JOURNAL OF VIROLOGY, Nov. 1992, p. 6587–6600
0022-538X/92/116587-14$02.00/0

Vol. 66, No. 11

Complete Nucleotide Sequence, Genome Organization, and Biological Properties of Human Immunodeficiency Virus Type 1 In Vivo: Evidence for Limited Defectiveness and Complementation

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Received 8 June 1992/Accepted 7 August 1992

Previous studies of the genetic and biologic characteristics of human immunodeficiency virus type 1 (HIV-1) have by necessity used tissue culture-derived virus. We recently reported the molecular cloning of four full-length HIV-1 genomes directly from uncultured human brain tissue (Y. Li, J. C. Kappes, J. A. Conway, R. W. Price, G. M. Shaw, and B. H. Hahn, J. Virol. 65:3973–3985, 1991). In this report, we describe the biologic properties of these four clones and the complete nucleotide sequences and genome organization of two of them. Clones HIV-1_{YU-2} and HIV-1_{YU-10} were 9,174 and 9,176 nucleotides in length, differed by 0.26% in nucleotide sequence, and except for a frameshift mutation in the *pol* gene in HIV-1_{YU-10}, contained open reading frames corresponding to 5′-*gag-pol-vif-vpr-tat-rev-vpu-env-nef*-3′ flanked by long terminal repeats. HIV-1_{YU-2} was fully replication competent, while HIV-1_{YU-10} and two other clones, HIV-1_{YU-21} and HIV-1_{YU-32}, were defective. All three defective clones, however, when transfected into Cos-1 cells in any pairwise combination, yielded virions that were replication competent and transmissible by cell-free passage. The cellular host range of HIV-1_{YU-2} was strictly limited to primary T lymphocytes and monocyte-macrophages, a property conferred by its external envelope glycoprotein. Phylogenetic analyses of HIV-1_{YU-2} gene sequences revealed this virus to be a member of the North American/European HIV-1 subgroup, with specific similarity to other monocyte-tropic viruses in its V3 envelope amino acid sequence. These results indicate that HIV-1 infection of brain is characterized by the persistence of mixtures of fully competent, minimally defective, and more substantially altered viral forms and that complementation among them is readily attainable. In addition, the limited degree of genotypic heterogeneity observed among HIV-1_{YU} and other brain-derived viruses and their preferential tropism for monocyte-macrophages suggest that viral replication within the central nervous system may differ from that within the peripheral lymphoid compartment in significant and clinically important ways. The availability of genetically and biologically well characterized HIV-1 clones from uncultured human tissue should facilitate future studies of virus-cell interactions relevant to viral pathogenesis and drug and vaccine development.

Although human immunodeficiency virus type 1 (HIV-1) is known to be the cause of AIDS, the pathogenic mechanisms responsible for its many disease manifestations remain enigmatic. Among the most important unanswered questions relating to AIDS pathogenesis are the basic virologic and cellular processes underlying both $CD4^+$ lymphocyte depletion and the neurologic dysfunction associated with AIDS dementia complex (ADC). Many different possible explanations have been considered for these pathologic outcomes, including both virally mediated and immunopathologically mediated processes (3, 24, 35, 43, 62, 67, 93, 98). There is increasing evidence that virus replication and the accumulated tissue burden of virus and viral products play an important and direct role in AIDS pathogenesis. For example, there is a direct relationship between high-level viral replication and rapid decline in $CD4^+$ cells in primary HIV-1 infection (15, 18, 101) and between increasing virus burden and accelerated disease progression in late infection (16, 42, 65, 84, 89). Similarly, a positive correlation exists between the level of HIV-1 expression in the central nervous system (CNS) and the clinical severity of ADC (30, 41, 66, 98). Finally, treatment of adults and children with AZT (azidothymidine) inhibits viral replication, as measured by circulating p24 antigen, and leads to increases in $CD4^+$ lymphocyte counts, improvement in neurologic function, and delayed progression to AIDS and death (19, 26, 27, 61, 80, 109). Together, these observations argue for the importance of viral replication and, by inference, virally encoded gene products in disease pathogenesis.

In addition to viral replication, genetic and biologic variations of HIV-1 are other properties that are linked directly to disease pathogenesis (14, 25, 64, 85, 91, 92, 103, 112). Mutation in the reverse transcriptase (RT) gene selected by clinical treatment with AZT (11, 49, 54, 55) or other RT inhibitors (22) represents a clear-cut example of biologically important HIV-1 variation in vivo. Immunologic escape mutants of HIV-1 in experimentally infected chimpanzees (72) and humans (1, 79) are additional examples. Another manifestation of HIV-1 variability with potential pathogenic relevance is the generation and persistence of defective viral forms (46, 60, 64, 107). HIV-1 genomes defective in the function of *tat* (a gene representing less than 3% of the

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provirus) have been reported to comprise as much as 10 to 15% of the virus population in vivo, thus implying that most viral sequences are multiply defective (64). While other studies (6, 53) suggest that the prevalence of defective forms may be less than this extreme number, it is conceivable that defective viral genomes play an important role in modulating the biologic expression of HIV-1 infection. In related AIDS animal model systems, replication-defective forms of feline leukemia virus (75) and murine leukemia virus (5, 13) play a central role in disease pathogenesis.

Virus replication and burden, genotypic variation, and biologic diversity are thus all important determinants of AIDS pathogenesis which are inseparably linked to the genetic composition of HIV-1 in vivo. Analysis of the genetic and biologic characteristics of HIV-1 from infected individuals at various stages of disease and with different clinical presentations has therefore been a focus of scientific attention leading to the identification, for example, of macrophage- and T-cell-tropic virus strains (14, 32, 33, 45, 52, 60, 91), syncytium-inducing and non-syncytium-inducing virus strains (25, 92, 103, 104), and the quasi-species nature of viral infection in general (34, 37, 64, 85, 94, 95, 112). However, as a consequence of the generally low abundance of viral DNA in patient tissues (94, 95), the molecular analysis of HIV-1 in vivo has, by necessity, been limited to the study of culture-amplified virus isolates, proviral DNA clones derived from them, and polymerase chain reaction (PCR)-amplified subgenomic viral fragments. These approaches can be problematic since even brief periods of culture can lead to selection of virus subpopulations that do not accurately represent the predominant species in vivo (53, 64). In the extreme case, isolation and propagation of virus in immortalized T-cell lines has yielded virus strains with biologic properties (e.g., stability of the gp120/41 envelope complex and its affinity for CD4) which differ significantly from those of primary isolates (17, 68). Similarly, PCR-amplified subgenomic fragments must, by definition, be studied outside the context of the individual interactive viral unit from which they were derived (53).

On the basis of these considerations, we sought to clone full-length viral DNA genomes directly from primary human tissue. From our earlier studies of HIV-1 DNA sequences in uncultured human tissues (94, 95), we suspected that it might be possible to clone unintegrated circular forms from such material, thereby avoiding any possibility of culture-related selection pressures. Infected brain tissue was chosen as a cloning source because of its relatively high abundance of viral DNA (76, 77, 95) and the possibility that this compartment might harbor viral strains with unique genetic and biologic properties relevant to the pathogenesis of ADC (32, 93, 98). In a previous report (60), we described the generation of 10 recombinant HIV-1 DNA clones from brain tissue, including four full-length HIV-1 genomes, one chromosomally integrated proviral half-genome, and five incomplete genomes with deletions or rearrangements. In the present study, we analyzed the replicative properties of the four full-length HIV-1 clones and determined the complete nucleotide sequences, genome organization, and biologic properties of two of them. This approach allowed us, for the first time, to examine directly the genomic organization, molecular complexity, and biologic properties of HIV-1 in vivo.

MATERIALS AND METHODS

Derivation of molecular HIV-1 clones. The viral clones HIV-1_{YU-2}, HIV-1_{YU-10}, HIV-1_{YU-21}, and HIV-1_{YU-32} analyzed in this study were obtained as described previously (60) directly from uncultured brain tissue of a man who died in 1986 at the age of 40 with severe (stage 3) ADC (66, 81). Although the date of initial HIV-1 infection of this individual was unknown, he first presented with an AIDS-defining illness (Kaposi's sarcoma) in 1983 and then developed extraoral candidiasis, cytomegalovirus retinitis, and *Pneumocystis carinii* pneumonia in 1985. He never received AZT or other antiretroviral therapy. Immunohistological examination of the brain described previously (66) revealed severe multinucleated giant cell encephalitis and widespread HIV-1 infection of macrophages and microglia. Because the HIV-1 clones were derived from this tissue by recombinant lambda phage cloning of permuted circular viral DNA intermediate forms, the viral inserts were excised by using unique restriction endonucleases (*Sal*I, *Sph*I, and *Eco*RI) and reconstructed in the plasmid vector pTZ so as to yield full-length nonpermuted viral genomes (described in reference 60). The resulting plasmid constructs were termed pYU-2, pYU-10, pYU-21, and pYU-32. Plasmid pYU-2 has been provided to the AIDS Reference and Reagent Program. The T-cell-tropic and monocyte-tropic HIV-1 strains, pHXB2d (28) and pNFN-SX (73), were used as relevant control viruses; the latter clone was provided by Irvin Chen.

