JOURNAL OF VIROLOGY, Aug. 2004, p. 7883–7893 Vol. 78, No. 15
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.15.7883–7893.2004

Compartmentalization of Human Immunodeficiency Virus Type 1 between Blood Monocytes and CD4$^+$ T Cells during Infection

Jennifer A. Fulcher,[1]† Yon Hwangbo,[1]† Rafael Zioni,[1] David Nickle,[2] Xudong Lin,[1] Laura Heath,[2] James I. Mullins,[1,2] Lawrence Corey,[1,2,3] and Tuofu Zhu[1,2]*

Departments of Laboratory Medicine[1] and Microbiology,[2] University of Washington School of Medicine, Seattle, Washington 98195, and Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, Seattle, Washington 98109[3]

Received 4 September 2003/Accepted 23 March 2004

Distinct sequences of human immunodeficiency virus type 1 (HIV-1) have been found between different tissue compartments or subcompartments within a given tissue. Whether such compartmentalization of HIV-1 occurs between different cell populations is still unknown. Here we address this issue by comparing HIV-1 sequences in the second constant region through the fifth hypervariable region (C2 to V5) of the surface envelope glycoprotein (Env) between viruses in purified blood CD14$^+$ monocytes and CD4$^+$ T cells obtained longitudinally from five infected patients over a time period ranging from 117 to 3,409 days postseroconversion. Viral populations in both cell types at early infection time points appeared relatively homogeneous. However, later in infections, all five patients showed heterogeneous populations in both CD14$^+$ monocytes and CD4$^+$ T cells. Three of the five patients had CD14$^+$ monocyte populations with significantly more genetic diversity than the CD4$^+$ T-cell population, while the other two patients had more genetic diversity in CD4$^+$ T cells. The cellular compartmentalization of HIV-1 between CD14$^+$ monocytes and CD4$^+$ T cells was not seen early during infections but was evident at the later time points for all five patients, indicating an association of viral compartmentalization with the time course of HIV-1 infection. The majority of HIV-1 V3 sequences indicated a macrophage-tropic phenotype, while a V3 sequence-predicted T-cell tropic virus was found in the CD4$^+$ T cells and CD14$^+$ monocytes of two patients. These findings suggest that HIV-1 in CD14$^+$ monocytes could disseminate and evolve independently from that in CD4$^+$ T cells over the course of HIV-1 infection, which may have implications on the development of new therapeutic strategies.

Human immunodeficiency virus type 1 (HIV-1) gene sequences develop a large degree of variation between and within infected individuals (4, 13, 14, 19, 20, 34, 43, 45, 48, 54, 55, 83, 85, 97, 103–105, 112). In the initial period after infection, most individuals evaluated to date have shown homogeneous sequence populations of the HIV-1 surface envelope glycoprotein gene (*env*) (54, 64, 106, 109, 112) and a low level of variation in other structural genes, including *gag* p17 (109, 112) and *gp41*/*nef* (112). However, some individuals, especially women, have relatively diverse HIV-1 populations at or before seroconversion (49, 63, 68, 69, 112, 114). After this initial period, divergent HIV-1 variants with different but related genetic sequences emerge and turn over throughout the course of infection (4, 13, 14, 19, 20, 34, 39, 43, 45, 48, 54–56, 58, 83, 85, 97, 103–105, 112). Consequently, chronically infected patients typically have heterogeneous HIV-1 populations, resulting in distinct but related viral quasispecies. Virus replication is associated with the generation of sequence variation (16, 64), particularly in the hypervariable regions of the *env* gene (46). The amount of subsequent viral genetic diversity seen in a given individual coincides, at least in part, with selective pressures for structural change imposed by the immune system (7, 15, 48, 50, 96, 105), antiretroviral drug pressure (44, 57), or preferential tropism for and replication in target cells (6, 10, 30, 80, 89).

Analyses of HIV-1 *env* sequences from donor-recipient pairs (sexually transmitted virus) have revealed genetic distinctions among viral populations in peripheral blood mononuclear cells (PBMC), plasma, seminal cells, and seminal fluid (114). Other studies have shown differential representations of groups of viral sequences from blood, semen (18, 114), and genital secretions from women (68, 114). Likewise, drug resistance mutations in HIV-1 populations are unequally distributed in the blood and semen (42). Furthermore, marked differences exist among HIV-1 sequences from different tissue compartments, such as the blood, brain, spleen, lymph nodes, and other tissues (5, 22, 31, 35, 37, 40, 52, 82, 107, 108), as well as from subcompartments of a given tissue, such as the white pulp and red pulp of the spleen (30) or the frontal lobe, basal ganglia, medial temporal lobe, and nonmedial temporal lobe of the brain (81). Until now, the possibility of compartmentalization among different cell types has not been demonstrated.

It has been well documented that macrophage-tropic (M-tropic) HIV-1 variants dominate in the establishment and early stages of HIV-1 infection (72, 93, 109, 112). Given that macrophages originate from blood monocytes, it is important to determine the pathogenic significance of HIV-1 in monocytes in the establishment and persistence of HIV-1 infection. We have therefore characterized and compared HIV-1 sequences corresponding to the second constant region through the fifth hypervariable region (C2 to V5) of envelope *gp120* between the cell compartments of CD14$^+$ monocytes and CD4$^+$ T cells

* Corresponding author. Mailing address: Department of Laboratory Medicine, University of Washington School of Medicine, Box 358070, 1959 NE Pacific St., Seattle, WA 98195-8070. Phone: (206) 732-6079. Fax: (206) 732-6109. E-mail: tzhu@u.washington.edu.

† J.A.F. and Y.H. contributed equally to this study.

TABLE 1. Characterization of HIV-1-infected patients and samples

Patient no.	Sample	Time (days)		No. of $CD4^+$ cells/ mm^3	No. of HIV-1 RNA copies/ ml of plasma[c]
		Sampling[a]	ART[b]		
1	I	127	None	900	499
	II	912	None	495	3155
	III	990	None	548	<500
	IV	1,046	None	430	1,426
2	I	117	None	262	25,140
	II	1,490	638	255	<50
3	I	925	None	824	<10,000
	II	1,079	None	497	<10,000
	III	1,285	None	546	861
	IV	2,535	None	686	90
4	I	2,193	1,124	ND[d]	133
	II	2,823	1,754	207	<50
5	I	167	None	1,564	14,910
	II	999	None	986	ND[d]
	III	3,212	None	ND[d]	26,943
	IV	3,409	55	375	<50

[a] Number of days postseroconversion.
[b] Number of days on antiretroviral therapy.
[c] Viral RNA levels in plasma were measured by a commercial reverse transcription-PCR assay (Amplicor Ultrasensitive HIV-1 monitor assay; Roche) or a bDNA assay (Chiron Corporation, Emeryville, Calif.).
[d] ND, not done.

in the peripheral blood obtained longitudinally from patients after seroconversion. Our results indicate that both cellular compartmentalization and nonparallel evolution of HIV-1 between blood monocytes and $CD4^+$ T cells occur over the course of infection.

(This work was presented in part at the 9th Conference on Retroviruses and Opportunistic Infections, Seattle, Wash., 24 to 28 February 2002.)

MATERIALS AND METHODS

Study patients. Three heterosexual women (patients 1 to 3) and two homosexual men (patients 4 and 5) were recruited from the University of Washington Primary Infection Clinic (75, 76). The enrollment criteria, detailed demographic characteristics, and definitions of the onset of infection and of seroconversion of this primary infection cohort were reported previously (75, 76). Patients were randomly selected based on sample availability. Longitudinal samples obtained for the present study from the five patients were collected over a time period ranging from 117 to 3,409 days postseroconversion (Table 1). Two of the five patients were naïve to therapy. Both patient 2 and patient 4 were on zidovudine (600 mg/day) and lamivudine (300 mg/day) beginning 420 days (patient 2) and 3 years (patient 4) after HIV-1 infection. Patient 2 initiated highly active antiretroviral therapy (HAART) containing lamivudine (300 mg/day), stavudine (80 mg/day), and nelfinavir (2,500 mg/day) at day 1,026 postseroconversion. Patient 4 switched to HAART containing stavudine (80 mg/day), didanosine (400 mg/day), and amprenivir (2,400 mg/day) after 3 years for 474 days before changing from stavudine to zidovudine (600 mg/day). Patient 5 initiated HAART at year 8 postseroconversion.

Isolation of $CD14^+$ monocytes and $CD4^+$ T cells. $CD14^+$ monocytes and $CD4^+$ T cells were purified from PBMC samples by a two-step column purification method (Miltenyi Biotec, Auburn, Calif.). Specifically, $CD14^+$ monocytes were first purified by use of a negative selection kit containing a cocktail of antibodies against CD3, CD7, CD19, CD45RA and CD56 and an anti-immunoglobulin E (IgE) antibody to remove T cells, B cells, NK cells, granulocytes, dendritic cells, and basophils. This monocyte-enriched population was further purified by positive selection with CD14 MicroBeads. $CD4^+$ T cells were purified by positive selection with CD3 MicroBeads followed by another positive selection with CD4 MicroBeads. The purity of isolated cells was then analyzed by flow cytometry with a FACSCaliber instrument (Becton Dickinson, San Jose, Calif.), and the data were analyzed with Cell Quest software (Becton Dickinson).

