JOURNAL OF VIROLOGY, Feb. 1998, p. 1092–1102 Vol. 72, No. 2
0022-538X/98/$04.00+0

Conservation of an Intact *vif* Gene of Human Immunodeficiency Virus Type 1 during Maternal-Fetal Transmission

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Received 21 August 1997/Accepted 20 October 1997

The human immunodeficiency virus type 1 (HIV-1) *vif* gene is conserved among most lentiviruses, suggesting that *vif* is important for natural infection. To determine whether an intact *vif* gene is positively selected during mother-to-infant transmission, we analyzed *vif* sequences from five infected mother-infant pairs following perinatal transmission. The coding potential of the *vif* open reading frame directly derived from uncultured peripheral blood mononuclear cell DNA was maintained in most of the 78,912 bp sequenced. We found that 123 of the 137 clones analyzed showed an 89.8% frequency of intact *vif* open reading frames. There was a low degree of heterogeneity of *vif* genes within mothers, within infants, and between epidemiologically linked mother-infant pairs. The distances between *vif* sequences were greater in epidemiologically unlinked individuals than in epidemiologically linked mother-infant pairs. Furthermore, the epidemiologically linked mother-infant pair *vif* sequences displayed similar patterns that were not seen in *vif* sequences from epidemiologically unlinked individuals. The functional domains, including the two cysteines at positions 114 and 133, a serine phosphorylation site at position 144, and the C-terminal basic amino acids essential for *vif* protein function, were highly conserved in most of the sequences. Phylogenetic analyses of 137 mother-infant pair *vif* sequences and 187 other available *vif* sequences from HIV-1 databases revealed distinct clusters for *vif* sequences from each mother-infant pair and for other *vif* sequences. Taken together, these findings suggest that *vif* plays an important role in HIV-1 infection and replication in mothers and their perinatally infected infants.

The majority of AIDS cases in children occur as a result of mother-to-infant transmission of human immunodeficiency virus type 1 (HIV-1), at an estimated rate of more than 30% (1, 8, 12, 13, 24, 29, 37, 38, 50, 54, 61, 65). However, the molecular mechanisms and the factors involved in perinatal transmission are not known, making it difficult to develop strategies for the prevention and treatment of HIV-1 infection in children. Several maternal factors, including the advanced clinical stage of the mother, low $CD4^+$ cell counts, maternal immune response to HIV-1 antigenemia, recent infection, high levels of circulating HIV-1, and maternal disease progression, have been implicated in an increased risk of mother-to-infant transmission of HIV-1 (1, 5, 7, 8, 13, 20, 22, 49, 50). Furthermore, the possibility of viral determinants associated with maternal transmission cannot be discounted, since more than half of the children born to HIV-1-infected mothers are uninfected.

In addition to the usual retroviral *gag*, *pol*, and *env* genes, HIV-1 has several regulatory and accessory genes, including *tat*, *rev*, *nef*, *vif*, *vpu*, and *vpr*. Genetic variability in HIV-1 has been observed in several regions of the genome but mainly in the variable regions of the envelope gene within infected individuals (41). Little sequence information is available on HIV-1 accessory and regulatory genes within and among infected individuals (41). HIV-1 variants arise during retroviral replication by errors made during reverse transcription (11, 47, 48) because of immunologic pressure for change, alteration in cell tropism, and replication efficiency (23, 34, 55, 57). Several studies have shown a correlation between viral dynamics and HIV-1 disease progression (19, 25, 31, 70).

To understand the molecular mechanisms involved in mother-to-infant transmission of HIV-1, we (2) and others (39, 40, 53, 69) have shown that the minor HIV-1 genotypes of infected mothers are transmitted to their infants. This conclusion was based on an analysis of HIV-1 *env* sequences from mother-infant pairs following perinatal transmission. The sequences from mothers were found to be more heterogeneous than the sequences from infants. Initially, the minor genotypes in the infants predominated as a homogeneous sequence but became more diverse as the infants grew older (2, 39). Recently, greater HIV-1 genetic distances relative to the time of infection were shown for infected children with low virion-associated RNA levels and slow disease progression relative to children with high virion-associated RNA levels and rapid disease progression (19). In addition, selective transmission was demonstrated for sexual transmission of HIV-1 from transmitters to recipients, including a homogeneous sequence population present in the recipients (10, 35, 45, 68, 72–74).

Genetic analysis of HIV-1 sequences in other regions of the genome, in addition to the variable regions of *env*, from mother-infant pairs following perinatal transmission has been very limited. The possibility exists, however, that several other regions or motifs in the HIV-1 genome are involved in mother-to-infant transmission and are critical determinants of perinatal transmission. Since the accessory gene *vif* is highly conserved and functional during natural infection (60, 66), there should be a high prevalence of intact *vif* open reading frames in HIV-1 maternal-fetal isolates that are involved in perinatal transmission. Therefore, we sought to examine *vif*

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TABLE 1. Demographic, clinical, and laboratory findings for HIV-1-infected mother-infant pairs

Patient	Age	Sex[a]	Race[b]	$CD4^+$ lymphocytes/mm^3 (%)	Antiretroviral drug[c]	Clinical evaluation[d]
Mothers						
A	36 yr		B	706	None	Asymptomatic
B	28 yr		B	509	None	Asymptomatic
C	23 yr		W	818	None	Asymptomatic
D	31 yr		W	480	None	Asymptomatic
E	26 yr		B	395	ZDV	Symptomatic AIDS
Infants						
A	6 wk	F	B	2,994 (53)	None	Asymptomatic, P1A
B	4.75 mo	M	B	1,942 (42)	None	Asymptomatic, P1A
C	14 mo	F	W	772 (26)	ZDV	Symptomatic AIDS, P-2A, D1, 3, F
D	28 mo	M	W	46 (8)	ddC	Symptomatic AIDS, P-2A, B, F, failed to respond to ZDV therapy
E	34 mo	M	B	588 (34)	ZDV	Symptomatic AIDS, P-2A, B

[a] F, female; M, male.
[b] B, black; W, white.
[c] ZDV, zidovudine; ddC, zalcitibine.
[d] Evaluation for infants was based on the criteria in reference 9.

sequences from mother-infant pairs following perinatal transmission.

The *vif* open reading frame is conserved among most lentiviruses (42) and facilitates HIV-1 infection and cytopathogenicity (15, 17, 36, 51, 58, 59, 62). In addition, *vif* is required for HIV-1 replication in primary lymphocytes and macrophages (15, 17, 18, 51, 59, 64). These studies suggest that an intact *vif* gene may be required during natural HIV-1 infection. Sova et al. (60) performed sequence analysis of the HIV-1 *vif* gene in DNA from infected patient peripheral blood mononuclear cells (PBMC) and found limited sequence variability and a high level of conservation of *vif* during natural infection. However, an analysis of *vif* sequences following HIV-1 mother-to-infant transmission has not been performed. Since HIV-1 in sexual (73) and vertical (33) transmissions seems to be macrophage tropic and since *vif* is required for viral replication in macrophages, *vif* should have a role in HIV-1 infection and replication in mothers and infants and in perinatal transmission.

In this study, we analyzed *vif* sequences from five mother-infant pairs following perinatal transmission. We show that the *vif* open reading frame was conserved in most of the mother-infant pair sequences. The functional domains required for *vif* function in terms of viral infectivity and replication were also present in most of the mother-infant pair sequences. The data presented here support the notion that an intact *vif* open reading frame is necessary for HIV-1 infection and replication in maternal-fetal isolates that are involved in perinatal transmission.

MATERIALS AND METHODS

Patient population and sample collection. This study was approved by the Human Subjects Committee of the University of Arizona, Tucson, and the Institutional Review Board of the Children's Hospital Medical Center, Cincinnati, Ohio. Written informed consent was obtained for participation in the study. We studied five HIV-1-infected mother-infant pairs. Blood samples were collected from mother-infant pairs; the infants' ages at the time of specimen collection were 6 weeks (infant A), 4.75 months (infant B), 14 months (infant C), 28 months (infant D), and 34 months (infant E). The demographic, clinical, and laboratory findings for the HIV-1-infected mother-infant pairs are summarized in Table 1.

Isolation of DNA from PBMC. PBMC were isolated by a single-step Ficoll-Paque procedure (Pharmacia-LKB) from the whole blood of HIV-1-positive mothers and their infants. DNA was isolated according to a modification of the procedure described by Oram et al. (44). Approximately 10^6 PBMC were centrifuged at 12,000 rpm (Eppendorf model 5417C centrifuge) for 2 min, and the cell pellet was resuspended in 0.5 ml of TNE buffer (0.5 M Tris-HCl [pH 7.5], 0.1 M NaCl, 1 mM EDTA). The suspension was treated with 0.5% sodium dodecyl sulfate and 10 μg of proteinase K (Boehringer Mannheim Biochemicals) per ml at 60°C for 3 h, followed by several extractions with phenol and chloroform. The DNA was precipitated with ethanol, dissolved in 50 to 100 μl of TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA), and sheared by repeated pipetting.

