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Independent Variation and Positive Selection in *env* V1 and V2 Domains within Maternal-Infant Strains of Human Immunodeficiency Virus Type 1 In Vivo

SUSANNA L. LAMERS,[1]† JOHN W. SLEASMAN,[1,2] JIN XIONG SHE,[1,3] KIMBERLY A. BARRIE,[1] STEVEN M. POMEROY,[1] DOUGLAS J. BARRETT,[1,2] AND MAUREEN M. GOODENOW[1,2,3]*

Department of Pathology and Laboratory Medicine,[1] Department of Pediatrics, Division of Immunology,[2] and the Center for Mammalian Genetics,[3] University of Florida College of Medicine, Box 100275, Gainesville, Florida 32610-0275

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Multiple targets for immune recognition and cellular tropism are localized to the V1 and V2 hypervariable regions in the amino portion of human immunodeficiency virus type 1 (HIV-1) gp120^{env}. We have assessed genetic diversity in *env* V1 and V2 hypervariable domains in vivo within epidemiologically related strains of HIV-1. Our strategy was to analyze longitudinal samples from two seropositive mothers and multiple children infected by perinatal transmission. Although the V1 and V2 domains are closely linked in the HIV-1 genome, nucleotide sequences in V1 and in V2 evolved independently in maternal-infant viruses in vivo. A high proportion of the nucleotide substitutions would introduce amino acid diversity in V1 and in V2. A significant excess of nonsynonymous over synonymous substitutions was identified in HIV-1 *env* V1 and V2 peptides in the mothers and in two older children but was not generally apparent in HIV-1 sequences in infants. An excess of nonsynonymous over synonymous substitutions indicated that there is positive selection for independent genetic variation in the V1 and V2 domains in vivo. It is likely that there are host responses to complex determinants in the V1 or V2 hypervariable domain of HIV-1 gp120.

Genetic variability within the human immunodeficiency virus type 1 (HIV-1) genome is most pronounced in *env*, in which nucleotide substitutions, duplications, and deletions produce extensive amino acid diversity within five hypervariable domains in the envelope glycoprotein (7). The hypervariable domains, designated V1 through V5, are interspersed with conserved regions along the gp120 molecule (42, 44, 70). Sequence variation in *env* can affect an array of functional parameters of the virus in vivo and in culture (8, 9, 14, 16, 28, 52, 57, 59, 61, 62, 74). Major determinants critical for HIV-1 tropism, for cytopathology in culture, and for host immunological responses to HIV-1 infection have been mapped to the carboxyl half of gp120, where V3, V4, and V5 are localized (14, 15, 24, 35). The V3 hypervariable region in particular encodes the principal neutralizing domain in gp120 (25). Nucleotide variability in the V3 loop is characterized by a high proportion of nonsynonymous substitutions indicative of positive genetic selection (1, 60, 71). A principal mechanism contributing to evolution of genetic variability in the V3 domain of gp120 is the host immune response (6, 21, 30, 39).

Complex functional determinants are not restricted to the V3 region of gp120. Sequences associated with HIV-1 tropism for macrophages (49, 69) or encoding targets for immune recognition (13, 17, 26, 32, 67) are also localized in the amino half of *env* gp120 in the V1 and V2 hypervariable regions. Genetic variation results in V1 and V2 domains that are distinctly different in nucleotide sequence, in deduced amino acid composition, and in peptide length among epidemiologically unrelated isolates of HIV-1 (19, 44), yet only limited genetic variability develops in V1 and V2 after short-term cultivation of HIV-1 isolates in peripheral blood mononuclear cells (19, 29, 68). These results suggest that the environment in vivo contributes to the evolution of diversity in V1 and V2. In contrast to the extensive analyses of V3 variability, there is very little information about V1 and V2 variability in vivo (2, 11, 23, 29). If extensive genetic variation in V1 and V2 evolves in vivo, selective pressures would contribute to an excess of nonsynonymous nucleotide substitutions in the region. If there is evidence for selective pressures, direct associations between genetic diversity in the V1 and V2 domains and functional epitopes that map to the region become an aspect of virus-host interactions that requires examination.

V1 and V2 may evolve coordinately as a single hypervariable region or independently as two distinct domains. In contrast to V3, V4, and V5, which are separated from each other in gp120 by a minimum of 50 conserved amino acids, V1 and V2 are closely linked, with no more than 12 conserved amino acids interspersed between the two clusters of hypervariable sequences (42, 44, 70). Secondary structure predicted for V1 and V2 domains on the basis of disulfide linkages between cysteine residues is a complex double-loop structure rather than the single-loop configuration of V3 or V4 (31). The extent to which genetic variation develops in each of the V1 and V2 hypervariable domains in vivo has not been examined.

We have assessed genetic variability in *env* V1 and V2 sequences over time in infected individuals. Our studies are focused on maternal-infant strains of HIV-1 following perinatal transmission of the virus. There are a number of advantages to studying transmission and genetic variation of HIV-1 in infected women and children. A principal advantage is that the source of HIV-1 infection in the infant is unambiguous. Another is that evolution of genetic diversity in related strains of HIV-1 can be examined both in adults and in the context of the developing immune system of the

* Corresponding author.
† Present address: Gene Genie, Inc., Palm Coast, FL 32137.

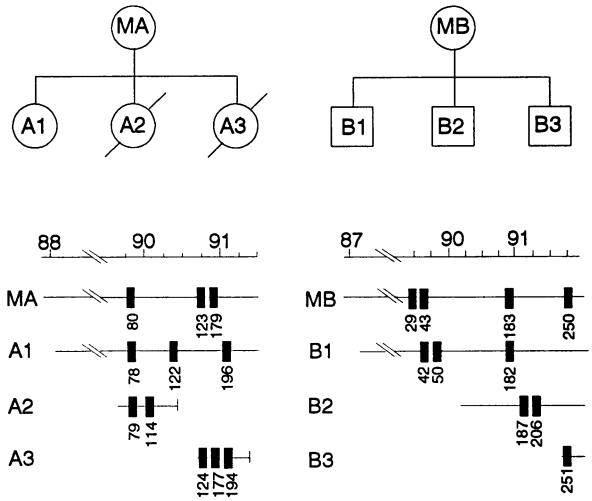

FIG. 1. Internal variability in HIV-1 *env* V1 and V2 hypervariable domains in two mothers and their children. Twenty-one samples obtained over a 2-year period were analyzed. Time in years is represented by the top line, with 1990 (90) and 1991 (91) indicated. Vertical boxes along the time lines indicate when samples were obtained. Numbers under boxes designate samples. The precise timing of HIV-1 infection in the mothers (MA and MB) is not known but preceded the birth of oldest child (A1, born in January 1988; B1, born in February 1987). For the four other children, time lines start at their births and, in two cases (A2 and A3), end with a vertical line at the time of their deaths.

child. Our strategy was to evaluate longitudinal samples that have been obtained from two families, each with multiple children infected by maternal transmission of HIV-1. Analyses of HIV-1 sequences in longitudinal samples provides an overview of the dynamics of genetic variation within infected individuals not apparent from analyses at a single point in time (19, 22, 37, 60, 65, 71). Our goals were to determine whether V1 and V2 are linked or independent genetic domains in *env* and to assess whether sequence variation in the region reflects positive selection. A total of 120 nucleotide sequences were evaluated from eight individuals over a period of 2 years. V1 and V2 domains were found to vary independently in mothers and children. An excess of nonsynonymous over synonymous nucleotide substitutions indicated that genetic variation in V1 and in V2 reflected selective pressures in vivo.

