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Diversity of the HIV-1 Long Terminal Repeat Following Mother-to-Child Transmission

Jason T. Blackard,* Boris Renjifo,* Beth Chaplin,* Gernard Msamanga,† Wafaie Fawzi,‡,§ and Max Essex*,[1]

*Department of Immunology and Infectious Diseases, ‡Department of Nutrition, and §Department of Epidemiology, Harvard School of Public Health, 651 Huntington Avenue, Boston, Massachusetts 02115-6017; and †Department of Community Health, Muhimbili Medical Centre, Dar es Salaam, Tanzania

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A study of the human immunodeficiency virus Type 1 (HIV-1) 5′ long terminal repeat (LTR) was performed to determine the extent of variation found within the LTR from 19 mother–infant pairs in Tanzania and to assess whether the LTR is useful in distinguishing maternal sequences that were transmitted to infants. HIV-1 subtypes A, C, and D as well as intersubtype recombinant LTR sequences were detected in mothers and infants. The LTR subtype was 100% concordant between mothers and their infants. Diversity calculations showed a significant reduction in LTR variation in infants compared to their mothers. However, the overall magnitude of LTR variation was less than that found in the *env* gene from the same individuals. These data suggest a selective constraint active upon the 5′ long terminal repeat that is distinct from immune selective pressure(s) directed against HIV-1 structural genes. Detection of maternal LTR variants that were transmitted to infants may yield important information concerning nonstructural determinants of HIV-1 transmission from mother to infant. © 2000 Academic Press

INTRODUCTION

Transmission rates of the human immunodeficiency virus Type 1 (HIV-1) from mother to infant vary significantly from 13 to 43% (Working Group on Mother-To-Child Transmission of HIV, 1995). Differences in maternal disease status, mode of delivery, breastfeeding, and the availability of antiviral therapies may contribute to the varying rates of HIV-1 perinatal transmission (John and Kreiss, 1996). Identification of specific viral features of maternal HIV-1 variants that are transmitted to infants may aid in designing more effective approaches to prevent perinatal transmission. Viral factors such as phenotype (Ometto *et al.,* 1995; Scarlatti *et al.,* 1993a) and genotype (Renjifo *et al.,* 1999) may contribute to the transmission of HIV-1 variants from a mother to her infant.

Because of the error-prone nature of the HIV reverse transcriptase enzyme, extensive variation within the viral genome is generated. HIV within an individual exists as a complex of related but distinct genotypic variants, termed the quasispecies (Wain-Hobson, 1992). As intrapatient variation exists, so does interpatient variation. Based on phylogenetic analyses of samples from diverse geographic locations, at least 11 major HIV-1 subtypes, termed HIV-1 A–K, have been identified to date (Korber *et al.,* 1998; McCutchan *et al.,* 1996; Roques *et al.,* 1999). Intrasubtype variation is typically higher than intrapatient variation but lower than intersubtype variation. *Gag* and *env* sequences from different subtypes show that intersubtype variation may be as high as 14 and 30%, respectively (Burke and McCutchan, 1997). HIV-1 subtypes also have distinct geographic locations. For instance, HIV-1 subtype B is the most prevalent subtype in the Americas and Western Europe, subtype E is the most prevalent subtype in Southeast Asia, and subtypes A, C, and D are the most prevalent subtypes in Africa. Despite accounting for the vast majority of HIV-1 infections globally, genotypic data from epidemiologically related individuals infected with non-B HIV-1 subtypes are extremely limited.

Currently, the number and identity of viral loci that contain potential determinants associated with perinatal transmission of HIV-1 are unknown. Multiple studies have shown that *env* is significantly more diverse in mothers than in newborns (Ahmad *et al.,* 1995; Mulder-Kampinga *et al.,* 1995; Scarlatti *et al.,* 1993b; Wike *et al.,* 1992; Wolinsky *et al.,* 1992). The low heterogeneity of infant *env* sequences has led some researchers to hypothesize that viruses able to escape the maternal immune response or those with specific phenotypic properties are preferentially transmitted to infants (Wike *et al.,* 1992; Wolinsky *et al.,* 1992). Distinct glycosylation patterns in the V3 region of envelope were found to be associated with perinatal transmission. However, subsequent studies have been unable to confirm these findings (Ahmad *et al.,* 1995; Contag *et al.,* 1997; Mulder-Kampinga *et al.,* 1993; Scarlatti *et al.,* 1993b). Similar analyses with *vif* (Yedavalli *et al.,* 1998a) and *vpr* (Ye-

[1] To whom correspondence and reprint requests should be addressed. Fax: (617) 739-8348. E-mail: messex@hsph.harvard.edu.