Isolation and quantification of HIV-1 DNA. Genomic DNAs were isolated from purified cells by use of a QIAmp DNA mini kit (Qiagen, Valencia, Calif.) according to the manufacturer's protocol. Patient DNAs diluted in fivefold series in triplicate were used for nested PCRs to amplify HIV-1 sequences. After limiting dilution PCRs to amplify the HIV-1 C2 to V5 region (see below), HIV-1 DNA copies were quantified with the computer program QUALITY (71, 111, 113). DNA copy numbers calculated from HIV-1 gp120 limiting dilution PCRs (see below) were multiplied by 4.89 to compensate for the difference in sensitivities of the assays (111–114).

PCRs, sequencing, and sequence analyses. Cellular DNAs and cDNAs, which were reverse transcribed from plasma viral RNAs as described previously (113, 114), were used in nested PCRs with outer primers PE0 and PE3 (residues 8029 to 8000 of the HIV-1 HXB2 sequence in GenBank; 5′-GGTGCAAATGAGTT TTCCAGAGCAACCCCA-3′) and inner primers PE1 and PE2 (113, 114) to amplify HIV-1 *env* gp120 and with outer primers P5-2 (112) and PE3 and inner primers P5 (112) and PE2 to amplify the C2 to V5 region of gp120. The PCR conditions used were described previously (111–114). To avoid template resampling (47), we performed limiting dilution PCRs (see above) and additional end-point PCRs in which <25% of the PCR amplifications of patient DNAs diluted to the end point were HIV-1 positive, as described previously (84, 111), and employed a combination of sequencing procedures, as follows. (i) For samples with low levels of HIV-1 DNA (<250 copies/1 million cells), individual end-point PCR products were sequenced directly or cloned; only one sequence per PCR product was used for analysis. (ii) For samples that possibly contained homogeneous sequences, such as cells from an early stage of infection, end-point PCR products were sequenced directly or one clone-derived sequence was used per PCR product amplified from 25 or fewer HIV-1 copies. (iii) For samples with high numbers of viral copies, one sequence was used per PCR product from 25 or fewer amplifiable copies and five individual clone sequences were used per PCR product from 125 or more amplifiable copies from limiting dilution PCRs. Additional end-point PCR amplifications were then performed and sequenced directly to obtain more individual sequences. Sequences from each of the samples from the five patients were aligned with Clustal W (90) and edited with MacClade software (51) for misaligned insertions-deletions by examining the amino acid alignment. Phylogenetic trees were built with PAUP*4.0b10 software (Sinauer Associates, Inc., Sunderland, Mass.). Maximum-likelihood trees were constructed by using the HKY85 model of nucleotide substitution, with the transition/transversion ratio and alpha shape parameter for a gamma distribution estimated on a neighbor-joining tree. The resulting model was used in a heuristic search using SPR branch swapping to find the best maximum-likelihood tree. Branch-length distances calculated by PAUP* were used to calculate diversity by using the HKY85+G+I model of evolution in PAUP*. The total pairwise distances of monocytes were compared to the total pairwise distances of $CD4^+$ T cells for each given time point. *P* values were obtained by using the unpaired *t* test with Welch's correction (GraphPad Software, Inc., San Diego, Calif.). Compartmentalization was evaluated by Slatkin and Maddison's method for measuring the restriction of gene flow among populations (86), which was slightly modified for HIV-1 populations (60, 61, 66).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been submitted to GenBank and were given accession numbers AY614758 to AY615119.

RESULTS

We combined negative selection for monocytes with CD14 positive selection to purify monocytes, which greatly increased the purity of the isolated cell population compared to either method used singly (data not shown). Similarly, for $CD4^+$ T-cell isolation, a combination of CD3 positive selection followed by CD4 positive selection resulted in much more pure results than the use of a single $CD4^+$ T-cell negative selection kit (data not shown). As shown in Fig. 1, both cell purification strategies generated extremely pure cell populations, with no detectable cross-contamination, when analyzed by fluorescence-activated cell sorting and by reverse transcription-PCR (88) of T-cell receptor mRNA (data no shown). DNAs extracted from purified $CD14^+$ monocytes and $CD4^+$ T cells were used in limiting dilution PCRs to amplify HIV-1 *env* gp120 or the C2 to V5 region as described previously (111–114). Numbers of HIV-1 provirus DNA copies were, on average, 4.5-fold (ranging from 0.84- to 23.51-fold) higher in $CD4^+$

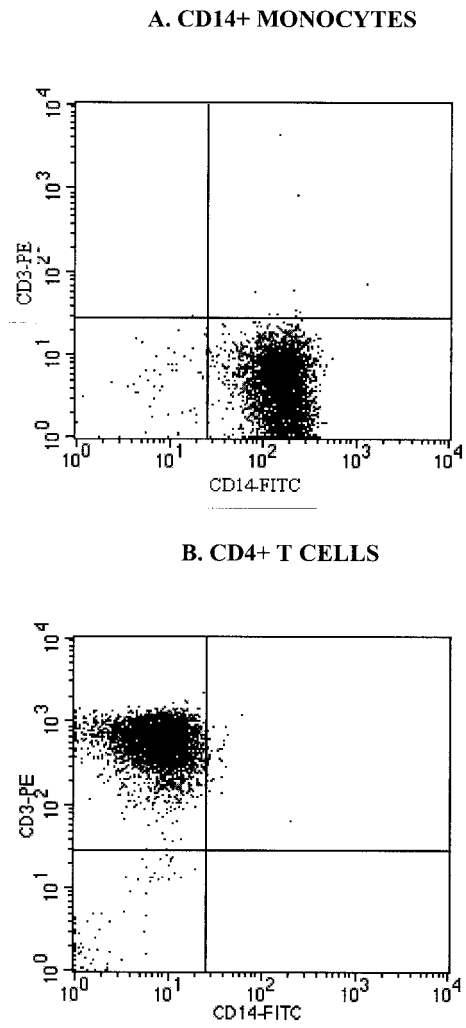

FIG. 1. Two-color fluorescence analysis of isolated $CD14^+$ monocytes (A) and $CD4^+$ T cells (B) from sample II from patient 2. Purified cells were stained with a fluorescein isothiocyanate-conjugated anti-CD14 or phycoerythrin-conjugated anti-CD3 monoclonal antibody. The cells were also stained with fluorescein isothiocyanate- or phycoerythrin-conjugated isotype IgG2a to control for nonspecific binding. The purity of $CD14^+$ monocytes was 98.2% ± 0.94% and the purity of $CD4^+$ T cells was 98.87% ± 0.36%. The purified cells were also shown to be free from $CD8^+$ T cells and B cells (data not shown).

T cells than in monocytes (Table 2). A 700-bp C2-to-V5 region of gp120 was sequenced. As described above (see Materials and Methods), we employed a combination of limiting dilution and end-point PCRs and a sequencing procedure in order to exclude the resampling of provirus that may occur during the PCR-cloning-sequencing process (47). Analyses of sequences were conducted by using several phylogenetic approaches. Sequences from all five patients grouped with HIV-1 subtype B reference sequences from the database (data not shown). Moreover, sequences from each patient clustered together (supported by 100% of the bootstrap samples). Figure 2 depicts the phylogenetic trees generated for each of the five patients. The horizontal branches reflect the relative genetic distances between sequences. Samples from early infections (117 to 167 days postseroconversion) were obtained from three of the five patients studied (Table 1); early samples of the other two patients were not sufficient for the present study, which typically required 100 million or more PBMC for cell purification. As expected for early infections (106, 109, 112), sequences obtained shortly after seroconversion (samples I for patients 1, 2, and 5 in Fig. 2) were relatively homogeneous in both monocyte and $CD4^+$ T-cell populations, as reflected by the tight clustering and short horizontal branches among the sequences on the phylogenetic tree for each of the patients. This homogeneity is unlikely due to the resampling of a few proviruses, as described above. The diversity of HIV-1 nucleotide sequences obtained during early infections (sample I) was very low for patients 1 and 5 in both the monocyte and $CD4^+$ T-cell populations (Table 2) and was higher for patient 2 (Table 2) due to sequence variation in the fourth hypervariable region of gp120 (data not shown). The average levels of diversity of HIV-1 nucleotide sequences around the time of seroconversion of sample I from the three patients was similar in blood monocytes and $CD4^+$ T cells (0.546% ± 0.081% and 0.579% ± 0.0807%, respectively).