Sequence determination and genomic analysis of HIV-1_{YU-2} and HIV-1_{YU-10}. Overlapping restriction fragments corresponding to the full-length HIV-1_{YU-2} and HIV-1_{YU-10} genomes cloned in lambda (60) were subcloned into M13mp18 and M13mp19. Dideoxynucleotide sequencing was performed, using Sequenase version 2.0 (U.S. Biochemical, Cleveland, Ohio) and HIV-1-specific oligonucleotide primers evenly spaced throughout the viral genome. All sequence differences between HIV-1_{YU-2} and HIV-1_{YU-10} were confirmed by double-stranded sequence determinations. The nucleotide sequences were aligned and examined for potential open reading frames (ORFs) by Microgenie (Beckman) and University of Wisconsin Genetics Computer Group software packages, using Staden's methods (99).

Phylogenetic analyses. Phylogenetic trees were constructed for the *env* gene of HIV-1_{YU-2}, HIV-1_{YU-10}, and other HIV-1 viruses from North America (BAL, JRFL, JRCSF, SC, BRVA, MN, ALA1, SF2, ADA, SF33, CDC451, SF162, NY5, and JFL), Europe (LAI and HAN), Africa (Z321, U455, Z84, NDK, ELI, and OYI), Haiti (WMJ2 and RF), and Japan (JH3) (70). Phylogenetic trees were also constructed for *gag*, *pol*, and *nef* genes of these same viruses when all sequences were available (70). Predicted protein sequences were first aligned by using the CLUSTALV program (39, 40), and the distances between gene sequences were then determined as a function of K, the estimated number of nucleotide substitutions per site, according to the method of Li et al. (59). K is the weighted average of the numbers of substitutions per synonymous site and per nonsynonymous site, each corrected for multiple hits, allowing for different rates of transitions and transversions. Phylogenetic relationships among the sequences were estimated by the neighbor-joining method (87) applied to these distances. The neighbor-joining method allows for unequal rates of evolution in different lineages and appears to be one of the most efficient methods of phylogenetic inference (86).

DNA transfection and analysis of viral replication. Plasmid DNAs (10 μg of each) containing HIV-1_{YU-2}, HIV-1_{YU-10}, HIV-1_{YU-21}, HIV-1_{YU-32}, HIV-1_{HXB2d}, and HIV-1_{NFN-SX} genomes were transfected into Cos-1 cells by the calcium phosphate precipitation method (4). Supernatants were har-

vested at 24, 48, and 72 h for p24 antigen and RT determinations and for cell-free transmissibility studies. Alternatively, phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) were added directly to Cos-1 cultures 24 h after transfection to allow cell-to-cell spread and amplification of virus. Forty-eight hours later, PBMCs were transferred to T25 flasks and cocultured with additional PHA-stimulated PBMCs to further amplify virus. The replication competence of these virus stocks was then analyzed by clarification of culture supernatants (600 × *g* for 10 min), filtration (1.2-μm-pore-size filters), and passage onto various human cell types. These samples included primary PBMCs, macrophages, and lymphocytes; the immortalized $CD4^+$ T-cell lines Molt4 clone 8, Jurkat, CEM, CEMx174, SupT1, and HUT78; the myeloid-derived cell line U937; the fibrous histiocytoma cell line GCT (21); the fetal glial cell line N370FG (33a); and the glioblastoma cell lines D54 (10) and CH235 (9). PBMCs were prepared as described previously (15, 97) with PHA stimulation for 24 to 48 h and maintenance in complete RPMI medium (see below) supplemented with interleukin-2 (30 U/ml). Monocytes (51, 97) were prepared in six-well (35-mm-diameter) tissue culture plates, using Hanks balanced salt solution with calcium and magnesium and supplemented with 10% human AB serum to facilitate monocyte adherence. Purified lymphocytes were prepared by negative selection, using two sequential cell adherence steps in Hanks balanced salt solution with calcium and magnesium and 10% human AB serum to remove monocytes (51, 96). Primary PBMCs, monocytes, lymphocytes, and immortalized T-cell lines were cultured in complete RPMI medium (RPMI 1640 medium supplemented with fetal bovine serum [15%], glutamine [2 mM], penicillin [50 U/ml], and streptomycin [50 μg/ml]). Cos-1 cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum. The remaining cell lines were maintained in a 1:1 mixture of Dulbecco modified Eagle medium and Ham's nutrient mixture F-12 supplemented with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.2), 2 mM glutamine, and 10% fetal bovine serum.

RT assays were performed as follows. A 25-μl volume of culture supernatant was mixed with 75 μl of RT cocktail containing 67 mM Tris-HCl (pH 8.0), 6.7 mM dithiothreitol, 6.7 mM $MgCl_2$, 200 mM KCl, 0.13% Triton X-100, 0.66 mM EGTA, 1.25 μg of poly(A)-oligo(dT), and 5 μCi of [^{35}S]TTP. The reaction was performed for 1.5 h at 37°C and stopped by the addition of 50 μl of 0.2 M $Na_4P_2O_7$. Each sample was blotted onto an NA 45 membrane (Schleicher & Schuell, Inc., Keene, N.H.) by vacuum. Membranes were washed twice (0.3 M NaH_2PO_4, 0.46 M Na_2HPO_4, pH 6.8), air dried, and analyzed for [^{35}S]thymidine incorporation by radioanalytic imaging (AMBIS System, Inc., San Diego, Calif.). HIV-1 p24 antigen capture assays were performed by using a commercial assay (Coulter Diagnostics, Hialeah, Fla.).

Complementation studies. To explore the possibility of retroviral complementation among the four clones (HIV-1_{YU-2}, HIV-1_{YU-10}, HIV-1_{YU-21}, and HIV-1_{YU-32}), the respective plasmids were mixed together in all possible combinations (5 μg of each) and transfected into Cos-1 cells by the calcium phosphate precipitation method (4). Twenty-four hours after transfection, 5 × 10^6 PHA-stimulated normal human PBMCs were added to the Cos-1 monolayer and cocultivated for an additional 48 h. Nonadherent PBMCs were then transferred from six-well plates to T25 flasks, cocultivated with additional PHA-stimulated PBMCs, and monitored for RT activity. After 7 to 10 days, the culture supernatants were equilibrated for RT levels, clarified by low-speed centrifugation (600 × *g* for 10 min), filtered (1.2-μm-pore-size filters), and transmitted cell free to uninfected PHA-stimulated PBMCs. The medium was changed weekly, new PBMCs were added, and the culture supernatants were checked for p24 antigen and RT activity. Viral replication, transmission, and complementation experiments were repeated three to five times, with similar results; representative data are shown in the figures.

Nucleotide sequence accession numbers. The sequence data reported have been assigned GenBank accession numbers M93258 (HIV-1_{YU-2}) and M93259 (HIV-1_{YU-10}).

RESULTS

Nucleotide sequence and genome organization of HIV-1_{YU-2} and HIV-1_{YU-10}. The complete nucleotide sequences of HIV-1_{YU-2} and HIV-1_{YU-10} were determined (Fig. 1). The viral genomes extended 9,174 and 9,176 nucleotides (nt), respectively, from the beginning of the long terminal repeat (LTR) R sequence (corresponding to the mRNA start site) to the terminus of R in the 3′ LTR (the site of polyadenylation). The potential ORFs deduced from the two sequences (Fig. 2) were identical except for a truncated polymerase gene in HIV-1_{YU-10} that resulted from a single nucleotide deletion at position 2440.