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davalli *et al.,* 1998b) have shown these genes to be highly conserved during perinatal transmission but have found no signature sequences/motifs associated with transmission.

Although viral populations may be influenced by the immunological response of the host, they may also be influenced by the transcriptional activation of the viral quasispecies. The long terminal repeat (LTR) region of HIV-1 is essential for proviral synthesis, integration of the proviral DNA into the host cell's genome, and regulation of HIV-1 transcription [reviewed in Al-Harthi *et al.* (1998); Gaynor (1992)]. The LTR's central role in the regulation of HIV-1 gene expression suggests that variations within this region of the HIV genome may dramatically affect proviral regulation and expression. A number of studies have suggested that point mutations, insertions, and deletions in the LTR may influence the levels of LTR transactivation (Delassus *et al.,* 1991; Estable *et al.,* 1996; Kirchhoff *et al.,* 1997; Michael *et al.,* 1994). Others have demonstrated that insertions and even single point mutations in the LTR may lead to viruses with a better capacity to replicate and/or direct gene expression (Chene *et al.,* 1999; Golub *et al.,* 1990; Verhoef *et al.,* 1999). Subtype-specific differences in the LTR response to *rel* proteins (Montano *et al.,* 1997; Naghavi *et al.,* 1999) and the cytokine TNF-α (Jeeninga *et al.,* 2000; Montano *et al.,* 2000) have also been shown, further suggesting that LTR variation is more extensive than previously thought.

To date, no data on LTR sequences associated with maternal–infant transmission and few data on the extent of LTR variation within individuals or between individuals infected with non-B subtypes have been collected. It is also unknown whether maternal LTR variants that were transmitted to infants could be identified and whether such variants had common sequence characteristics. We have analyzed 5′ LTR sequences from 19 HIV-1-positive mother–infant pairs from Tanzania to determine whether the LTR might also vary between mother–infant pairs and to determine the relevance of nonstructural regulatory regions to perinatal transmission of HIV-1.

RESULTS

LTR subtype classification

A previous study of the 5′ long terminal repeat from 24 infants in Dar es Salaam, Tanzania, identified HIV-1 subtypes A, C, and D as well as intersubtype recombinant LTRs with multiple recombination patterns (Blackard *et al.,* 1999). In the current study, we have further explored the genetic diversity of the HIV-1 long terminal repeat by amplifying the 5′ LTR from multiple mother–infant pairs infected with different HIV-1 subtypes as well as intersubtype recombinant viruses. The consensus LTR for each individual of 17 of 19 pairs clustered together with bootstraps of greater than 99%, indicating epidemiological linkage of these samples (Fig. 1). The consensus LTR for pair 3 grouped together but with a bootstrap of less than 70%, and the consensus LTR for pair 16 formed a nonsignificant grouping with the consensus sequences of pair 17. Interestingly, these pairs were subsequently shown to possess transmitting maternal LTR variants. This suggests that the presence of multiple maternal sequences in the infant may influence the generation of an accurate consensus LTR sequence in the infant. In all cases, the consensus LTR subtype for the mother matched that of the infant. Additionally, all clones from a mother and her infant typically grouped together with high bootstrap values, suggesting that mothers were unlikely to be infected with multiple LTR subtypes or recombination patterns (data not shown). Five mother–infant pairs (Nos. 1, 2, 3, 4, and 5) were infected with subtype A, 7 pairs (Nos. 13, 14, 15, 16, 17, 18, and 19) were infected with subtype C, 5 pairs (Nos. 7, 8, 9, 10, and 11) were infected with subtype D, and 2 pairs (No. 6 with A/D and No. 12 with D/A/D) were infected with intersubtype recombinant LTRs.

Intrapatient variation

To determine the extent of LTR diversification in epidemiologically linked individuals, multiple clones from each mother or each infant were aligned and used to calculate the LTR intrapatient divergence for each mother–infant pair. The range of intrapatient LTR diversity varied from 0.6 to 2.4% for the 19 mothers, while the range of intrapatient diversity for infants varied from 0.3 to 1.3% (Fig. 2A). For 16 of 19 pairs (84%), the intrapatient LTR divergence of the mother was greater than that of the infant. For one pair (5%), the intrapatient divergence of the infant—14 weeks of age at the time of sample collection—was greater than that of the mother. For two pairs (11%), the LTR intrapatient divergence was equal in the mother and the infant (Table 1). The mean LTR intrapatient variation of the mothers was significantly higher than the mean LTR intrapatient variation of the infants (1.42% versus 0.70%, respectively; $P = 0.0003$) despite collection of infant samples at various times from 6 to 50 weeks after delivery (Fig. 2C). The maternal quasispecies typically displayed more variation than that found within the infant regardless of the HIV-1 subtype.