PCR amplification. A two-step PCR amplification, first with outer primers and then with nested or inner primers, was performed to detect the presence of HIV-1 in infected patient PBMC (2). An equal amount of PBMC DNA was used for each HIV-1-infected patient, as determined by end-point dilution (16). For pairs A and B, we used DNA oligonucleotide primers VF4937 (5′-GGACCAG CAAAGCTCCTCTGGAAAGT, nucleotides [nt] 4937 to 4962, sense), VF5710 (5′-CAGTGCAAAAAATTCCCCTCCACAATT, nt 5710 to 5735, antisense), VF52 (5′-GAGAAGCTTTAATACAAGATAATAGTGACAT, nt 4979 to 4999, sense), and VF32 (5′-CTCGGATCCCATAAGTTTCATAGATATGTTG, nt 5988 to 5707, antisense), provided by David Volsky, St. Luke's-Roosevelt Hospital Center (60). The nucleotides are numbered as in HXB-2 (41). PCRs were performed according to the procedure of Ahmad et al. (2–4) with a 25-μl reaction mixture containing 2.5 μl of 10× PCR buffer (100 mM Tris-HCl [pH 8.3], 100 mM KCl, 0.02% Tween 20), 2.5 mM $MgCl_2$, 40 μM (each) dATP, dCTP, dGTP, and dTTP, a 0.2 to 1.0 μM concentration of each outer primer pair, and 2.5 U of ULTma DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). The reactions were carried out at 94°C for 30 s, 45°C for 45 s, and 72°C for 1 min for 35 cycles. In an attempt to amplify a larger fragment encompassing other regions in addition to *vif*, for pairs C, D, and E, we used DNA oligonucleotide primers VIF5 (5′-TGGCAGCAATTTCACCGGTACTA, nt 4580 to 4602, sense) and VPR1 (5′-CAACTTGGCAATGAAAGCAACAC, nt 5916 to 5939, antisense) as outer primers and VIF6 (5′-TCAAGCAGGAATTTGGAA TTCCC, nt 4633 to 4655, sense) and VPR2 (5′-GGTACAAGCAGTTTAGGC TGACT, nt 5875 to 5898, antisense) as inner primers; these primers were synthesized according to the published HIV-1 NL4-3 sequence (41). PCRs were performed as described above with a 25-μl reaction mixture containing 2.5 μl of 10× buffer (25 mM tris-(hydroxymethyl)-methylaminopropanesulfonic acid, sodium salt [TAPS] [pH 9.3]), 50 mM KCl, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 400 μM (each) dATP, dCTP, dGTP, and TTP, a 0.2 μM concentration of each outer primer, and 2.5 U of TaKaRa LA *Taq* polymerase (TaKaRa Biomedicals, Shiga, Japan). The reactions were carried out at 95°C for 30 s, 50°C for 45 s, and 72°C for 3 min for 35 cycles. The amplified DNA products were analyzed by electrophoresis on a 1.2% agarose gel. Negative controls consisting of DNA from PBMC of seronegative individuals were included in each set of reactions and were negative in all the assays. After the first round of PCR, 1 μl of the product was amplified for 25 cycles with the corresponding inner primers at 95°C for 30 s, 55°C for 45 s, and 72°C for 1 min (for inner primers VF52 and VF32) and 95°C for 30 s, 55°C for 45 s, and 72°C for 3 min (for inner primers VIF6 and VPR2). The PCR products were analyzed by electrophoresis on a 1.2% agarose gel. To avoid contamination, all the samples, reagents, and first- and second-round PCR products were kept separately and dispensed in a separate room free from all laboratory DNAs. We also included the known HIV-1 NL4-3 sequence for PCR amplifications as a control to assess errors generated by ULTma DNA polymerase and TaKaRa LA *Taq* polymerase.

Cloning and DNA sequencing. The PCR products amplified by inner primer pairs VF52-VF32 and VIF6-VPR2 were blunt ended with DNA polymerase I (Gibco-BRL, Gaithersburg, Md.), treated with T4 polynucleotide kinase (Gibco-BRL), and cloned into the *Sma*I site of the pGem 3Zf (+) vector (Promega Corp., Madison, Wis.). Individual bacterial colonies were screened for the presence of recombinants by restriction enzyme analysis of plasmid DNA. The clones

with the correct sizes of inserts were selected and propagated for DNA preparation, followed by nucleotide sequencing of 10 to 19 clones for each patient according to the Sequenase protocol (U.S. Biochemical Corp., Cleveland, Ohio) as reported before (2, 4).

Computer alignment and analysis of HIV-1 sequences. The nucleotide sequences of the *vif* genes (576 bp) from the five mother-infant pairs were translated to corresponding amino acid sequences (192 amino acids). Alignments were performed manually, as no positions contained gaps. Pairwise distances, defined as the percentages of mismatches between two aligned nucleotide sequences, were used to study the extent of genetic variability within an individual and between mother and infant. For intraindividual variability (within mother and infant sequence sets), pairwise distances were calculated for all possible comparisons of pairs of sequences within the sets. For interindividual variability (between related mother-infant sets and between epidemiologically unlinked individual sets), each sequence from one set was compared with each sequence from the other set. The selection pressure was calculated as the ratio of nonsynonymous to synonymous substitutions (42) by comparison of all possible pairs of sequences between related mother-infant sets. The phylogenetic analysis was performed with PHYLIP version 3.5 software (14). The tree was built from a distance matrix (function DNADIST) by use of the neighbor-joining method (function NEIGHBOR). The robustness of the neighbor-joining tree was assessed by bootstrap resampling of the multiple alignments (function SEQBOOT). One tree was generated for the 137 sequences of the five mother-infant pairs and the reference HIV-1 NL4-3 sequence (GenBank accession no. U26942), used as a root for the tree display. Another tree contained the sequences of the five mother-infant pairs and 187 outgroup sequences extracted from genetic databases, including 152 *vif* sequences published in three recent studies of the variability of the *vif* gene (60, 63, 66). These outgroup sequences were extracted with Entrez version 6.04 (56), a program for retrieving data from databases, and Sequin version 2.20 (26), a program for managing the data. Entrez allowed us to retrieve and align all the sequences encompassing the *vif* gene present in the genetic databases. Sequin was used to merge the alignment of our sequences with the alignment of the outgroup sequences and to save them in a format compatible with PHYLIP.

Nucleotide sequence accession numbers. The sequences have been submitted to GenBank with accession numbers AF019419 to AF019555.

RESULTS

PCR amplification of the HIV-1 *vif* gene from mother-infant pair PBMC DNA. PCR amplification of the *vif* gene was performed as a two-step procedure as described by Ahmad et al. (2). The first round of amplification was performed with primer pairs VF4937-VF5710 and VIF5-VPR1, followed by nested PCR amplification with primer pairs VF52-VF32 (54) and VIF6-VPR2. The inner primer pairs yielded 728- and 1,246-bp fragments from PBMC DNA of infected mother-infant pairs (data not shown). HIV-1 was not detected in PBMC DNA from a normal donor. An equal amount of PBMC DNA from each HIV-infected patient was used in PCR amplifications, as determined by end-point dilution (16). In addition, we confirmed the PCR results with another set of primers from the *gag* (6) and *env* (2) regions, and the PCR results from the *gag* and *env* regions correlated with those from the *vif* region. To determine errors generated by ULTma DNA polymerase and TaKaRa LA *Taq* polymerase, we included a known HIV-1 sequence (NL4-3) for PCR amplification and sequencing.

Coding potential of the *vif* open reading frame in maternal-fetal isolates. The multiple alignments of the deduced amino acid sequences (192 amino acids) of the *vif* gene of HIV-1 from PBMC DNA of the five mother-infant pairs are shown in Fig. 1A to E. The amino acid sequences were deduced from the nucleotide sequences of 137 different *vif* sequences and were aligned with reference to the subtype B consensus sequence (Fig. 1A to E). The coding potential of the *vif* open reading frame was maintained in most of the 78,912 bp sequenced. We analyzed 137 different *vif* clones, and 123 clones contained an intact *vif* open reading frame, an 89.8% frequency of conservation of the intact *vif* open reading frame. The frequency of defective *vif* genes in our five mother-infant pair sequences was 10.2%. A total of 13 clones contained stop codons, and 1 clone, in mother C (CM09), had a 150-bp (50-amino-acid) deletion at