Inspection of the LTR regions of HIV-1_{YU-2} and HIV-1_{YU-10} (Fig. 1) indicated that the principal regulatory elements, including the TATA box, Sp1 binding sites (47), NFκB sites (71), AP1 sites (29), LBP-1 sites (48), and the *cis*-acting *tat*-responsive element (83), were conserved. Similarly, the primer binding site, polyadenylation signal (AATAA), central and 3′ polypurine tracts, consensus splice donor and acceptor sites, and consensus RNA packaging signal were present (12, 57, 70, 106).

Coding sequences corresponding to p17, p24, p9, and p7 in *gag* and p10 (protease), p66 (RT), and p32 (integrase) in *pol* were similar in length to sequences in the *gag* and *pol* genes of previously characterized HIV-1 proviruses (63, 106, 108). The myristylation site for p17 *gag* (amino-terminal glycine) and two copies of the zinc finger motif (Cys-X_2-Cys-X_4-His-X_4-His-X_4-Cys) (7, 8, 63, 108) in the p9 nucleocapsid protein were also present. The p17 matrix protein and the p24 major capsid protein each contained potential serine-linked phosphorylation sites (Arg-X-Ser) (63, 108), as shown in Fig. 1.

HIV-1_{YU-2} and HIV-1_{YU-10} differed importantly in the *pol* gene. The latter sequence contained a one base pair deletion at position 2440, resulting in premature termination of the ORF at position 2502, 714 amino acids short of the normal terminus of the polyprotein precursor. In the RT gene of HIV-1_{YU-2} (and in the corresponding region of HIV-1_{YU-10}), the codons at amino acid positions 41 (Met), 67 (Asp), 70 (Lys), 103 (Lys), 181 (Tyr), 215 (Thr), and 219 (Lys) (nt 2219, 2297, 2306, 2405, 2639, 2741, and 2753 in Fig. 1) corresponded to wild-type sequences found in viruses from drug-naive patients which are sensitive to AZT (11, 49, 54, 55) and the non-nucleoside RT inhibitors BI-RG-587, R82913, and L697,661 (22).

The *tat* genes of HIV-1_{YU-2} and HIV-1_{YU-10} comprised two exons, one 216 nt in length preceding the *env* gene and the other 87 nt long overlapping *env*. The second *tat* exon, 29 codons in length, was similar to the exons of a number of HIV-1 isolates but longer than others (70, 106). Like other HIV-1 strains, there was a stop codon immediately following the splice donor site of the first *tat* exon, suggesting that a truncated 72-amino-acid form of *tat* may be expressed from

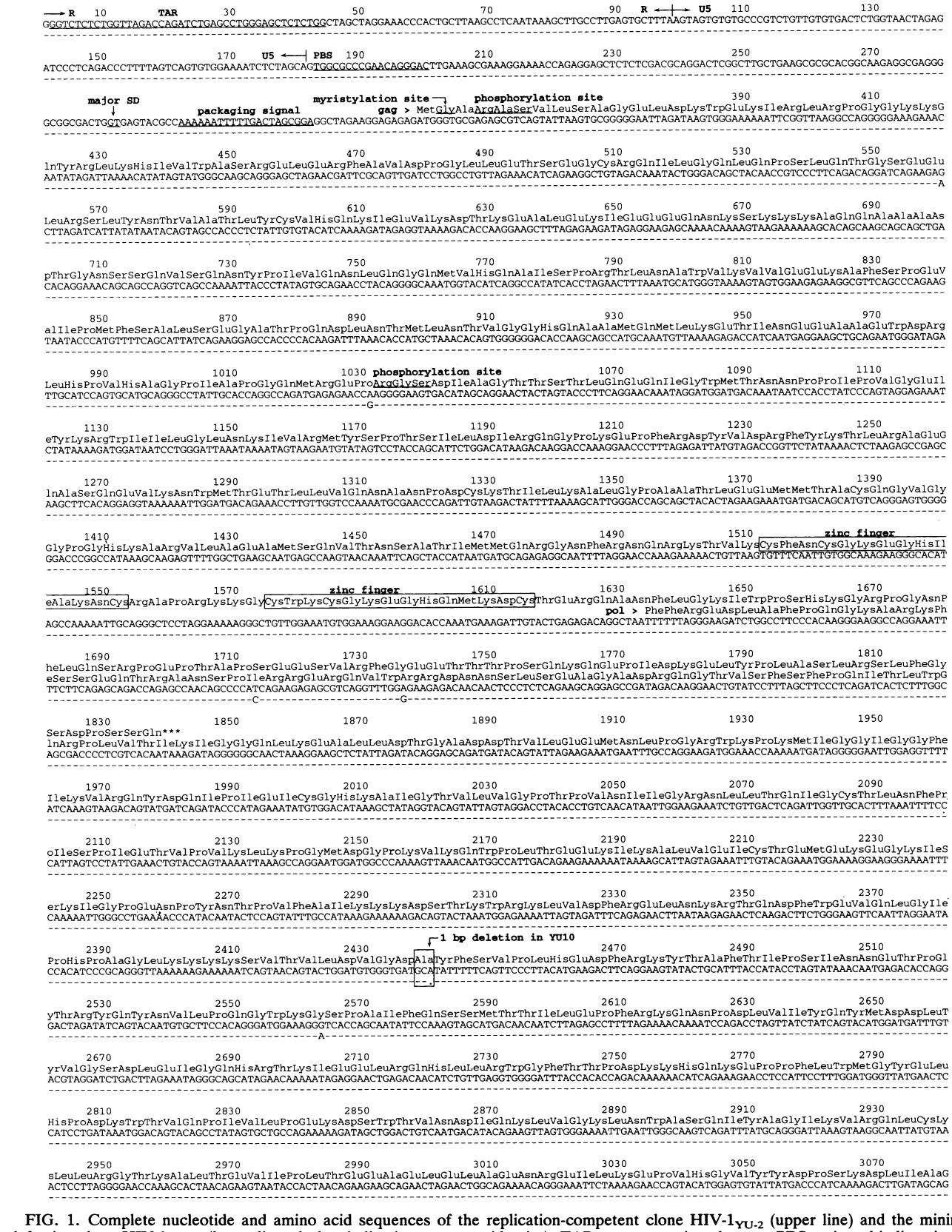

FIG. 1. Complete nucleotide and amino acid sequences of the replication-competent clone HIV-1_{YU-2} (upper line) and the minimally defective clone HIV-1_{YU-10} (lower line; dashes indicating sequence identity). TAR, *tat*-responsive element; PBS, primer binding site; SD, splice donor; PPT, polypurine tract; SA, splice acceptor.