MATERIALS AND METHODS

Clinical samples. Twenty-one samples of peripheral blood mononuclear cells were collected over a period of 16 to 24 months from eight individuals in two families (Fig. 1). The mothers were infected with epidemiologically unrelated viruses by heterosexual transmission from independent sources. During the period of these studies, the clinical status of mothers MA and MB progressed from asymptomatic to AIDS. Samples were obtained simultaneously from the mothers and from three newborns: A2 at 7 and 18 weeks of age; A3 at 5 days, 9.5 weeks, and 14 weeks; and B3 at 4 weeks. Clinical staging of the three newborns was Centers for Disease Control classification P1 at the time the first sample was obtained (5). A2 and A3 progressed rapidly to clinical stage P2AD and died within the first year of life. Samples were obtained from a fourth child, B2, at about 1 year of age, when he was clinical stage P2ABD, and from the oldest child in each family, A1 (at 21, 28, and 36 months of age) and B1 (at age 29, 31, and 49 months), each of whom was stage P2A. The studies were carried out under a protocol approved by the Institutional Review Board of the University of Florida. Informed consent was obtained.

Nucleic acid extraction, amplification, and sequencing. Cell separation, DNA purification, and amplification procedures were carried out in a separate laboratory free of cultured HIV-1 isolates or cloned HIV-1 sequences. DNA was prepared from Ficoll-Hypaque-separated peripheral blood mononuclear cells according to standard procedures (55).

HIV-1 sequences were amplified by the polymerase chain reaction (PCR), and nested oligonucleotide primers were prepared in the DNA synthesis core facility of the Interdisciplinary Center for Biotechnology Research at the University of Florida. Sequences for all primers were located in the highly conserved regions of *env*, which flank V1 and V2 in an array of HIV-1 isolates from diverse geographical locations (19, 44). The outer primers were ENV5 (5′-GGTAGAACA GATGCATGAGGAT-3′) and ENV6 (5′-CCATGTGTA CATTGTACTGTGCT-3′), located at nucleotides 6102 to 6123 and 6544 to 6566 in the HIV_{LAI} genome (44). The inner primers were SK122 and SK123 (19). HIV-1 *env* sequences were amplified from 1 μg of DNA in 100-μl reactions with PCR buffer (1.75 mM $MgCl_2$, 50 mM KCl, 20 mM Tris [pH 8.4], 0.1% bovine serum albumin), 100 pmol of each primer, 200 μmol of each deoxynucleoside triphosphate, and 2.5 U of AmpliTaq (Perkin Elmer) overlaid with 25 μl of mineral oil. Reagent controls and DNA from uninfected cells were included in every reaction. Amplification reactions were carried out in an automated 48-well thermal cycler (Perkin Elmer Cetus) programmed for one cycle of denaturation (94°C for 10 min), 25 cycles of amplification (each cycle included 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C), and one cycle at 72°C for 10 min. After passage through a Centricon 100 filter (Amicon), 1/10 of the product was used as template in a second round of amplification for 20 cycles with inner primers and the same profile as specified above. Controls from the first round of amplification, including multiple reagent controls, were included in the second round of amplification.

Amplified products of approximately 400 to 450 bp included V1, V2, and 100 bp of C2, the conserved region adjacent to V2 (44). DNA fragments were isolated from agarose gels and blunt-end ligated into pGEM-3Z plasmid vectors (Promega). Multiple recombinant plasmids from each sample were sequenced by the dideoxy-chain termination method with SP6 and T7 primers, [^{35}S]dATP, and Sequenase version 2.0 (U.S. Biochemical) (56). The error rate of *Taq* polymerase during two rounds of amplification under the conditions of our experiments was approximately 10^5, as calculated by diluting 1 ng of plasmid DNA containing the V1-V2-C2 sequences in 1 μg of genomic DNA, amplifying the DNA, and then cloning and sequencing 20 recombinants.

Analyses of nucleotide sequences. Sequences from each individual within a family were aligned to give minimum evolutionary distances, using the multiple aligned sequence editor program (D. Faulkner and Y. Jerka, Molecular Biology Computer Research Resource, Harvard University). After translation of nucleotide sequences by using Microgenie (Intelligenetics) to maximize amino acid alignments, nucleotide alignments were subsequently edited and gaps were removed by hand. The program SYNO (kindly provided by M. Nei, Pennsylvania State University) was used to

TABLE 1. Distances in the V1-V2-C2 region of *env* over time within individuals in each family

Family A				Family B			
Sample set[a]	Date (mo/yr)	No. of clones	Distance (% [±SE])[b]	Sample set	Date (mo/yr)	No. of clones	Distance (% [±SE])
MA ALL		15	3.7 (0.4)	MB ALL		21	7.4 (0.7)
.80	10/89	5	8.2 (1.1)	.29	6/89	5	7.0 (0.9)
.123	9/90	5	0.2 (0.1)	.43	7/89	5	7.3 (1.0)
.179	11/90	5	1.6 (0.4)	.183	11/90	6	4.6 (0.7)
				.250	10/91	5	4.5 (0.7)
A3 ALL		22	3.6 (0.4)	B3 .251	10/91	4	8.6 (1.1)
.124	9/90	8	1.3 (0.3)				
.177	11/90	9	3.2 (0.4)				
.194	1/91	5	8.4 (1.2)				
A2 ALL		12	0	B2 ALL		11	4.3 (0.5)
.79	10/89	5	0	.187	2/91	5	2.0 (0.5)
.114	1/90	7	0	.206	4/91	6	5.6 (0.7)
A1 ALL		16	6.6 (0.6)	B1 ALL		19	5.5 (0.5)
.78	10/89	6	2.1 (0.5)	.42	7/89	8	5.9 (0.7)
.122	5/90	5	7.4 (0.9)	.50	9/89	3	5.6 (1.0)
.196	1/91	5	4.8 (0.8)	.182	11/90	8	4.3 (0.6)

[a] Multiple clones for each sample set were sequenced.
[b] Calculated for all sequences from an individual and for sequences from each time point.

calculate nucleotide distances transformed by the Jukes-Cantor formula, as well as nonsynonymous and synonymous substitutions (d_N and d_S) according to Nei and Gojobori (33, 47). Means and standard errors of d_N and d_S values were calculated by using the program SEND (48). Distance calculations were carried out on intrapatient sequences, that is, those found within each individual. Student's *t* test was used to evaluate the significance of differences within each individual between overall nucleotide distances or between d_N and d_S values.

Nucleotide sequence accession numbers. Sequences have been submitted to GenBank with accession numbers L15085 to L15187. Alignments of sequences have been provided to the Human Retroviruses and AIDS Database.

RESULTS

Variation in nucleotide sequences within individuals. The subgenomic region of HIV-1 *env* that was amplified extended approximately 400 to 450 bp and included V1 and V2 (240 to 300 bp) flanked 5′ and 3′ by 30 and 130 bp, respectively, of conserved sequences. The extent of genetic variability or distance across the amplified region ranged from less than 1 to 8.5% within any set of sequences from each member of family A and family B (Table 1). Distance in V1-V2-C within sample sets was similar to variation found for other regions of *env* in related viruses (34, 44, 54, 71, 72). The number of clones in a sample set ranged from three to nine, with most (19 of 21) of the samples having five or more sequences (Table 1). Extent of diversity detected in the amplified region within an individual at any time point does not appear to be related to the number of sequences determined for a sample.

Variation identified between samples in most individuals was not simply a consequence of time (Table 1). For example, mean distances in HIV-1 variability were significantly different between samples obtained a year or more apart from MA.80 and MA.123 ($P < 0.001$) or MB.43 and MB.183 ($P < 0.05$). Likewise, significant differences in variation of HIV-1 sequences could be detected between samples obtained at 6- to 8-month intervals from child A1 ($P < 0.001$ for A1.78 and A1.122; $P < 0.05$ for A1.122 and A1.196) or only 7 weeks apart from child B2 (B2.187 and B2.206, $P < 0.001$). In contrast, samples obtained 12 months apart from child B1 (B1.50 and B1.182) were not significantly different from one another in the extent of their genetic variation.