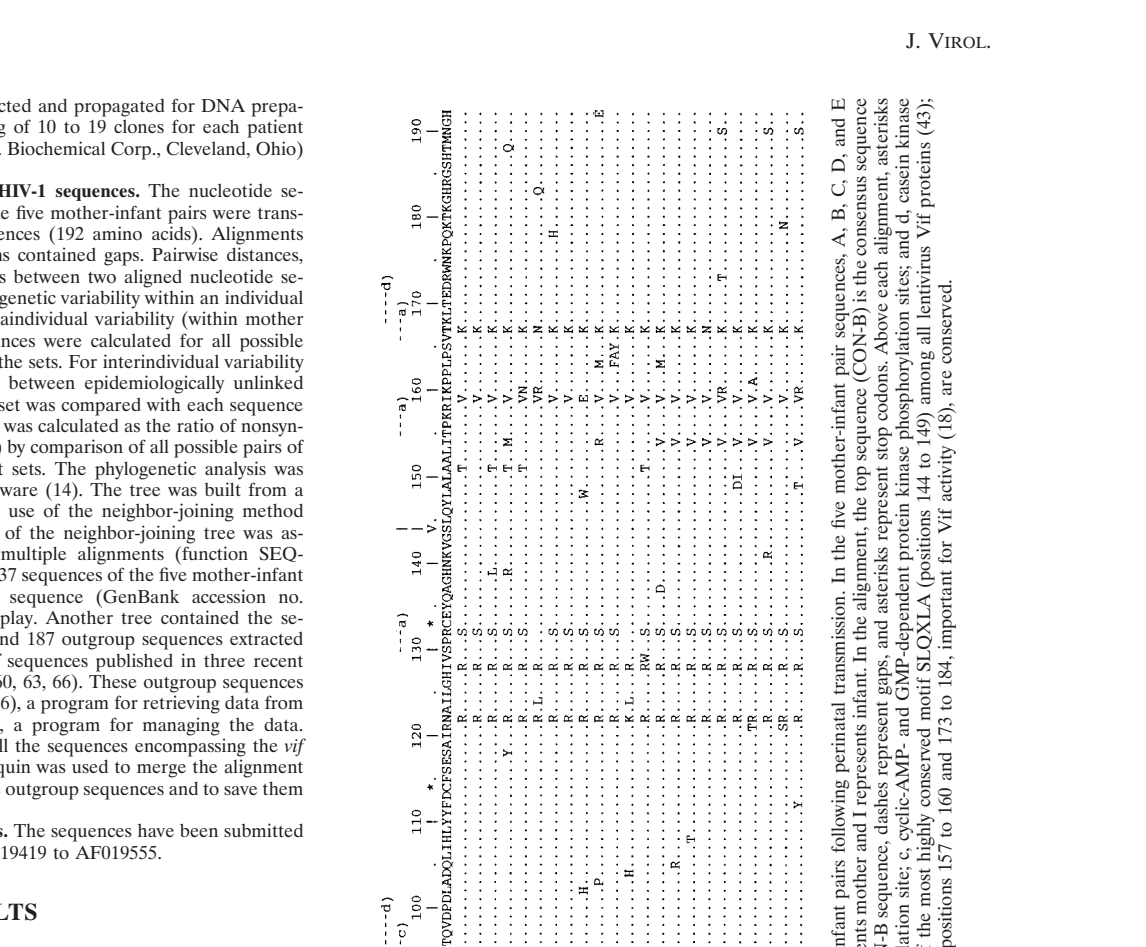
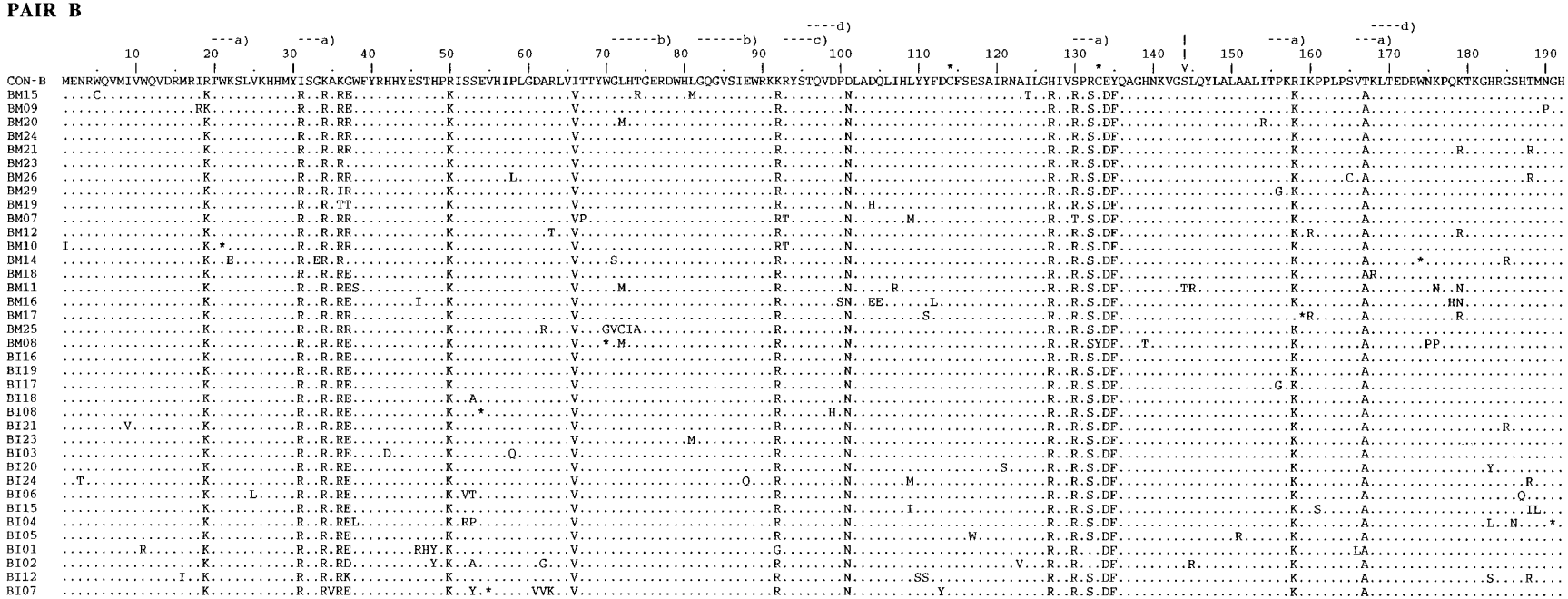


FIG. 1. Multiple alignments of deduced amino acid sequences for the *vif* gene of HIV-1 from five mother-infant pairs following perinatal transmission. In the five mother-infant pair sequences, A, B, C, D, and E correspond to mother-infant pairs A, B, C, D, and E, respectively. In each mother-infant pair sequence, M represents mother and I represents infant. In the alignment, the top sequence (CON-B) is the consensus sequence of the clade or subtype B, as defined elsewhere (41, 60). Dots indicate amino acids identical to those in the CON-B sequence, dashes represent gaps, and asterisks represent stop codons. Above each alignment, asterisks locate the two cysteine residues critical for Vif function; a, protein kinase C phosphorylation site; b, N myristoylation site; c, cyclic-AMP- and GMP-dependent protein kinase phosphorylation sites; and d, casein kinase II phosphorylation site. In addition, above each alignment, a vertical arrow at position 144 indicates a serine of the most highly conserved motif SLQXLA (positions 144 to 149) among all lentivirus Vif proteins (43); the serine at position 144 is critical for Vif function (71). The basic amino acid (lysine and arginine) motifs at positions 157 to 160 and 173 to 184, important for Vif activity (18), are conserved.

PAIR B



PAIR C

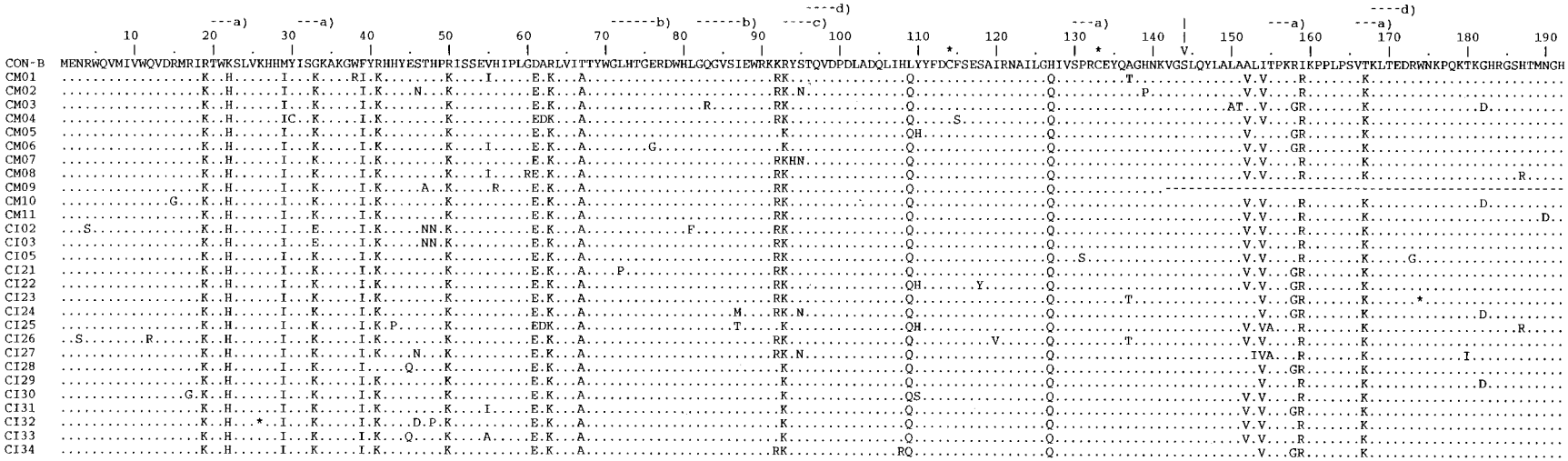
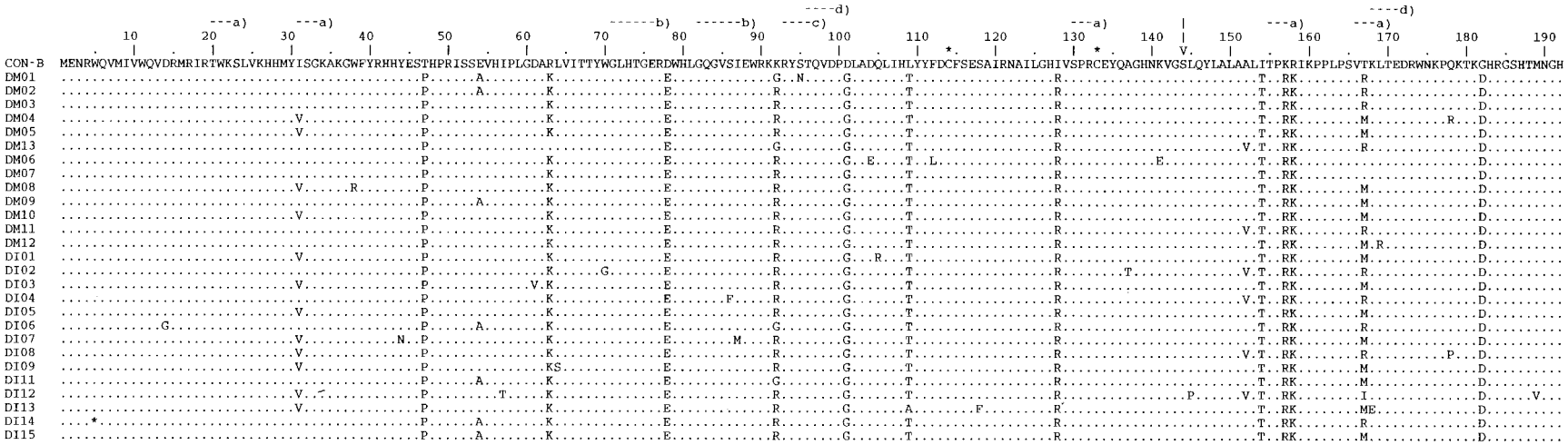


