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In-utero transmission of quasispecies among human immunodeficiency virus type 1 genotypes

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Abstract

Samples from infants infected in-utero by the human immunodeficiency virus type 1 (HIV-1) subtypes A, C, D, and recombinants from Dar es Salaam, Tanzania, were examined for the presence of viral genetic quasispecies. HIV-1 envelope diversity was measured on peripheral blood mononuclear cells collected within the first 48 h of life from 53 infants. Phylogenetic analysis of C2–C5 envelope nucleotide sequences was used for HIV-1 subtype classification. Forty-two of 53 samples (79%) showed a heteroduplex mobility assay (HMA) suggestive of transmission of a single quasispecies, while 21% showed infection with multiple quasispecies. No differences among HIV-1 subtypes were found in the proportion of single to multiple quasispecies transmitted in-utero (Likelihood ratio test, $P = 0.83$), nor were differences found among single to multiple quasispecies transmitted and maternal viral load (Mann–Whitney test, $P = 0.44$). This suggests that differences in perinatal transmission between subtypes we previously observed in this cohort could not be associated with the likelihood for multiple independent infections during in-utero infections.
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Introduction

Transmission of HIV-1 from mother to infant through in-utero, intra-partum or breastfeeding routes is the main source of pediatric infections and AIDS worldwide (Newell, 1998; The Working Group, 1995). While treatment of pregnant mothers with antiretroviral drugs during gestation and delivery has limited mother-to-child transmission (MTCT) of HIV-1 in developed countries, vertical transmission of HIV-1 in Africa is still a significant source of infection in the pediatric population (Connor et al., 1994). Since non-B subtypes of HIV-1 viruses are responsible for the majority of infections in Africa, the characterization of phenotypes associated with distinct subtypes may give new insights into the development or implementation of therapeutic regimens aimed at decreasing the rates of MTCT in Africa.

The development of genetic viral variants, known as quasispecies, within an infected individual is the result of mutations and recombinations introduced by HIV-1 reverse transcriptase during viral replication (Domingo et al., 1985). The number and prevalence of quasispecies within an individual at any given time is modulated by the high viral population turnover, changes in cellular tropism, host immune response, and antiviral therapy (Coffin, 1995). Since only one or a few closely related quasispecies are transmitted to the newborn infant, it has been hypothesized that viruses transmitted perinatally were capable of escaping maternal immune responses or might have tropism for infant cells exposed during birth or both (Lathey et al., 1999; Newell, 1998; Wilson et al., 1999). Given that HIV-1 non-B

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Table 1
Distribution of quasispecies among HIV-1 env subtypes

Env subtype	Single	Multiple	Total
A	15 (79%)	4 (21%)	19
C	16 (80%)	4 (20%)	20
D	5 (83%)	1 (17%)	6
Recombinant	6 (100%)	0 (0%)	6
C and D	0 (0%)	2 (100%)	2
Total	42 (79%)	11 (21%)	53

genetic subtypes cause the vast majority of MTCT in Africa, we were interested in determining whether the quasispecies in mothers infected with distinct HIV-1 subtypes are subjected to similar selection pressures during in-utero transmission. In this report we found that in a MTCT cohort in Dar es Salaam, Tanzania, the presence of quasispecies among infants infected in-utero is similar between HIV-1 envelope subtypes A, C, D or intersubtype recombinants.

Results

Fifty-four of 873 infants (6.2%) tested positive for HIV-1 infection by polymerase chain reaction (PCR) within 48 h of birth. The fact that their samples, taken within the first 48 h of life, tested positive for HIV-1 suggested that they were infected in-utero, according to the classification proposed to determine the time of transmission (Bryson et al., 1992). These samples were further used for envelope subtype classification and the presence of genetic quasispecies. Nucleotide sequencing and a determination of phylogenetic classification showed that 19 samples had HIV-1 subtype A envelopes, 20 samples had subtype C envelopes, six samples had subtype D envelopes and six samples had intersubtype recombinant envelopes including one CA, two DA, and three CD. In addition, two samples showed evidence of co-infection with HIV-1 subtype C and subtype D envelopes (Table 1).

