102–104] and blood [109, 110]. These results seemingly contradict the epidemiologic and in vitro data presented above, which suggest the importance of cell-associated virus in MTCT. This discrepancy is particularly true after short-course treatments used for prevention of MTCT [103].

If cell-associated virus is important in transmission, then it is unclear why short-course ARVs are so effective. One explanation may be that infant prophylaxis, discussed above, is the major mediator of protection. Alternatively, the number of activated infected cells that produce infectious virus may represent a small proportion of the total number of infected cells measured by HIV DNA. Thus, while maternal treatment may lead to rapid decline of the infected, activated $CD4^+$ T cells (which would lead to a rapid decline in HIV RNA as these are the cells that produce the majority of free virus), a reservoir of primarily latently infected T cells and/or macrophages would remain. These cells are potentially less infectious, but still detected by HIV DNA assays. In this case, part of the discrepancy may be explained by the method used to quantify cell-associated HIV. Provirus levels detected by DNA, typically used to enumerate cell-associated HIV, have been shown to be poorly predictive of the number of latently infected cells capable of producing infectious virus [30, 111]. In fact, in patients on long-term ARV treatment, DNA levels gave infected cell frequencies greater than 100× those levels predicted by viral outgrowth assays [111]. Thus, it is possible that the majority of cells in maternal fluids that harbor HIV DNA after ARV treatment encode defective virus or virus that cannot be induced, and are therefore not an appropriate measure of cell-associated virus capable of mediating transmission [30].

A more relevant measure of the transmissible form of cell-associated virus may be cell-associated RNA, which is likely more indicative of infectious virus associated with cells. Lehman and colleagues measured the impact of antiretroviral treatment on cell-free RNA, cell-associated RNA, and cell-associated DNA levels in breast milk. While treatment had no measurable impact on cell-associated DNA, it did significantly reduce the levels of cell-free and cell-associated RNA [103]. These data suggest that cell-associated HIV RNA levels may be a more important measure of cell-associated virus in breast milk, and future studies should determine if cell-associated RNA levels in maternal fluids correlate with infant infection risk.

CONCLUSIONS

A majority of mother-to-child transmission events are believed to occur across epithelial barriers; however, the relative contribution of cell-free versus cell-associated virus in transmission is still unclear. A number of epidemiologic studies of breastfeeding and intrapartum transmission suggest that cell-associated virus may be relatively more important than cell-free virus. Similarly, in vitro studies examining the infection of polarized epithelial cell layers

(including placental trophoblasts), suggest that cell-associated HIV is more efficient and infectious. Alternatively, data from nonhuman primates have provided proof-of-concept that cell-free virus can result in infant infection, although these studies often use very high levels of virus relative to those levels which infants are naturally exposed. Supporting the cell-free virus transmission hypothesis, data from human studies have shown that HIV RNA is drastically reduced following antiretroviral treatment, and this drop in viral load is associated with a reduction in transmission. Contrary to the epidemiologic and in vitro studies, cell-associated viral loads remain relatively constant despite treatment. While this discrepancy may be explained by the methods used to measure cell-free and cell-associated virus, there is need for future studies in this area.

A number of considerations should be taken into account when planning future MTCT studies. First, as antiretroviral treatment and prophylaxis is scaled up, it is of interest to better define the viral reservoir in women receiving ARV treatment and to clarify the potential of these cells to produce infectious virus. Along these lines, as highlighted by latent reservoir studies, it is important to consider a better measure of cell-associated virus that accounts for the potential of cells to transmit virus. A measure that is more indicative of the levels of infectious virus associated with infected cells, such as cell-associated RNA levels, should be utilized in epidemiologic and treatment studies. Similarly, both cell-associated and cell-free virus levels should be measured in these studies to allow for a more direct comparison of their relative contributions to infection. Finally, animal models of MTCT should also incorporate cell-associated virus in challenge inoculum. The data garnered from such research will ultimately provide a clearer picture of the relative importance of cell-free and cell-associated virus in HIV mother-to-child transmission.

Notes

Acknowledgments. The authors would like to thank Dr Grace John-Stewart and Dr Dara Lehman for reading the paper and providing advice and suggestions.

Financial support. This work was supported by the National Institutes of Health (NIH) (R01 AI076105), and C. M. was supported by an NIH training grant (T32 AI083203).

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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