FIG. 1—*Continued.*

PAIR D



PAIR E

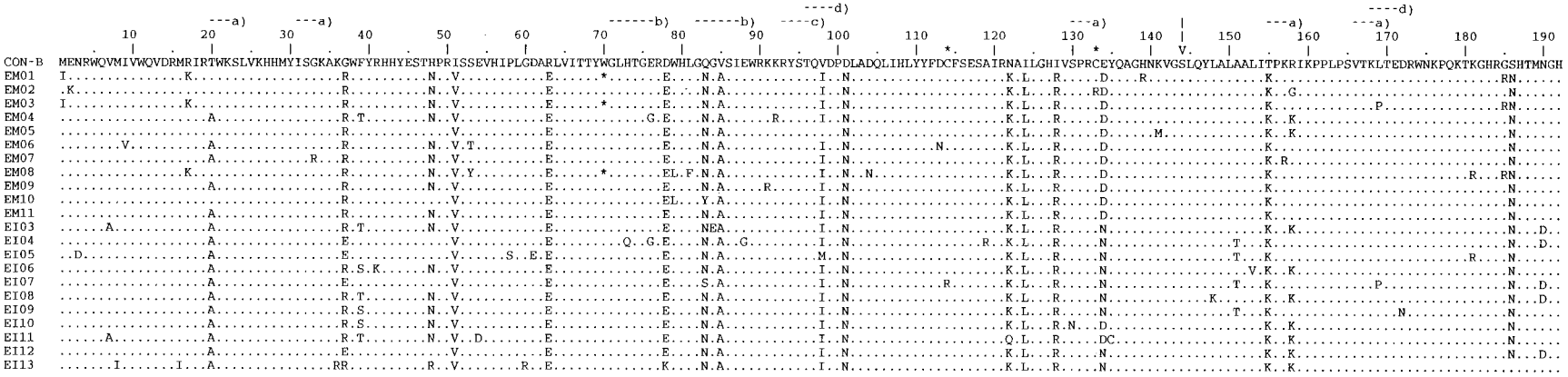


FIG. 1—*Continued.*

TABLE 2. Distances in the *vif* sequences within mother sets, within infant sets, and between mother and infant sets

Sequence	Pair[a]	% Distances[b]								
		Within mother set			Within infant set			Between mother and infant sets		
		Min	Median	Max	Min	Median	Max	Min	Median	Max
Nucleotide	A	1.2	2.6	4.5	1.0	2.5	3.8	1.2	3.1	5.0
	B	0.3	2.1	4.5	0.2	1.8	4.3	0.5	2.3	4.7
	C	0.5	1.6	2.6	0.3	1.9	3.1	0.3	1.8	3.5
	D	0.2	0.9	1.6	0.3	1.2	2.1	0	1.1	2.1
	E	0.3	1.8	3.3	0.9	2.4	4.0	0.9	2.7	4.9
	Total	0.2	1.8	4.5	0.2	2.0	4.3	0	2.2	5.0
Amino acid	A	1.0	4.6	7.3	2.1	4.7	8.3	1.0	5.2	9.9
	B	0.5	3.7	7.3	0	3.3	7.3	5.2	3.7	7.3
	C	1.4	3.0	4.7	1.0	3.5	6.3	0.5	3.2	6.8
	D	0	1.7	3.6	0.5	2.4	4.2	0.5	3.7	7.3
	E	0.5	3.6	6.8	1.6	4.7	7.8	1.6	5.8	9.4
	Total	0	3.3	7.3	0	3.7	8.3	0.5	4.3	9.9

[a] Totals were calculated for all pairs together.
[b] Min, minimum; max, maximum.

the 3′ end. In addition, three clones (BM10, EM01, and EM03) that contained stop codons also lacked initiation codons. These data demonstrated that *vif* sequences directly derived from the five HIV-1-infected mother-infant pairs following perinatal transmission were conserved. The high frequency of intact *vif* open reading frames observed here is in agreement with the data of Sova et al. (60) and Wieland et al. (66), who found intact *vif* in 87 and 90% of clones analyzed, respectively. It is interesting to note that *vif*-encoded amino acid sequences from each mother-infant pair displayed a pattern that was not seen in epidemiologically unlinked pairs (Fig. 1). No common signature sequence was seen in all mother-infant pair sequences. These data suggested that an intact *vif* open reading frame is conserved in maternal-fetal isolates following perinatal transmission.

Comparison of *vif* sequences from epidemiologically linked maternal-fetal isolates. To determine the degree of variability of the *vif* gene from five mother-infant pairs, we analyzed variations in nucleotide and amino acid sequences as shown in Table 2. The nucleotide sequences of the *vif* genes in the mother sets (mothers A, B, C, D, and E) differed by 2.6, 2.1, 1.6, 0.9, and 1.8% (median values), respectively (range, 0.2 to 4.5%). The variability in the infant set (infants A, B, C, D, and E) was similar to that in the mother set: 2.5, 1.8, 1.9, 1.2, and 2.4% (median values), respectively (range, 0.2 to 4.3%). Interestingly, the variability between mother and infant sets (epidemiologically linked pairs A, B, C, D, and E) was also on the same order, 3.1, 2.3, 1.8, 1.1, and 2.7% (median values), respectively (range, 0 to 5%). The median values of amino acid sequence variability for *vif* products for mothers A, B, C, D, and E were 4.6, 3.7, 3.0, 1.7, and 3.6%, respectively; within infants, respective values were 4.7, 3.3, 3.5, 2.4, and 4.7%; and between epidemiologically linked mother-infant pairs, respective values were 5.2, 3.7, 3.2, 3.7, and 5.8%. There was no difference in the variability of *vif* sequences with increasing infant age. However, the variability in general was greater between mother-infant pairs than within mothers or within infants. These results indicated that the sequence variability within and among *vif* clones obtained from mother-infant pairs in our study is on the same order as reported before for *vif* (60, 63) and *gag* (35, 52, 72) genes from infected individuals. We also determined whether the low variability of the *vif* gene from maternal-fetal isolates was due to errors generated by ULTma DNA polymerase or TaKaRa LA *Taq* polymerase. We rarely found any errors generated by ULTma DNA polymerase or TaKaRa LA *Taq* polymerase when using the known sequence of HIV-1 NL4-3 for PCR amplifications and DNA sequencing of the *vif* gene. Despite the low variability in the deduced amino acid sequences for *vif* from the five mother-infant pairs, the sequences from the infants displayed amino acid patterns similar to those of the sequences from their mothers.

Comparison of *vif* sequences from epidemiologically unlinked maternal-fetal isolates. Figure 2 shows histograms of the distributions of the *vif* nucleotide sequence distances within mothers, within infants, between epidemiologically linked mother-infant pairs, and between two epidemiologically unlinked mothers; the medians of the distributions were 1.9, 1.9,

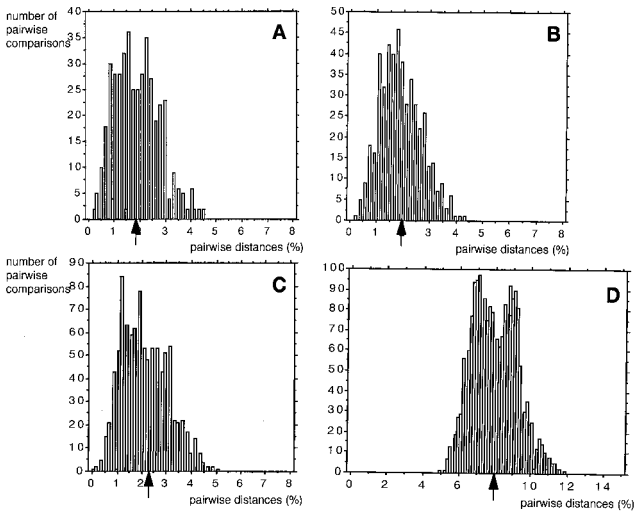


FIG. 2. Distribution of the *vif* gene nucleotide distances between epidemiologically linked and unlinked mother-infant pairs. The percentages of mismatches were calculated between nucleotide sequences within mother sets (A), within infant sets (B), between epidemiologically linked mother-infant pair sequences (C), and between epidemiologically unlinked mother sequences (D). The distance percentages were rounded off to the nearest decimal. The numbers on the *y* axes represent the total numbers of pairwise comparisons that yielded the corresponding percent nucleotide distances. The median values of the distributions (indicated by the arrows) were 1.9% (A), 1.9% (B), 2.1% (C), and 8.0% (D).

2.1, and 8.0%, respectively. The data suggested that *vif* sequences from epidemiologically linked mother-infant pairs were closer than those from epidemiologically unlinked individuals, keeping in mind the fact that a low degree of variability of the *vif* gene was observed in sequences from our five mother-infant pairs and other infected individuals (60, 66). By using the sequence distances for a conserved region such as *vif*, we were able to easily differentiate the epidemiologically unlinked individuals from the epidemiologically linked mother-infant pairs (Fig. 2D). Interestingly, the *vif* sequences from older infants (28 and 34 months old) were closer to those from their mothers (pairs D and E) than to those from epidemiologically unlinked individuals, suggesting that epidemiologically linked viral sequences can be identified even in older infants.