We then used the same procedures to examine HIV-1 C2-to-V5 sequences in blood monocytes and $CD4^+$ T cells isolated at later time points from each of the five patients, up to 3,409 days postseroconversion (Table 1). As shown in Table 2, HIV-1 genetic diversity increased in all five study patients over the course of HIV-1 infection. The diversity of HIV-1 nucleotide sequences was found to be significantly different between monocyte and $CD4^+$ T-cell populations at the last time point

TABLE 2. HIV-1 DNA copies and C2-to-V5 sequence diversity in monocytes and $CD4^+$ T cells

Patient no.	Sample	Cell type	No. of HIV-1 DNA copies/10^6 cells (mean ± SE)	Diversity	
				Mean % ± SE	*P* value[a]
1	I	Monocytes	206 ± 109	0.543 ± 0.124	
		$CD4^+$ T cells	854 ± 436	0.543 ± 0.124	1.000
	II	Monocytes	235 ± 145	1.432 ± 0.067	
		$CD4^+$ T cells	5,532 ± 3,908	1.095 ± 0.083	0.002[b]
	III	$CD4^+$ T cells	1,966 ± 1,640	1.772 ± 0.130	
	IV	Monocytes	932 ± 769	1.743 ± 0.103	
2	I	Monocytes	2,146 ± 1,519	0.809 ± 0.074	
		$CD4^+$ T cells	5,632 ± 4,573	0.988 ± 0.066	0.077
	II	Monocytes	171 ± 108	0.231 ± 0.245	
		$CD4^+$ T cells	2,322 ± 1,938	2.293 ± 0.141	<0.001[c]
3	I	$CD4^+$ T cells	3,276 ± 1,937	2.345 ± 0.118	
	II	Monocytes	258 ± 187	3.934 ± 0.246	
	III	Monocytes	624 ± 333	2.491 ± 0.245	
		$CD4^+$ T cells	988 ± 527	2.499 ± 0.141	0.977
	IV	Monocytes	196 ± 76	4.811 ± 0.247	
		$CD4^+$ T cells	1,222 ± 1,033	2.637 ± 0.204	<0.001[b]
4	I	Monocytes	153 ± 128	2.642 ± 0.398	
		$CD4^+$ T cells	161 ± 48	1.978 ± 0.642	0.386
	II	Monocytes	84 ± 47	5.047 ± 0.311	
		$CD4^+$ T cells	307 ± 206	3.633 ± 0.455	0.012[b]
5	I	Monocytes	338 ± 127	0.286 ± 0.055	
		$CD4^+$ T cells	284 ± 60	0.205 ± 0.052	0.879
	II	Monocytes	300 ± 225	2.786 ± 0.235	
		$CD4^+$ T cells	281 ± 103	3.405 ± 0.235	0.092
	IV	Monocytes	493 ± 89	2.786 ± 0.235	
		$CD4^+$ T cells	4,894 ± 3,416	3.405 ± 0.235	0.021[b]
All		Monocytes	472 ± 115	0.4381 ± 0.0041	0.7813
		$CD4^+$ T cells	2,132 ± 328	0.6237 ± 0.0021	

[a] If the P value is <0.05, there is a significant difference in diversity between the monocyte and $CD4^+$-T-cell populations.
[b] Diversity is significantly higher in monocytes than in $CD4^+$ T cells.
[c] Diversity is significantly higher in $CD4^+$ T cells than in monocytes.

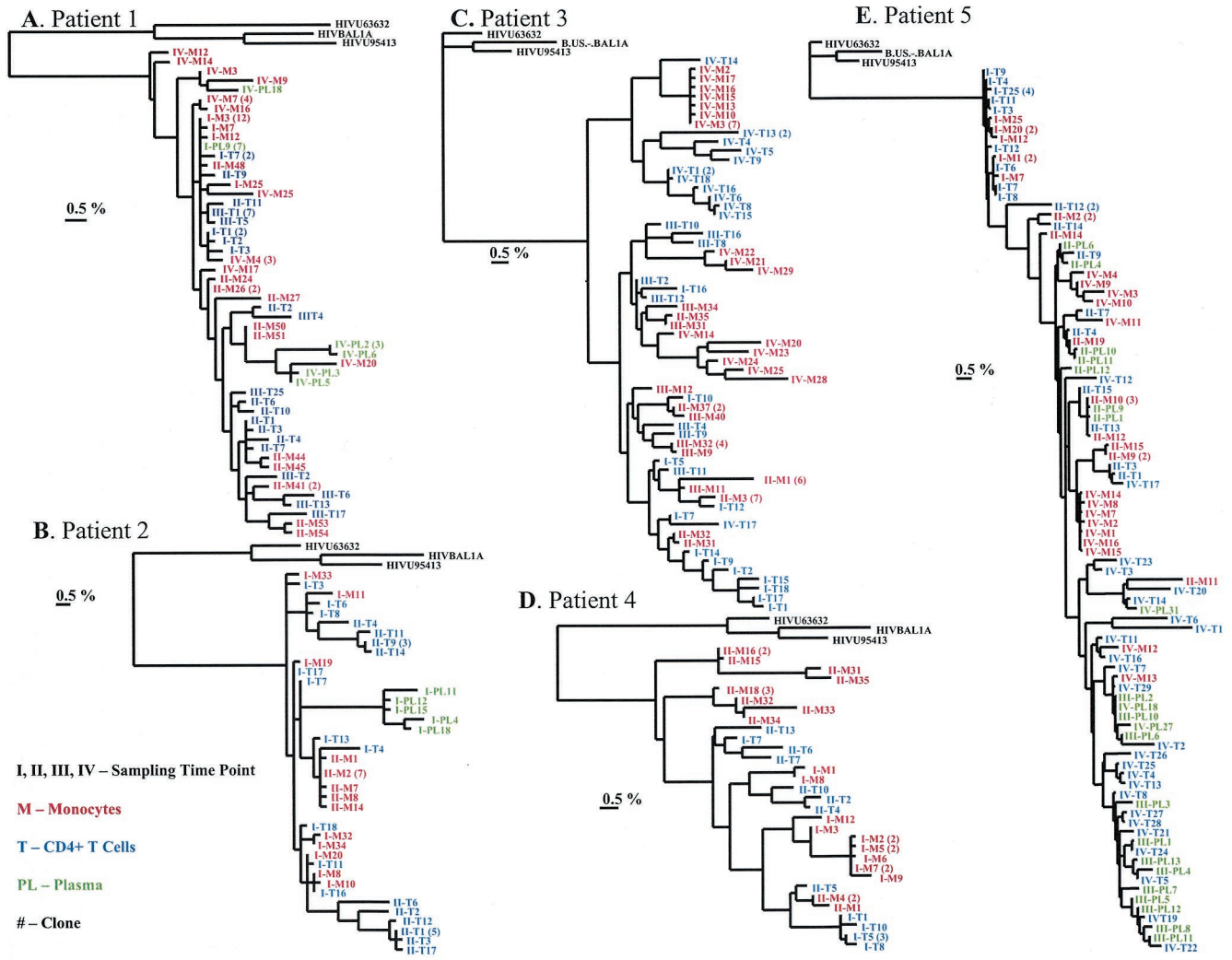

FIG. 2. Phylogenetic trees based on the maximum-likelihood method showing the evolutionary relationship between HIV-1 C2-to-V5 sequences from blood monocytes (M) and $CD4^+$ T cells (T) from each of the five study patients. Panels A to E are for patients 1 to 5, respectively. I to IV, sampling time points shown in Table 1. The scale bar for each tree indicates the percent genetic distance. The numbers in parentheses represent the number of clones with identical sequences.

for four study patients (patients 2 to 5) and in sample II from patient 1 (Table 2). $CD14^+$ monocytes had more viral diversity than $CD4^+$ T cells in sample II (990 days postseroconversion) from patient 1 ($P = 0.0023$), sample IV (2,535 days postseroconversion) from patient 3 ($P < 0.001$), and sample II (2,823 days postseroconversion) from patient 4 ($P = 0.012$). However, patients 2 and 5 showed significantly more diversity in $CD4^+$ T cells than in $CD14^+$ monocytes at the later time points (sample II from patient 2 and sample IV from patient 5).

To assess the evolutionary relationship of HIV-1 strains in the two cellular compartments, we constructed phylogenetic trees for each patient (Fig. 2). Again, an increased heterogeneity of HIV-1 populations was evident in both the blood monocyte and $CD4^+$ T-cell compartments of all five patients at the later time points, as indicated by the longer branches of the sequences on the phylogenetic trees (Fig. 2). The viral sequences from monocytes tended to form cell-specific subclusters within the radiation of viral sequences from $CD4^+$ T cells. This cell-specific grouping was most obvious for patients 2 and 4 (Fig. 2B and 2D). For the other patients, there seemed to be evidence for a similar cell-specific grouping, though this pattern was less obvious. We then evaluated the HIV-1 compartmentalization between monocytes and $CD4^+$ T cells by using Slatkin and Maddison's method for measuring the restriction of gene flow among populations (60, 61, 66, 86). Starting with a maximum-likelihood tree, we assigned each compartment an unordered character (for example, all sequences from monocytes were called "1" and all sequences from $CD4^+$ T cells were called "2"), and then we traced these characters to determine the minimum number of steps, or interlocality migration events, allowed by the tree (we assumed "hard" polytomies). We then generated 1,000 random trees and compared the distribution of steps created to those for our original tree. If there were a compartmentalization, the number of steps from our original tree would fall well outside the random distribution (MacClade generates a P value to assess this). As seen in Table 3, compartmentalization of HIV-1 was not found during early infection for all five patients, including patient 2, who had relatively diverse virus populations in sample I. However, significant compartmentalization was gradually evident

TABLE 3. Analysis of compartmentalization of HIV-1

Patient no.	Sampling time (days post-SC[a])	*P* value for compartmentalization
1	127	0.153
	912	0.159
	1,046[b]	0.045
	All sequences	<0.001
2	117	0.331
	1,490	<0.001
	All sequences	<0.001
3	925[c]	0.457
	1,285	0.103
	2,535	0.003
	All sequences	<0.001
4	2,193	0.013
	2,823	0.003
	All sequences	<0.001
5	167	0.365
	999	0.321
	3,409	0.002
	All sequences	<0.001