We also calculated the *env* intrapatient variation using at least five maternal clones or at least three infant clones per pair. The *env* intrapatient variation in mothers ranged from 0.2 to 7.9% (Fig. 2B). The *env* intrapatient variation in infants ranged from 0.2 to 2.8%. In contrast to the LTR intrapatient data, only 12 mothers (63%) had higher *env* variation than their infants. The mean *env* intrapatient variation was significantly higher in mothers than in infants (3.11% versus 0.98%, respectively; $P = 0.011$). The mean LTR variation was also significantly lower than the mean *env* variation for mothers ($P = 0.03$)

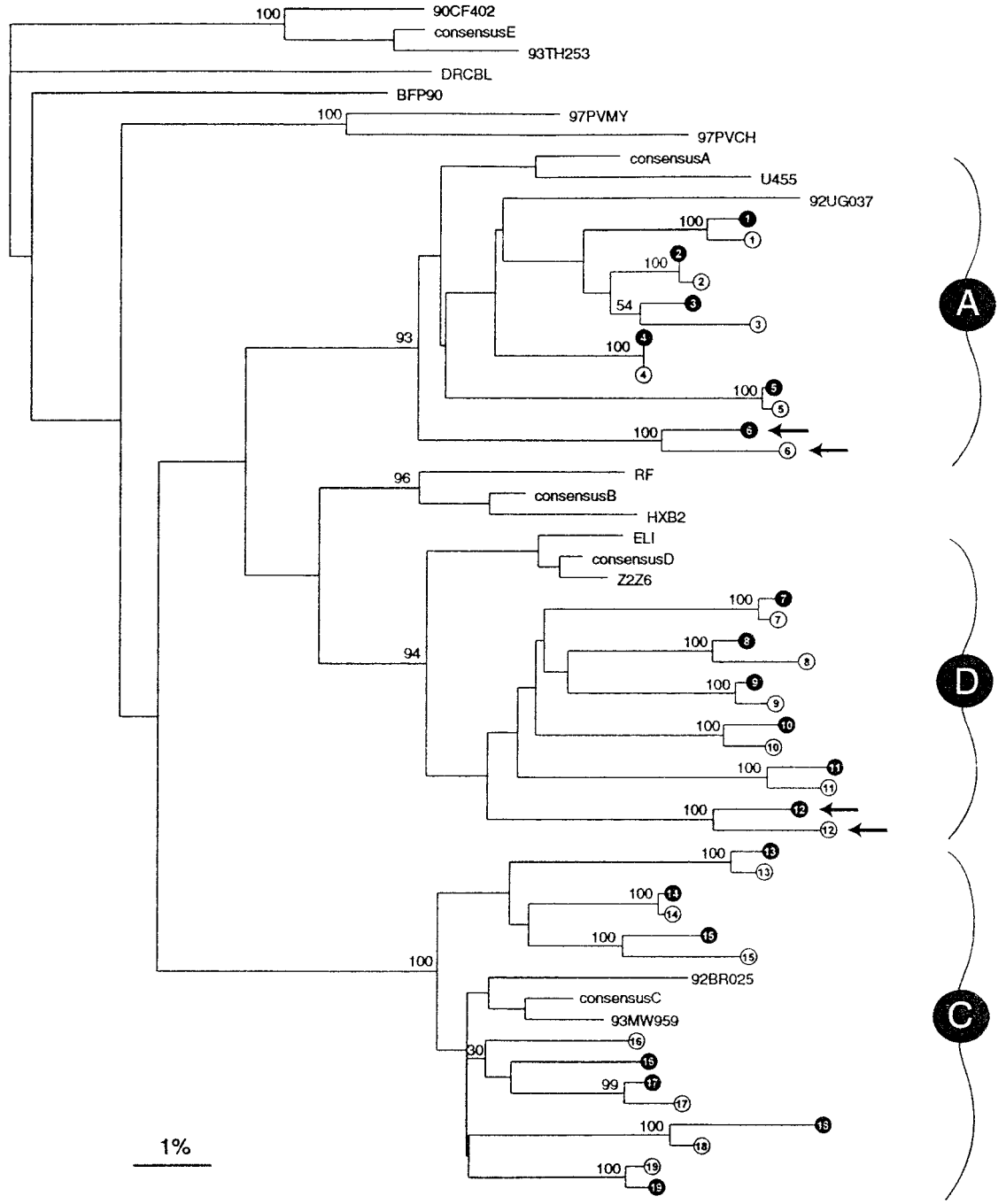
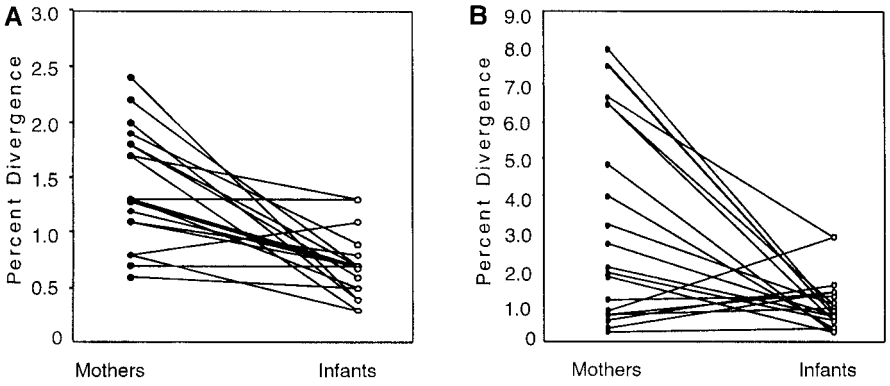