Rates of accumulation of synonymous and nonsynonymous substitutions. The selective pressure on mother-infant pair *vif* sequences was determined by calculating the ratios of nonsynonymous to synonymous substitutions (42). Several HIV-1 sequence analyses have suggested that nonsynonymous/synonymous substitution ratios of more than 1 indicate positive selective pressures by immune responses selecting for escape variants (2, 25, 69, 70, 72). The selective pressure on the *vif* gene, quantified as the ratio of nonsynonymous to synonymous substitutions, showed no evidence for positive selection pressure for change. Comparisons of infant sequences with mother sequences from pairs A, B, C, D, and E gave ratios of nonsynonymous to synonymous substitutions of 0.4, 0.5, 0.2, 0.9, and 0.5, respectively. Thus, there was very little selection pressure (a ratio of <1) on *vif* sequences to change. These values are comparable to (although greater than) those found for the *gag* gene (72).

Phylogenetic tree analysis of *vif* sequences of maternal-fetal isolates. The similarity relationships among the 137 *vif* sequences from the five mother-infant pairs and 187 other *vif* sequences from infected individuals (in HIV-1 databases) were traced and are shown in Fig. 3 and 4. The phylogenetic tree analysis performed as described in Materials and Methods for the 137 *vif* sequences from the five mother-infant pairs revealed that the five mother-infant pairs were well discriminated, separated, and confined within subtrees (Fig. 3), indicating the absence of PCR product cross-contamination (27, 28). These subtrees were equidistant from each other. The root of the tree was the reference HIV-1 NL4-3 sequence. High bootstrap values further emphasized the separation of the subtrees into distinct clusters. Bootstrap analysis performed by resampling the data sets 100 times formed the same clusters of the sequences all 100 times for the five mother-infant pairs. Furthermore, the five subtrees showed homogeneous *vif* sequences in which some mother and infant sequences were intermingled.

In the second phylogenetic tree analysis (Fig. 4), the 137 *vif* sequences from the five mother-infant pairs and 187 other available *vif* sequences were included. These 187 additional *vif* sequences were the result of three independent analyses of the *vif* gene from infected individuals (60, 63, 66) and included 35 sequences belonging to subtypes B, A, A/E, C, and D (in HIV-1 databases). The tree grouped the 324 *vif* sequences in a manner similar to that for the *env* and *gag* genes (2, 35, 41, 69, 70, 72). The largest, starlike cluster contained all subtype B sequences. The subtype A, A/E, and C sequences diverged in a common lineage. Subtype D sequences (ELI and NDK) diverged independently from the center of the tree. In the subtype B cluster, the *vif* sequences from each individual and from each mother-infant pair were grouped closely together in subtrees. The average genetic distance between two sequences of subtype B (intra- and interindividual distances included) was

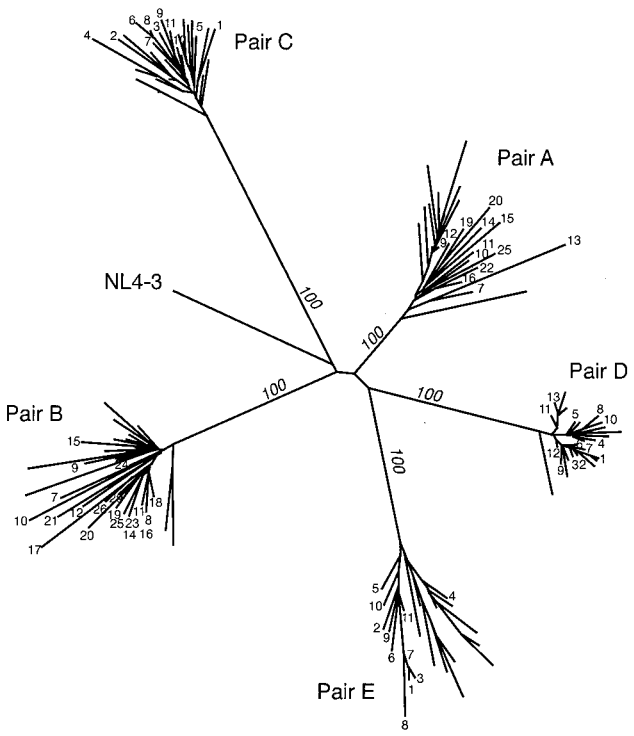


FIG. 3. Phylogenetic tree of 137 *vif* sequences from five mother-infant pairs (A, B, C, D, and E). The distances were calculated between the nucleotide sequences from the five mother-infant pairs. Each leaf of the tree represents one *vif* sequence. The mother sequences in each pair are labeled with the number of the clones (Fig. 1), whereas the infant sequences are unlabeled. The root of the tree was the reference HIV-1 NL4-3 sequence (41). The numbers at branch points indicate the numbers of occurrences of branches over 100 bootstrap resamplings of the data sets. The mother-infant pairs formed a distinct cluster and were discriminated, separated, and confined within subtrees, indicating the absence of PCR product cross-contamination (27, 28).

6.9% (range, 0 to 11.8%). The average pairwise distance within the cluster containing sequences of subtypes A and C and recombinants was 11% (range, 2.8 to 15.1%). The average distance between sequences of subtype B and sequences of the cluster of subtypes A and C and recombinants was 12.8% (range, 8.3 to 16.3%). These values did not change when the two sequences of subtype D were included in the subtype B cluster. This analysis further suggested that the *vif* sequences from the five mother-infant pairs were more closely related to subtype B than to any other subtype. The *vif* sequences from our five mother-infant pairs were distinct from each other and from *vif* sequences from other infected individuals (in HIV-1 databases, including HIV-1 NL4-3, which is used in our laboratory).

Conservation of functional domains required for Vif protein function in maternal-fetal isolates. We next examined the presence of functional domains essential for Vif function in deduced Vif amino acid sequences from five mother-infant pairs. Ma et al. (32) showed that cysteines present at positions 114 and 133 (HXB2 clone numbering) are essential for viral infectivity. Both cysteines were present in 134 of the 137 Vif sequences (Fig. 1A to E). In one clone from mother B (BM08) and in two clones from mother-infant pair E (EM02 and EI07), cysteine was replaced with tyrosine and arginine, respectively. These data indicated a strong selection for the cysteine residues that are critical for *vif*-mediated viral infectivity in maternal-fetal isolates during perinatal transmission. We also identified protein kinase C phosphorylation, N myristoylation, and

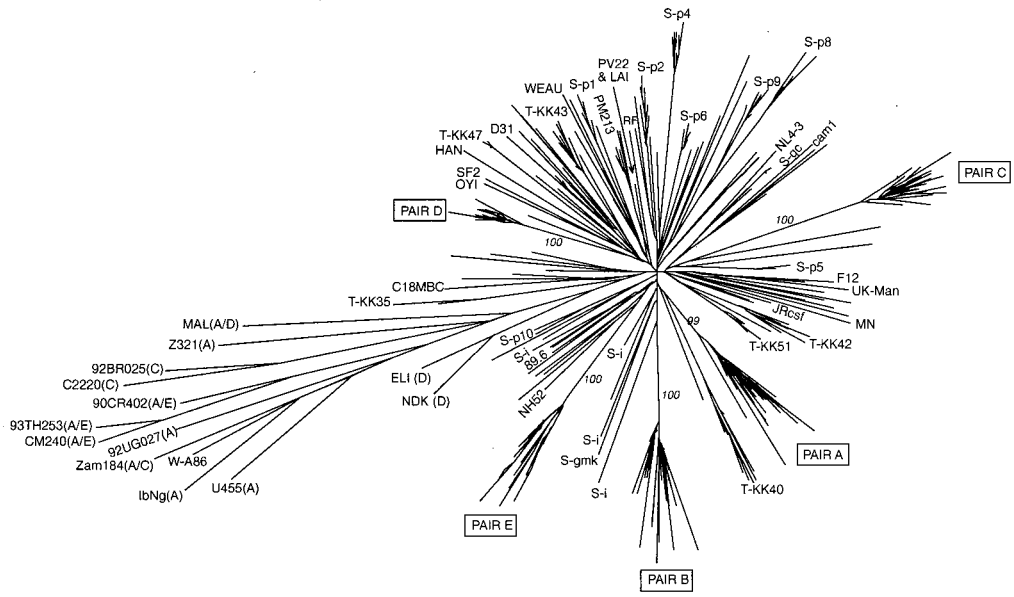


FIG. 4. Phylogenetic tree of 137 mother-infant pair (A, B, C, D, and E) sequences and 187 other *vif* sequences from HIV-1 databases. The sequences from Sova et al. (60) are labeled S-p1 to S-p10, S-gc, S-i, and S-gmk (p, gc, i, and gmk represent clusters of sequences from patients); the sequences from Wieland et al. (66) are unlabeled, except for W-A86 (GenBank no. Z30637); and the sequences from Tominaga et al. (63) are labeled T. The other 35 sequences from the HIV-1 databases belong to the following HIV-1 subtypes: B, LAI (GenBank accession no. K02013), MN (M17449), CAM1 (D10112), Jrcsf (M38429), Jrfl (U63632), NL4-3 (U26942), NY5 (M19921), NH52 (L07424), RF (M17451), B clade (U26546), MCK1 (D86068), PM213 (D86069), PV22 (K02083), OYI (M26727), SF2 (K02007), HAN (U43141), D31 (U43096), WEAU (U21135), UK-Manchester (U23487), F12 (Z11530), 89.6 (U39362), and C18MBC (U37270); A, U455 (M62320), Z321 (U76035), IbNg (L39106), and 92UG027 (U51190); C, 92BR025 (U52953) and C2220 (U46016); A/C (recombinant), Zam184 (U86780); A/D (recombinant), MAL (X04415); A/E (recombinant), CM240 (U54771), 93TH253 (U51189), and 90CR402 (U51188); and D, ELI (K03454) and NDK (M27323). The values in italics are the numbers of occurrences of the corresponding branches over 100 bootstrap resamplings of the data sets. The tree grouped the 324 *vif* sequences, and the largest, starlike cluster contained all subtype B sequences. Subtypes other than B are indicated in parentheses after the isolate names. The five mother-infant pair sequences clustered with subtype B sequences.