[a] Days post-SC, days postseroconversion.
[b] Blood monocytes were obtained at day 1,046, while $CD4^+$ T cells were obtained from the blood at day 990.
[c] $CD4^+$ T cells were obtained at 925 days postseroconversion, while monocytes were obtained at 1,079 days postseroconversion.

over the course of HIV-1 infection in all five patients. For example, for patient 1 there was no difference at days 127 and 912 postseroconversion, but a marginally significant difference was present between virus populations of monocytes and $CD4^+$ T cells at day 1,046 postseroconversion (Table 1). Similarly, for patient 2, compartmentalization of HIV-1 between monocytes and $CD4^+$ T cells was significant at day 1,490, but not at day 117, postseroconversion. For patient 3, viral compartmentalization was not seen at days 925 and 1,285, but was only found at day 2,535 postseroconversion. For patient 4, only two late time points were studied, and significantly different virus populations between the cell types were found. For patient 5, viral compartmentalization was not seen at days 107 and 999, but was evident at day 3,409. Analyses of all sequences from each patient found that all five patients had evidence of compartmentalization between $CD14^+$ monocytes and $CD4^+$ T lymphocytes ($P < 0.001$). We also performed a correction analysis for multiple significance determinations. This was done by taking our target alpha value (the *P* value that we considered to be significant) and dividing it by the number of patients ($n = 5$) studied ($0.05/5 = 0.01$). Thus, to find significant compartmentalization at a level of 0.05, the *P* value generated from each test would have to be 0.01 or less. Alternatively, we used the number of tests performed for each individual and the combined number of time points ($n = 18$) in the denominator rather than 5. In this case, our target *P* value would have to be 0.003 or less. Except for one result from patient 1 at the 1,046/999-day time point ($P = 0.045$), the significance of compartmentalization at other individual and combined late time points remained the same (Table 3). The observed compartmentalization of HIV-1 during late-stage infections was not likely due to the persistence of "old" sequences dating back to early infection, at least for patient 5, because none of the HIV-1 sequences from sample IV was identical or close to the old sequences from sample I (Fig. 2E). These results, together with our findings of nonparallel diversity and evolution of HIV-1 between the two cell compartments, suggest that compartmentalization of HIV-1 developed and increased during the course of infection.

HIV-1 strains isolated from newly infected individuals are largely M-tropic and non-syncytium-inducing (NSI) (72, 93, 112), although those from some of the corresponding chronically infected donors are both syncytium-inducing (SI) and NSI (112). The NSI and SI phenotypes of HIV-1 are predicted by a variant at positions 11 and 25 of the amino acids in the third hypervariable region (V3) (12, 25, 72). Amino acids with a positive charge (R and K) at one or two of these positions predict the SI and T-cell-tropic (T-tropic) phenotype. Most sequences from all five patients showed V3 sequences corresponding to an M-tropic, NSI phenotype (Fig. 3). However, a V3-predicted T-tropic SI virus was detected as a minor variant in two of the five subjects (Fig. 3). For patient 3, a V3-predicted T-tropic virus was identified in $CD4^+$ T cells in samples I (925 days postseroconversion; clone I-T2 in Fig. 3) and III (1,285 days postseroconversion; clone III-T16). Additionally, three predicted T-tropic clones were identified in monocytes at day 2,535 after seroconversion (sample IV; clone IV-M21-like). Similarly, T-tropic clones were detected in both $CD4^+$ T cells and monocytes at day 999 (sample II; clones II-T1-like and II-M9-like) and day 3,409 (sample IV; clones IV-M1-like and IV-T17) for patient 5. The existence of possible SI and T-tropic HIV-1 strains in $CD14^+$ monocytes needs to be verified with further in vitro studies, but it could provide evidence for the ability of $CD14^+$ monocytes to support the infection and replication of HIV-1 strains with various phenotypes.

DISCUSSION

Previous studies comparing HIV-1 genotypes were conducted at the level of anatomic compartments, such as the peripheral blood, brain, spleen, lymphoid tissue, or other tissues (5, 18, 22, 31, 35, 37, 40, 52, 68, 82, 107, 108, 114). We demonstrated here for the first time that compartmentalization of HIV-1 occurred at the cellular level within blood cells in all five study patients. Viral compartmentalization was not seen during early infections, when viral populations were relatively homogeneous, but was found at later time points, when heterogeneous virus populations emerged. Coincidently, there was evidence for the nonparallel evolution of HIV-1 *env* sequences between blood monocytes and $CD4^+$ T cells over the course of HIV-1 infection. While M-tropic NSI viruses dominated in both $CD4^+$ T cells and monocytes for all five study subjects, V3 loop-predicted T-tropic SI viruses were seen in both monocytes and T cells at later time points for two patients (time points III and IV for patient 3 and time point II for patient 5).

The mechanism of cellular compartmentalization remains to be defined. Several possible explanations could account for the nonuniform distribution of HIV-1. The initial mechanism driving compartmentalization could occur upon viral entry into the target cell. Selective tropism via coreceptor specificity could enhance the infectivity of certain HIV-1 variants to specific target cells such as monocytes or $CD4^+$ T cells. The V3 loop of the HIV-1 envelope gp120 protein interacts with cellular receptors, making it an important domain for cellular tropism (12, 25, 33, 59, 70, 72). All sequences from our five patients showed dominant V3 sequences corresponding to the M-tropic,

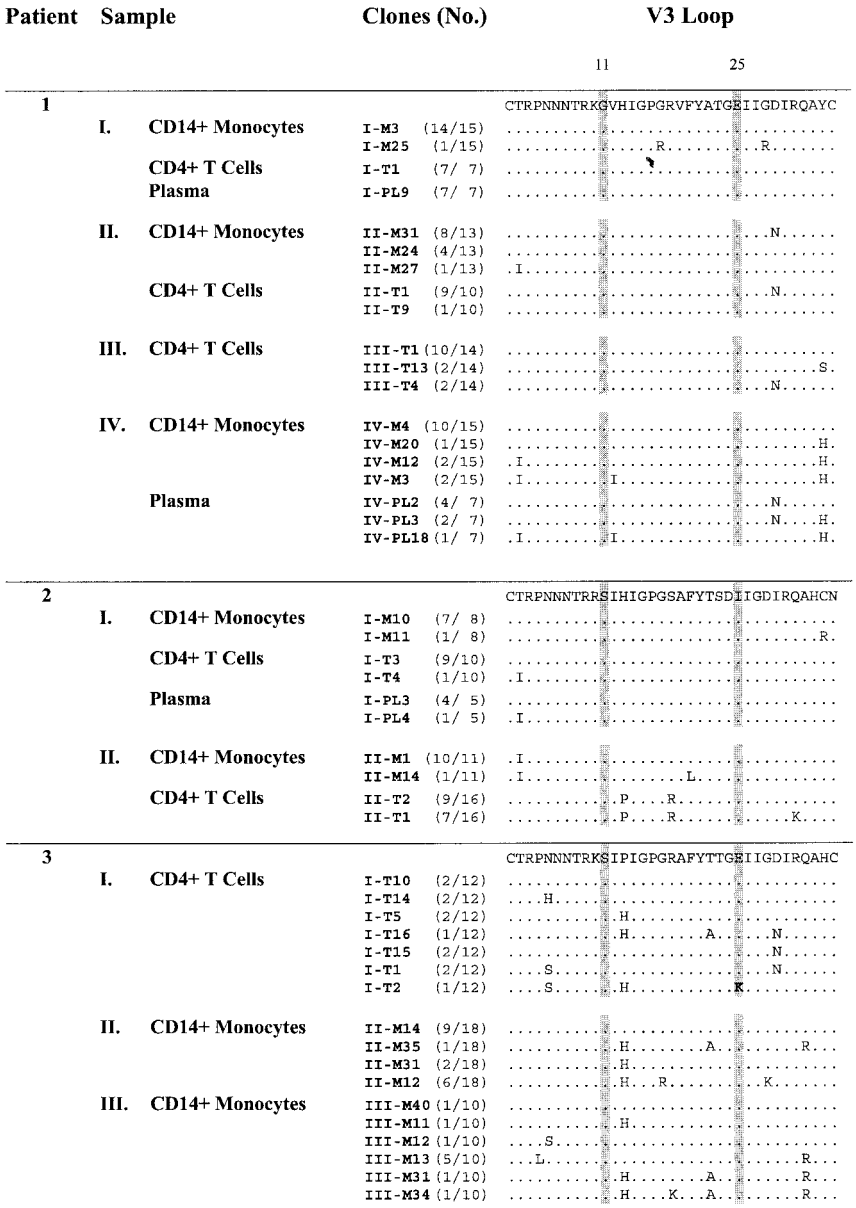

FIG. 3. Deduced amino acid sequences of HIV-1 V3 loops from patients 1 to 5. The amino acid sequences have been aligned with the consensus sequences of both monocytes and $CD4^+$ T cells from sample I from each patient. Dots indicate identity with the consensus sequences and dashes indicate deletions. Amino acids at positions 11 and 25 of the V3 loop are shaded; positively charged amino acids (R or K) at these two positions are shown in bold.