FIG. 1. Consensus LTR sequences were aligned in Clustal W (Thompson *et al.,* 1994) and compared to reference database LTR sequences for HIV-1 subtypes A through E, G, I, and J to assign an HIV-1 subtype. Closed circles denote maternal LTR sequences, while open circles denote infant LTR sequences. Arrows indicate intersubtype recombinant LTRs. Bootstrap values (out of 100) showing each mother–infant pair are indicated. Similar results were obtained using maximum parsimony (data not shown).

(Fig. 2C). However, a similar comparison for infants found that LTR and *env* showed equivalent levels of intrapatient diversity ($P = 0.26$).

Transmission of LTR variants

To address the issue of LTR variants that were successfully transmitted from the maternal blood to her infant, separate phylogenetic analyses were performed for each mother–infant pair. The 10 clones from the mother and the 5 clones from the infant for each pair were aligned and phylogenetic trees of each pair demonstrated the presence of multiple maternal genotypes in most mothers regardless of LTR subtype. In contrast, most infants were infected with a highly homogenous



C

	LTR	ENV	
Mothers	1.42% (0.6 - 2.4)	3.11% (0.2 - 7.9)	p = 0.03
Infants	0.70% (0.3 - 1.3)	0.98% (0.2 - 2.8)	p = 0.26

FIG. 2. (A) The LTR intrapatient variation was calculated using 10 maternal clones or 5 infant clones. The percentage divergence is indicated as a closed circle (mother) or open circle (infant); however, due to similar values in some individuals, several circles represent more than one individual. Each horizontal line represents one mother–infant pair. (B) Envelope C2–C5 intrapatient variation was calculated using at least 5 maternal clones or at least 3 infant clones. (C) Mean LTR and *env* intrapatient variation for mothers and infants is shown in boldface type with ranges in parentheses.

LTR population. The relationship between maternal viral genotypes and those actually transmitted to infants was assessed by phylogenetic tree analyses. Transmitted maternal LTR sequences were defined as those sequences within the mother that formed a monophyletic grouping with all infant LTR sequences. Transmitted maternal LTR variants were detected in 7 mothers (Fig. 3a). For these 7 pairs, 1 to 5 maternal clones were highly related to infant variants and termed transmitted maternal variants. For example, 2 maternal LTR variants that form a subcluster (93 of 100 bootstrap values) with all infant LTR sequences were detected in pair 1. For pair 3,

TABLE 1

Pair name	HIV-1 subtype		Infant age at draw (in weeks)	Highest variability	
	LTR	*env*		LTR	*env*
1*	A	A	6	Mother	Mother
2	A	A	26	Equal	Mother
3*	A	A	10	Mother	Mother
4*	A	DCD	14	Mother	Mother
5	A	D	26	Mother	Infant
6	AD	A	39	Mother	Mother
7	D	D	39	Mother	Infant
8*	D	A	26	Mother	Infant
9	D	A	8	Mother	Mother
10	D	D	6	Mother	Mother
11	D	D	50	Mother	Mother
12	DAD	A (mother) & D (infant)	10	Mother	Mother
13	C	C	6	Mother	Infant
14*	C	C	6	Mother	Mother
15	C	A	6	Mother	Infant
16*	C	C	6	Mother	Infant
17	C	C	6	Mother	Mother
18*	C	C	14	Infant	Infant
19	C	C	14	Equal	Mother

Note. For 16 of 19 mother–infant pairs, the mother was the more heterogeneous individual of the pair when the LTR was analyzed. For 12 mother–infant pairs, the mother was the more heterogeneous individual when *env* was analyzed. Asterisks denote mother–infant pairs for which transmitting maternal LTR variants were detected.