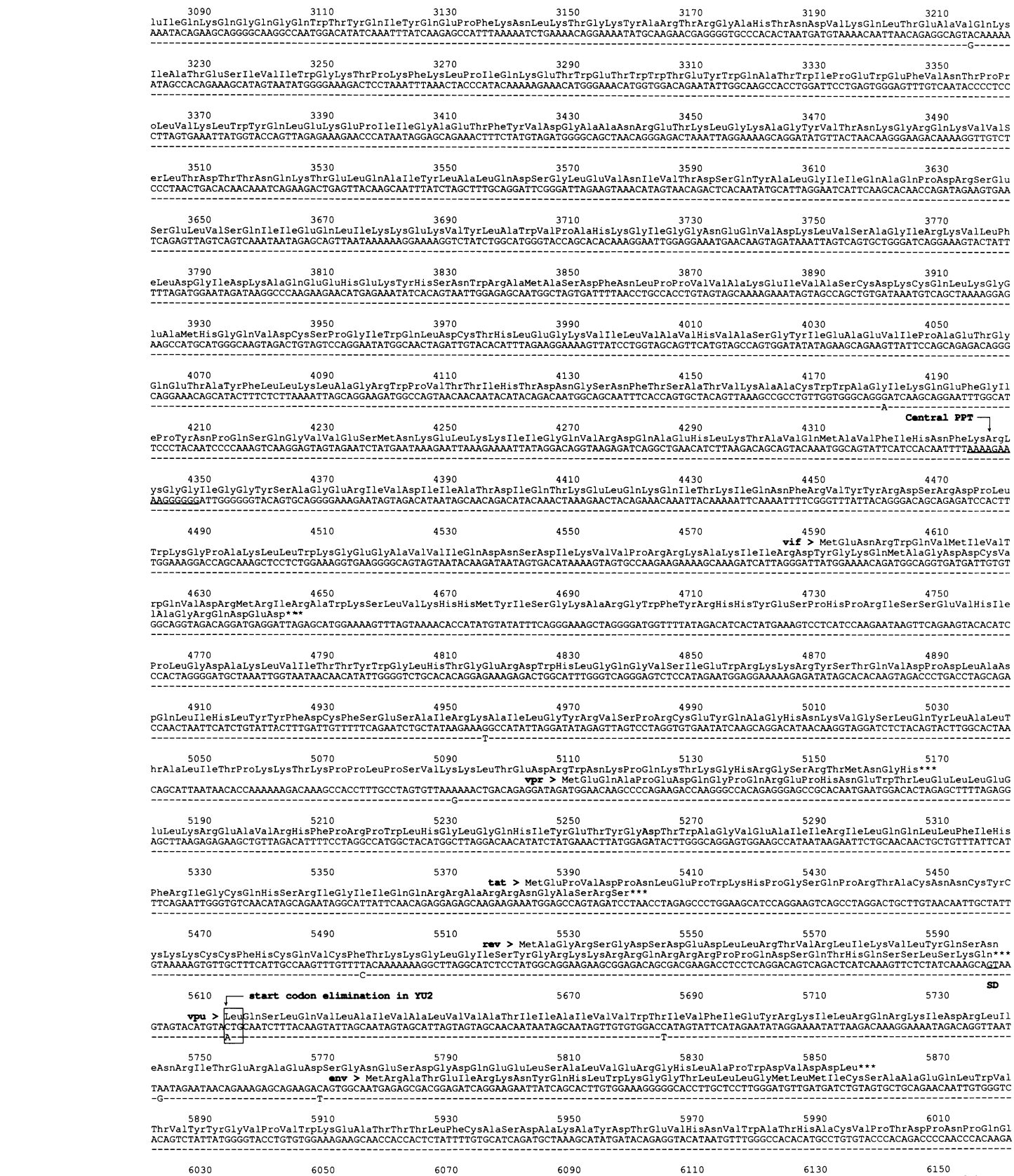

FIG. 1—*Continued.*

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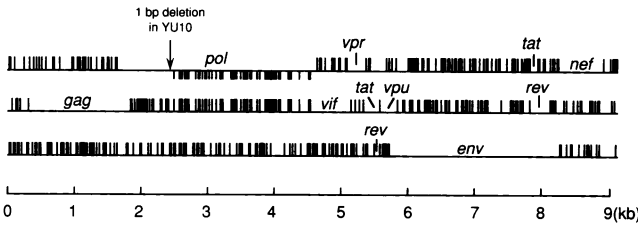

FIG. 2. ORFs for HIV-1_{YU-2} and HIV-1_{YU-10}. Vertical bars indicate positions of termination codons. Because of the high degree of sequence similarity between HIV-1_{YU-2} and HIV-1_{YU-10} (99.74%), the positions of termination codons and the organization of ORFs were identical except in *pol*, in which a single nucleotide deletion in HIV-1_{YU-10} resulted in a frameshift and truncation of the ORF (indicated by vertical bars below the line). The *vpu* ATG initiation codon in HIV-1_{YU-2} but not HIV-1_{YU-10} is missing (see text for details).

TABLE 1. Nucleotide sequence differences between HIV-1_{YU-2} and HIV-1_{YU-10}

Gene	Sequence length (nt)	No. (%) of nucleotide differences	No. nonsynonymous/ no. synonymous
LTR	630	1 (0.16)	
gag	1,398	4 (0.29)	1/3
pol	2,841	3 (0.11)	0/3
vif	576	2 (0.35)	1/1
vpr	291	0 (0.00)	0/0
tat	303	1 (0.33)	0/1
rev	351	0 (0.00)	0/0
vpu	243	4 (1.65)	2/2
env	2,529	7 (0.28)	5/2
nef	642	2 (0.31)	1/1
Total	9,085[a]	24 (0.26)	10/13

[a] Excludes reading frame overlaps, the duplicated LTR, and four unpaired nucleotides.

the first exon alone. Three functionally important domains of *tat* (acidic [ExxDxxExx], cysteine-rich [CxxCxCxxCCxx CxxC], and basic, [xRKKRRxRRR]) (106) were highly conserved in HIV-1_{YU-2} and HIV-1_{YU-10}. The bicistronic *rev* gene was also identified, and the nuclear localization signal NRRRRW and a leucine-rich active domain LPPLERLTLD were highly conserved (106).

The *vif* and *vpr* genes were 576 and 291 nt long, respectively, with deduced polypeptide sequences similar to those of other HIV-1 isolates. The *vpu* ORF, however, differed between the two clones, with HIV-1_{YU-2} lacking a methionine (ATG) initiation codon. Importantly, the *nef* ORF (2, 20, 50), which was 642 nt in length, was conserved in both viral genomes in a nontruncated form. A proposed glycine myristylation site at the amino terminus of *nef* (36) was also conserved.

The length and hydrophobicity profile of the *env* ORF (2529 nt; 843 amino acids) and the number and position of its cysteine residues and potential N-linked glycosylation sites (21 and 27, respectively), were very similar between HIV-1_{YU-2} and HIV-1_{YU-10} and previously characterized HIV-1 strains (70). In hypervariable region V3 (corresponding to amino acid positions 292 to 326 from the *env* methionine initiation codon; Fig. 1), the predicted amino acid sequences of HIV-1_{YU-2} and HIV-1_{YU-10} were remarkably similar to those of other previously reported monocyte-tropic strains, differing in only one (HIV-1_{BAL}, His-304→Asn) or two (HIV-1_{JRFL} and HIV-1_{ADA}; His-304→Asn and Phe-311→Leu) positions (45, 70, 111). Compared with the North American consensus V3 sequence (56, 70), HIV-1_{YU-2} and HIV-1_{YU-10} also differed in only two amino acids (His-304→Asn and Phe-311→Leu). In contrast, HIV-1_{YU-2} and HIV-1_{YU-10} differed from nine T-cell line-adapted North American/European viruses in this region by an average of 8 amino acids (range, 5 to 11). Amino acid residues known to be involved in gp120-CD4 binding (Ser-252 [nt 6529], Thr-253 [nt 6532], Asp-363 [nt 6862], Glu-365 [nt 6868], Tyr-379 [nt 6910], Trp-414 [nt 7015], Val-417 [nt 7024], Ser-434 [nt 7075], Asp 444 [nt 7105], Asp 464 [nt 7165], Glu 469 [nt 7180], Leu 470 [nt 7183], and Tyr 471 [nt 7186] [74,105]) were conserved without exception in HIV-1_{YU-2} and HIV-1_{YU-10}.

Genotypic variation of HIV-1_{YU-2} and HIV-1_{YU-10}. Alignment of HIV-1_{YU-2} and HIV-1_{YU-10} sequences revealed identity at 9,061 of 9,085 positions (99.74%) (Table 1). Generally, nucleotide differences between these clones were evenly dispersed throughout the genome, although *vpu* contained a higher proportion of changes than did other genes (4 bp; 1.65%; $P < 0.01$, Fisher's exact test). There was also a trend for a higher proportion of nonsynonymous changes in *env* (and possibly in some of the nonstructural genes) than in *gag* and *pol*. HIV-1_{YU-10} differed from HIV-1_{YU-2} in having a 3-bp insertion in U3/*nef* and in the previously described single base pair deletion in *pol*. Of 24 mismatches between HIV-1_{YU-2} and HIV-1_{YU-10}, 15 involved G-to-A substitutions, 8 of which occurred in a GpA nearest-neighbor motif (107).

Replication potential and cell tropism of HIV-1_{YU} clones. To determine the replicative potential of the HIV-1_{YU-2}, HIV-1_{YU-10}, HIV-1_{YU-21}, and HIV-1_{YU-32} genomes, plasmids containing each of the four clones were transfected individually into Cos-1 cells, which were then cocultivated with PHA-stimulated normal human PBMCs to amplify any virus that might be produced. On the basis of serial measurements of RT activity and p24 antigen released into the culture supernatants as well as the ability of these supernatants to transmit infection cell free to other uninfected PBMCs, it was determined that only HIV-1_{YU-2} was replication competent. In PBMCs, HIV-1_{YU-2} produced syncytia and led to cell death, as determined by enumeration of cells that

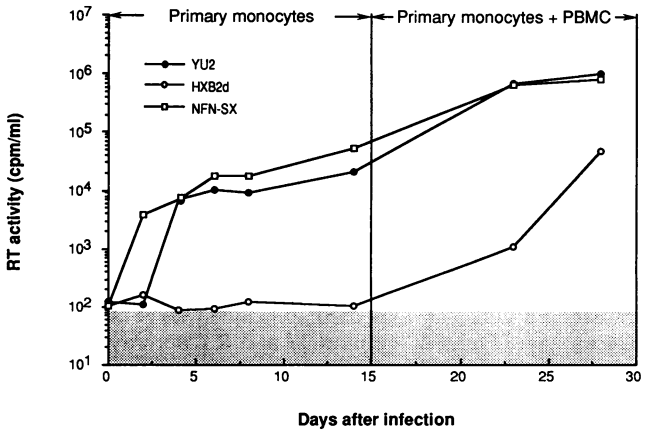

FIG. 3. Replication of HIV-1_{YU-2}, HIV-1_{NFN-SX}, and HIV-1_{HXB2D} in primary monocytes (days 0 to 15) and in PBMCs (days 15 to 28). The shaded area represents assay background (<100 cpm).