NSI phenotype, with the exception of minor variants predicting a T-tropic, SI phenotype (Fig. 3). The emergence of possible T-cell-tropic HIV-1 variants in blood monocytes could indicate the ability of blood monocytes to support infection by HIV-1 strains with various phenotypes, perhaps enhancing compartmentalization. However, the finding of an HIV-1 provirus with predicted T-cell tropism does not necessarily indicate that a T-tropic virus would replicate in monocytes/macrophages, since a previous study showed that the replication of T-tropic virus in macrophages was blocked after entry (77). Alternatively, precursors such as $CD34^+$ progenitor cells in the bone marrow could serve as target cells and be infected by HIV-1 variants with different phenotypes (17, 24, 38, 53, 73, 74, 95, 99) and carry the virus through differentiation into blood monocytes (reviewed in reference 110). Peripheral blood-derived $CD34^+$ $CD4^+$ cells express both CCR5 and CXCR4 coreceptors and are susceptible to active HIV-1 infection by multiple viral isolates (73). It is plausible that peripheral blood monocytes could become infected at this early stage and carry the virus through differentiation into tissue macrophages.

Another possible mechanism may occur in the cell, as HIV-1 variants may replicate or evolve preferentially in either mono cytes/macrophages or $CD4^+$ T cells. The genetic diversity of HIV-1 is believed to be due to the high level of rapid and mutation-prone replication that occurs during active HIV-1 infection. Our finding that HIV-1 diversity patterns were not

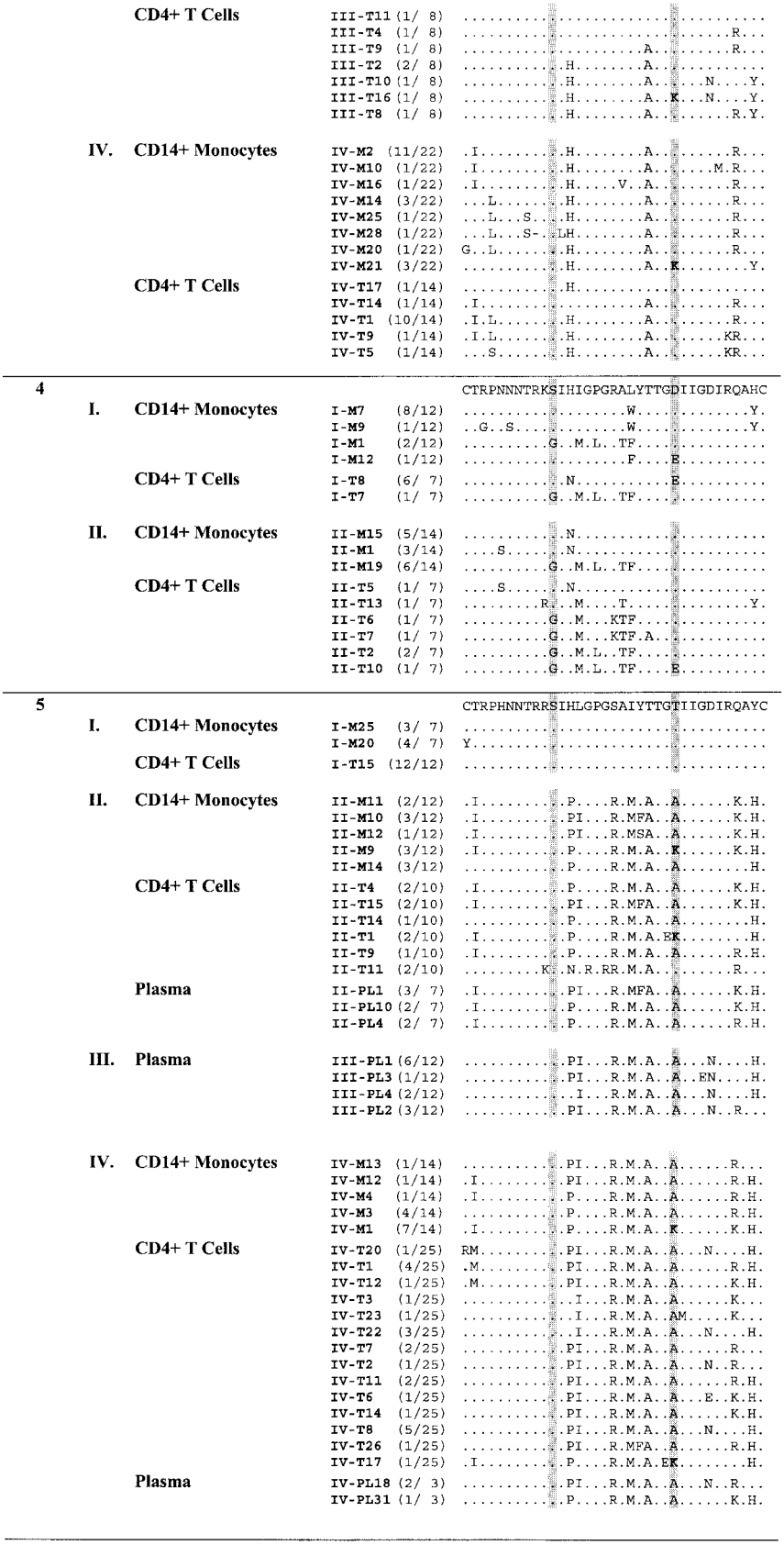

FIG. 3—*Continued*.

parallel between the $CD14^+$ monocytes and $CD4^+$ T cells implies some degree of independent replication and/or evolution, which may contribute to cellular compartmentalization. Local amplification in blood monocytes (113) or $CD4^+$ T cells might also account for the nonrandom distributions of HIV-1 populations. Alternatively, provirus-bearing monocytes may carry the virus and differentiate into various tissue macrophages, in which a larger amount of virus may be produced (reviewed in reference 110). Our finding of the independent replication and evolution of HIV-1 strains in $CD14^+$ monocytes compared to $CD4^+$ T cells suggests that these cells play a role in harboring different viruses and trafficking them into various tissue compartments where HIV-1 may replicate extensively. Macrophages in nonlymphoid tissues, including the brain (the resident parenchyma imbroglio and the perivascular macrophages), the lung (alveolar macrophages), the liver (Kupffer's cells), and the gut, are infected productively and serve as a source of virus, especially in patients with end-stage disease (8, 9, 21, 26, 27, 32, 62, 65, 78, 79, 87, 94, 100). In simian-human immunodeficiency virus-infected rhesus macaques, macrophages in lymph nodes, the spleen, the gastrointestinal tract, the liver, and the kidneys all supported virus production in the absence of $CD4^+$ T cells (36). As an upstream precursor, the blood monocyte compartment could actually seed virus in these macrophages and thereby establish the reservoir. Similarly, blood monocytes are likely the precursors to brain macrophages (91, 92) and serve as a carrier of HIV-1 to the central nervous system (23, 101, 102). Brain-derived isolates show enhanced macrophage replication competence relative to those simultaneously derived from blood (11, 28, 29, 41), possibly reflecting a phenotypic requirement for passage into the brain within infected macrophages. The compartmentalization of HIV-1 observed between the brain, where the principle target cells are of a monocyte/macrophage lineage, and lymphoid tissue, where lymphocytes are the predominant cell type infected (28, 98), could be an extension of the compartmentalization observed between $CD14^+$ monocytes and $CD4^+$ T cells after trafficking of the virus from these two cell types. The relative similarity and relatedness of HIV-1 sequences among brain and other nonlymphoid tissues (28, 98), in which macrophages are the major target of infection, might be due to the fact that the infected macrophages were from related blood monocytes.

In summary, our studies examining HIV-1 sequence variation over the course of infection in five patients indicate that there is cellular compartmentalization of HIV-1 strains between $CD14^+$ monocytes and $CD4^+$ T cells. Furthermore, HIV-1 in blood monocytes could disseminate and evolve independently from $CD4^+$ T cells over the course of an HIV-1 infection. Such compartmentalization and independent evolution of HIV-1 in $CD14^+$ monocytes and $CD4^+$ T cells present challenges to the treatment of HIV-1 infection because HIV-1 populations in monocytes and macrophages are poorly suppressed (1, 3, 67; for a review, see reference 2) compared to those in $CD4^+$ T cells and are replicate relatively actively (113) in patients undergoing seemingly effective HAART. The occurrence of cellular compartmentalization in patients 2 and 4 while they were on HAART indicates that a drug regimen does not prevent this phenomenon and suggests that the role of cellular compartments should be considered in attempts to develop new therapeutic strategies.

ACKNOWLEDGMENTS

We thank H. Zhu, H. Zhao, F. Feng, and T. Andrus for technical and editorial assistance as well as E. Peterson and J. Maenza for coordinating clinical samples and data for this study. We thank all of the individuals for their participation.

This work was supported by the National Institutes of Health grants AI45402, AI055336, and AI49109 (to T.Z.) and grant AI41535 (to L.C.).