Variation in *env* ranging from 1.3 to 8.4% was detected in independent samples obtained during the first 14 weeks of life in infant A3. Likewise, in infant B3, genetic variation was 8.6% at 4 weeks of age. However, there was a marked absence of heterogeneity in HIV-1 *env* sequences from two independent sample sets obtained 11 weeks apart in a second newborn, A2. Restricted variation in V1 and V2 sequences found in infant A2 did not necessarily reflect virus latency because results of p24 antigen assays were positive at both time points, indicating virus replication at some site in the infant. Apparent homogeneity in V1-V2-C2 did not reflect overall virus homogeneity in infant A2 because variation was detected in other subgenomic regions of HIV-1 in the infant (20). It is unlikely that this particular HIV-1 genome amplified preferentially because a highly related sequence, together with three additional genotypes, was identified in one maternal sample set, MA.80. The results from infant A2 suggested that HIV-1 replication in vivo need not introduce detectable genetic variation in the V1 and V2 hypervariable domains. When sequence variation in the V1-V2-C2 region was found in vivo within infected individuals, it appeared to be unrelated to age, clinical status, or duration of infection.

Independent variation in V1 and V2 nucleotide sequences. Genetic variation in vivo was not random across the amplified sequences but mapped primarily to V1 and V2 rather than the conserved region (data not shown). The proximity of V1 and V2 in *env* raised a question as to whether genetic variation in the two domains fluctuated coordinately or independently in individuals infected with related viruses. To address the question, distances within nucleotide sequences in V1 (approximately 75 to 90 bp), V2 (120 to 145 bp), and C2 (130 bp) were analyzed separately for each of 19 samples.

Results indicated that mean variation in the V1 or V2 domain ranged from 2% to more than 20% between any two sequences in the same sample set (Fig. 2). In contrast, mean variation in the conserved sequences of *env* did not exceed

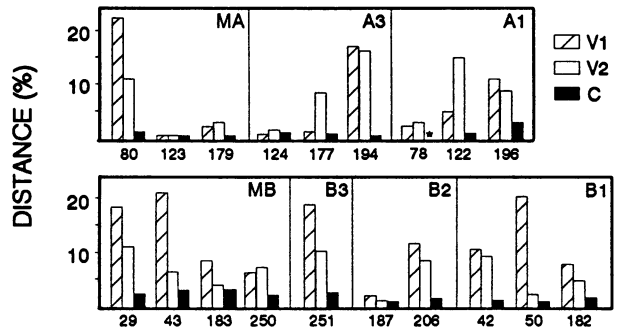

FIG. 2. Independent variability in HIV-1 V1, V2, and C (conserved region 3′ of V2) regions of *env* in members of family A (upper panels) and family B (lower panels). Two samples from infant A2 were not included because there was no internal variation in the amplified region. The vertical axis indicates variability as percent distance. Vertical bars represent variability within V1 (diagonal lines), within V2 (open), and within C (solid). Numbers under bars designate samples. The asterisk indicates that partial C sequences were not determined in A1.78.

4%. Among the 14 samples in which overall internal variability was greater than 2%, mean distances in V1 and V2 were similar in 6 samples. Two samples appeared in family A (A3.194 and A1.196), while four samples were identified among members of family B (MB.250, B2.206, B1.42, and B1.182).

Significant differences in mean variation between V1 and V2 were identified in eight samples from six individuals. Variation in V1 was two- to fourfold greater than in V2 in MA.80 ($P < 0.001$), in B3.251 ($P < 0.001$), and at three time points in MB (MB.29 and MB.43, $P < 0.001$; MB.183, $P < 0.05$). V1 sequences could be almost 10-fold more variable than sequences in V2, as identified in one sample, B1.50, from child B1. However, V1 was not always more variable than V2. In samples from two children in family A, A1.122 and A3.177, V2 domains were four- or sevenfold more variable than their respective V1 domains ($P < 0.001$). Scatter plot analysis revealed no significant correlation between V1 and V2 variation in these sample sets (data not shown). The results suggest that V1 and V2 are independent domains in *env* that can and do vary independently within related strains of HIV-1 in vivo.

Amino acid substitutions. The impact of nucleotide diversity in the amplified region would be reflected in multiple amino acid substitutions clustered within V1 or V2 in each sequence within a sample set (Fig. 3). The V1 and V2 domains are defined by the cysteine residues that are proposed to form disulfide linkages in the region (31). Because all sequences from members of a family were aligned relative to the most recent maternal sequences, nonsynonymous substitutions were apparent within sequence sets from an individual and between sample sets from different individuals. In family A, 40 different genotypes were identified among 65 clones sequenced; in family B, 53 of 55 clones were different. Sequences appeared to be representative of the population of the HIV-1 sequences within most of the individuals because repeated V1-V2-C sequences were identified in 13 of 21 sample sets.

Nonsynonymous substitutions were only one source of amino acid variation in V1 and V2. Imprecise duplications of nucleotides would introduce in-frame insertions of 3 to 5 amino acids in V1 sequences in MA or 8 to 17 amino acids in the V2 domain in child A1. An additional source of variation was contributed by recombination of V1 and V2 sequences within sample sets. For example, in MA.80.2 and A3.177.7, closely related V2 genotypes were linked to different V1 sequences. Conversely, in sample MA.80.3, a unique V1 sequence was associated with the predominant V2 genotype. The frequencies of different V1-V2 combinations in sample MA.80 (one of five clones, or 20%) and in sample A3.177 (one of nine clones, or 11%) were higher than the 5% recombination reported for PCR artifacts (23, 40, 41, 58). However, we cannot exclude the possibility that our amplification conditions are more likely to generate recombination in vitro. Defective genomes were not a feature of HIV-1 sequences in vivo in either family. Among a total of 120 sequences, only one, MB.29.1, had a translation termination codon introduced by a nucleotide substitution (Fig. 3B). There were no single nucleotide insertions or deletions that would shift the open reading frame in the amplified region.

Twelve conserved amino acids were localized between the hypervariable sequences in V1 and V2 (Fig. 3). The conserved amino acids were essentially invariant in all HIV-1 strains in vivo as a result of little, if any, nucleotide diversity. Conserved regions flanking V1 and V2 were also relatively homogeneous in peptide sequence, although nucleotides encoding the conserved regions were not invariant.

Nonsynonymous and synonymous substitutions. To assess the impact of genetic variation in this region of HIV-1 *env*, distances for nonsynonymous and synonymous changes were calculated. A portion of the C2 constant region extended 44 amino acids adjacent to V2 and was characterized by a low frequency of both nonsynonymous (d_N) and synonymous (d_S) changes within all individuals in either family (Table 2). For most sample sets, mean d_N and d_S values ranged from 1 to 5%. An excess of nonsynonymous over synonymous substitutions was not detected in the constant region of any sample set. The ratio d_N/d_S in C2 was always less than 1 (Table 2).