TABLE 2. Replication of HIV-1_{YU-2}, HIV-1_{HXB2d}, and HIV-1_{NFN-SX} in different cell types

Virus	Replication[a]												
	Primary target cells			Immortalized cell lines									
	PBMC	Macrophages	Primary T cells	Molt4 clone 8	U937	Jurkat	CEM	CEMx174	SupT1	HUT78	D54	CH235	GCT	N370FG
YU-2	4+	4+	4+	−[b]	−	−	−	−	−	−	−	−	−	−
HXB2d	4+	−	4+	4+	4+	4+	4+	4+	4+	4+	−	−	−	−
NFN-SX	4+	4+	4+	−[b]	−	−	−	−	−	−	−	−	−	−

[a] Assessed 10, 15, and 20 days after exposure of cells to a virus inoculum of 100,000 RT cpm, corresponding to approximately 10,000 PBMC tissue culture infectious doses. −, <200 RT cpm; 4+, >10,000 RT cpm.

[b] Although virus could not be successfully transmitted to Molt4 clone 8 cells by cell-free passage, virus could be transmitted to these cells by cell-cell cocultivation (60).

excluded trypan blue. Figure 3 illustrates the growth curve of HIV-1_{YU-2} following transfection into Cos-1 cells and cell-free passage to purified monocyte monolayers. The growth kinetics of HIV-1_{YU-2} and the macrophage-tropic control virus HIV-1_{NFN-SX} were very similar and distinctly different from those of HIV-1_{HXB2d}, a T-cell-adapted virus that does not replicate efficiently in monocytes. The addition of unfractionated PBMCs to the monocyte monolayers at day 15 led to a further increase in viral titers, suggesting that HIV-1_{YU-2} and HIV-1_{NFN-SX} were replicating actively in both monocytes and primary T cells. The replication capacity of HIV-1_{YU-2} was then examined separately in purified primary lymphocytes and monocytes as well as in various immortalized human cell lines, including T cells (Molt4 clone 8, Jurkat, CEM, CEMx174, SupT1, and HUT78), myeloid cells (U937), glioma cells (D54 and CH235), fibrous histiocytoma cells (GCT), and fetal glial cells (N370FG). As shown in Table 2, clone HIV-1_{YU-2} replicated well in primary PBMCs, monocytes, and monocyte-depleted lymphocytes but failed to replicate in any of the immortalized cell lines, including N370FG cells that express galactocerebroside, a molecule reported to bind specifically to HIV-1 gp120 (38).

Limited defectiveness and complementation of HIV-1_{YU} genomes. Inspection of the HIV-1_{YU-10} sequence revealed only a single obvious lethal mutation, although other less conspicuous changes that might also render the clone defective could not be excluded. Nonetheless, the very few differences observed between HIV-1_{YU-10} and HIV-1_{YU-2} suggested that most ORFs in all of the defective clones might actually be intact and functional. Therefore, to assess the extent of defectiveness and the potential for complementation in HIV-1_{YU-10} and the other noncompetent clones, all possible combinations of the viral DNAs were mixed and transfected into Cos-1 cells, and culture supernatants were analyzed for p24 antigen release (Fig. 4A). PBMCs were then added to amplify any virus that might be produced, and culture supernatants were harvested after 7 to 10 days. After equilibration for RT content (or for volume if no detectable RT activity was present), viruses were passaged cell free (filtered) onto PBMCs and subsequently tested for RT production after 8 and 16 days of culture (Fig. 4B). HIV-1_{YU-2}-transfected cells, as expected, produced large amounts of p24 antigen (Fig. 4A, column 1) and RT activity (Fig. 4B, column 1), comparable to the two control clones pHXB2d and pNFN-SX. HIV-1_{YU-10}-transfected Cos-1 cells also produced large amounts of p24 antigen (Fig. 4A, column 2) but no RT activity (Fig. 4B, column 2), consistent with the finding of a frameshift mutation in the *pol* gene of this clone. Cells transfected with either HIV-1_{YU-21} or HIV-1_{YU-32} alone produced no p24 antigen or RT activity (Fig. 4A and B, columns 3 and 4). However, when HIV-1_{YU-10}, HIV-1_{YU-21}, and HIV-1_{YU-32} were transfected in any combination, p24 antigen and RT activity were produced and viral replication ensued (Fig. 4A and B, columns 10 to 13). There was no evidence that cotransfection of any of the defective clones in

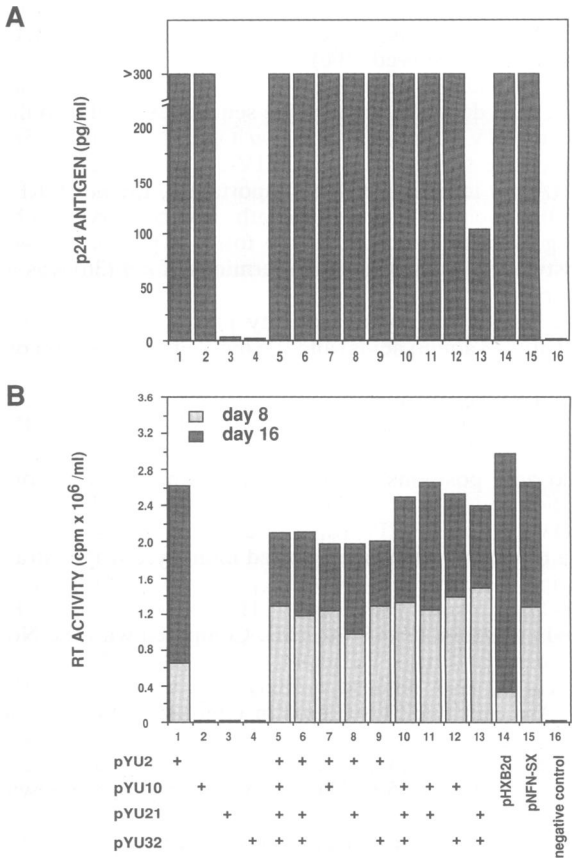

FIG. 4. (A) HIV-1 p24 antigen release into culture supernatants at 48 h following transfection of Cos-1 cells with individual HIV-1 DNA clones (or combinations of clones) designated pYU2, pYU10, pYU21, and pYU32. (B) Supernatant HIV-1 RT activity at days 8 and 16 following exposure of uninfected PHA-stimulated PBMCs to filtered supernatants from transfected Cos-1/PBMC cocultures. The virus inocula that were applied to uninfected cells were equilibrated on the basis of RT activity (columns 1 and 5 to 15) or, if no RT activity was present, then by volume (columns 2 to 4).

combination with HIV-1_{YU-2} led to inhibition of the latter virus strain's replicative or cytopathic activity in PBMCs (Fig. 4, columns 5 to 9).

Phylogeny of HIV-1_{YU-2}. Phylogenetic analyses of the four major genes (*gag*, *pol*, *env*, and *nef*) were performed to ascertain the relationship of HIV-1_{YU-2} to other HIV-1 strains of African (U455, ELI, NDK, and OYI) and non-African (LAI, MN, JRFL, JRCSF, SF2, HAN, and RF) origin for which full-length sequences have been determined (70). Previous phylogenetic analyses (58, 70) indicated that all North American and European strains form a cluster quite separate from all strains of African origin except for OYI. For each of the four genes analyzed, HIV-1_{YU-2} clustered within the North American/European subgroup (data not shown but available on request). In addition, whereas certain viruses (HIV-1_{MAL} and HIV-2_{7312A} [31, 58]) exhibit mosaic genomes, suggesting previous recombination between viruses of disparate phylogenetic origin, only minor (insignificant) differences in branching orders of the four major HIV-1_{YU-2} genes were found, thereby providing no evidence that this virus is a recombinant. Finally, the similarity between HIV-1_{YU-2} and a large number of other isolates for which *env* sequences are known, including other monocyte-tropic viruses (JRFL, BAL, and ADA), was examined specifically. Although the amino acid sequence of the envelope V3 region of HIV-1_{YU-2} was most similar to that of other monocyte-tropic viruses (see above), the overall envelope nucleotide sequence of HIV-1_{YU-2} did not indicate a specific clustering with these viruses (Fig. 5).