cyclic AMP- and cyclic GMP-dependent protein kinase phosphorylation sites in the Vif sequences (Fig. 1A to E). The 10 potential phosphorylation sites identified in the subtype B consensus Vif sequence were conserved in most of the mother-infant pair Vif sequences (Fig. 1A to E). Yang et al. (71) showed that phosphorylation of Vif by a serine/threonine protein kinase(s) plays an important role in regulating HIV-1 replication and infectivity. Serine at position 144 is present in the motif SLQXLA (positions 144 to 149), which is the most highly conserved sequence among all lentivirus Vif proteins (43). Mutation of serine to alanine at position 144 resulted in 90% inhibition of HIV-1 replication (71). The SLQXLA motif at positions 144 to 149 and the serine at position 144 were examined in Vif amino acid sequences and found to be highly conserved in 135 of the 137 clones (Fig. 1A to E). The other important domain essential for Vif function, for membrane localization during HIV-1 replication, requires basic amino acids at the C terminus (21). The C terminus of Vif contains a high density of basic amino acids, such as lysine and arginine, clustered at positions 157 to 160 and 173 to 184 (21, 41). Mutations of these basic amino acids to alanine impaired Vif function and HIV-1 replication (21). We investigated the presence of these basic amino acid domains in the C termini of the deduced amino acid sequences for the mother-infant pair *vif* sequences (Fig. 1A to E). The basic amino acids (lysine and arginine) at positions 157 to 160 and 173 to 184 in the C termini were highly conserved (Fig. 1A to E), supporting the significance of these essential domains for Vif function (21) during mother-to-infant transmission. The data on the conservation of the functional domains required for Vif function suggested that functional Vif is essential for HIV-1 infection and replication in maternal-fetal isolates.

Importance of *vif* in mother-infant HIV-1 infection. The *vif* open reading frame was generally conserved in sequences from five mother-infant pairs following perinatal transmission (Fig. 1A to E). We found that 123 of the 137 clones analyzed had a conserved intact *vif* open reading frame. The frequency of an intact *vif* open reading frame in the five mother-infant pair sequences analyzed was 89.8%. The functional domains required for Vif function (in terms of viral infectivity and replication) including the two cysteines at positions 114 and 133, a serine phosphorylation site at position 144, and the basic amino acids at the C terminus, were highly conserved in mother and infant sequences (Fig. 1). The variability of the *vif* gene within mothers, within infants, and between mother-infant pairs was low, but there was a distinction between epidemiologically linked and unlinked individuals (Fig. 2). Our data showed that there was no difference in *vif* sequence variability with increasing age of the infants (Tables 1 and 2), contrary to the situation for V3 region sequences (2). There was no obvious correlation between *vif* intactness and the disease status of the mothers or infants (Table 1 and Fig. 1). Considering the complete sequence analysis of the *vif* gene from five mother-infant pairs, an intact *vif* gene with functional sites conserved is important for HIV-1 infection and replication in maternal-fetal isolates that are involved in perinatal transmission, as shown before for natural HIV-1 infection (60, 66).

DISCUSSION

We have performed a complete analysis of HIV-1 *vif* sequences from five mother-infant pairs following perinatal transmission. The *vif* sequences directly derived from the DNA of uncultured PBMC, the closest in vivo situation, revealed an

89.8% frequency of conserved intact *vif* open reading frames in maternal-fetal isolates. The two cysteines at positions 114 and 133 (32), the serine phosphorylation site at position 144 (71), and the C-terminal basic amino acid domains (21), required for *vif*-dependent viral infectivity and replication, were highly conserved in most of the mother-infant pair Vif sequences. Our results also showed a low degree of variability of *vif* sequences following mother-to-infant transmission. However, the epidemiologically linked mother-infant pair *vif* sequences were easily distinguishable from those of the epidemiologically unlinked individuals. These findings indicate that an intact *vif* open reading frame is required for HIV-1 infection and replication in mothers and infants and suggest a role in perinatal transmission. Our results are consistent with the earlier published analysis of *vif* sequences from HIV-1-infected individuals (60, 66), which suggested that *vif* plays a critical role in natural HIV-1 infection.

The coding potential of the *vif* gene was maintained in most of the sequences (78,912 bp were sequenced), except for 13 sequences containing stop codons and 1 having a deletion (Fig. 1A to E). A total of 89.8% of the *vif* clones obtained from uncultured PBMC DNA from five mother-infant pairs contained intact *vif* open reading frames. Similar observations of 87% (60) and 90% (66) conservation of intact *vif* open reading frames in infected individuals and 83% (60) conservation in short-term virus cultures have been reported. The frequency of inactive *vif* genes in isolates from our five mother-infant pairs was 10.2%, on the same order as the 13% (60) and 10% (66) frequencies observed for uncultured PBMC DNA from HIV-1-infected individuals. In contrast, a frequency of 31% defective *vif* genes in infected individuals has been reported (63). These differences could be attributed to the time of sampling, clinical stage, or geographic origin. The frequency of inactivating mutations found in our five mother-infant pair *vif* sequences was higher than those observed for *gag* (1.5%) (30, 52) and *nef* (3.3%) (46) genes. However, the possibility exists that the inactive *vif* genes that persisted in the mothers may have been transmitted to their infants at a very low rate.

Phylogenetic analysis performed on the five mother-infant pair *vif* sequences and 187 other *vif* sequences from HIV-1 databases clearly demonstrated that the five pairs were well discriminated, separated, and confined within subtrees (Fig. 4), indicating the absence of PCR product cross-contamination (27, 28). In addition, our five mother-infant pair *vif* sequences formed clusters distinct from those of all the other sequences, including HIV-1 NL4-3, which is used in our laboratory (Fig. 3). The variability of the *vif* gene within mothers, within infants, between mother-infant pairs, and between epidemiologically unlinked mothers was 1.9, 1.9, 2.1, and 8.0%, respectively, suggesting that the epidemiologically linked sequences were closer than the epidemiologically unlinked sequences. Our data also suggested that the low variability of *vif* sequences was not due to errors generated by ULTma or TaKaRa polymerases. Therefore, the low variability of *vif* sequences observed in mother-infant pairs persisted in vivo and was in agreement with those reported for infected individuals (60, 66).

The data presented here do not provide evidence for positive selection pressure for change in *vif* sequences. This finding is in contrast to the situation for *env* V3 region sequences from maternal-fetal isolates, for which positive selection pressure for change was observed (2, 39, 53, 69). The V3 region sequence population is known to be variable and to contain several variants or genotypes (2, 41, 69, 70). In a V3 region sequence analysis for mother-infant pairs, it was shown that HIV-1 minor genotypes were transmitted from mothers to infants (2, 39, 40, 53, 69). The minor genotypes predominated initially as a homogeneous virus population in the infants and then became heterogeneous as the infants grew older (2, 39), supporting the notion that there was strong pressure on the V3 region sequences to change. On the contrary, *vif* (like *gag* [72]) evolves with little selection, and variants from mothers persist during transmission. Evidence for this conclusion is provided by the low variability of and little selection pressure for the five mother-infant pair *vif* sequences.

The most interesting observation was the high conservation of the functional domains essential for Vif function in mother-infant pair sequences. The two cysteines at positions 114 and 133 were present in 134 of the 137 clones (Fig. 1A to E), confirming the finding of Ma et al. (32), who demonstrated that the two cysteines in HIV-1 Vif are critical for Vif-mediated viral infectivity. Phosphorylation of Vif by serine protein kinases at position 144 plays an important role in regulating HIV-1 replication and infectivity (71). Mutation of serine to alanine at position 144 in the motif SLQXLA (positions 144 to 149) (43) results in a loss of Vif activity and 90% inhibition of HIV-1 replication (71). The mother-infant pair Vif sequences contained the motif SLQXLA and the serine residue at position 144, supporting the preservation of these elements in the Vif protein (43, 71). In addition, Vif sequences contained the basic amino acids (lysine and arginine) at positions 157 to 160 and 173 to 184 of the C terminus (Fig. 1A to E) that are required for membrane localization-dependent Vif activity and HIV-1 replication (21). Our data also supported the importance of these basic amino acids at the C terminus (21) during maternal-fetal transmission of HIV-1. These motifs were also preserved in *vif* sequences from HIV-1-infected individuals (60, 63, 66, 67), consistent with our results. The conservation or selection of functional domains, such as cysteines, serine phosphorylation sites, and motifs of basic amino acids at the C terminus that are required for Vif function in maternal-fetal isolates suggests that a functional *vif* gene is required for HIV-1 replication in maternal-fetal isolates.

The molecular mechanisms of maternal transmission of HIV-1 are not known. The demonstration of selective transmission of HIV-1 minor genotypes or variants from mothers to infants was a significant first step in understanding the molecular mechanisms involved in perinatal transmission of HIV-1 (2, 39, 40, 53, 69). In addition, the elucidation of viral factors or determinants influencing maternal transmission may provide useful information for the development of strategies for the prevention and treatment of HIV-1 infection in children. Since the *vif* gene is conserved among most lentiviruses (41, 43) and is required for viral replication in primary lymphocytes and macrophages (15, 17, 18, 51, 59, 64), its role in HIV-1 transmission seems logical. The data presented here, showing a high frequency (89.8%) of intact *vif* open reading frames and conserved functional domains for Vif function, suggest that *vif* is involved in instituting HIV-1 infection and replication in mothers and their perinatally infected infants and may be one of the viral determinants of mother-to-infant transmission. This information may be helpful in the development of strategies for the prevention of HIV-1 mother-to-infant transmission by means of perinatal interventions.