In the V1 and V2 hypervariable domains, the extent of nonsynonymous substitutions was greater than in the adjacent conserved region (Table 2). To assess the implication of nonsynonymous substitutions within independent hypervariable domains, the ratio d_N/d_S in V1 and in V2 was calculated for all sample sets in each individual. Values of d_N that were significantly greater than values of d_S within a hypervariable domain are highlighted in Table 2. A significant excess of nonsynonymous over synonymous substitutions was identified in all samples from two individuals. In mother MA ALL,

FIG. 3. Deduced amino acid sequences of V1, V2, and C over time in maternal-infant strains of HIV-1 from family A (A) and family B (B). Sequences from all individuals within a family are aligned with the most recent set from the mother as the reference. Multiple samples from an individual are arranged from the most recent sequence set to the earliest set. Sample numbers are as in Fig. 1. Sequences encoding amino acid differences within a sample set are numbered. Sequences of the overall region that were identified more than once in a set are designated by n = followed by the number of clones with that sequence. Dashes represent amino acid identity with the reference sequence. Spaces were introduced to maximize alignment. The asterisk in the V1 sequence of MB.29.1 designates a translation termination codon. Dots in A1.78 indicate that conserved sequences were not determined. Boxes delineate V1 and V2 domains as defined by cysteine residues (31). Amino acids in bold between V1 and V2 are conserved.

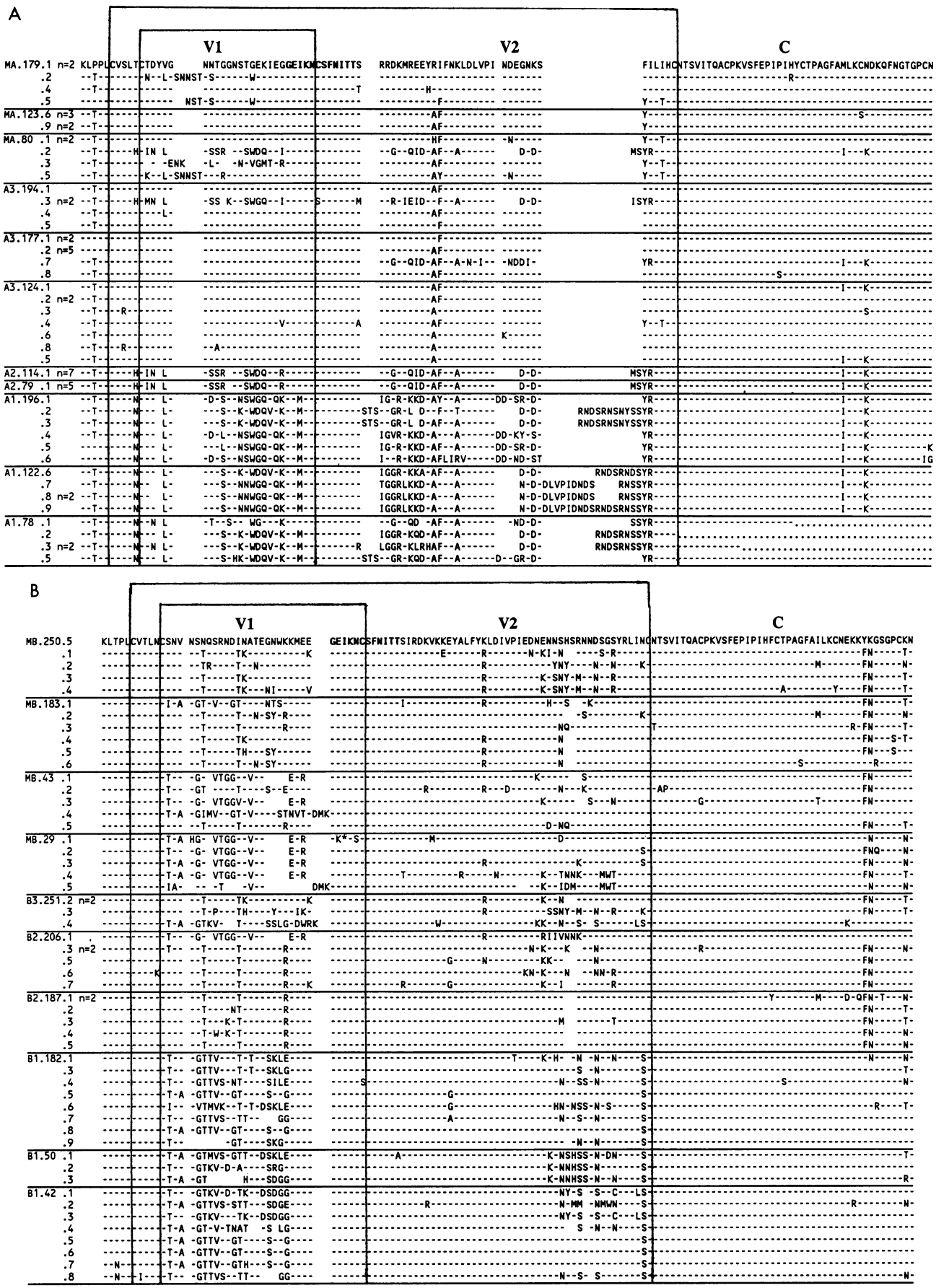
A
V1
V2
C
B
V1
V2
C

TABLE 2. Means and standard errors of nonsynonymous and synonymous substitutions within V1, V2, and C2 in individuals at different time points

Family	Sample set	Extent of substitution (mean % [± SE])[a]								
		V1			V2			C2		
		d_N	d_S	d_N/d_S	d_N	d_S	d_N/d_S	d_N	d_S	d_N/d_S
A	MA ALL	8.3 (1.4)	4.8 (1.0)	**1.7**	6.6 (1.2)	4.5 (0.8)	1.5	0.8 (0.4)	1.0 (0.4)	0.8
	.80	24.0 (3.9)	16.2 (3.4)	**1.5**	11.8 (2.3)	12.5 (2.2)	0.9	1.0 (0.6)	3.4 (1.2)	0.3
	.123	<0.1 (0.01)	<0.1 (0.05)		0	<0.1 (0.6)		0.6 (0.5)	<0.1 (0.02)	
	.179	3.0 (1.2)	<0.1 (0.08)	**>3.0**	2.7 (1.0)	2.9 (0.9)	0.9	0.4 (0.4)	<0.1 (0.08)	
	A3 ALL	4.3 (0.7)	4.2 (0.9)	1.0	8.1 (1.0)	6.2 (0.7)	1.3	0.8 (0.4)	2.5 (0.6)	0.3
	.124	1.4 (0.7)	<0.1 (0.7)	**>3.0**	1.9 (0.6)	<0.1 (0.07)	**>3.0**	1.1 (0.6)	3.3 (0.9)	0.3
	.177	1.0 (0.5)	2.4 (0.7)	0.4	8.3 (1.2)	11.4 (1.4)	0.7	0.8 (0.4)	1.5 (1.5)	0.5
	.194	17.1 (3.4)	15.4 (3.2)	1.1	19.3 (2.8)	6.2 (1.6)	**3.1**	0	3.4 (1.2)	
	A1 ALL	8.2 (1.8)	3.4 (0.7)	**2.4**	15.0 (1.8)	4.7 (1.0)	**3.2**	2.5 (0.7)	7.6 (1.3)	0.3
	.78	3.2 (1.2)	<0.1 (0.1)	**>3.0**	3.5 (0.9)	4.0 (1.0)	0.8	ND	ND	
	.122	5.9 (1.8)	<0.1 (0.1)	**>3.0**	18.2 (2.7)	4.8 (1.3)	**3.8**	0	5.4 (1.4)	
	.196	11.2 (2.3)	10.3 (2.2)	1.0	10.1 (1.9)	6.2 (1.3)	**1.6**	1.8 (0.7)	4.3 (1.2)	0.4
B	MB ALL	21.6 (3.0)	19.8 (1.4)	1.1	8.2 (1.2)	7.1 (1.2)	1.1	2.7 (0.7)	4.9 (0.9)	0.6
	.29	19.3 (3.4)	17.2 (3.1)	1.1	11.7 (2.3)	6.0 (1.7)	**1.9**	1.9 (0.8)	4.7 (1.2)	0.4
	.43	23.2 (3.7)	14.1 (3.0)	**1.6**	5.7 (1.5)	8.5 (2.2)	0.7	2.7 (0.9)	5.3 (1.4)	0.5
	.183	9.5 (1.9)	8.4 (2.2)	1.1	3.8 (1.1)	5.8 (1.5)	0.6	2.8 (0.9)	7.3 (1.8)	0.4
	.250	5.7 (1.6)	9.1 (2.0)	0.6	7.5 (1.9)	4.3 (1.4)	**1.7**	2.7 (0.9)	4.9 (0.4)	0.6
	B3 .251	19.3 (3.6)	21.4 (3.6)	0.9	10.0 (2.3)	12.7 (2.6)	0.8	1.6 (0.9)	5.0 (1.5)	0.3
	B2 ALL	11.5 (1.6)	11.5 (1.6)	1.0	7.1 (1.2)	10.0 (1.7)	0.7	1.0 (0.4)	2.8 (0.8)	0.4
	.187	1.9 (0.9)	3.1 (1.1)	0.6	1.5 (0.8)	0.9 (0.6)	1.1	0.9 (0.6)	1.0 (0.5)	0.9
	.206	11.2 (2.2)	16.0 (2.4)	0.7	8.5 (1.7)	9.7 (2.3)	0.9	0.1 (0.6)	2.2 (1.0)	0.5
	B1 ALL	7.0 (1.3)	9.5 (0.8)	0.7	6.6 (1.3)	6.5 (1.7)	1.0	1.1 (0.5)	1.5 (0.7)	0.7
	.42	11.2 (2.1)	10.4 (2.1)	1.1	8.0 (1.6)	13.1 (2.3)	0.6	0.7 (0.4)	2.5 (1.0)	0.3
	.50	19.3 (3.6)	30.3 (5.3)	0.6	2.0 (1.2)	6.1 (1.9)	0.3	1.0 (0.7)	2.1 (1.1)	0.5
	.182	8.6 (1.9)	5.3 (1.4)	**1.6**	5.2 (1.2)	7.4 (1.7)	0.7	1.4 (0.6)	3.3 (0.8)	0.4