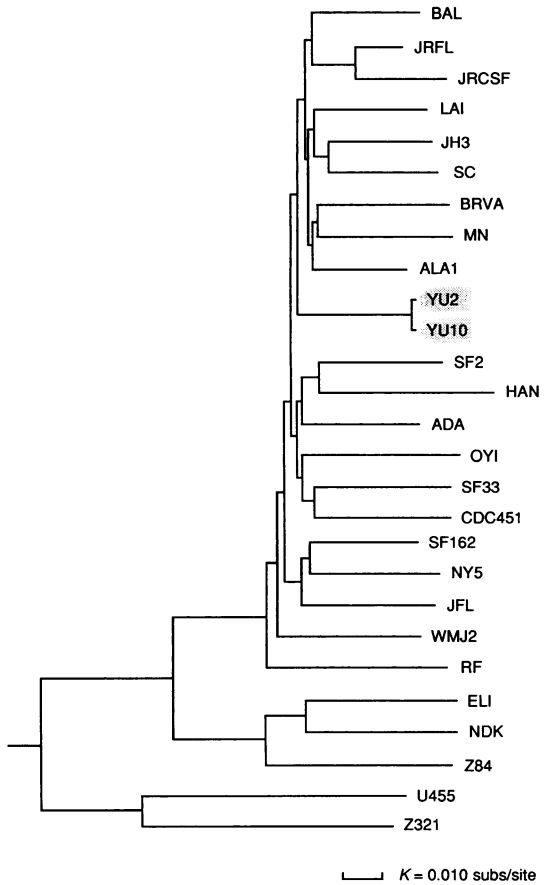

FIG. 5. Phylogenetic relationship of YU-2 and YU-10 to other HIV-1 isolates, derived from comparisons of *env* gene sequences. The neighbor-joining method was applied to a pairwise distance matrix of values of *K*, the estimated number of nucleotide substitutions per site. Three short regions of problematical sequence alignment (70) were excluded from the analysis, and an average of 736 codons were compared between sequences. Horizontal branch lengths are drawn to scale (the bar represents $K = 0.010$ substitutions per site, or 1% divergence); vertical separation is for clarity only. The tree was rooted by using simian immunodeficiency virus strain CPZ as the outgroup. The derivation of HIV-1 sequences is summarized in Materials and Methods and elsewhere (70).

DISCUSSION

The results of this study provide a unique view of the genetic and biologic characteristics of HIV-1 in vivo and the natural history of HIV-1 infection. The viral clones analyzed were obtained from uncultured brain tissue of a man who died with ADC. Thus, unlike all other full-length HIV-1 clones, they were not subjected to any form of tissue culture selection, reflecting instead only the consequences of viral replication, persistence, and natural selection in this individual and those infected before him.

Previous studies of the genomic organization of highly passaged prototype HIV-1 strains (HIV-1_{IIIB}, HIV-1_{LAV}, and HIV-1_{ARV-2}) identified a complex array of genes that is now recognized to include 5'-LTR-*gag-pol-vif-vpr-tat-rev-vpu-env-nef*-LTR-3' (69, 82, 88, 110). Subsequent studies confirmed this general organization but also revealed a high frequency of base substitutions, deletions, and insertions which often led to obvious disruptions of certain of these ORFs (34, 37, 64, 100). Deletion or truncation of the major structural genes *gag*, *pol*, and *env* and the nuclear regulatory genes *tat* and *rev* resulted in replication-defective virus, and these genes were therefore described as essential (20). Disruption of the *vif*, *vpr*, *vpu*, or *nef* ORF, on the other hand, still allowed viral replication to proceed in vitro, although such viruses were frequently altered phenotypically. These genes, along with the *vpx* gene of HIV-2, were therefore described as nonessential accessory (or auxiliary) genes (20). However, the striking evolutionary conservation of these accessory genes, and the recent demonstration of the importance of *nef* to simian immunodeficiency virus pathogenesis in rhesus macaques (50), argues that they possess a biologically important function in vivo. The results of our studies showing the integrity of at least eight of the nine ORFs in both HIV-1_{YU-2} and HIV-1_{YU-10} support this conclusion by demonstrating that throughout the natural course of HIV-1 infection, even to the last stages of clinical illness, there remains a significant positive selection for viruses with intact *vif*, *vpr*, *vpu*, and *nef* genes. On the other hand, not one of the four full-length HIV-1 clones examined in this study nor any of four other HIV-1 clones with obvious deletions that were derived from the same tissue (60) contained all nine transcriptional units completely intact. This finding suggests that complementation of minimally defective genomes may be a frequent and biologically important occurrence in vivo.

We examined the nucleotide sequences of the LTR and the structural and accessory genes of HIV-1_{YU-2} and HIV-1_{YU-10} for the presence of elements with known functions. In the LTR regions of HIV-1_{YU-2} and HIV-1_{YU-10} (Fig. 1) as well as of two other HIV-1_{YU} clones that we also sequenced (YU-3, YU-4 [60]), all regulatory elements, including TATA, Sp1, NFκB, AP1, LBP-1, and the *tat*-responsive element, were highly conserved. Functional regions within the viral coding regions were also conserved, with the exception of

the *vpu* ATG start codon in HIV-1_{YU-2}. In the case of *vpu*, we demonstrated by radioimmunoprecipitation assay analysis using a *vpu*-specific polyclonal antiserum (102) that this protein is in fact not produced in HIV-1_{YU-2}-infected cells (data not shown). The envelope gene of HIV-1_{YU-2} was previously shown to encode SU and TM proteins of 120 and 41kDa (60). The number and position of cysteine residues and potential N-linked glycosylation sites were conserved in comparison with prototype HIV-1 isolates, as were the amino acids known to be involved in CD4 receptor binding (74, 105). Interestingly, while the overall *env* DNA sequence of HIV-1_{YU-2} was approximately equidistantly related to T-cell-tropic and monocyte-tropic North American/European isolates (Fig. 5), the amino acid sequence of the V3 region was specifically similar to those of three other well-studied monocyte-tropic viruses, HIV-1_{BAL} (33), HIV-1_{JRFL} (52), and HIV-1_{ADA} (111), differing in only one or two amino acids. In separate studies (111), the V3 envelope sequence of HIV-1_{YU-2} was found to represent a functional domain responsible for conferring monocyte tropism to this and heterologous (chimeric) virus strains. These data thus suggest that despite their different phylogenetic backgrounds, tissues of origin (lung versus brain), and methods of cloning (cultured versus uncultured), HIV-1_{YU-2}, HIV-1_{BAL}, HIV-1_{JRFL}, and HIV-1_{ADA} all represent a common subtype of virus that replicates preferentially in primary T cells, monocyte-macrophages, and cells of microglial lineage (32, 33, 52, 60, 93).

Because of the unique derivation of HIV-1_{YU-2} and its potential utility as a reagent for studies of viral pathogenesis and drug and vaccine development, we investigated its phylogenetic relationships with other HIV-1 isolates. Phylogenetic trees for each of the *gag*, *pol*, *env*, and *nef* genes placed HIV-1_{YU-2} clearly within the North American/European subgroup of HIV-1, though not particularly close to any one isolate within that subgroup. Thus, HIV-1_{YU-2} appears to be generally representative of non-African isolates and probably represents one of the earlier viral strains present in the United States, since the subject from whom it was derived had already developed an AIDS-defining illness by 1983.