To investigate whether distinct HIV-1 genetic quasispecies were transmitted to the infant in-utero, a denaturation and renaturation cycle of the purified envelope PCR product followed by polyacrylamide gel separation was performed on 53 of the 54 samples classified as in-utero infection. HMA could not be done in the last sample due to insufficient amount available for testing and therefore is not included in the analysis shown on Table 1. The presence of quasispecies was determined by counting the number of bands in each sample lane. A maximum of three bands could be visible in a gel when a genetically homogenous sample is subjected to a denaturation and renaturation cycle. The fastest migrating band corresponded to the homoduplexes while the two slower migrating bands corresponded to single-stranded products. The presence of four or more distinguishable bands was considered compatible with heteroduplexes formed by at least two distinct quasispecies (Fig. 1). Samples with three or fewer bands were classified as transmission of single HIV-1 quasispecies while samples containing four or more bands were classified as transmission of multiple quasispecies. Due to the inability of the HMA to differentiate between the presence of two subtypes or the presence of multiple quasispecies of the same subtype, samples with phylogenetic evidence of dual infections could not be included in the comparison among subtypes. Multiple heteroduplex bands were detected in infant samples and the band patterns are shown in Fig. 1.

The envelope subtype and the presence of single or multiple quasispecies of 53 infants is shown in Table 1. Overall, 42 of the 53 samples showed an HMA pattern compatible with transmission of single quasispecies. Logistic regression analysis showed no significant differences in the proportion of samples with single quasispecies between

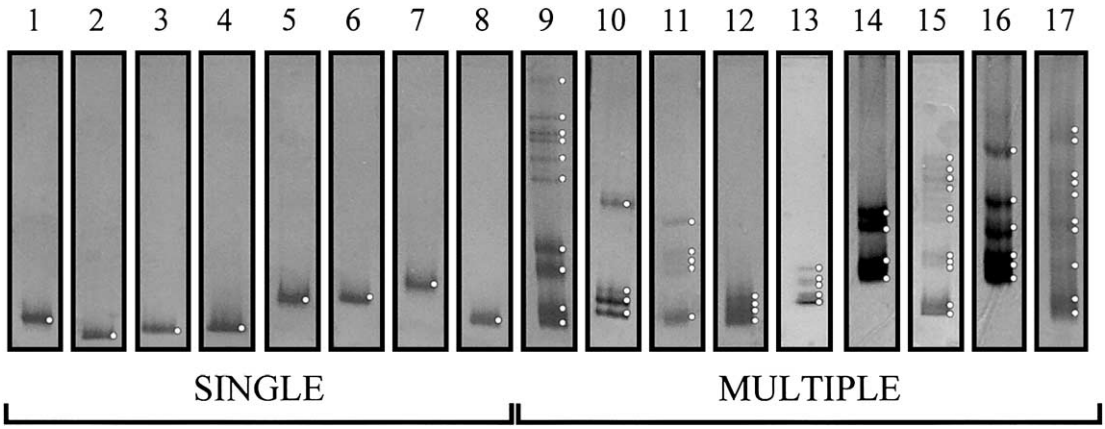


Fig. 1. Heteroduplex mobility analysis of HIV-1 quasispecies from representative samples of infants infected in utero. Purified envelope amplicons were subjected to a denaturation and renaturation cycle followed by separation on polyacrylamide gels. Silver staining was used for visualization of migrating DNA complexes and each visible band was indicated by a white dot. Lanes 1 to 8 are representative samples with one migrating band corresponding to a single quasispecies. Lanes 9 to 17 correspond to the nine samples with evidence of multiple quasispecies. The presence of multiple bands represents heteroduplexes formed between divergent quasispecies. Lanes 9 to 12, 13 to 16 and 17 correspond to samples with subtype A, C, or D envelopes, respectively.

subtype A, C, D, or recombinant envelope (Likelihood test, $P = 0.83$). Eighty percent of the samples with either subtype A, subtype C, or subtype D envelopes showed single transmission. Even though none of the six samples with recombinant envelopes showed evidence of multiple quasispecies, they were not statistically different from samples with non-recombinant envelopes. The two samples from infants that were co-infected with subtype C and subtype D were excluded from the analysis among subtypes but they truly represented transmission of multiple quasispecies.