[a] Boldface numbers indicate d_N/d_S ratios that are significantly greater than 1, as determined by Student's t test. ND, not determined. Data for A2 are not included.

nonsynonymous substitutions occurred more frequently than synonymous changes in V1 ($P < 0.05$) but not in V2. In child A1 ALL, d_N was greater than d_S in V1 ($P < 0.05$) and in V2 ($P < 0.001$).

Excess d_N/d_S values in overall *env* hypervariable sequences actually reflected temporal differences in excess nonsynonymous over synonymous substitutions within each individual. For example, d_N values were significantly greater than d_S values in samples MA.80 ($P < 0.001$) and MA.179 ($P < 0.05$), in contrast to sample MA.123. In child A1, there were points in time when nonsynonymous changes were no more frequent than synonymous changes, as in the V2 domain in A1.78 and in the V1 domain in A1.196. Likewise, in MB and her oldest child B1, significant differences in d_N/d_S became apparent only when sample sets from specific time points were analyzed (Table 2).

Nonsynonymous substitutions were significantly greater than synonymous substitutions in one infant A3 at 5 days (A3.124) and 14 weeks (A3.194) of age. The excess nonsynonymous substitutions occurred in both the V1 and V2 domains. In contrast, there was no excess of nonsynonymous changes in either V1 or V2 among three additional infants (A2, B2, and B3), even though mean values for both d_N and d_S were high in B2.206 and B3.251 (Table 2).

A significant excess of d_N/d_S in a hypervariable domain was not always related to overall genetic variation within a sample set. Among 18 samples which displayed d_N and d_S values greater than 1%, a significant excess of nonsynonymous substitutions was identified in the hypervariable domains of 11 sample sets. In general, d_N values were usually higher than d_S values in either one or the other hypervariable domains. There was no significant correlation for nonsynonymous or synonymous substitutions between V1 and V2 within any individual, consistent with the overall independent genetic variation in the two domains. The results indicate a role for selective pressures in amino acid diversity and suggest that V1 and V2 are responsive to different selective pressures.

DISCUSSION

HIV-1 genetic variation, pathogenesis, perinatal transmission, and host interactions cannot be examined directly in an animal model. Maternal transmission of HIV-1 provides a natural alternative because the source of virus transmitted to the infected infant is unambiguous and because evolution of related HIV-1 genotypes can be followed in genetically related individuals.

In our studies of two HIV-1-infected families, localization of genetic variation in the 5′ portion of *env* in vivo is not random but maps almost exclusively to the hypervariable domains V1 and V2. In contrast to V3, V4, and V5, which are organized as distinct domains, V1 and V2 have a close physical linkage within gp120. The evidence from our studies establishes that the V1 and V2 hypervariable domains are distinct genetic entities that can vary independently of each other and the surrounding constant regions. Although the sequences that we analyzed were by no means exhaustive of virus populations in vivo, we found that V1 and V2 fluctuated over time and diverged independently in related individuals infected with epidemiologically related viruses. Only a limited number of studies have evaluated subgenomic

sequence variability in HIV-1 in vivo from longitudinal samples (1, 10, 22, 37, 40, 71). Nonetheless, a picture is emerging of fluctuations in HIV-1 provirus sequences in peripheral blood cells that were shown in one study to reflect the sequences found in plasma virus (60).

It is possible that fluctuations in genetic variation in vivo accumulate stochastically during virus replication and do not necessarily reflect the influence of selective or adaptive pressures (4, 64, 73). If this were the case, one could predict that genetic variation in vivo and in culture might be similar. However, our results indicate that there are distinct differences in V1 and V2 domains between viruses in vivo and those in culture. The extent of variation in V1 or V2 that we found in infected individuals exceeds genetic variation that accumulates when HIV-1 isolates are passaged in peripheral blood mononuclear cells in culture (19, 29). In contrast to virus strains in culture, which can accumulate defective genomes as a result of stop codons in V1 and V2 (19), defective genomes in vivo were not a feature of the HIV-1 sequences in our studies. Variation in V1 or V2 in vivo can be dramatically different within related viruses infecting members of the same family. Moreover, a significant excess of nonsynonymous over synonymous changes in vivo became apparent when V1 and V2 were analyzed at different points in time within an infected individual.

The significant excess of nonsynonymous over synonymous changes in V1 and V2 hypervariable domains of *env* is characteristic of positive genetic selection (22, 23) and suggests that the host environment plays a role in virus variation. However, host factors are apparently unrelated to age, mode or duration of infection, or time interval between samples. There was not an obvious relationship between extent or type of genetic variation in V1 or V2 and clinical status of the individuals in our study. Perhaps changes in V1 and V2 are irrelevant in the clinical course of HIV-1 disease. Because all of the individuals progressed to AIDS during the course of our study, variation in V1 and V2 during prolonged clinical latency is not known. Fluctuations in V1 and V2 variation may reflect periodic restriction in virus populations that would require analyses of more frequent samples sets to detect. Alternatively, a relationship between specific genotypic changes in V1 or V2 and clinical status of the host may not be obvious in view of the extent of diversity in HIV-1 sequences in the region. Associations between lentivirus *env* genotypes and disease progression have been made in animal models by using molecular clones as inocula (27, 50). Nonetheless, a multiplicity of genotypes evolve and can be detected late in disease. In humans, associations between V3 genotype and HIV-1 phenotype were not immediately obvious. As a result of a larger data base and functional analyses, substitutions in specific amino acid positions in the V3 loop which are significant for virus phenotype and can be associated with disease progression have been identified (30, 65).