ACKNOWLEDGMENTS

We thank John J. Marcahlonis, Department of Microbiology and Immunology, College of Medicine, The University of Arizona, for support, encouragement, and review of the manuscript. We also thank Raymond C. Baker, Division of General Pediatrics, Children's Hospital Medical Center, Cincinnati, Ohio, for providing HIV-1-infected mother-infant pair blood samples; Scott Martin for technical help;

Tobias Hahn and Mohammad Husain, Department of Microbiology and Immunology, The University of Arizona, for reviewing the manuscript; and the AIDS Research and Reference Reagent Program for providing HIV-1 NL4-3 (contributed by Malcolm Martin).

This work was supported by grants to N.A. from the National Institutes of Health (AI 40378) and the Arizona Disease Control Research Commission (9601).

REFERENCES

1. **Ahmad, N.** 1996. Maternal-fetal transmission of human immunodeficiency virus. J. Biomed. Sci. **2:**238–250.
2. **Ahmad, N., B. M. Baroudy, R. C. Baker, and C. Chappey.** 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.
3. **Ahmad, N., G. M. Schiff, and B. M. Baroudy.** 1993. Detection of viremia by a one step polymerase chain reaction method in hepatitis C virus infection. Virus Res. **30:**303–315.
4. **Ahmad, N., I. K. Kuramoto, and B. M. Baroudy.** 1993. A ribonuclease protection assay for the direct detection and quantitation of hepatitis C virus RNA. Clin. Diagn. Virol. **1:**233–244.
5. **Albert, J., H. Gaines, A. Sonnerborg, G. Nystrom, P. O. Pehrson, F. Chiodi, M. V. Sydow, L. Moberg, K. Lidman, B. Christensson, B. Asjo, and E. M. Fenyo.** 1987. Isolation of human immunodeficiency virus (HIV) from plasma during primary HIV infection. J. Med. Virol. **23:**67–73.
6. **Albert, J., and E. M. Fenyo.** 1990. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 in clinical specimens by polymerase chain reaction with nested primers. J. Clin. Microbiol. **28:**1560–1564.
7. **Anderson, R. M., and G. F. Medley.** 1989. Epidemiology of HIV infection and AIDS: incubation and infectious periods, survival and vertical transmission. AIDS **2:**S57–S63.
8. **Blanche, S., C. Rouzious, M.-I. Guihard Moscato, F. Veber, M.-J. Mayaux, J. Jacomet, A. Crepy, D. Douard, M. Robin, C. Courpotin, N. Ciraru-Vigeneran, F. Deist, C. Griscelli, and the French Collaborative Group.** 1989. A prospective study of infants born to women seropositive for human immunodeficiency virus type 1. N. Engl. J. Med. **320:**1643–1648.
9. **Centers for Disease Control.** 1987. Classification system for HIV in children under 13 years of age. Morbid. Mortal. Weekly Rep. **36:**225–236.
10. **Cichutek, K., H. Merget, S. Norley, R. Linde, W. Kreuz, M. Gahr, and R. Kurth.** 1992. Development of quasispecies of human immunodeficiency virus type 1 in vivo. Proc. Natl. Acad. Sci. USA **89:**7365–7369.
11. **Dougherty, J., and H. Temin.** 1988. Determination of the rate of base-pair substitution and insertion mutations in retrovirus replication. J. Virol. **62:**2817–2822.
12. **Ehrnst, A., S. Lindergren, M. Dictor, B. Johanon, A. Sonnerborg, J. Czajkowski, G. Sundin, and A.-E. Bohlin.** 1991. HIV in pregnant women and their offsprings: evidence for late transmission. Lancet **338:**203–207.
13. **European Collaborative Study.** 1988. Mother to child transmission of HIV-1. Lancet **ii:**1039–1042.
14. **Felsenstein, J.** 1989. Phylip phylogenetic inference package. Cladiatice **5:**164–166.
15. **Fisher, A. G., B. Ensoli, L. Ivanoff, M. Chamberlain, S. Petteway, L. Ratner, R. C. Gallo, and F. Wong-Staal.** 1987. The sor gene of HIV-1 is required for efficient virus transmission in vitro. Science **237:**888–892.
16. **Furtado, M. R., L. A. Kingsley, and S. M. Wolinsky.** 1995. Changes in the viral mRNA expression pattern correlate with a rapid rate of $CD4^+$ T-cell number decline in human immunodeficiency virus type 1-infected individuals. J. Virol. **69:**2092–2100.
17. **Gabuzda, D. H., K. Lawrence, E. Langhoff, E. Terwilliger, T. Dorfman, W. A. Haseltine, and J. Sodroski.** 1992. Role of *vif* in replication of human immunodeficiency virus type 1 $CD4^+$ T lymphocytes. J. Virol. **66:**6489–6495.
18. **Gabuzda, D. H., H. Li, K. Lawrence, B. S. Vasir, K. Crawford, and E. Langhoff.** 1994. Essential role of vif in establishing productive HIV-1 infection in peripheral blood T lymphocytes and monocytes/macrophages. J. Acquired Immune Defic. Syndr. **7:**908–915.
19. **Ganeshan, S., R. E. Dickover, B. T. M. Korber, Y. J. Bryson, and S. M. Wolinsky.** 1997. Human immunodeficiency virus type 1 genetic evolution in children with divergent rates of development of disease. J. Virol. **71:**663–677.
20. **Goedert, J. J., A. M. Duliege, C. I. Amos, S. Felton, R. J. Biggar, and the International Registry of HIV-Exposed Twins.** 1991. High risk of HIV-1 infection for first born twins. Lancet **338:**1471–1475.
21. **Gonclaves, J., B. Shi, X. Yang, and D. Gabuzda.** 1995. Biological activity of human immunodeficiency virus type 1 Vif requires membrane targeting by C-terminal basic domains. J. Virol. **69:**7196–7204.
22. **Hira, S., J. Kamanga, G. J. Bhat, C. Mwale, G. Tembo, N. Luo, and P. L. Perine.** 1989. Perinatal transmission of HIV-1 in Zambia. Br. Med. J. **299:**1250–1252.
23. **Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen.** 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism. Science **253:**71–74.
24. **Italian Multiculture Study.** 1988. Epidemiology, clinical features and prognostic factors of pediatric HIV infection. Lancet **ii:**1043–1046.
25. **Iversen, A. K., E. G. Shpaer, A. G. Rodrigo, M. S. Hirsch, B. D. Walker, H. W. Sheppard, T. C. Merigan, and J. I. Mullins.** 1995. Persistence of attenuated *rev* genes in human immunodeficiency virus type 1-infected asymptomatic individuals. J. Virol. **69:**5743–5753.
26. **Kans, J. A.** 1996. Sequin, p. 114. *In* Conference Proceedings of the Cold Spring Harbor Laboratory Genome Mapping and Sequencing Meeting. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Korber, B. T. M., G. Learn, J. I. Mullins, B. H. Hahn, and S. M. Wolinsky.** 1995. Protecting HIV databases. Nature **378:**242–243.
28. **Learn, G. H., B. T. M. Korber, B. Foley, B. H. Hahn, S. M. Wolinsky, and J. I. Mullins.** 1996. Maintaining the integrity of human immunodeficiency virus sequence databases. J. Virol. **70:**5720–5730.
29. **Lepage, P., P. Vande Perre, M. Carael, F. Nsengumuremyi, J. Nkurunziza, J.-P. Butzler, and S. Sprecher.** 1987. Postnatal transmission of HIV from mother to child. Lancet **i:**400.
30. **Louwagie, J., F. E. McCurchan, M. Peeters, T. P. Brennan, E. Sanders-Buell, E. Eddy, G. van der Groen, K. Fransen, G.-M. Gershy-Damet, R. Deleys, and D. S. Burke.** 1993. Phylogenetic analysis of *gag* genes from 70 international HIV-1 isolates provides evidence for multiple genotypes. AIDS **7:**769–780.
31. **Lu, S.-L., T. Schacker, L. Musey, D. Shriner, M. J. McElrath, L. Corey, and J. I. Mullins.** 1997. Divergent patterns of progression to AIDS after infection from the same source: human immunodeficiency virus type 1 evolution and antiviral responses. J. Virol. **71:**4284–4295.
32. **Ma, X., P. Sova, and D. J. Volsky.** 1994. Cysteine residues in the Vif protein of human immunodeficiency virus type 1 are essential for viral infectivity. J. Virol. **68:**1714–1720.
33. **Matala, E., V. R. K. Yedavalli, and N. Ahmad.** Unpublished data.
34. **McNearny, T., P. Westervelt, B. J. Thielan, D. B. Trowbridge, J. Garcia, R. Whittler, and L. Ratner.** 1990. Limited sequence heterogeneity among biologically distinct human immunodeficiency virus type 1 isolates from individuals involved in a clustered infectious outbreak. Proc. Natl. Acad. Sci. USA **87:**1917–1922.
35. **McNearny, T., Z. Hornickora, R. Markham, A. Birdnell, M. Arnes, A. Saah, and L. Ratner.** 1992. Relationship of human immunodeficiency virus type 1 sequence heterogeneity to stage of disease. Proc. Natl. Acad. Sci. USA **89:**10247–10251.
36. **Michaels, F., N. Hattori, R. Gallo, and G. Franchini.** 1993. The HIV-1 Vif protein is located in the cytoplasm of infected cells and its effect on virus replication is equivalent in HIV-2. AIDS Res. Hum. Retroviruses **9:**1025–1029.
37. **Mok, J. Q., C. Giaquinto, A. DeRossi, I. Gruch-Worner, A. E. Ades, and C. S. Pekham.** 1987. Infants born to mothers seropositive for human immunodeficiency virus—preliminary findings from a multiculture European study. Lancet **i:**1164–1168.
38. **Muary, W., B. Potts, and A. B. Rabson.** 1989. HIV-1 infection of first trimester and term human placental tissue, a possible mode of maternal-fetal transmission. J. Infect. Dis. **460:**583–588.
39. **Mulder-Kampinga, G. A., C. Kuiken, J. Dekker, H. J. Scherpbier, K. Boer, and J. Goudsmit.** 1993. Genomic human immunodeficiency virus type 1 RNA variation in mother and child following intrauterine virus transmission. J. Gen. Virol. **74:**1747–1756.
40. **Mulder-Kampinga, G. A., A. Simonon, C. L. Kuiken, J. Dekker, H. J. Scherpbier, P. van de Perre, K. Boer, and J. Goudsmit.** 1995. Similarity in *env* and *gag* genes between genomic RNAs of human immunodeficiency virus type 1 (HIV-1) from mother and infant is unrelated to time of HIV-1 RNA positivity in the child. J. Virol. **69:**2285–2296.
41. **Myers, G., B. Korber, B. H. Hahn, K.-T. Jeang, J. W. Mellors, F. E. McCutchan, L. E. Henderson, and G. N. Pavlakis.** 1995. Human retroviruses and AIDS database. Los Alamos National Laboratory, Los Alamos, N.Mex.
42. **Nei, M., and T. Gojobori.** 1986. Simple methods for estimating the number of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. **3:**418–426.
43. **Oberste, M. S., and M. A. Gonda.** 1992. Conservation of amino acid sequence motifs in lentivirus Vif proteins. Virus Genes **6:**95–102.
44. **Oram, J. D., R. G. Downing, M. Roff, N. Sernankambo, J. C. S. Clegg, A.-S. R. Featherstone, and J. C. Booth.** 1991. Sequence analysis of the V3 loop regions of the env genes of Ugandian human immunodeficiency proviruses. AIDS Res. Hum. Retroviruses **7:**605–614.
45. **Pang, S., Y. Shlesinger, E. S. Darr, T. Moudgh, and D. D. Ho.** 1992. Rapid generation of sequence variation during primary HIV-1 infection. AIDS **6:**453–460.
46. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
47. **Peterson, B. D., B. J. Poresz, and J. A. Loeb.** 1988. Fidelity of HIV-1 reverse transcriptase. Science **242:**1168–1171.
48. **Roberts, J. D., B. D. Preston, L. A. Johnston, A. Soni, L. A. Loeb, and T. A. Kunkel.** 1989. Fidelity of two retroviral reverse transcriptases during DNA-dependent DNA synthesis in vitro. Mol. Cell. Biol. **9:**469–476.
49. **Rossi, P., V. P. A. Moschese, C. Broliden, I. Fundaro, A. Quinti, C. Plebani, P. Giaquinto, K. Tovo, K. Ljunggren, and J. Rosen.** 1989. Presence of maternal antibodies to human immunodeficiency virus 1 envelope glycopro-