REFERENCES

1. **Aquaro, S., R. Calio, E. Balestra, P. Bagnarelli, A. Cenci, A. Bertoli, B. Tavazzi, D. Di Pierro, M. Francesconi, D. Abdelahad, and C. F. Perno.** 1998. Clinical implications of HIV dynamics and drug resistance in macrophages. J. Biol. Regul. Homeost. Agents **12:**23–27.
2. **Aquaro, S., R. Calio, J. Balzarini, M. C. Bellocchi, E. Garaci, and C. F. Perno.** 2002. Macrophages and HIV infection: therapeutical approaches toward this strategic virus reservoir. Antivir. Res. **55:**209–225.
3. **Aquaro, S., C. F. Perno, E. Balestra, J. Balzarini, A. Cenci, M. Francesconi, S. Panti, F. Serra, N. Villani, and R. Calio.** 1997. Inhibition of replication of HIV in primary monocyte/macrophages by different antiviral drugs and comparative efficacy in lymphocytes. J. Leukoc. Biol. **62:**138–143.
4. **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.
5. **Ball, J. K., E. C. Holmes, H. Whitwell, and U. Desselberger.** 1994. Genomic variation of human immunodeficiency virus type 1 (HIV-1): molecular analyses of HIV-1 in sequential blood samples and various organs obtained at autopsy. J. Gen. Virol. **75:**67–69.
6. **Bonhoeffer, S., E. C. Holmes, and M. A. Nowak.** 1995. Causes of HIV diversity. Nature **376:**125.
7. **Borrow, P., G. M. Shaw, et al.** 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat. Med. **3:**205–211.
8. **Cavert, W., and A. T. Haase.** 1998. A national tissue bank to track HIV eradication and immune reconstruction. Science **280:**1865–1866.
9. **Cavert, W., D. W. Notermans, K. Staskus, S. W. Wietgrefe, M. Zupancic, K. Gebhard, K. Henry, Z. Q. Zhang, R. Mills, H. McDade, J. Goudsmit, S. A. Danner, and A. T. Haase.** 1997. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. Science **276:**960–964.
10. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biological features of HIV-1 that correlate with virulence in the host. Science **240:**80–82.
11. **Cheng-Mayer, C., C. Weiss, D. Seto, and J. A. Levy.** 1989. Isolates of human immunodeficiency virus type 1 from the brain may constitute a special group of the AIDS virus. Proc. Natl. Acad. Sci. USA **86:**8575–8579.
12. **Chesebro, B., J. Nishio, S. Perryman, A. Cann, W. O'Brien, I. S. Y. Chen, and K. Wehrly.** 1991. Identification of human immunodeficiency virus envelope gene sequences influencing viral entry into CD4-positive HeLa cells, T-leukemic cells and macrophages. J. Virol. **65:**5782–5789.
13. **Cichutek, K., H. Merget, S. Norley, R. Linde, W. Kreuz, M. Gahr, and R. Kurth.** 1992. Development of a quasispecies of human immunodeficiency virus type 1 in vivo. Proc. Natl. Acad. Sci. USA **89:**7365–7369.
14. **Coffin, J. M.** 1992. Genetic diversity and evolution of retroviruses. Curr. Top. Microbiol. Immunol. **176:**143–164.
15. **Coffin, J. M.** 1995. HIV population dynamics in vivo: implications for genetic variation, pathogenesis and therapy. Science **267:**483–489.
16. **Connor, R. I., and D. D. Ho.** 1994. Human immunodeficiency virus type 1 variants with increased replicative capacity develop during the asymptomatic stage before disease progression. J. Virol. **68:**4400–4408.
17. **Davis, B. R., D. H. Schwartz, J. C. Marx, C. E. Johnson, J. M. Berry, J. Lyding, T. C. Merigan, and A. Zander.** 1991. Absent or rare human immunodeficiency virus infection of bone marrow stem/progenitor cells in vivo. J. Virol. **65:**1985–1990.
18. **Delwart, E. L., J. I. Mullins, P. Gupta, G. H. Learn, Jr., M. Holodniy, D. Katzenstein, B. D. Walker, and M. K. Singh.** 1998. Human immunodeficiency virus type 1 populations in blood and semen. J. Virol. **72:**617–623.
19. **Delwart, E. L., H. W. Sheppard, B. D. Walker, J. Goudsmit, and J. I. Mullins.** 1994. Human immunodeficiency virus type 1 evolution in vivo tracked by DNA heteroduplex mobility assays. J. Virol. **68:**6672–6683.
20. **Delwart, E. L., E. G. Shpaer, F. E. McCutchan, J. Louwagie, M. Grez, H. Rübsamen-Waigmann, and J. I. Mullins.** 1993. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 *env* genes. Science **262:**1257–1261.
21. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lympho-

