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Viral Sequence Analysis from HIV-Infected Mothers and Infants: Molecular Evolution, Diversity, and Risk Factors for Mother-To-Child Transmission

Philip L. Bulterys, BSc^{1,2}, Sudeb C. Dalai, MSc^{3,4}, and David A. Katzenstein, MD³

¹Department of Biology, Stanford University, Stanford, CA

²David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA

³Division of Infectious Disease, Stanford University School of Medicine, Stanford, CA

⁴Division of Epidemiology, University of California-Berkeley School of Public Health, Berkeley, CA

Abstract

Synopsis

Great progress has been made in understanding the pathogenesis, treatment, and transmission of HIV and the factors influencing the risk of mother-to-child transmission (MTCT). However, many questions regarding the molecular evolution and genetic diversity of HIV in the context of MTCT remain unanswered. Further research to identify the selective factors governing which variants are transmitted, how the compartmentalization of HIV in different cells and tissues contributes to transmission, and the influence of host immunity, viral diversity and recombination on MTCT may provide insight into new prevention strategies and the development of an effective HIV vaccine.

Keywords

HIV; mother-to-child transmission; sequence; molecular evolution; diversity; risk factor

Introduction

In most wealthy or industrialized countries, the frequency of mother-to-child transmission (MTCT) of Human Immunodeficiency Virus Type 1 (HIV-1) has been reduced to less than

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Author contact information: David Katzenstein, Division of Infectious Disease, Stanford University Medical Center, S-141 Grant Bldg., 300 Pasteur Drive, Stanford California 94305, tel: 650-725-8304, mobile: 650-868-7250, davidkk@stanford.edu

Sudeb Dalai, Stanford University School of Medicine, S-141 Grant Bldg., 300 Pasteur Drive, Stanford California 94305
sdalai@stanford.edu

Philip Bulterys, David Geffen School of Medicine, UCLA, 10833 Le Conte Ave, Los Angeles, CA 90095, tel: 310-484-4196,
bulterys@ucla.edu

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2% as a result of prevention strategies including access to highly active antiretroviral therapy (HAART), replacement of breast-feeding, elective caesarean section, or a combination thereof [1]. However, in many resource-limited countries with high levels of endemic infection, transmission of HIV-1 from mother to child is recognized as a leading cause of infant and child mortality, where in the absence of antiretroviral treatment (ART) and prophylaxis, more than 25% of infants born to HIV-positive women become infected with HIV [2,3].

MTCT may be influenced by a number of maternal characteristics, including high plasma viral load, low CD4+ T-lymphocyte count, other cervico-vaginal infections at delivery, and concentration of virus in breast-milk [4–6]. In settings where prolonged breast-feeding is practiced, infection rates may rise to nearly 40%. Social/behavioral risk factors for MTCT include lack of access to prevention-of-mother-to-child-transmission (PMTCT) services and high-risk sexual behavior or injection drug use during the second and third trimesters of pregnancy [7].

Consensus conventions adopted to classify the presumed timing of transmission distinguish between *in utero*, intrapartum/early postpartum, and late postpartum acquisition of infant infection. Infants are classified as having acquired *in utero* infection on the basis of polymerase chain reaction (PCR) detection of HIV-1 RNA or DNA from samples obtained within 72 hours of birth. Infants negative by PCR at birth but in whom infection is detectable by 2–6 weeks are regarded as cases of intra-partum or early postpartum transmission. In these infants, transmission of virus is thought to have occurred during very late gestation, labor, or delivery, potentially through oral and mucosal exposure to maternal virus in blood, cervical secretions, or colostrum via early breast feeding. Infants with undetectable infection by screening assays within 6–8 weeks of birth who later acquire infection are deemed to have late postpartum infection presumed to be transmitted through breast-feeding [8,9].

Whether cell-associated provirus or viral RNA from blood, cervical secretions, or breast-milk is the primary source of infection in each of these settings is not well understood. The early observation that the first-born twin of an HIV-infected mother is much more likely to acquire infection than the subsequently born twin supported the idea that trauma and contact with maternal blood and secretions is important in MTCT [10], although a more recent study did not corroborate this finding [11]. When maternal ART is initiated in the second trimester at 24–28 weeks, less than 2% of infants acquire HIV infection, suggesting that early infection *in utero* occurs much less frequently than infection in later stages [12]. The most striking reductions in MTCT are seen when HIV RNA is suppressed by ART and postpartum prophylaxis is given to the newborn. Without ART there is more than a two-fold increase in transmission for every log increase in maternal virus load [13]. Transmission from mothers with less than 1,000 copies/ml of plasma HIV RNA is unusual [14]. Similarly, there is an independent association of higher maternal CD4 cell numbers with reduced transmission, suggesting that the maternal immune response plays a role in limiting MTCT [15].