tein gp120 epitopes correlates with uninfected status of children born to seropositive mothers. Proc. Natl. Acad. Sci. USA **86:**8055–8058.
50. **Ryder, R. W., W. Nsa, S. E. Hassig, F. Behets, M. Rayfield, E. Kungola, A. Nelson, U. Mulenda, H. Francis, K. Mwandagalirwa, F. Davachi, M. Rogers, N. Nzilambi, A. Greenberg, J. Mann, T. C. Quinn, P. Piot, and J. W. Curran.** 1988. Perinatal transmission of human immunodeficiency virus type 1 to infants of seropositive women in Zaire. N. Engl. J. Med. **320:**1637–1642.
51. **Sakai, K., S. Dewhurst, X. Ma, and D. J. Volsky.** 1988. Differences in cytopathogenicity and host cell range among infectious molecular clones of human immunodeficiency virus type 1 simultaneously isolated from an individual. J. Virol. **62:**4078–4085.
52. **Salmien, M., A. Nykanen, H. Brummer-Korvenkontio, M. L. Kantenen, K. Liitsola, and P. Leinikki.** 1993. Molecular epidemiology of HIV-1 based on phylogenetic analysis of in vivo gag p7/p9 direct sequences. Virology **195:** 185–194.
53. **Scarlatti, G., T. Leitner, E. Hapi, J. Wahlberg, P. Marchisi, M. A. Clerici-Schoeller, H. Wigzell, E. M. Fenyo, J. Albert, M. Uhlen, and P. Rossi.** 1993. Comparison of variable region 3 sequences of human immunodeficiency virus type 1 from infected children with RNA/DNA sequences of virus populations of their mothers. Proc. Natl. Acad. Sci. USA **90:**1721–1725.
54. **Scott, G. B., M. A. Fischl, N. Khmas, M. A. Fletcher, G. M. Dickinson, R. S. Levine, and W. P. Parks.** 1985. Mothers of infants with acquired immunodeficiency syndrome: evidence for both symptomatic and asymptomatic carriers. JAMA **253:**363–366.
55. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell line tropism of HIV-1 is determined by specific regions of the envelope gp120 gene. Nature **349:**167–169.
56. **Shuler, G. D., J. A. Epstein, H. Ohkawa, and J. A. Kans.** 1996. Entrez: molecular biology database and retrieval system. Methods Enzymol. **266:** 141–162.
57. **Siliciano, R. F., T. Lawton, R. W. Knall, R. W. Karr, P. Berman, T. Gregory, and E. L. Reinherz.** 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for CD4+ cell depletion. Cell **54:**561–575.
58. **Sodroski, J., W. C. Goh, C. Rosen, A. Tartar, D. Portetella, A. Bumy, and W. A. Haseltine.** 1986. Replicative and cytopathic potential of HTLV III/LAV with sor gene deletions. Science **231:**1549–1553.
59. **Sova, P., and D. J. Volsky.** 1993. Efficiency of viral DNA synthesis during virus infection of permissive and nonpermissive cells with *vif*-negative human immunodeficiency virus type 1. J. Virol. **67:**6322–6326.
60. **Sova, P., M. van Rannst, P. Gupta, R. Balachandran, W. Chao, S. Itescu, G. McKinley, and D. J. Volsky.** 1995. Conservation of an intact human immunodeficiency virus type 1 *vif* gene in vitro and in vivo. J. Virol. **69:**2557–2564.
61. **Sprecher, S., G. Soumenkoff, F. Puissant, and M. Degueldre.** 1986. Vertical transmission of HIV in 15 week fetus. Lancet **ii:**288–289.
62. **Strebel, K., D. Daughtery, K. Clouse, D. Cohen, T. Folks, and M. A. Martin.** 1987. The HIV 'A' (sor) gene product is essential for virus infectivity. Science **328:**728–730.
63. **Tominaga, K., S. Kato, M. Negishi, and T. Takano.** 1996. A high frequency of defective vif genes in peripheral blood mononuclear cells from HIV type 1-infected individuals. AIDS Res. Hum. Retroviruses **12:**1543–1549.
64. **von Schwedler, U., J. Song, C. Aiken, and D. Trono.** 1993. *vif* is critical for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J. Virol. **67:**4945–4955.
65. **Weinbreck, P., V. Loustand, F. Denis, B. Vidal, M. Muvnier, and I. DeLumley.** 1988. Postnatal transmission of HIV infection. Lancet. **i:**482.
66. **Wieland, U., J. Hartman, H. Suhr, B. Salzberger, H. J. Eggers, and J. E. Kuhn.** 1994. In vivo genetic variability of the HIV-1 vif gene. Virology **203:**43–45.
67. **Wieland, U., A. Seelhoff, A. Hofmann, J. E. Kuhn, H. J. Eggers, P. Mugyeny, and S. Schwander.** 1997. Diversity of the vif gene of human immunodeficiency virus type 1 in Uganda. J. Gen. Virol. **78:**393–400.
68. **Wolfs, T. F. W., G. Zwart, M. Bakker, and J. Goudsmit.** 1992. HIV-1 genomic RNA diversification following sexual and parental virus transmission. Virology **189:**103–110.
69. **Wolinsky, S. M., C. M. Wike, B. T. M. Korber, C. Hutto, W. P. Parks, L. L. Rosenblum, K. J. Kuntsman, M. R. Furtado, and I. L. Munoz.** 1992. Selective transmission of human immunodeficiency virus type 1 variants from mother to infants. Science **255:**1134–1137.
70. **Wolinsky, S. M., B. T. M. Korber, A. U. Neumann, M. Daniels, K. J. Kunstman, A. J. Whetsell, M. R. Furtado, Y. Cao, D. D. Ho, J. T. Safrit, and R. A. Koup.** 1996. Adaptive evolution of human immunodeficiency virus-type 1 during the natural course of infection. Science **272:**537–542.
71. **Yang, X., J. Goncalves, and D. Gabuzda.** 1996. Phosphorylation of Vif and its role in HIV-1 replication. J. Biol. Chem. **271:**10121–10129.
72. **Zhang, J., L. Q. MacKenzie, A. Cleland, E. C. Holms, A. J. Leigh Brown, and P. Simmonds.** 1993. Selection for specific sequences in the external envelope protein of human immunodeficiency virus type 1 upon primary infection. J. Virol. **67:**3345–3356.
73. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. Science **261:**1179–1181.
74. **Zhu, T., N. Wang, A. Carr, S. Nam, R. Moor-Jankowski, D. Cooper, and D. D. Ho.** 1996. Genetic characterization of human immunodeficiency virus type 1 in blood and genital secretions: evidence for viral compartmentalization and selection during sexual transmission. J. Virol. **70:**3098–3107.