An excess of nonsynonymous over synonymous substitutions in V1 and V2 is reminiscent of the V3 hypervariable domain, for which there is evidence that immune selection contributes to the evolution of sequence variation (22, 71, 75). An excess of nonsynonymous substitutions in V1 or V2 within infected individuals would alter the virus phenotype and could result in the emergence of antigenic variants of HIV-1. The possibility that positive selection in V1 or V2 results at least in part from immune pressures is a plausible hypothesis for several reasons. Although V3 is an immunodominant domain in gp120, recognition of V3 epitopes can be modulated by genetic changes outside the V3 hypervariable domain (38, 43, 45, 46). Host immune responses against HIV-1 are not restricted to determinants in V3 but map to regions within V1 and V2 as well. For example, antibodies cross-reactive with linear V1 epitopes can be identified in individuals infected with HIV-1 (67). Likewise, epitopes in V2 can provide targets for conformation-dependent and -independent antibodies that neutralize homologous strains of HIV-1 (17). Because V1 and V2 are independent domains that could be involved in multiple functions, positive selection in V1 or V2 need not be restricted to neutralization antibodies but may also include T-cell recognition epitopes (13), antibodies that enhance infection (26, 63), or cell tropism (69). Isolates of HIV-1 develop multiple nonsynonymous substitutions in V2 after propagation in a macrophage cell line that do not appear when the strains are cultivated on a T-cell line (68). Moreover, determinants that, in conjunction with sequences in V3, are critical for macrophage infectivity by HIV-1 in vitro have been localized to V2 (69). The full extent of functional interplay between epitopes in V3 and domains in V2 or V1 remains to be examined directly.

A significant excess of nonsynonymous over synonymous changes in V1 or V2 was not apparent in all of the infants. Genetic variability and the extent of nonsynonymous and synonymous substitutions could fluctuate, indicating that virus populations in the infants were not static. Even in one infant with homogeneous V1 and V2 sequences, there was evidence of active viremia. Therefore, although genetic variation in HIV-1 sequences occurs in infants, it does not necessarily reflect the extent of selective pressures observed in older children and adults. Positive selection may not be a factor in infants because of where the virus is replicating, because of the naive state of the neonatal immune system, or because of impaired immune function induced by neonatal HIV-1 infection.

Antibody titers and affinities for epitopes in gp120 have been implicated as factors protecting infants from perinatal transmission of HIV-1 (12, 18, 51, 53, 66) or in delaying disease progression in infected children (3, 36). Conformation-dependent or -independent epitopes that are localized within the principal neutralization domain or are located outside the V3 loop can contribute to maternal humoral immune responses (66). There is evidence to suggest that determinants in V1 and V2 can elicit immune responses in HIV-1-infected individuals (67). Therefore, effective immune strategies to prevent perinatal HIV-1 infection should consider the extent to which protective immunity involves V1 and V2 epitopes of maternal HIV-1 strains.

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REFERENCES

1. **Balfe, P., P. Simmonds, C. A. Ludlam, J. O. Bishop, and A. J. Leigh Brown.** 1990. Concurrent evolution of human immunodeficiency virus type 1 in patients infected from the same source: rate of sequence change and low frequency of inactivating mutations. J. Virol. **64**:6221–6233.
2. **Becker, M. L. B., S. Engelbrecht, G. deJager, and W. B. Becker.** 1992. Sequence variation in the *env* gene of HIV-1 strains from Southern Africa, p. 197–199. *In* F. Brown, R. M. Chanock,