It is hypothesized that infant infection acquired at different stages may result from distinct modes of viral transmission associated with unique virus sub-populations, or quasi-species, derived from the diversity of viruses present during chronic infection in the mother. The extent to which viral genetic and evolutionary characteristics influence the risk of MTCT of HIV remains unclear. Investigation of the genetic composition and evolution of HIV in the context of mother-to-child transmission (MTCT) may elucidate viral risk factors for transmission, infection, and disease progression. As HIV sequence data become more widely available, analysis of these data will provide a more complete understanding of the factors influencing MTCT and enable development of additional prevention strategies.

Selection and Bottlenecks in MTCT of HIV

Cases of MTCT represent an opportunity to identify the virologic characteristics associated with transmission. Some studies have investigated specific characteristics of viral genes while others have analyzed the diversity of HIV-1 quasi-species, comparing the range of virus diversity in maternal samples to those obtained from the infant. These studies suggest that infection in the infant is usually initiated by a single or limited number of maternal viruses, indicating the presence of a bottleneck in vertical HIV transmission [16]. Such a bottleneck may provide an opportunity for intervention strategies in MTCT and transmission in general.

The extensive diversity of viruses present in prolonged HIV-1 infection within individuals, including pregnant women, is the result of continuing viral expansion, selection, and evolution in the face of immune and/or drug pressures. In chronic infection up to 10^{12} new virions are produced each day in an infected individual [17], with a high rate of evolutionary change driven primarily by the poor fidelity of both reverse transcriptase and RNA polymerase. The rate of erroneous nucleotide substitution during reverse transcription is approximately one nucleotide per 10^5 base pairs [18]. This is accompanied by the absence of mis-sense correction mechanisms in the reverse transcription and synthesis of the viral genome.

The viral diversity generated by mutation, nucleotide substitution and point mutation, insertion/deletions, and duplication, is acted upon by selective pressure from the host humoral and cellular immune systems and when present, antiretroviral drug therapy. In addition, the phenomenon of strand switching, in which the viral reverse transcriptase switches within or between viral RNA template strands in synthesizing a DNA transcript, enables frequent recombination events. A robust host immune response exerts a strong selective force on the viral population, and patients with longer asymptomatic phases typically show evidence of greater positive selection [19,20], defined by a per-site rate of non-synonymous mutation (d_N) exceeding that of synonymous mutation (d_S) [19,21,22]. Vertical HIV transmission events are shaped by extensive diversity in maternal virus, genetic bottlenecks in MTCT, and potential founder effects in the infected infant resulting in relative viral homogeneity of early infant infection, although the extent of diversity may vary by mode of transmission [16,23]. MTCT may result in the transmission of a minor subset of maternal viruses with subsequently limited viral diversity in infant viral sequences compared to the maternal sequences [24–26]. In some settings there is evidence that

infection *in utero* may involve transmission of a greater diversity of maternal viral variants compared to transmission at delivery [24,27].

The associations among the strength of positive selection, progression of maternal disease, and HIV transmission are not well-defined. Numerous small studies suggest that viral variants transmitted from mother-to-child are derived from a minority virus population which has effectively escaped the maternal immune response [24,26,28,29–32]. In addition, transmitted viruses may derive from the major maternal variant in compartments including blood, placenta, or cervical secretions in the birth canal. Differences between maternal and infant immunogenetics, particularly human leukocyte antigen (HLA) specificity in the recognition of cytotoxic T lymphocyte (CTL) epitopes by maternal and infant immune responses, likely play a role in selection. Additionally, some transmitted viruses may have a replication advantage in the infant and may therefore be naturally selected [33]. Alternatively, success of a particular variant may result from stochastic effects unrelated to selection [34]. In a phylogenetic analysis of maternal and infant C2V3 envelope sequences, Ceballos *et al.* found that during early pregnancy a single minor viral variant was transmitted to the infant followed by subsequent evolution, suggesting either a selection process or a stochastic event, whereas in later maternal infection (during the final trimester or via breast feeding), maternal and infant sequences were intermingled, suggesting repeated transmission of multiple viral variants [34]. This analysis did not detect positive selection ($d_N/d_S > 1$) in the maternal and infant sequences, suggesting that stochastic effects accounted for the viral quasispecies that were successfully transmitted.