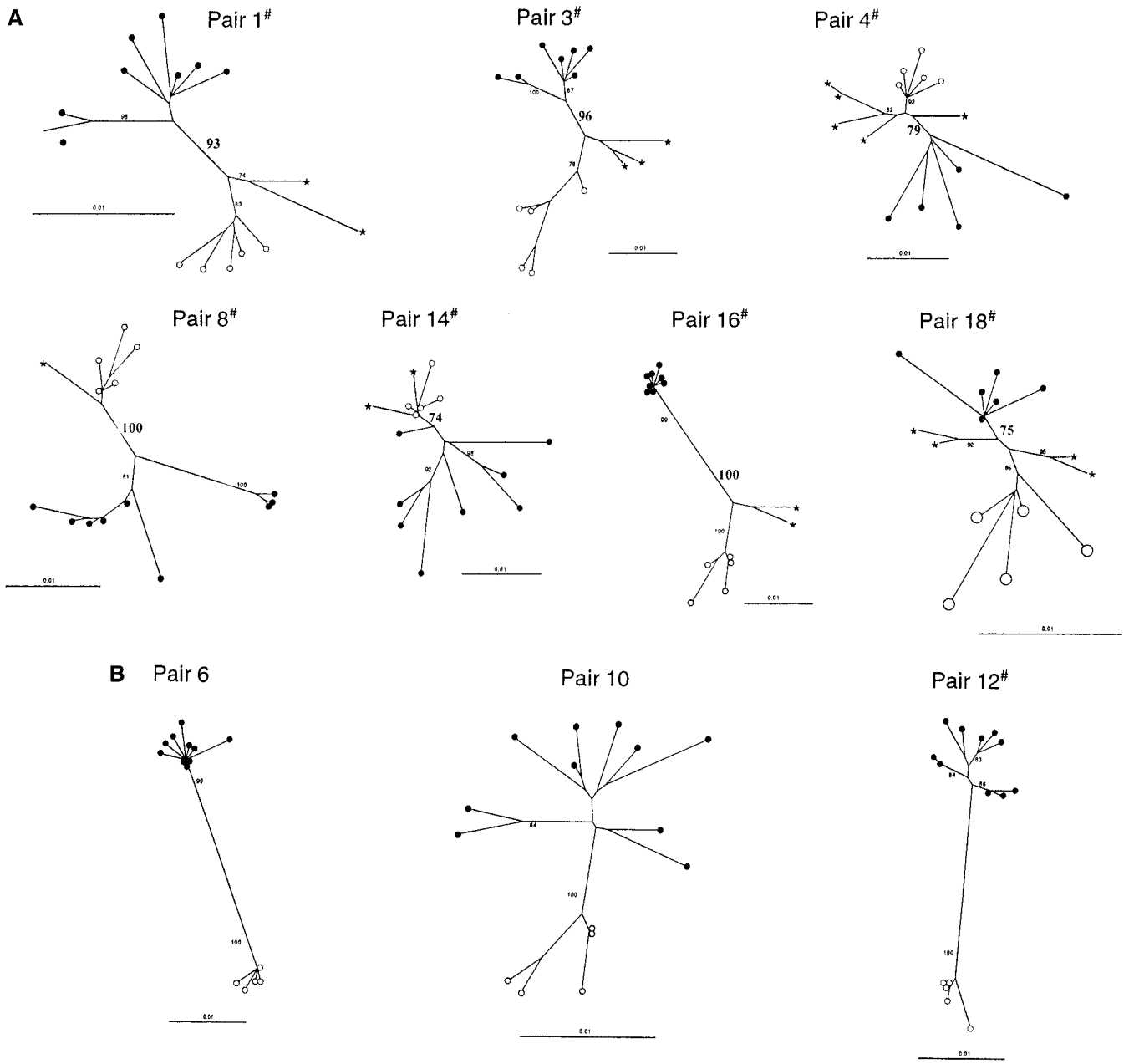


FIG. 3. (a) Maternal LTR variants that were transmitted were determined by aligning the 10 clones per mother and 5 clones per infant for each pair. Only mother sequences that clustered monophyletically with infant sequences with a bootstrap larger than 70 were considered significant. Closed circles denote maternal LTR sequences, while open circles denote infant LTR sequences. Relevant bootstrap values greater than 70% are given. Asterisks denote maternal LTR variants that were transmitted. A pound sign denotes the presence of multiple maternal LTR genotypes. (b) Three representative pairs showing no detectable maternal LTR variants that were transmitted to infants are given.