The availability of full-length sequences of HIV-1_{YU-2} and HIV-1_{YU-10}, as well as previously reported partial envelope sequences from 6 additional lambda phage-derived and 12 PCR-derived clones from the same subject's brain tissue (60), allowed us to analyze the genetic composition of the HIV-1 quasi-species in vivo in ways not previously possible. While all 20 clones were derived from the same tissue, the 8 lambda clones represented unintegrated HIV-1 DNA intermediates whereas the PCR clones represented both unintegrated and integrated viral forms. The HIV-1_{YU-2} and HIV-1_{YU-10} sequences differed from each other by surprisingly few nucleotide changes, 24 of 9,085 (0.26%). This limited degree of variation was clearly representative of the overall population of viral sequences within the brain compartment of this individual, since the 6 other lambda phage-derived clones and 11 of the 12 PCR-derived clones differed to a similar degree, 0 to 1.2%. These data thus provide rather striking evidence that the extent of HIV-1 variability in brain tissue is considerably less than that in the peripheral lymphoid compartment, where the range in HIV-1 variability within individual patients has generally been reported to be between 1.0 and 9.2% (6, 53). The degree of variation among brain-derived HIV-1 sequences that we observed is in the same range as that described in other studies of intrapatient variation of brain-derived HIV-1 (23, 76; unpublished data). Less extensive variability of HIV-1 within the CNS than in peripheral lymphoid tissues may be explained by less intensive immune selective pressures within the CNS, lower overall rates of viral replication (despite the high accumulated burden of viral sequences), and/or a more restricted viral host cell range. Differences in HIV-1 variability between central and peripheral compartments may have important consequences in regard to viral pathogenesis and response to clinical treatments such as AZT that are subject to the development of viral resistance.

The availability of four full-length HIV-1 genomes also enabled us to examine the phenomenon of G-to-A hypermutation in vivo (34, 107). Since all four full-length clones that we studied were either replication competent or only minimally defective, they were not expected to have excessive G-to-A changes because such changes invariably lead to multiple premature termination codons as a result of alterations in tryptophan codons (i.e., TGG to TGA, TAG, or TAA). In the HIV-1_{YU-2} and HIV-1_{YU-10} sequences, there was suggestive evidence for preferential G-to-A substitution but not hypermutation (i.e., 15 of 24 nucleotide differences [60%] involved G-to-A substitutions, whereas the expected frequency of such changes in a genome of this base composition would be approximately 20%). Eight of these fifteen changes occurred in a GpA motif which has been suggested to be a preferential site for G-to-A hypermutation on the basis of dislocation mutagenesis (107). Thus, our analysis of this individual's brain tissue revealed a trend toward preferential G-to-A substitution between two full-length clones and extreme G-to-A hypermutation in 1 of 12 PCR-derived clones (60). The generality of G-to-A hypermutation in vivo and its biologic significance remain unknown.

A striking finding of our analyses was that any combination of the defective clones HIV-1_{YU-10}, HIV-1_{YU-21}, and HIV-1_{YU-32} resulted in prompt complementation and wild-type levels of viral replication, as measured by p24 antigen and RT production, virus-induced syncytium induction, and production of virus that could be passaged cell free onto normal donor PBMCs. The fact that HIV-1_{YU-10} could be complemented by either HIV-1_{YU-21} or HIV-1_{YU-32} suggests that these latter viruses have an intact *pol* gene to complement the defective *pol* gene in HIV-1_{YU-10} (Fig. 1 and 2). Similarly, HIV-1_{YU-21} and HIV-1_{YU-32} are likely to be defective in different genes, since they can complement each other. However, it cannot be determined from our experiments whether the observed complementation was due to homologous recombination between heterotypic viral genomes or to phenotypic mixing and continued transmission of defective genomes. Hu and Temin (44) have recently shown that as many as 30 to 40% of dimeric viral genomes recombine during a single cycle of infection, making this a distinct possibility in our system. Complementation by defective HIV-1 genomes has also been reported by Inoue et al. (46), although their results were based on an analysis of viral clones derived from a heavily passaged laboratory-adapted strain of HIV-1_{IIIB}.

The biologic significance of viral defectiveness and complementation in HIV-1 pathogenesis is currently unknown, and even the extent of defectiveness of HIV-1 in vivo is uncertain. Meyerhans et al. reported (64) that 10 to 15% of *tat* genes (which represent less than 3% of the viral genome) were defective, implying that virtually all genomes in vivo must be multiply defective. Conversely, Balfe et al. (6) and Kusumi et al. (53) have independently estimated that HIV-1 genomes in vivo contain, on average, one or fewer obviously lethal mutations. Our data for HIV-1_{YU-2}, HIV-1_{YU-10}, HIV-

1_{YU-21}, and HIV-1_{YU-32} are more consistent with the latter estimates. These different results may reflect the limited numbers of subjects analyzed, differences in the stages of their clinical illnesses, and/or the different tissues analyzed. It has been suggested that because the copy number of HIV-1 proviruses in infected PBMCs is low (approximately one), functional complementation of even minimally defective genomes would not generally be possible and therefore complementation is unlikely to be important in viral pathogenesis (6). However, other considerations suggest a different interpretation. First, viral DNA burden and expression are higher, transmission by cell-cell contact is more likely, and the opportunity for complementation is therefore greater in lymphoid organs and brain tissue than in circulating PBMCs (76–78, 94, 95). Second, cells that harbor HIV-1 in vivo have been shown to maintain their expression of CD4 (90), and consequently there is the opportunity for multiple infection events at a single-cell level. Thus, we believe that the question of the relative importance of defective HIV-1 genomes in viral pathogenesis remains open and worthy of consideration.

In conclusion, the results of this study provide new insight into the genetic and biologic characteristics of the HIV-1 quasi-species that evolves and persists within the CNS compartment and provide new viral reagents for analyzing gene structure-function relationships. The HIV-1_{YU-2} clone, in particular, with its monocyte-tropic phenotype, near-consensus V3 envelope sequence, and wild-type polymerase sequence with regard to AZT and non-nucleoside analog drug sensitivity, may be particularly useful for studies related to viral pathogenesis and drug and vaccine development.

ACKNOWLEDGMENTS

We thank Donna M. Burton, Sandra Williams-Jackson, and A. Jan Nicholson for careful preparation of the manuscript and G. Y. Gillespie for providing the N370FG cell line. The *vpu* antiserum was provided by F. Maldarelli and K. Strebel through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID. Phylogenetic analyses were performed using the facilities of the Irish National Center for Bioinformatics.

This work was supported by the National Institutes of Health (grants NS25701, AI27290, and AI27767), the Life and Health Insurance Medical Research Fund, the U.S. Army Medical Research Acquisition Activity, and the UAB Center for AIDS Research. George M. Shaw is a Pew Scholar of the Biomedical Sciences.