We previously described that lower CD4+ cell counts at enrollment were associated with higher rate of mother-to-child transmission, but that CD4+ cell counts did not explain the differences in transmission observed among HIV-1 subtypes (Renjifo et al., 2001). In the same way, no differences were found among subtype A, C, D, or recombinants in their likelihood of transmitting multiple quasispecies when we adjusted for CD4+ cell counts at enrollment (data not shown). Since maternal viral load had been associated with a higher risk for MTCT (Fawzi et al., 2001; Garcia et al., 1999; Newell, 1998), we quantified the plasma viral load on mothers with available samples. The log viral load range on 29 mothers who transmitted single quasispecies to their infants ranged from 3.667 to 6.234 (mean 4.908, SD 0.726) while in four mothers who transmitted multiple quasispecies to their infants, it ranged from 4.150 to 5.031 (mean 4.755, SD 0.407). Based on this data, no differences in log viral load were detected between these two groups of mothers with available sample (Mann–Whitney test, $P = 0.44$).

Discussion

Not all infants born to HIV-1 infected mothers acquired HIV-1 infection, even if the mothers had high viral loads (Hoelscher et al., 2001). This raises the possibility that selection may play a role during mother-to-child transmission. That one or a few closely related genetic variants can be detected in the blood of newborns during the first month of life has been used as an argument supporting the selection of HIV-1 variants in MTCT. Although genetic variants representing a minor subset of the maternal quasispecies had been detected in the infant in the majority of studies, transmission of multiple variants has also been reported (Ahmad et al., 1995; Dickover et al., 2001; Lamers et al., 1994; Pasquier et al., 1998; Wade et al., 1998; Wolinsky et al., 1992).

Since most HIV-1 infections in Africa are caused by non-B subtypes, we have been interested in determining the role of different HIV-1 genetic subtypes in MTCT. A population in which several HIV-1 subtypes co-circulate is a unique resource for the evaluation of the impact of HIV-1 subtypes in the epidemic. Several reports showed that in Tanzania several subtypes co-circulate in the population (Blackard et al., 2001; Hoelscher et al., 2001; Holme-Hansen et al., 1996; Renjifo et al., 2000; Renjifo et al., 2001; Renjifo et al., 1999). We have shown that in Dar es Salaam, HIV-1 subtypes A, C, D, and their recombinants are circulating in pregnant women and they are being transmitted perinatally (Blackard et al., 2001; Renjifo et al., 1999; Renjifo et al., 2000; Renjifo et al., 2001). Previously we reported that mothers infected with HIV-1 subtype C or intersubtype recombinants were significantly more likely to transmit HIV-1 to their infants compared to mothers infected with subtype D viruses (Blackard et al., 2001; Renjifo et al., 2001). Whether these differences are caused by different selection pressures during in-utero, intra-partum or breastfeeding transmission merits further investigation.

In contrast to other studies that determined whether transmitted viruses are representative of major or minor quasispecies in the maternal blood or genital secretions, this report aimed at establishing whether infants infected in-utero by distinct HIV-1 subtypes have evidence of infection with more than one quasispecies. According to the presence of multiple bands in an HMA, 42 of 53 (79%) infants infected in-utero in this cohort showed a single homogeneous genetic species compatible with transmission of one quasispecies. These results agree with previous findings that transmission in-utero may be associated with selective pressures (Dickover et al., 2001). In addition, transmission at the time of birth has been reported to be primarily associated with single quasispecies (Ahmad et al., 1995; Wolinsky et al., 1992). While transmission of single quasispecies predominated in-utero, our study also showed that transmission of multiple quasispecies occurred in 21% of the cases. This is in agreement with other studies that have demonstrated the in-utero transmission of multiple quasispecies (Lamers et al., 1994; Newell, 1998; Pasquier et al., 1998). Even though there was no difference in log viral load between mothers with single or multiple transmission of quasispecies (Mann–Whitney test, $P = 0.44$), the limited number of mothers with multiple quasispecies and viral load in the analysis limited our ability to draw conclusive evidence regarding the role of viral load and the transmission of multiple quasispecies in-utero.