Role of Viral Recombination and Diversity in MTCT of HIV

HIV has an estimated recombination rate of three events per genome per replication, one of the highest rates of all organisms [35]. Recombination occurs as a result of host cell superinfection by distinct viruses [36–38]. Inter- and intra-subtype recombination are major driving forces of HIV genetic diversity [39,40] and have resulted in a wide distribution of circulating recombinant forms which contribute significantly to the global pandemic (accounting for >25% of infections in some areas) [39,41–43]. Recombination events allow viruses to escape immune pressure, avoid accumulation of deleterious mutations, or jump between adaptive peaks [44–47]. Recombinant viruses may also have fitness and/or transmission advantages, potentially enhancing observed rates of MTCT [48]. Quan *et al.* reported that defective HIV provirus with a lethal mutation in *env* can be rescued by superinfection with either a wild-type virion or a second replication-defective virus with a lethally-mutated capsid protein [49]. These findings suggest that noninfectious HIV-1 variants may constitute a large proportion of *in vivo* HIV populations (9 out of 10 clones isolated from infected brain for example) and that the rescue of such defective variants may be attributable to recombination events [50].

Within geographic settings where multiple HIV subtypes circulate and recombine, subtype-specific differences have been observed in timing and rate of transmission. It has been suggested that geographic differences in rates of MTCT may be related to the genetic diversity of HIV in different settings [51,52]. HIV-1 subtypes and recombinant forms may have functional and phenotypic differences, including chemokine coreceptor usage,

replication efficiency, and viral load that may lead to differences in rates of vertical transmission [53–62]. Studies examining the rate of MTCT by maternal subtype or recombinant infection have yielded discrepant findings. Pádua *et al.* were unable to detect specific genetic forms in *env* or *nef* sequences associated with MTCT or a significant difference in RNA viral load by viral subtype, but found a greater diversity of genetic forms among non-transmitting mothers [52]. The latter suggests that increased maternal immune pressure may successfully limit transmission. Renjifo *et al.* found that MTCT was more common among Tanzanian mothers infected with viruses that included a subtype C envelope, compared to viruses with subtype A or D (or A/D recombinant) envelope [62]. In Kenya, Yang *et al.* found MTCT to be more common among mothers infected with subtype D or A/D recombinant viruses compared to subtype A [63]. In a separate study based on the C2–C5 envelope and 5'LTR regions, Renjifo *et al.* found that some intersubtype recombinant viruses are preferentially transmitted during breast feeding [6].

Whether and how recombinant viruses have a transmission advantage in MTCT warrants further investigation, particularly with the increasing prevalence of circulating recombinant forms of HIV-1. Studies interpreting the effect of viral genotype on the risk of MTCT may be enhanced by increased sample sizes, adjustment of confounding variables, and improved detection of recombinants on a population level [6,60–65]. Paradoxically, the success of ART and prevention limits the potential of MTCT studies as infant infection becomes less frequent in study populations.

Phylogenetics, Compartmentalization, and MTCT of HIV

Phylogenetic analyses of HIV genetic sequences provide insight into the genetic relatedness of multiple virus variants within or between individuals and across populations. These analyses can uncover trends within an epidemic and also provide insight into the origins, timing, and demographic history of transmitted viruses [66–68]. Phylogenetic comparisons of infant and maternal sequences from various compartments are crucial to understanding the viral evolution in these tissues and their respective contributions to the risk of MTCT.

Compartmentalization of HIV infection involves the formation of distinct genetic populations in specific organs, cells or tissues. Compartments of special importance to MTCT are the maternal genital tract, placenta, and breast milk. Multiple studies have shown that HIV variants from the genital tract appear distinct from the blood [69–81], although HIV-1 viral load is typically lower in these compartments compared to plasma [82–84]. Localized inflammation, co-infections, physical and cellular barriers, incomplete penetration of antiretroviral drugs into the genital tract or local immune responses may account for independent and divergent evolution within the maternal genital tract compared to plasma [70–72,85,86].

Bull *et al.* analyzed HIV-1 RNA and cell-associated HIV-1 DNA (*env*) from the blood and genital tract of women with chronic HIV-infection and reported low diversity of genital-tract-specific phylogenetic clades, particularly from the cervix, consistent with bursts of viral replication or the proliferation of infected cells in compartments [83]. However, the absence of tissue-specific genetic features and the phylogenetic overlap of genital tract HIV

clades with those from the blood suggest that HIV-1 flow is not restricted between the genital tract and blood, and that viral evolution may not occur independently within the two compartments [83].