cytes and macrophages by HIV during the incubation period of AIDS. Nature **362:**359–362.
22. **Epstein, L. G., C. Kuiken, B. M. Blumberg, S. Hartman, L. R. Sharer, M. Clement, and J. Goudsmit.** 1991. HIV-1 V3 domain variation in brain and spleen of children with AIDS: tissue-specific evolution within host-determined quasispecies. Virology **180:**583–590.
23. **Fischer-Smith, T., S. Croul, A. E. Sverstiuk, C. Capini, D. L'Heureux, E. G. Regulier, M. W. Richardson, S. Amini, S. Morgello, K. Khalili, and J. Rappaport.** 2001. CNS invasion by CD14+/CD16+ peripheral blood-derived monocytes in HIV dementia: perivascular accumulation and reservoir of HIV infection. J. Neurovirol. **7:**528–541.
24. **Folks, T. M., S. W. Kessler, J. M. Orenstein, J. S. Justement, E. S. Jaffe, and A. S. Fauci.** 1988. Infection and replication of HIV-1 in purified progenitor cells of normal human bone marrow. Science **242:**919–922.
25. **Fouchier, R. A. M., M. Groenink, N. A. Kootstra, M. Tersmette, H. G. Huisman, F. Miedema, and H. Schuitemaker.** 1992. Phenotype-associated sequence variation in the third variable domain (V3) of the human immunodeficiency virus type 1 gp120 molecule. J. Virol. **66:**3183–3187.
26. **Fox, C. H., K. Tenner-Racz, P. Racz, A. Firpo, P. A. Pizzo, and A. S. Fauci.** 1991. Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA. J. Infect. Dis. **164:**1051–1057.
27. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science **233:**215–219.
28. **Gorry, P. R., G. Bristol, J. A. Zack, K. Ritola, R. Swanstrom, C. J. Birch, J. E. Bell, N. Bannert, K. Crawford, H. Wang, D. Schols, E. De Clercq, K. Kunstman, S. M. Wolinsky, and D. Gabuzda.** 2001. Macrophage tropism of human immunodeficiency virus type 1 isolates from brain and lymphoid tissues predicts neurotropism independent of coreceptor specificity. J. Virol. **75:**10073–10089.
29. **Gorry, P. R., J. Taylor, G. H. Holm, A. Mehle, T. Morgan, M. Cayabyab, M. Farzan, H. Wang, J. E. Bell, K. Kunstman, J. P. Moore, S. M. Wolinsky, and D. Gabuzda.** 2002. Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate. J. Virol. **76:**6277–6292.
30. **Gratton, S., R. Cheynier, M. J. Dumaurier, E. Oksenhendler, and S. Wain-Hobson.** 2000. Highly restricted spread of HIV-1 and multiply infected cells within splenic germinal centers. Proc. Natl. Acad. Sci. USA **97:**14566–14571.
31. **Haggerty, S., and M. Stevenson.** 1991. Predominance of distinct viral genotypes in brain and lymph node compartments of HIV-1-infected individuals. Viral Immunol. **4:**123–131.
32. **Ho, D. D., T. R. Rota, and M. S. Hirsch.** 1986. Infection of monocyte/macrophages by human T lymphotropic virus type III. J. Clin. Investig. **77:**1712–1715.
33. **Hoffman, N. G., F. Seillier-Moiseiwitsch, J. Ahn, J. M. Walker, and R. Swanstrom.** 2002. Variability in the human immunodeficiency virus type 1 gp120 Env protein linked to phenotype-associated changes in the V3 loop. J. Virol. **76:**3852–3864.
34. **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 HIV-1 within a single infected patient. Proc. Natl. Acad. Sci. USA **89:**4835–4839.
35. **Hughes, E. S., J. E. Bell, and P. Simmonds.** 1997. Investigation of the dynamics of the spread of human immunodeficiency virus to brain and other tissues by evolutionary analysis of sequences from the p17 *gag* and *env* genes. J. Virol. **71:**1272–1280.
36. **Igarashi, T., C. R. Brown, Y. Endo, A. Buckler-White, R. Plishka, N. Bischofberger, V. Hirsch, and M. A. Martin.** 2001. Macrophages are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): implications for HIV-1 infections of humans. Proc. Natl. Acad. Sci. USA **98:**658–663.
37. **Itescu, S., P. F. Simonelli, R. J. Winchester, and H. S. Ginsberg.** 1994. Human immunodeficiency virus type 1 strains in the lungs of infected individuals evolve independently from those in peripheral blood and are highly conserved in the C-terminal region of the envelope V3 loop. Proc. Natl. Acad. Sci. USA **91:**11378–11382.
38. **Kaczmarski, R. S., F. Davison, E. Blair, S. Sutherland, J. Moxham, T. McManus, and G. J. Mufti.** 1992. Detection of HIV in haemopoietic progenitors. Br. J. Haematol. **82:**764–769.
39. **Kitrinos, K. M., N. G. Hoffman, J. A. Nelson, and R. Swanstrom.** 2003. Turnover of *env* variable region 1 and 2 genotypes in subjects with late-stage human immunodeficiency virus type 1 infection. J. Virol. **77:**6811–6822.
40. **Korber, B. T. M., K. J. Kunstman, B. K. Patterson, M. Furtado, M. M. McEvilly, R. Levy, and S. M. Wolinsky.** 1994. Genetic differences between blood- and brain-derived viral sequences from HIV-1-infected patients: evidence of conserved elements in the V3 region of the envelope protein of brain-derived sequences. J. Virol. **68:**7467–7481.
41. **Koyanagi, Y., S. Miles, R. T. Mitsuyasu, J. E. Merrill, H. V. Vinters, and I. S. Y. Chen.** 1987. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science **236:**819–822.
42. **Kroodsma, K. L., M. J. Kozal, K. A. Hamed, M. A. Winters, and T. C. Merigan.** 1994. Detection of drug resistance mutations in the human immunodeficiency virus type 1 (HIV-1) pol gene: differences in semen and blood HIV-1 RNA and proviral DNA. J. Infect. Dis. **170:**1292–1295.
43. **Kuiken, C., G. Zwart, E. Baan, R. Coutinho, J. Anneke, R. van den Hoek, and J. Goudsmit.** 1993. Increasing antigenic and genetic diversity of the HIV-1 V3 domain in the course of the AIDS epidemic. Proc. Natl. Acad. Sci. USA **90:**9061–9065.
44. **Larder, B. A., and S. D. Kemp.** 1989. Multiple mutations in HIV-1 reverse transcriptase confer high-level resistance to zidovudine (AZT). Science **246:**1155–1158.
45. **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 principal neutralizing determinant. Science **249:**932–935.
46. **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.
47. **Liu, S.-L., A. G. Rodrigo, R. Shankarappa, G. H. Learn, L. Hsu, O. Davidov, L. P. Zhao, and J. I. Mullins.** 1996. HIV quasispecies and resampling. Science **273:**415–416.
48. **Liu, 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.
49. **Long, E. M., H. L. Martin, Jr., J. K. Kreiss, S. M. Rainwater, L. Lavreys, D. J. Jackson, J. Rakwar, K. Mandaliya, and J. Overbaugh.** 2000. Gender differences in HIV-1 diversity at time of infection. Nat. Med. **6:**71–75.
50. **Lukashov, V. V., C. L. Kuiken, and J. Goudsmit.** 1995. Intrahost human immunodeficiency virus type 1 evolution is related to length of the immunocompetent period. J. Virol. **69:**6911–6916.
51. **Maddison, W. P., and D. R. Maddison.** 1992. MacClade—analysis of phylogeny and character evolution, version 3. Sinauer Associates, Inc., Sunderland, Mass.
52. **Marras, D., L. A. Bruggeman, F. Gao, N. Tanji, M. M. Mansukhani, A. Cara, M. D. Ross, G. L. Gusella, G. Benson, V. D. D'Agati, B. H. Hahn, M. E. Klotman, and P. E. Klotman.** 2002. Replication and compartmentalization of HIV-1 in kidney epithelium of patients with HIV-associated nephropathy. Nat. Med. **8:**522–526.
53. **McElrath, M. J., J. E. Pruett, and Z. A. Cohn.** 1989. Mononuclear phagocytes of blood and bone marrow: comparative roles as viral reservoirs in human immunodeficiency virus type 1 infections. Proc. Natl. Acad. Sci. USA **86:**675–679.
54. **McNearney, T., Z. Hornickova, R. Markham, A. Birdwell, M. Arens, 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.
55. **McNearney, T., P. Westervelt, B. J. Thielan, D. B. Trowbridge, J. Garcia, R. Whittier, 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:**1917–1921.
56. **Milich, L., B. Margolin, and R. Swanstrom.** 1993. V3 loop of the human immunodeficiency virus type 1 Env protein: interpreting sequence variability. J. Virol. **67:**5623–5634.
57. **Molla, A., M. Korneyeva, Q. Gao, S. Vasavanonda, P. J. Schipper, H.-M. Mo, M. Markowitz, T. Chernyavsky, P. Niu, N. Lyons, A. Hsu, G. R. Granneman, D. D. Ho, C. A. B. Boucher, J. M. Leonard, D. W. Norbeck, and D. J. Kempf.** 1996. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nat. Med. **2:**760–766.
58. **Nelson, J. A., F. Baribaud, T. Edwards, and R. Swanstrom.** 2000. Patterns of changes in human immunodeficiency virus type 1 V3 sequence populations late in infection. J. Virol. **74:**8494–8501.
59. **Nelson, J. A. E., S. A. Fiscus, and R. Swanstrom.** 1997. Evolutionary variants of the human immunodeficiency virus type 1 V3 region characterized by using a heteroduplex tracking assay. J. Virol. **71:**8750–8758.
60. **Nickle, D. C., M. A. Jensen, D. Shriner, S. J. Brodie, L. M. Frenkel, J. E. Mittler, and J. I. Mullins.** 2003. Evolutionary indicators of human immunodeficiency virus type 1 reservoirs and compartments. J. Virol. **77:**5540–5546.
61. **Nickle, D. C., D. Shriner, J. E. Mittler, L. M. Frenkel, and J. I. Mullins.** 2003. Importance and detection of virus reservoirs and compartments of HIV infection. Curr. Opin. Microbiol. **6:**410–416.
62. **Orenstein, J. M., C. Fox, and S. M. Wahl.** 1997. Macrophages as a source of HIV during opportunistic infections. Science **276:**1857–1861.
63. **Overbaugh, J., R. J. Anderson, J. O. Ndinya-Achola, and J. K. Kreiss.** 1996. Distinct but related human immunodeficiency virus type 1 variant populations in genital secretions and blood. AIDS Res. Hum. Retrovir. **12:**107–115.
64. **Pang, S., Y. Shlesinger, E. S. Daar, T. Moudgil, D. D. Ho, and I. S. Y. Chen.**