H. S. Ginsberg, and R. A. Lerner (ed.), Vaccines 92. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Broliden, P. A., V. Moschese, K. Ljunggren, J. Rosen, C. Fundaro, A. Plebani, M. Jondal, P. Rossi, and B. Wahren.** 1989. Diagnostic implications of specific immunoglobulin G patterns of children born to HIV-infected mothers. AIDS **3**:577–582.
4. **Brown, A. L., and P. Monaghan.** 1988. Evolution of the structural proteins of human immunodeficiency virus: selective constraints on nucleotide substitution. AIDS Res. Hum. Retroviruses **4**:399–407.
5. **Centers for Disease Control.** 1987. Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. Morbid. Mortal. Weekly Rep. **36**:833–845.
6. **Clements, J. E., S. L. Gdovin, R. C. Montelaro, and O. Narayan.** 1988. Antigenic variation in lentivirus diseases. Annu. Rev. Immunol. **6**:139–159.
7. **Coffin, J. M.** 1992. Genetic diversity and evolution of retroviruses. Curr. Top. Microbiol. Immunol. **176**:143–164.
8. **Cordonnier, A., L. Montagnier, and M. Emerman.** 1989. Single amino-acid changes in HIV envelope affect viral tropism and receptor binding. Nature (London) **340**:571–574.
9. **De Jong, J.-J., J. Goudsmit, W. Keulen, B. Klaver, W. Krone, M. Tersmette, and A. De Ronde.** 1992. Human immunodeficiency virus type 1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity. J. Virol. **66**:757–765.
10. **Delassus, S., R. Cheynier, and S. Wain-Hobson.** 1991. Evolution of human immunodeficiency virus type 1 *nef* and long terminal repeat sequences over 4 years in vivo and in vitro. J. Virol. **65**:225–231.
11. **Delassus, S., R. Cheynier, and S. Wain-Hobson.** 1992. Nonhomogeneous distribution of human immunodeficiency virus type 1 proviruses in the spleen. J. Virol. **66**:5624–5645.
12. **Devash, Y., T. Cavelli, D. Wood, K. Reagan, and A. Rubenstein.** 1990. Vertical transmission of human immunodeficiency virus is correlated with the absence of high-affinity/avidity maternal antibodies to the gp 120 principal neutralizing domain. Proc. Natl. Acad. Sci. USA **87**:3445–3449.
13. **Earl, P. L., S. Koenig, and B. Moss.** 1991. Biological and immunological properties of human immunodeficiency virus type 1 envelope glycoprotein: analysis of proteins with truncations and deletions expressed by recombinant vaccinia viruses. J. Virol. **65**:31–41.
14. **Fouchier, R. A. M., M. Groenink, N. A. Kootstra, M. Tersmette, H. G. Huisman, F. Miedema, and H. Schvitemaker.** 1992. Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. J. Virol. **66**:3183–3187.
15. **Freed, E. O., D. J. Myers, and R. Risser.** 1991. Identification of the principal neutralizing determinant of human immunodeficiency virus type 1 as a fusion domain. J. Virol. **65**:190–194.
16. **Fujita, K., J. Silver, and K. Peden.** 1992. Changes in both gp120 and gp41 can account for increased growth potential and expanded host range of human immunodeficiency virus type 1. J. Virol. **66**:4445–4451.
17. **Fung, M. S. C., C. Sun, W. L. Gordon, R.-S. Liou, T. W. Chang, W. Sun, E. S. Daar, and D. D. Ho.** 1992. Identification and characterization of a neutralization site within the second variable region of human immunodeficiency virus type 1 gp120. J. Virol. **66**:848.
18. **Goedert, J., H. Mendez, J. E. Drummond, M. Robert-Guroff, H. L. Minkoff, S. Holman, R. Stevens, A. Rubinstein, W. A. Blattner, A. Willoughby, and S. H. Landesman.** 1989. Mother-to-infant transmission of human immunodeficiency virus type 1: association with prematurity or low anti-gp120. Lancet **ii**:1351–1354.
19. **Goodenow, M., T. Huet, W. Saurin, S. Kwok, J. Sninsky, and S. Wain-Hobson.** 1989. HIV-1 isolates are rapidly evolving quasispecies: evidence for viral mixtures and preferred nucleotide substitutions. J. Acquired Immune Defic. Syndr. **2**:344–352.
20. **Goodenow, M. M., J. W. Sleasman, and S. L. Lamers.** Unpublished data.
21. **Goudsmit, J., K. Nicole, T. Back, and P. L. Nara.** 1991. Genomic diversity and antigenic variation of HIV-1: links between pathogenesis, epidemiology and vaccine development. FASEB J. **5**:2427–2436.
22. **Holmes, E. C., L. Q. Zhang, P. Simmonds, C. A. Ludlam, and A. J. Leigh-Brown.** 1992. Convergent and divergent sequence evolution in the surface envelope glycoprotein of human immunodeficiency virus type 1 within a single infected patient. Proc. Natl. Acad. Sci. USA **89**:4835–4839.
23. **Howell, R. M., J. E. Fitzgibbon, M. Noe, Z. Ren, D. J. Gocke, T. A. Schwartzer, and D. T. Dubin.** 1991. In vivo sequence variation of the human immunodeficiency virus type 1 *env* gene: evidence for recombination among variants found in a single individual. AIDS Res. Hum. Retroviruses **7**:869–876.
24. **Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. P. Cullen.** 1992. Identification of envelope V3 loop as the major determinant of CD4 neutralization sensitivity of HIV-1. Science **257**:535–537.
25. **Javaherian, K., A. J. Langlois, C. McDanal, K. L. Ross, L. I. Eckler, C. L. Jellis, A. T. Profy, J. R. Rusche, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1989. Principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein. Proc. Natl. Acad. Sci. USA **86**:6768–6772.
26. **Jiang, S., L. Kang, and A. R. Neurath.** 1991. Search for domains in envelope glycoproteins of HIV-1 eliciting antibodies enhancing HIV-1 infection. J. Exp. Med. **174**:151–155.
27. **Kestler, H. W., Y. Li, Y. M. Naidu, C. V. Butler, M. F. Ochs, G. Jaenel, N. W. King, M. D. Daniel, and R. C. Desrosiers.** 1988. Comparison of simian immunodeficiency virus isolates. Nature (London) **331**:619–621.
28. **Kuiken, C. L., J.-J. de Jong, E. Baan, W. Keulen, M. Tersmette, and J. Goudsmit.** 1992. Evolution of the V3 envelope domain in proviral sequences and isolates of human immunodeficiency virus type 1 during transition of the viral biological phenotype. J. Virol. **66**:4622–4627.
29. **Kusumi, K., B. Conway, S. Cunningham, A. Berson, C. Evans, K. Astrid, N. Iversen, D. Colvin, M. V. Gallo, S. Coutre, E. G. Shaper, D. V. Faulkner, A. DeRonde, S. Volkman, C. Williams, M. S. Hirsch, and J. I. Mullins.** 1992. Human immunodeficiency virus type 1 envelope structure and diversity in vivo and after cocultivation in vitro. J. Virol. **66**:875–885.
30. **LaRosa, G. J., J. P. Davide, K. Weinhold, J. A. Waterbury, A. T. Profy, J. A. Lewis, A. J. Langlois, G. R. Dreesman, R. N. Boswell, P. Shadduck, L. H. Holley, M. Karplus, D. P. Bolognesi, T. J. Matthews, E. A. Emini, and S. D. Putney.** 1990. Conserved sequence and structural elements in the HIV-1 principle neutralizing determinant. Science **249**:932–935.
31. **Leonard, C. K., M. W. Spellman, L. Riddle, R. J. Harris, J. N. Thomas, and T. J. Gregory.** 1990. Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. J. Biol. Chem. **265**:10373–10382.
32. **Lewis, G. K., Y. H. Abacioglu, T. Fouts, J. Sampson, M. Moorman, G. Bauer, R. Tuskan, G. A. Cole, and R. Kamin-Lewis.** 1991. Epitope dominance in the antibody response to recombinant gp 160 of HIV-IIIB, p. 157–163. *In* F. Brown, R. M. Chanock, H. S. Ginsberg, and R. A. Lerner (ed.), Vaccines 91. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Li, W.-H., M. Tanimura, and P. M. Sharp.** 1988. Rates and dates of divergence between AIDS virus nucleotide sequences. Mol. Biol. Evol. **5**:313–330.
34. **Li, Y., J. C. Kappes, J. A. Conway, R. W. Price, G. M. Shaw, and B. H. Hahn.** 1991. Molecular characterization of human immunodeficiency virus type 1 cloned directly from uncultured human brain tissue: identification of replication-competent and -defective viral genomes. J. Virol. **65**:3973–3985.
35. **Liu, Z.-Q., C. Wood, J. A. Levy, and C. Cheng-Mayer.** 1990. The viral envelope gene is involved in macrophage tropism of a human immunodeficiency virus type 1 strain isolated from brain tissue. J. Virol. **64**:6148–6153.
36. **Ljunggren, K., V. Moschese, and P. Proliden.** 1990. Antibodies mediating cellular cytootoxicity and neutralization correlate