It is plausible to envision at least two scenarios in which a sample collected from an infant at birth may contain multiple quasispecies. The first one is that the infant was infected by at least two independent and distinct quasispecies from the mother. It has been reported that the genetic variants in the infant could be assigned to distinct quasispecies present in the mother (Dickover et al., 2001; Lamers et al., 1994). The fact that we detected subtype C and subtype D sequences within the same sample is the most clear indication that multiple infection can occur in-utero. The second scenario includes the notion that the infant was infected by a single genetic variant that evolved into genetically distinct quasispecies within the fetus. Although few reports suggested that HIV-1 can be transmitted at any time during pregnancy, it appears that the majority of in-utero transmissions occur toward the end of the pregnancy (Newell, 1998). The time span between the infection in-utero to

the detection within the first 48 h of life may not be sufficient for a single genetic variant to evolve into genetically distinct quasispecies within the fetus. Therefore, the presence of quasispecies in the first hours of life in a newborn infected in-utero probably represents multiple infections by at least two quasispecies. However, the minutes to hours between delivery and sample collection may not be sufficient for the new infecting virus to expand and to become a significant genetic variant detectable by the HMA assay in this study.

Even though mothers infected with HIV-1 subtype C or intersubtype recombinants in this cohort transmitted HIV-1 more frequently to their infants than mothers infected with HIV-1 subtype D viruses, our analysis of in-utero transmission of quasispecies showed no differences between these subtypes (Blackard et al., 2001; Renjifo et al., 2001). If the differences reported in the overall MTCT between subtypes C and subtype D are due to higher transmission of subtype C in-utero, the number of quasispecies transmitted may not be an indication for better fitness for in-utero transmission. The differences in MTCT between subtypes could have been associated with differences in the rate of intra-partum and/or breastfeeding infections.

The lack of association between transmission of multiple quasispecies and viral load suggests that transmission of multiple quasispecies may be related to specific phenotypes shared by the related genetic variants within the infected mother. This finding is another indication that it is not uncommon for HIV-1 to transmit multiple independent viruses at the time of infection. It has been reported that multiple viruses may infect women during primary infection through the heterosexual route (Long et al., 2000; Poss et al., 1995). These results, as well as this study, provide evidence for the beneficial effect of carefully monitored antiretroviral treatments of all infected individuals, aimed at reducing the number of transmissible quasispecies. In addition, they suggest that an effective preventive vaccine must be able to induce an immunological response capable of broadly neutralizing numerous HIV-1 variants across and within different HIV-1 subtypes. A better understanding of factors responsible for the differences in transmission among HIV-1 subtypes may be helpful to establish priorities and strategies for the implementation of therapeutic interventions.

Materials and methods

Samples were collected from infants participating in a randomized double-blind trial to determine whether vitamin supplements reduce the rate of perinatal transmission of HIV-1 in Dar es Salaam, Tanzania (Fawzi et al., 2001; Renjifo et al., 2001). No mothers or infants received ZDV or other anti-retroviral medications during pregnancy, labor, or after delivery. Baseline demographic, clinical, and laboratory information was obtained for each pregnant woman at recruitment. Blood samples were obtained at enrollment and delivery and absolute CD4 lymphocyte counts were measured with the FACS count system (Becton-Dickinson, San Jose, CA). Two hundred microliters of plasma from 33 mothers was used for HIV-1 viral load determination using the HIV-1 Amplicor Monitor version 1.5 assay according to the manufacturer's instructions (Roche Diagnostic Systems, Inc., NJ). Values <2.6 log 10 (400 copies/mL) were considered to be below the level of detection. Infants' peripheral blood mononuclear cells (PBMC) were tested for HIV-1 infection using the Amplicor HIV-1 detection kit (Roche Diagnostic System, Branchburg, NJ). Samples that tested positive for HIV-1 were used for env (C2–C5) PCR amplification, cloning, sequencing, and phylogenetic classification (Renjifo et al., 1999, Renjifo et al., 2001). The nucleotide sequences have been deposited in the GenBank Sequence Database under the accession numbers AY116024 to AY116071, AF03091, AF038103, AF106334.

Heteroduplex mobility assay (HMA) was used to determine the presence of quasispecies in an infant PBMC sample (Delwart et al., 1993). The assumption was that the presence of more than four bands in a newborn sample indicates transmission of more than one viral genetic variant. Heteroduplexes were formed by denaturing 1 μl of a nested PCR at 94 C for 2 minutes in 100 mM NaCl, 10 mM Tris–HCL (pH 7.8) and 2 mM EDTA. Denatured samples were quickly cooled by immersion in an ice bath for 10 minutes. DNA duplexes were separated on a continuous 12.5% polyacrylamide gel using the PhastSystem (Amersham Biosciences, New Jersey). The parameters for gel separation were Sep 1.1, sample down, 100 V, 7 mA, 1 W, 15 C, 0010 Vh; Sep 1.2, sample up, 300 V, 10 mA, 2 W, 15 C, 330 Vh. The gels were stained in the PhastSystem Development Unit using the PhastGel Silver Staining Kit.