HIV compartmentalization between blood and breast milk is also not well-understood. Breast milk transmission is a major source of pediatric HIV infection [87,88], yet the few studies of viral compartmentalization in breast milk provide contradictory results [89–92]. In the absence of antiretroviral therapy HIV-1 viral load is 10–100 fold lower in breast milk than in plasma [82]. Whether this indicates limited exchange of virus between the two compartments or is instead a consequence of differential immune selection remains unclear. Immunologic elements such as HIV-specific T-cells, antibodies, cytokines, and chemokines appear to be highly compartmentalized in breast milk [93–95]. For example, Becquart *et al.* detected compartmentalization of the humoral IgG response to HIV in the mammary gland [96]. Despite these seemingly unique immunologic environments, Heath *et al.* recently examined the compartmentalization of HIV-1 between breast milk and blood in envelope sequences from 13 breast-feeding women and uncovered substantial genetic overlap [97]. Specifically, genetic compartmentalization in breast milk was only detected in one of six subjects with contemporaneously-collected samples available. The authors suggest that virologic selection in breast milk does not account for the genetic bottleneck associated with mother-to-child transmission [97]. Further studies will clarify the extent to which potential tissue-specific virologic divergence may influence the risk of transmission or the characteristics of the transmitted variant.

Implications for Drug Resistance and Vaccine Design in MTCT of HIV

The ability of HIV to accumulate and exchange drug resistance via single-nucleotide mutations and recombination presents a clinical dilemma in the use of ART, particularly single-dose nevirapine to prevent MTCT. Once drug resistance has been selected, it may persist in circulating viral RNA and within latent reservoirs of proviral DNA [47]. The persistence of maternal drug resistance mutations following drug exposure during pregnancy presents significant challenges to the design of optimal antiretroviral regimens that prevent MTCT without compromising the efficacy of HAART for mothers [98]. Short-course peripartum regimens in resource-poor settings, including maternal single-dose nevirapine and short-course zidovudine, significantly reduce MTCT (37–77% compared to no intervention) [99–105]. However, up to two-thirds of mothers treated with these regimens develop viral resistance to nonnucleoside reverse-transcriptase inhibitor (NNRTI) drugs [106]. Universal HAART, already a mainstay of maternal HIV treatment in developed or resource-rich countries, could mitigate some of the burden of drug resistance associated with peripartum regimens and has been recommended for pregnant and breast-feeding mothers in resource-poor settings [98].

Consideration of HIV molecular evolution and diversity is also important in the design of HIV vaccine strategies [22]. There is compelling rationale to develop a preventive HIV vaccine for use in infants to prevent vertical transmission via breast milk and provide a foundation for life-long immunity [107]. Several recent advances in HIV vaccine research and development, including the partial protection imparted by the ALVAC-AIDSVAX

vaccine in Thailand [108], identification of a novel HIV-1 vaccine target expressed on envelope protein [109], and vaccine-induced control of simian immunodeficiency virus in rhesus monkeys [110], have given momentum to renewed efforts to develop an HIV vaccine. The genetic bottlenecks observed in MTCT provide a model for selective transmission, which may inform the design of vaccines, the identification of target antigens, and potentially, the inclusion of infants and pregnant women in preventive vaccine trials. An understanding of the bottleneck(s) in transmission and specific characteristics of transmitted viruses coupled with innovative vaccine technologies, advances in HIV vaccinology, and recruitment of scientific talent are important steps towards achieving a successful HIV vaccine [111].

Conclusions and Future Directions

Great progress has been made in understanding the evolution of HIV and the factors influencing the risk of MTCT. Translation of these scientific advances, primarily in the use of antiretroviral drugs to prevent MTCT of HIV, has led to successful interventions and significant reductions in infant infection where preventive strategies have been made available. However, many questions regarding the impact of molecular evolution and extensive genetic diversity of HIV on MTCT remain unanswered. Studies of viral characteristics that contribute to the risk of vertical transmission may inform drug and vaccine prevention efforts. Further research to identify the selective factors governing which variants are transmitted, how the compartmentalization of HIV in different cells and tissues contributes to transmission, and the influence of viral diversity and recombination on the risk of MTCT, may provide insight into new therapeutic and preventive strategies.

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