3 distinct subclusters of maternal LTR variants were detected: one grouping of 2 variants (100 of 100 bootstrap values), a second grouping of 5 variants (87 of 100 bootstrap values), and a third grouping of 3 maternal variants with all 5 infant clones (96 of 100 bootstrap values). The consensus LTRs for these transmitting mothers were classified as 3 subtype As (pairs 1, 3, and 4), 3 subtype Cs (pairs 14, 16, and 18), and 1 subtype D (pair 8). The infants ranged from 6 to 26 weeks of age (average = 11.7 weeks) at the time of sample collection.

No transmitted maternal LTR variants, as defined in this study, were detected in the remaining 12 mothers despite the presence of multiple maternal variants. For instance, all 10 maternal LTR sequences from pair 6 form a tight subgroup (93 of 100 bootstrap values) that is distinct from all 5 infant sequences (Fig. 3b). These infants ranged from 6 to 50 weeks of age (average = 19.7 weeks) at the time of sample collection. Maternal LTR sequences were analyzed for potential sequence determinants of perinatal transmission. However, due to the

sample size, we were unable to detect features common to all maternally transmitted LTR variants (data not shown).

CONCLUSIONS

Despite its role in viral transcription and potential roles in cellular tropism (Chen *et al.,* 1984; DesGroseillers *et al.,* 1983a; Rosen *et al.,* 1985; Speck *et al.,* 1990b) and disease specificity (Chatis *et al.,* 1983; DesGroseillers and Jolicoeur, 1984; DesGroseillers *et al.,* 1983b; Speck *et al.,* 1990a; Stoye *et al.,* 1991), the extent of variation of the HIV-1 5′ long terminal repeat is largely unknown. Currently, the majority of perinatal transmission of HIV-1 occurs in Africa where non-B HIV-1 subtypes are the prevalent circulating viruses. In the current study of 19 mother–infant pairs from Tanzania, the LTR was classified as HIV-1 subtypes A, C, or D with two pairs having intersubtype recombinant LTRs.

The consensus sequences for a mother and her infant indicate that, using a phylogenetic approach, the 5′ long terminal repeat can be used to identify epidemiologically linked individuals such as mother–infant pairs and to identify maternal variants that were successfully transmitted to the infant. We noted 100% concordance between the mother LTR subtype and that of the infant. The monophyletic nature of LTR sequences from each mother or infant suggests that infections with multiple LTR subtypes are rare or that such infections were not detected because they represent a very small fraction of the total LTR quasispecies. We were able to show that maternal long terminal repeat diversity was generally higher than that of the infant, although there were several instances of transmission of multiple LTR variants to the infant. For instance, two or more maternal sequences were detected in infants of pairs 1, 3, 4, 14, 16, and 18. Maternal LTR diversity was high regardless of the infecting subtype or recombination pattern detected. The loss of sequence heterogeneity following transmission suggests that a limited number of maternal genomes selected during perinatal transmission are responsible for the subsequent infection of the infant. However, we did note that for a single pair, the infant LTR quasispecies (sample taken at 14 weeks) was more diverse than that of the mother, suggesting transmission of multiple maternal variants or multiple infant infections with HIV-1. The vertical transmission of multiple variants may be a function of the long exposure period of the infant *in utero,* the size of the viral inoculum to which the infant may be exposed during delivery, or subsequent reinfection of the infant during breastfeeding. In addition, we have shown that the variants that are transmitted from mother to infant may represent up to 50% of the maternal LTR quasispecies.

A study of variation over time in the 3′ LTR and the *nef* overlap coding region found that *nef* was significantly more diverse than the 3′ LTR (McNearney *et al.,* 1995). The authors concluded that the LTR was under greater selective constraint than *nef.* Using mother–infant pairs, we have also noted limited sequence diversity in the 5′ LTR. LTR intrapatient variation was significantly less than that found in a structural gene of mothers. Despite this limited LTR variation, we did find a statistically significant reduction in the 5′ LTR variation in infants compared to mothers following perinatal transmission. As the 5′ LTR itself does not encode proteins capable of interacting directly with the host immune system, maternal immune selection pressures do not contribute significantly to the low diversity of the infant LTR quasispecies found in the current study. However, immune selection pressures acting upon Tat and the Nef overlap region of the 3′ LTR may indirectly influence LTR diversity. One likely selection pressure influencing LTR quasispecies diversity—and subsequent transmission—is transcriptional fitness.