The presence of quasispecies was determined by counting the number of bands in each sample lane. A maximum of three bands was theoretically visible in a gel when a genetically homogenous sample was being subjected to a denaturation and renaturation cycle with the fastest migrating band corresponding to homoduplexes (Delwart et al., 1993). A sample containing four or more bands was considered to have at least two genetic variants.

Statistical analyses were performed using Stata v. 7.0 software (Stata Corporation, College Station, TX). Logistic regression models were used for evaluating whether the maternal viral subtype or CD4+ cell count at enrollment affected the odds of quasispecies transmission. All hypothesis tests were two-tailed with $P \leq 0.05$ indicating statistical significance. The Mann–Whitney test was used to evaluate whether maternal viral load was associated with transmission of multiple quasispecies.

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References

Ahmad, N., Baroudy, B.M., Baker, R.C., Chappey, C., 1995. Genetic analysis of human immunodeficiency virus type 1 envelope V3 region isolates from mothers and infants after perinatal transmission. J. Virol. 69, 1001–1012.

Blackard, J.T., Renjifo, B., Fawzi, W., Hertzmark, E., Msamanga, G., Mwakagile, D., Hunter, D., Spiegelman, D., Sharghi, N., Kagoma, C., Essex, M., 2001. HIV-1 LTR subtype and perinatal transmission. Virology 287, 261–265.

Bryson, Y.J., Luzuriaga, K., Sullivan, J.L., Wara, D., 1992. Proposed definitions for in utero versus intrapartum transmission of HIV-1. N. Engl. J. Med. 327, 1246–1247.

Coffin, J.M., 1995. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science 267, 483–489.

Connor, E.M., Sperling, R.S., Gelber, R., Kiselev, P., Scott, G., O'Sullivan, M.J., VanDyke, R., Bey, M., Shearer, W., Jacobson, R.L., Jimenez, E., O'Neill, E., Bazin, B., Delfraissy, J.F., Culnane, M., Coombs, R., Elkins, M., Moye, J., Stratton, P., Balsley, J., 1994. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N. Engl. J. Med. 331, 1173–1180.

Delwart, E.L., Shpaer, E.G., Louwagie, J., McCutchan, F.E., Grez, M., Rubsamen-Waigmann, H., Mullins, J.I., 1993. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. Science 262, 1257–1261.

Dickover, R.E., Garratty, E.M., Plaeger, S., Bryson, Y.J., 2001. Perinatal transmission of major, minor, and multiple maternal human immunodeficiency virus type 1 variants in utero and intrapartum. J. Virol. 75, 2194–2203.

Domingo, E., Martinez-Salas, E., Sobrino, F., de la Torre, J.C. , Portela, A., Ortin, J., Lopez-Galindez, C., Perez-Brena, P., Villanueva, N., Najera, R., Van de Pol, S., Steinhauer, D., DePolo, N., Holland, J., 1985. The quasispecies (extremely heterogeneous) nature of viral RNA genome populations: biological relevance—a review. Gene 40, 1–8.

Fawzi, W., Msamanga, G., Renjifo, B., Spiegelman, D., Urassa, E., Hashemi, L., Antelman, G., Essex, M., Hunter, D., 2001. Predictors of intrauterine and intrapartum transmission of HIV-1 among Tanzanian women. AIDS 15, 1157–1165.

Garcia, P.M., Kalish, L.A., Pitt, J., Minkoff, H., Quinn, T.C., Burchett, S.K, Kornegay, J., Jackson, B., Moye, J., Hanson, C., Zorrilla, C., Lew, J.F., For The Women and Infants Transmission Study Group, 1999. Maternal Levels of Plasma Human Immunodeficiency Virus Type 1 RNA and the Risk of Perinatal Transmission. N. Eng. Jour. Med. 341, 394–402.

Hoelscher, M., Kim, B., Maboko, L., Mhalu, F., von Sonnenburg, F., Birx, D.L., McCutchan, F.E., 2001. The UNAIDS Network for HIV Isolation and Characterization: high proportion of unrelated HIV-1 intersubtype recombinants in the Mbeya region of southwest Tanzania. AIDS 15, 1461–1470.