with a better clinical stage in children born to human immunodeficiency virus infected mothers. J. Infect. Dis. **161:**198–202.
37. **Martins, L. P., N. Chenciner, B. Asjo, A. Meyerhans, and S. Wain-Hobson.** 1991. Independent fluctuation of human immunodeficiency virus type 1 *rev* and gp41 quasispecies in vivo. J. Virol. **65:**4502–4507.
38. **McKeating, J., J. Moore, R. Willey, S. Putney, M. Martin, and R. Weiss.** 1989. Predicted interaction of V3 neutralization loop with other regions of the HIV-1 envelope, p. 195–202. *In* M. Girard and L. Valette (ed.), Proceedings of Quatrieme Colloque des Cent Gardes. Pasteur Vaccins, Paris.
39. **McKeating, J. A., J. Gow, J. Goudsmit, L. H. Pearl, C. Mulder, and R. A. Weiss.** 1989. Characterization of HIV-1 neutralization escape mutants. AIDS **3:**777–784.
40. **Meyerhans, A., R. Cheynier, J. Albert, M. Seth, S. Kwok, J. Sninsky, L. Morfeldt-Manson, B. Asjo, and S. Wain-Hobson.** 1989. Temporal fluctuations in HIV quasispecies in vivo are not reflected by sequential HIV isolations. Cell **58:**901–910.
41. **Meyerhans, A. F., J. P. Vartanian, and S. Wain-Hobson.** 1990. DNA recombination during PCR. Nucleic Acids Res. **18:**1687–1691.
42. **Modrow, S., B. H. Hahn, G. M. Shaw, R. C. Gallo, F. Wong-Staal, and H. de Wolf.** 1987. Computer-assisted analysis of envelope protein sequences of seven human immunodeficiency virus isolates: predictions of antigenic epitopes in conserved and variable regions. J. Virol. **61:**570–578.
43. **Moore, J. P., and P. L. Nara.** 1991. The role of the V3 domain of gp 120 in HIV infection. AIDS Suppl. **2:**521–533.
44. **Myers, G. M., B. Korber, J. A. Berzofsky, R. F. Smith, and G. N. Pavlakis.** 1991. Human retroviruses and AIDS. Los Alamos National Laboratory, Los Alamos, N.Mex.
45. **Nara, P. L., R. Garrity, A. Minassian, and J. Goudsmit.** 1991. Envelope plasticity outside the V3 and gp 120-CD4 binding epitopes results in resistance to neutralization and fusion without loss of antibody binding: implications to HIV-1 vaccine design. FASEB J. **5:**175–181.
46. **Nara, P. L., L. Smith, N. Dunlop, W. Hatch, M. Merges, D. Waters, J. Kelliher, R. C. Gallo, P. J. Fischinger, and J. Goudsmit.** 1990. Emergence of viruses resistant to neutralization by V3-specific antibodies in experimental human immunodeficiency virus type 1 IIIB infection of chimpanzees. J. Virol. **64:**3779–3791.
47. **Nei, M., and T. Gojobori.** 1986. Simple methods for estimating the number of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. **3:**418–426.
48. **Nei, M., and L. Jin.** 1989. Variances of the average numbers of nucleotide substitutions within and between populations. Mol. Biol. Evol. **6:**290–300.
49. **O'Brien, W. A., Y. Koyanag, A. Namazie, J. Zhao, A. Diagne, K. Idler, J. A. Zack, and I. S. Y. Chen.** 1990. HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4-binding domain. Nature (London) **348:**69–73.
50. **Overbaugh, J. M., and L. M. Rudensey.** 1992. Alterations in potential sites for glycosylation predominate during evolution of the simian immunodeficiency virus envelope gene in macaques. J. Virol. **66:**5937–5948.
51. **Parkeh, B., N. Shaffer, C. Pav, E. Abrams, P. Thomas, H. Pollack, M. Bamji, A. Kaul, G. Schochetman, M. Rogers, J. R. George, and the NYC Perinatal HIV Transmission Collaborative Group.** 1991. Lack of correlation between maternal antibodies to V3 loop peptides of gp 120 and perinatal HIV-1 transmission. AIDS **5:**1179–1184.
52. **Peden, K., M. Emerman, and L. Montagnier.** 1991. Changes in growth properties on passage in tissue culture of viruses derived from infectious molecular clones of HIV-1_{LAI}, HIV-1_{MAL}, and HIV-1_{ELI}. Virology **185:**661–672.
53. **Rossi, P., V. Moschese, P. A. Broliden, C. Fundaro, I. Quinti, A. Plebani, C. Giaquinto, P. Tovo, K. Ljunggren, and J. Rosen.** 1989. Presence of maternal antibodies to human immunodeficiency virus-1 envelope glycoprotein gp 120 epitopes correlates with the uninfected status of children born to seropositive mothers. Proc. Natl. Acad. Sci. USA **86:**8055–8056.
54. **Saag, M. S., B. H. Hahn, J. Gibbons, Y. Li, E. S. Parks, W. P. Parks, and G. M. Shaw.** 1988. Extensive variation of human immunodeficiency virus type-1 in vivo. Nature (London) **334:**440–444.
55. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
56. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
57. **Sharpless, N. E., W. A. O'Brien, E. Verdin, C. V. Kufta, S. Irvin, Y. Chen, and M. Dubois-Dalco.** 1992. Human immunodeficiency virus type 1 tropism for brain microglial cells is determined by a region of the *env* glycoprotein that also controls macrophage tropism. J. Virol. **66:**2588–2593.
58. **She, J. X., S. Boehme, T. W. Wang, F. Bonhomme, and E. K. Wakeland.** 1991. Amplification of major histocompatibility complex class II gene diversity by intra-exonic recombination. Proc. Natl. Acad. Sci. USA **88:**453–457.
59. **Shioda, T., J. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp 120 gene. Nature (London) **349:**167–169.
60. **Simmonds, P., P. Balfe, C. A. Ludlam, J. O. Bishop, and A. J. Leigh-Brown.** 1990. Discontinuous sequence change of human immunodeficiency virus (HIV) type 1 *env* sequences in plasma viral and lymphocyte-associated proviral populations in vivo: implications for models of HIV pathogenesis. J. Virol. **64:**5840–5850.
61. **Steimer, K. S., C. J. Scandella, P. V. Skiles, and N. L. Haigwood.** 1991. Neutralization of divergent HIV-1 isolates by conformation dependent human antibodies to gp120. Science. **254:**105–108.
62. **Stevenson, M., S. Haggerty, C. Lamonica, A. M. Mann, C. Meier, and A. Wasiak.** 1990. Cloning and characterization of human immunodeficiency virus type 1 variants diminished in the ability to induce syncytium-independent cytolysis. J. Virol. **64:**3792–3803.
63. **Takeda, A., J. E. Robinson, D. D. Ho, C. Debouck, N. L. Haigwood, and Francis A. Ennis.** 1992. Distinction of human immunodeficiency virus type 1 neutralization and infection enhancement by human monoclonal antibodies to glycoprotein 120. J. Clin. Invest. **89:**1952–1957.
64. **Temin, H. M.** Is HIV unique or merely different? J. Acquired Immune Defic. Syndr. **2:**1–9.
65. **Tersmette, M., R. A. Gruters, F. de Wolf, R. E. Y. de Goede, J. M. A. Lange, P. T. A. Schellekens, J. Goudsmit, H. G. Huisman, and F. Miedema.** 1989. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. J. Virol. **63:**2118–2125.
66. **Ugen, K. E., J. J. Goedert, J. Boyer, Y. Refaeli, I. Frank, W. V. Williams, A. Willoughby, S. Landesman, A. Rubinstein, J. Berzofsky, T. Kieber-Emmons, and D. B. Weiner.** 1992. Vertical transmission of HIV infection: maternal humoral immune responses to gp120 and gp41 peptides. J. Clin. Invest. **89:**183–189.
67. **van Tijn, D. A., C. A. Boucher, M. Bakker, and J. Goudsmit.** 1989. Antigenicity of linear B-cell epitopes in the C1, V1, and V3 region of HIV-1 gp 120. J. Acquired Immune Defic. Syndr. **2:**303–306.
68. **Vartanian, J.-P., A. Meyerhans, B. Asjo, and S. Wain-Hobson.** 1991. Selection, recombination, and G→A hypermutation of human immunodeficiency virus type 1 genomes. J. Virol. **65:**1779–1788.
69. **Westervelt, P., D. P. Trowbridge, L. G. Epstein, B. M. Blumber, Y. Li, B. H. Hahn, G. M. Shaw, R. W. Price, and L. Ratner.** 1992. Macrophage-tropic determinants of human immunodeficiency virus type 1. J. Virol. **66:**2577–2582.
70. **Willey, R. L., R. A. Rutledge, S. Dias, T. Folks, T. Theodore, C. E. Buckler, and M. A. Martin.** 1986. Identification of conserved and divergent domains within the envelope gene of the acquired immunodeficiency syndrome virus. Proc. Natl. Acad. Sci. USA **83:**5083–5042.

71. **Wolfs, T. F. W., J.-J. de Jong, H. vanden Berg, J. M. G. H. Tijnagel, W. J. A. Krone, and J. Goudsmit.** 1990. Evolution of sequences encoding the principal neutralization epitope of human immunodeficiency virus 1 is host dependent, rapid, and continuous. Proc. Natl. Acad. Sci. USA **87:**9938–9942.
72. **Wolinsky, S., C. M. Wike, B. T. M. Korber, C. Hutto, W. P. Parks, L. L. Rosenblum, K. J. Kunstman, M. R. Furtado, and J. L. Munoz.** 1992. Selective transmission of human immunodeficiency virus type 1 variants from mothers to infants. Science **255:**1134–1137.
73. **Yokoyama, S., L. Chung, and T. Gojobori.** 1988. Molecular evolution of the human immunodeficiency and related viruses. Mol. Biol. Evol. **5:**237–251.
74. **York-Higgins, D., C. Cheng-Mayer, D. Bauver, J. A. Levy, and D. Dino.** 1990. Human immunodeficiency virus type 1 cellular host range, replication, and cytopathicity are linked to the envelope region of the viral genome. J. Virol. **64:**4016–4020.
75. **Zwart, G., H. Langdijk, L. van der Hoek, J.-J. de Jong, T. F. W. Wolfs, C. Ramautarsing, M. Bakker, A. de Ronde, and J. Goudsmit.** 1991. Immunodominance and antigenic variation of the principal neutralization domain of HIV-1. Virology **181:**481–489.