The long terminal repeat is responsive to a number of cytokines, transcriptional activators, and viral proteins. However, like other HIV-1 loci, the LTR exists as a quasispecies of distinct LTR variants. Small nucleotide changes may impact interactions of the LTR with signaling molecules resulting in a range of transactivation from LTR sequences derived from a single individual. One study has demonstrated that a single point mutation in the LTR abolished responsiveness to one cellular protein while simultaneously increasing responsiveness to another protein (Verhoef *et al.,* 1999). These observations have led some researchers to suggest that HIV-1 gene regulation by the long terminal repeat may be linked to HIV-1 transmission (Al-Harthi *et al.,* 1998, 1999; Hashemi *et al.,* 1999; Montano *et al.,* 1997). Those LTR variants that consistently respond to the best to the host cell's signaling molecules will likely be transactivated more than less responsive LTRs, thereby leading to higher levels of transcription from these LTRs. We have previously demonstrated that HIV-1 subtypes respond differentially to nuclear factor κB (NF-κB)/*rel* proteins (Montano *et al.,* 1997) and the pro-inflammatory cytokine, TNF-α (Jeeninga *et al.,* 2000; Montano *et al.,* 2000). Similarly, transcriptional activation of particular LTR variants within an infected mother during pregnancy or during breastfeeding may increase the likelihood of transmission of these activated variants when compared to long terminal repeats with minimal transcriptional activity. Thus, persistent LTR variants found within infants may reflect increased transcriptional fitness of such variants in the mothers relative to LTR variants that were not transmitted. This hypothesis is compelling in light of the growing body of literature linking HIV-1 levels to sexually transmitted diseases and other components of the vaginal microflora. For instance, a factor identified in the female genital tract has been reported to increase HIV-1 gene expression via the viral long terminal repeat (Al-Harthi *et*

al., 1998; Spear *et al.,* 1997). Similar studies have demonstrated that *Treponema pallidum* lipoproteins (Theus *et al.,* 1998), bacterial vaginosis-associatd microorganisms (Al-Harthi *et al.,* 1999; Hashemi *et al.,* 1999), and components of the normal vaginal flora (Klebanoff *et al.,* 1999) may also induce HIV-1 expression. These data suggest the presence of mechanisms regulating HIV-1 gene expression that may be distinct from those influencing other genomic regions. For structural proteins, the maternal immune system is the most likely selection pressure influencing perinatal transmission. However, for nonstructural regions of the HIV-1 genome such as the long terminal repeat, immunoselective pressures are less likely to impact variability and/or transmission. Factors present in the female reproductive tract may influence both genital tract viral load and the likelihood of sexual and perinatal transmission of HIV-1.

To our knowledge, this is the largest number of mother–infant pairs studied to date and the first study to analyze LTR variants associated with perinatal transmission. Further analysis of viral variants transmitted from mothers to infants may further aid researchers in the development of new strategies for the prevention and treatment of HIV. It has been suggested recently that HIV-1 *env* variants from blood and genital secretions of the same individual may represent distinct viral populations (Overbaugh *et al.,* 1996; Poss *et al.,* 1995; Shaheen *et al.,* 1999; Zhu *et al.,* 1996). Similarly, LTR variants in the peripheral blood may not adequately reflect the transmitted virus population; therefore, further inclusion of sequences from vaginal fluid or breast milk is needed for future studies. The use of phylogenetic methods to distinguish transmitted viral variants from nontransmitted variants may be particularly useful in establishing HIV-1 sequences important for transmission.

In conclusion, our results suggest that multiple host–virus interactions may occur during perinatal transmission. We propose that there are multiple selection pressures that act upon different genomic regions of HIV-1. These pressures may include the host immune system acting upon structural regions as well as transcriptional activation and fitness acting upon nonstructural regions of the HIV-1 genome such as the 5′ long terminal repeat.