Holm-Hansen, C., Ayehumie, S., Johansson, B., Nkya, W., Shao, J., Haukenes, G., 1996. HIV-1 proviral DNA sequences of *env* gp41 PCR amplificates from Tanzania. APMIS 104, 459–464.

Lamers, S.L., Sleasman, J.W., She, J.X., Barrie, K.A., Pomeroy, S.M., Barrett, D.J., Goodenow, M.M., 1994. Persistence of multiple maternal genotypes of human immunodeficiency virus type I in infants infected by vertical transmission. J. Clin. Invest. 93, 380–390.

Lathey, J.L., Tsou, J., Brinker, K., Hsia, K., Meyer 3rd, W.A., Spector, S.A., 1999. Lack of autologous neutralizing antibody to human immunodeficiency virus type 1 (HIV-1) and macrophage tropism are associated with mother-to-infant transmission. J. Infect. Dis. 180, 344–350.

Long, E.M., Martin, H.K., Kreiss, J.K., Rainwater, S.M., Lavreys, L., Jackson, D.J., Rakwar, J., Mandaliya, K., Overbaugh, J., 2000. Gender differences in HIV-1 diversity at time of infection. Nat. Med. 6, 71–75.

Newell, M.L., 1998. Mechanism and timing of mother-to-child transmission of HIV-1. AIDS 12, 831–837.

Pasquier, C., Cayrou, C., Blancher, A., Tourne-Petheil, C., Berrebi, A., Tricoire, J., Puel, J., Izopet, J., 1998. Molecular evidence for mother-to-child transmission of multiple variants by analysis of RNA and DNA sequences of human immunodeficiency virus type 1. J. Virol. 72, 8493–8501.

Poss, M., Martin, H.L., Kreiss, J.K., Granville, L., Chohan, B., Nyange, P., Mandaliya, K., Overbaugh, J., 1995. Diversity in virus populations from genital secretions and peripheral blood from women recently infected with human immunodeficiency virus type 1. J. Virol. 69, 8118–8122.

Renjifo, B., Gilbert, P., Chaplin, B., Vannberg, F., Mwakagile, D., Msamanga, G., Hunter, D., Fawzi, W., Essex, M., 1999. Emerging recombinant human immunodeficiency viruses: uneven representation of the envelope V3 region. AIDS 13, 1613–1621.

Renjifo, B., Chaplin, B., Mwakagile, D., Shah, P., Vannberg, F., Msamanga, G., Hunter, D., Fawzi, W., Essex, M., 2000. Epidemic expansion of HIV type 1 subtype C and recombinant genotypes in Tanzania. AIDS Res. Hum. Retroviruses 14, 635–638.

Renjifo, B., Fawzi, W., Mwakagile, D., Hunter, D., Msamanga, G., Spiegelman, D., Garland, M., Kagoma, C., Kim, A., Chaplin, B., Hertzmark, E., Essex, M., 2001. Differences in perinatal transmission among human immunodeficiency virus type 1 genotypes. J. Hum. Virol. 4, 16–25.

The Working Group on Mother-to-child Transmission of HIV, 1995. Rates of mother-to-child transmission of HIV-1 in Africa, America, and Europe: results from 13 perinatal; studies. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. 8, 506–510.

Wade, C.M., Lobidel, D., Brown, A.J., 1998. Analysis of human immunodeficiency virus type 1 env and gag sequence variants derived from a mother and two vertically infected children provides evidence for the transmission of multiple sequence variants. J. Gen. Virol. 79, 1055–1068.

Wilson, C.C., Brown, R.C., Korber, B.T., Wilkes, B.M., Ruhl, D.J., Sakamoto, D., Kunstman, K., Luzuriaga, K., Hanson, I.C., Widmayer, S.M., Wiznia, A., Clapp, S., Ammann, A.J., Koup, R.A., Wolinsky, S.M., Walker, B.D., 1999. Frequent detection of escape from cytotoxic T-lymphocyte recognition in perinatal human immunodeficiency virus (HIV) type 1 transmission: the ariel project for the prevention of transmission of HIV from mother to infant. J. Virol. 73, 3975–3985.

Wolinsky, S.M., Wike, C.M., Korber, B.T., Hutto, C., Parks, W.P., Rosenblum, L.L., Kunstman, K.J., Furtado, M.R., Munoz, J.L., 1992. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. Science 255, 1134–1137.