1992. Rapid generation of sequence variation during primary HIV-1 infection. AIDS **6:**453–460.
65. **Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci.** 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature **362:**355–358.
66. **Paranjpe, S., J. Craigo, B. Patterson, M. Ding, P. Barroso, L. Harrison, R. Montelaro, and P. Gupta.** 2002. Subcompartmentalization of HIV-1 quasispecies between seminal cells and seminal plasma indicates their origin in distinct genital tissues. AIDS Res. Hum. Retrovir. **18:**1271–1280.
67. **Perno, C. F., F. M. Newcomb, D. A. Davis, S. Aquaro, R. W. Humphrey, R. Calio, and R. Yarchoan.** 1998. Relative potency of protease inhibitors in monocytes/macrophages acutely and chronically infected with human immunodeficiency virus. J. Infect. Dis. **178:**413–422.
68. **Poss, M., H. L. Martin, J. K. Kreiss, L. Granville, B. Chohan, P. Nyange, K. Mandaliya, and J. Overbaugh.** 1995. Diversity in virus populations from genital secretions and peripheral blood from women recently infected with HIV-1. J. Virol. **69:**8118–8122.
69. **Poss, M., and J. Overbaugh.** 1999. Variants from the diverse virus population identified at seroconversion of a clade A human immunodeficiency virus type 1-infected woman have distinct biological properties. J. Virol. **73:**5255–5264.
70. **Resch, W., N. Hoffman, and R. Swanstrom.** 2001. Improved success of phenotype prediction of the human immunodeficiency virus type 1 from envelope variable loop 3 sequence using neural networks. Virology **288:**51–62.
71. **Rodrigo, A. G., P. C. Goracke, K. Rowhanian, and J. I. Mullins.** 1997. Quantitation of target molecules from polymerase chain reaction-based limiting dilution assays. AIDS Res. Hum. Retrovir. **13:**737–742.
72. **Roos, M., J. Lange, R. de Goede, R. Coutinho, P. Schellekens, F. Miedema, and M. Tersmette.** 1992. Viral phenotype and immune response in primary human immunodeficiency virus type 1 infection. J. Infect. Dis. **165:**427–432.
73. **Ruiz, M. E., C. Cicala, J. Arthos, A. Kinter, A. T. Catanzaro, J. Adelsberger, K. L. Holmes, O. J. Cohen, and A. S. Fauci.** 1998. Peripheral blood-derived CD34+ progenitor cells: CXC chemokine receptor 4 and CC chemokine receptor 5 expression and infection by HIV. J. Immunol. **161:**4169–4176.
74. **Scadden, D. T., M. Zeira, A. Woon, Z. Wang, L. Schieve, K. Ikeuchi, B. Lim, and J. E. Groopman.** 1990. Human immunodeficiency virus infection of human bone marrow stromal fibroblasts. Blood **76:**317–322.
75. **Schacker, T., A. C. Collier, J. Hughes, T. Shea, and L. Corey.** 1996. Clinical and epidemiologic features of primary HIV infection. Ann. Intern. Med. **125:**257–264.
76. **Schacker, T. W., J. P. Hughes, T. Shea, R. W. Coombs, and L. Corey.** 1998. Biological and virologic characteristics of primary HIV infection. Ann. Intern. Med. **128:**613–620.
77. **Schmidtmayerova, H., M. Alfano, G. Nuovo, and M. Bukrinsky.** 1998. Human immunodeficiency virus type 1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-mediated pathway: replication is restricted at a postentry level. J. Virol. **72:**4633–4642.
78. **Schnittman, S. M., H. C. Lane, J. Greenhouse, J. S. Justement, M. Baseler, and A. S. Fauci.** 1990. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc. Natl. Acad. Sci. USA **87:**6058–6062.
79. **Schrager, L. K., and M. P. D'Souza.** 1998. Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. JAMA **280:**67–71.
80. **Schuitemaker, H., M. Koot, N. A. Kootstra, M. W. Dercksen, R. E. de Goede, R. P. van Steenwijk, J. M. Lange, J. K. Schattenkerk, F. Miedema, and M. Tersmette.** 1992. Biological phenotype of HIV-1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population. J. Virol. **66:**1354–1360.
81. **Shapshak, P., D. M. Segal, K. A. Crandall, R. K. Fujimura, B. T. Zhang, K. Q. Xin, K. Okuda, C. K. Petito, C. Eisdorfer, and K. Goodkin.** 1999. Independent evolution of HIV type 1 in different brain regions. AIDS Res. Hum. Retrovir. **15:**811–820.
82. **Sheehy, N., U. Desselberger, H. Whitwell, and J. K. Ball.** 1996. Concurrent evolution of regions of the envelope and polymerase genes of human immunodeficiency virus type 1 during zidovudine (AZT) therapy. J. Gen. Virol. **77:**1071–1081.
83. **Simmonds, P., P. Balfe, C. A. Ludlam, J. O. Bishop, and A. J. Leigh Brown.** 1990. Analysis of sequence diversity in hypervariable regions of the external glycoprotein of HIV-1. J. Virol. **64:**5840–5850.
84. **Simmonds, P., P. Balfe, J. F. Peutherer, C. A. Ludlam, J. O. Bishop, and A. J. Leigh Brown.** 1990. Human immunodeficiency virus-infected individuals contain provirus in small numbers of peripheral mononuclear cells and at low copy numbers. J. Virol. **64:**864–872.
85. **Simmonds, P. P., L. Q. Zhang, F. McOmish, P. Balfe, C. A. Ludlam, and A. J. Leigh Brown.** 1991. 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. **65:**6266–6276.
86. **Slatkin, M., and W. P. Maddison.** 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics **123:**603–613.
87. **Sonza, S., A. Maerz, N. Deacon, J. Meanger, J. Mills, and S. Crowe.** 1996. Human immunodeficiency virus type 1 replication is blocked prior to reverse transcription and integration in freshly isolated peripheral blood monocytes. J. Virol. **70:**3863–3869.
88. **Sonza, S., H. P. Mutimer, R. Oelrichs, D. Jardine, K. Harvey, A. Dunne, D. F. Purcell, C. Birch, and S. M. Crowe.** 2001. Monocytes harbour replication-competent, non-latent HIV-1 in patients on highly active antiretroviral therapy. AIDS **15:**17–22.
89. **Tersmette, M., J. M. Lange, R. E. de Goede, F. de Wolf, J. K. Eeftink-Schattenkerk, P. T. Schellekens, R. A. Coutinho, J. G. Huisman, J. Goudsmit, and F. Miedema.** 1989. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet **1:**983–985.
90. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
91. **Ulvestad, E., K. Williams, R. Bjerkvig, K. Tiekotter, J. Antel, and R. Matre.** 1994. Human microglial cells have phenotypic and functional characteristics in common with both macrophages and dendritic antigen-presenting cells. J. Leukoc. Biol. **56:**732–740.
92. **Ulvestad, E., K. Williams, S. Mork, J. Antel, and H. Nyland.** 1994. Phenotypic differences between human monocytes/macrophages and microglial cells studied in situ and in vitro. J. Neuropathol. Exp. Neurol. **53:**492–501.
93. **van't Wout, A. B., N. A. Kootstra, G. A. Mulder-Kampinga, N. Albrecht-van Lent, H. J. Scherpbier, J. Veenstra, K. Boer, R. A. Coutinho, F. Miedema, and H. Schuitemaker.** 1994. Macrophage-tropic variants initiate HIV-1 infection after sexual, parenteral, and vertical transmission. J. Clin. Investig. **94:**2060–2067.
94. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.** 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science **280:**427–431.
95. **Voulgaropoulou, F., B. Tan, M. Soares, B. Hahn, and L. Ratner.** 1999. Distinct human immunodeficiency virus strains in the bone marrow are associated with the development of thrombocytopenia. J. Virol. **73:**3497–3504.
96. **Wain-Hobson, S.** 1993. The fastest genome evolution ever described: HIV variation in situ. Curr. Biol. **3:**878–883.
97. **Wain-Hobson, S.** 1992. Human immunodeficiency virus type 1 quasispecies in vivo and ex vivo. Curr. Top. Microbiol. Immunol. **176:**181–193.
98. **Wang, T. H., Y. K. Donaldson, R. P. Brettle, J. E. Bell, and P. Simmonds.** 2001. Identification of shared populations of human immunodeficiency virus type 1 infecting microglia and tissue macrophages outside the central nervous system. J. Virol. **75:**11686–11699.
99. **Weiser, B., H. Burger, P. Campbell, S. Donelan, and J. Mladenovic.** 1996. HIV type 1 RNA expression in bone marrows of patients with a spectrum of disease. AIDS Res. Hum. Retrovir. **12:**1551–1558.
100. **Wiley, C. A., R. D. Schrier, J. A. Nelson, P. W. Lampert, and M. B. A. Oldstone.** 1986. Cellular localization of human immunodeficiency virus infection within the brain of acquired immune deficiency syndrome patients. Proc. Natl. Acad. Sci. USA **83:**7089–7093.
101. **Williams, K. C., S. Corey, S. V. Westmoreland, D. Pauley, H. Knight, C. deBakker, X. Alvarez, and A. A. Lackner.** 2001. Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. J. Exp. Med. **193:**905–915.
102. **Williams, K. C., and W. F. Hickey.** 2002. Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. Annu. Rev. Neurosci. **25:**537–562.
103. **Wolfs, T. F., G. Zwart, M. Bakker, M. Valk, C. L. Kuiken, and J. Goudsmit.** 1991. Naturally occurring mutations within HIV-1 V3 genomic RNA lead to antigenic variation dependent on a single amino acid substitution. Virology **185:**195–205.
104. **Wolfs, T. F. W., J. J. de Jong, H. van den 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.
105. **Wolinsky, S. M., B. T. M. Korber, A. U. Neumann, M. Daniels, K. J. Kuntsman, A. J. Whetsell, M. R. Furtado, Y. Cao, D. D. Ho, J. T. Safrit, and R. A. Koup.** 1996. Adaptive evolution of HIV-1 during the natural course of infection. Science **272:**537–542.
106. **Wolinsky, S. M., C. M. Wike, B. T. M. Korber, C. Hutto, W. P. Parks, L. L. Rosenblum, K. J. Kunstman, M. R. Furtado, and J. L. Muñoz.** 1992. Selective transmission of HIV-1 variants from mother to infants. Science **255:**1134–1137.
107. **Wong, J. K., C. C. Ignacio, F. Torriani, D. Havler, N. J. S. Fitch, and D. D. Richman.** 1997. In vivo compartmentalization of human immunodeficiency

virus: evidence from the examination of *pol* sequences from autopsy tissues. J. Virol. **71:**2059–2071.

108. **Zhang, L., L. Rowe, T. He, C. Chung, J. Yu, W. Yu, A. Talal, M. Markowitz, and D. D. Ho.** 2002. Compartmentalization of surface envelope glycoprotein of human immunodeficiency virus type 1 during acute and chronic infection. J. Virol. **76:**9465–9473.
109. **Zhang, L. Q., P. MacKenzie, A. Cleland, E. C. Holmes, A. J. Leigh Brown, and P. Simmonds.** 1993. Selection for specific sequences in the external envelope protein of HIV-1 upon primary infection. J. Virol. **67:**3345–3356.
110. **Zhu, T.** 2002. HIV-1 in peripheral blood monocytes: an underrated viral source. J. Antimicrob. Chemother. **50:**309–311.
111. **Zhu, T., L. Corey, Y. Hwangbo, J. M. Lee, G. H. Learn, J. I. Mullins, and M. J. McElrath.** 2003. Persistence of extraordinarily low levels of genetic homogeneous human immunodeficiency virus type 1 in exposed seronegative individuals. J. Virol. **77:**6108–6116.
112. **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.
113. **Zhu, T., D. Muthui, S. Holte, D. Nickle, F. Feng, S. Brodie, Y. Hwangbo, J. I. Mullins, and L. Corey.** 2002. Evidence for human immunodeficiency virus type 1 replication in vivo in $CD14^{+}$ monocytes and its potential role as a source of virus in patients on highly active antiretroviral therapy. J. Virol. **76:**707–716.
114. **Zhu, T., N. Wang, A. Carr, D. S. Nam, R. Jankowski-Moor, D. A. 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.