MATERIALS AND METHODS

Study population and sample collection

Mothers and infants participated in a randomized double-blind trial to determine whether vitamin supplements may reduce the rate of perinatal transmission of HIV-1 in Dar es Salaam, Tanzania (Fawzi *et al.,* 1998, 2000). All mothers were classified as stage 1 (84%) or stage 2 (16%) according to the World Health Organization HIV disease stage classification. To determine the correlation between the HIV-1 subtype in the infant and that of the mother, 22 pairs were selected. Whole blood was collected from mothers at delivery and from infants between 6 and 50 weeks of age. Peripheral blood mononuclear cells (PBMCs) were separated by centrifugation on Ficoll density gradients. Mother and infant samples were coded before amplifications and uncoded after phylogenetic analyses had been performed to ensure quality control of all sequences. Mother and infant samples were processed separately to avoid potential contamination. BLAST searches were performed to confirm that no contamination between samples or known viral isolates had occurred (Altschul *et al.,* 1997).

LTR and envelope amplifications

PBMC crude cell lysates were used in a heminested polymerase chain reaction to amplify a 725-nucleotide fragment including the 5′ long terminal repeat and the 5′ untranslated region. Primers used for the first-round amplification were LTR-A (nucleotides 63–92 of HXB2) (Korber *et al.,* 1998) and PBS-B (nucleotides 790–815). Primers for the second round amplification were LTR-A and PBS-D (nucleotides 764–787). Amplification conditions were 2 min at 94°C, followed by 31 cycles of 1 min at 94°C, 1 min at 56°C, 1 min at 72°C, and a final extension step of 5 min at 72°C. One reaction with HIV-1-negative cell extracts and another reaction with no DNA were included in every PCR amplification. The 5′ LTR from two maternal samples and one infant sample could not be amplified; therefore, sequences from 19 mother–infant pairs were further analyzed. Amplification of the envelope C2–C5 region has been previously described (Renjifo *et al.,* 1999). PCR amplicons were gel purified and ligated into the PCR2.1-TOPO vector. Plasmids were propagated and purified according to standard laboratory protocols prior to sequencing using an ABI 373 automated sequencer.

Phylogenetic analyses

A consensus LTR for each individual was generated using 10 clones per mother or 5 clones per infant. Manual editing of consensus alignments was performed and consensus LTR sequences for each individual were aligned with reference LTR sequences using the neighbor-joining method of Clustal W (Thompson *et al.,* 1994). The database reference LTR sequences for HIV-1 subtypes A through E, G, I, and J used to assign the HIV-1 subtype include the following: A, consensusA, U455, 92UG037; B, consensusB, HXB2, RF; C, consensusC, 92BR025, 93MW959; D, consensusD, Z2Z6, EL1; E, consensusE, 93TH253, 90CR402; G, DRCBL; I, 97PVMY, 97PVCH; and J, BFP90 (Korber *et al.,* 1998). Consensus sequences were analyzed using the Recombinant Identification Program (RIP) with a 100-bp window and gap stripping (Siepel *et al.,* 1995). A four-sequence alignment including the putative recombinant sequence, the con-

sensus sequence of the two putative parental subtypes, and an outlier (consensus O) was used to localize breakpoints for putative intersubtype LTR recombinant sequences. Additional phylogenetic analyses with HIV-1 subtype A, C, and D references were performed to confirm that sequences upstream (5′) of the recombinant breakpoint belonged to one subtype while sequences downstream (3′) of the breakpoint belonged to a different subtype. Only when agreement occurred between both RIP and the breakpoint analysis were sequences considered to be intersubtype LTR recombinants. Intrapatient pairwise distances were calculated using the Kimura method of MegAlign (DNASTAR, Inc., Madison, WI) with separate alignments of the 10 maternal or 5 infant clones. The maternal LTR sequences that were transmitted were determined by aligning the 10 clones per mother and 5 clones per infant for each pair in Clustal W (Thompson *et al.,* 1994). Statistical robustness and reliability of the branching order within each phylogenetic tree were confirmed by bootstrap analysis (Felsenstein, 1985). Only maternal sequences that formed a monophyletic grouping with infant sequences with a bootstrap value larger than 70% were considered significant. Here we use maternal "variants" to refer to maternal LTR sequences that were significantly associated with infant LTR sequences, thereby distinguishing maternal transmitted sequences from those not transmitted. Consensus nucleotide sequences have been submitted to GenBank under Accession Nos. AF239621–AF239658.

Statistical analyses

Intrapatient variation within both mothers and infants was not normally distributed; therefore, nonparametric analyses were performed. The Wilcoxon sign rank test was used to test the hypothesis that the median difference in intrapatient variation between mothers and infants was zero. *P* values of less than 0.05 were considered statistically significant.

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