REFERENCES

1. **Albert, J., B. Abrahamsson, K. Nagy, E. Aurelius, H. Gaines, G. Nyström, and E. M. Fenyö.** 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. AIDS **4**:107–112.
2. **Allan, J. S., J. E. Coligan, T. H. Lee, M. F. McLane, P. J. Kanki, J. E. Groopman, and M. Essex.** 1985. A new HTLV-III/LAV encoded antigen detected by antibodies from AIDS patients. Science **230**:810–813.
3. **Ameisen, J. C., and A. Capron.** 1991. Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. Immunol. Today **12**:102–105.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1987. Current protocols in molecular biology, p. 9.1.1–9.1.4. John Wiley & Sons, New York.
5. **Aziz, D., Z. Hanna, and P. Jolicoeur.** 1989. Severe immunodeficiency disease induced by a defective murine leukaemia virus. Nature (London) **338**:505–508.
6. **Balfe, P., P. Simmonds, C. A. Ludlam, J. O. Bishop, and A. J. L. 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.
7. **Bathurst, I. C., N. Chester, H. L. Gibson, A. F. Dennis, K. S. Steimer, and P. J. Barr.** 1989. N myristylation of the human immunodeficiency virus type 1 *gag* polyprotein precursor in *Saccharomyces cerevisiae*. J. Virol. **63**:3176–3179.
8. **Bess, Jr., J. W., P. J. Powell, H. J. Issaq, L. J. Schumack, M. K. Grimes, L. E. Henderson, and L. O. Arthur.** 1992. Tightly bound zinc in human immunodeficiency virus type 1, human T-cell leukemia virus type I, and other retroviruses. J. Virol. **66**:840–847.
9. **Bethea, J. R., I. Y. Chung, S. M. Sparacio, G. Y. Gillespie, and E. N. Benveniste.** 1992. Interleukin-1β induction of tumor necrosis factor-alpha gene expression in human astroglioma cells. J. Neuroimmunol. **36**:179–191.
10. **Bethea, J. R., G. Y. Gillespie, I. Y. Chung, and E. N. Benveniste.** 1990. Tumor necrosis factor production and receptor expression by a human malignant glioma cell line, D54-MG. J. Neuroimmunol. **30**:1–13.
11. **Boucher, C. A. B., E. O'Sullivan, J. W. Mulder, C. Ramautarsing, P. Kellam, G. Darby, J. M. A. Lange, J. Goudsmit, and B. A. Larder.** 1992. Ordered appearance of zidovudine resistance mutations during treatment of 18 human immunodeficiency virus-positive subjects. J. Infect. Dis. **165**:105–110.
12. **Charneau, P., and F. Clavel.** 1991. A single-stranded gap in human immunodeficiency virus unintegrated linear DNA defined by a central copy of the polypurine tract. J. Virol. **65**:2415–2421.
13. **Chattopadhyay, S. K., H. C. Morse III, M. Makino, S. K. Ruscetti, and J. W. Hartley.** 1989. Defective virus is associated with induction of murine retrovirus-induced immunodeficiency syndrome. Proc. Natl. Acad. Sci. USA **86**:3862–3866.
14. **Cheng-Mayer, C., D. Seto, M. Tateno, and J. A. Levy.** 1988. Biologic features of HIV-1 that correlate with virulence in the host. Science **240**:80–82.
15. **Clark, S. J., M. S. Saag, W. D. Decker, S. Campbell-Hill, J. L. Roberson, P. J. Veldkamp, J. C. Kappes, B. H. Hahn, and G. M. Shaw.** 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. N. Engl. J. Med. **324**:954–960.
16. **Coombs, R. W., A. C. Collier, J.-P. Allain, B. Nikora, M. Leuther, G. F. Gjerset, and L. Corey.** 1989. Plasma viremia in human immunodeficiency virus infection. N. Engl. J. Med. **32**:1626–1631.
17. **Daar, E. S., W. L. Li, T. Moudgil, and D. D. Ho.** 1990. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc. Natl. Acad. Sci. USA **87**:6574–6578.
18. **Daar, E. S., T. Moudgil, R. D. Meyer, and D. D. Ho.** 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N. Engl. J. Med. **324**:961–964.
19. **DeCarli, C., L. Fugate, and J. Fallon.** 1991. Brain growth and cognitive improvement in children with human immunodeficiency virus-induced encephalopathy after 6 months of continuous infusion zidovudine therapy. J. Acquired Immune Defic. Syndr. **4**:585–592.
20. **Desrosiers, R. C.** 1992. HIV with multiple gene deletions as a live attenuated vaccine for AIDS. AIDS Res. Hum. Retroviruses **8**:411–421.
21. **DiPersio, J. F., J. K. Brennan, M. A. Lichtman, and B. L. Speiser.** 1978. Human cell lines that elaborate colony-stimulating activity for the marrow cells of man and other species. Blood **51**:507–519.
22. **Emini, E. A., W. A. Schleif, V. W. Byrnes, A. Rhodes, D. L. Titus, J. A. Waterbury, J. C. Quintero, L. Gotlib, C. L. Schneider, C. Uncapher, O. L. Laskin, F. E. Massari, J. C. Kappes, G. M. Shaw, and M. S. Saag.** Rapid selection of human immunodeficiency virus type 1 resistant variants during experimental therapy with the nonnucleoside reverse transcriptase inhibitor L697,661. Submitted for publication.

23. **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.
24. **Fauci, A. S., S. M. Schnittman, G. Poli, S. Koenig, and G. Pantaleo.** 1991. Immunopathogenic mechanisms in human immunodeficiency virus (HIV) infection. Ann. Intern. Med. **114**: 678–693.
25. **Fenyo, E. M., L. Morfeldt-Manson, F. Chiodi, B. Lind, A. von Gegerfelt, J. Albert, E. Olausson, and B. Asjo.** 1988. Distinct replicative and cytopathic characteristics of human immunodeficiency virus isolates. J. Virol. **62**:4414–4419.
26. **Fischl, M. A., D. D. Richman, M. H. Grieco, et al.** 1987. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N. Engl. J. Med. **317**:185–191.
27. **Fischl, M. A., D. D. Richman, N. Hansen, et al.** 1990. The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type 1 (HIV) infection: A double-blind, placebo-controlled trial. Ann. Intern. Med. **112**:727–737.
28. **Fisher, A. G., E. Collalti, L. Ratner, R. C. Gallo, and F. Wong-Staal.** 1985. A molecular clone of HTLV-III with biological activity. Nature (London) **316**:262–265.
29. **Franza, Jr., B. R., F. J. Rauscher III, S. F. Josephs, and T. Curran.** 1988. The fos complex and fos-related antigens recognize sequence elements that contain AP-1 binding sites. Science **239**:1150–1152.
30. **Gabuzda, D. H., D. D. Ho, S. M. de la Monte, M. S. Hirsch, T. R. Rota, and R. A. Sobel.** 1986. Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. Ann. Neurol. **20**:289–295.
31. **Gao, F., L. Yue, A. T. White, P. G. Pappas, J. Barchue, A. P. Hanson, B. M. Greene, P. M. Sharp, G. M. Shaw, and B. H. Hahn.** 1992. Human infection by genetically diverse SIV_{SM}-related HIV-2 in West Africa. Nature (London) **358**:495–499.
32. **Gartner, S., P. Markovits, D. M. Markovitz, R. F. Betts, and M. Popovic.** 1986. Virus isolation from and identification of HTLV-III/LAV-producing cells in brain tissue from a patient with AIDS. JAMA **256**:2365–2371.
33. **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.

33a. **Gillespie, G. Y.** Unpublished data.

34. **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.
35. **Greene, W. C.** 1991. The molecular biology of human immunodeficiency virus type 1 infection. N. Engl. J. Med. **324**:308–317.
36. **Guy, B., M. P. Kieny, Y. Riviere, C. Le Peuch, K. Dott, M. Girard, L. Montagnier, and J.-P. Lecocq.** 1987. HIV F/3′ *orf* encodes a phosphorylated GTP-binding protein resembling an oncogene product. Nature (London) **330**:266–269.
37. **Hahn, B. H., G. M. Shaw, M. E. Taylor, R. R. Redfield, P. D. Markham, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, E. S. Parks, and W. P. Parks.** 1986. Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk for AIDS. Science **232**:1548–1553.
38. **Harouse, J. M., S. Bhat, S. L. Spitalnik, M. Laughlin, K. Stefano, D. H. Silberberg, and F. Gonzalez-Scarano.** 1991. Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. Science **253**:320–323.
39. **Higgins, D. G., A. J. Bleasby, and R. Fuchs.** 1992. CLUSTALV: improved software for multiple sequence alignment. Comp. Appl. Biosci. **8**:189–191.
40. **Higgins, D. G., and P. M. Sharp.** 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene **73**:237–244.
41. **Ho, D. D., D. E. Bredesen, H. V. Vinters, and E. S. Daar.** 1989. The acquired immunodeficiency syndrome (AIDS) dementia complex. Ann. Intern. Med. **111**:400–410.
42. **Ho, D. D., T. Moudgil, and M. Alam.** 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N. Engl. J. Med. **321**:1621–1625.
43. **Ho, D. D., R. J. Pomerantz, and J. C. Kaplan.** 1987. Pathogenesis of infection with human immunodeficiency virus. N. Engl. J. Med. **317**:278–286.
44. **Hu, W.-S., and H. M. Temin.** 1990. Genetic consequences of packaging two RNA genomes in one retroviral particle: pseudodiploidy and high rate of genetic recombination. Proc. Natl. Acad. Sci. USA **87**:1556–1560.
45. **Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen.** 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science **253**:71–74.
46. **Inoue, M., J. A. Hoxie, M. V. R. Reddy, A. Srinivasan, and E. P. Reddy.** 1991. Mechanisms associated with the generation of biologically active human immunodeficiency virus type 1 particles from defective proviruses. Proc. Natl. Acad. Sci. USA **88**:2278–2282.
47. **Jones, K. A., J. J. Kadonaga, P. A. Luciw, and R. Tjian.** 1986. Activation of the AIDS retrovirus promoter by the cellular transcription factor, Sp1. Science **232**:755–759.
48. **Jones, K. A., P. Luciw, and N. Duchange.** 1988. Structural arrangements of transcription control domains within the 5′-untranslated leader regions of the HIV-1 and HIV-2 promoters. Genes Dev. **2**:1101–1114.
49. **Kellam, P., C. A. B. Boucher, and B. A. Larder.** 1992. Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. Proc. Natl. Acad. Sci. USA **89**:1934–1938.
50. **Kestler, H. W., D. J. Ringler, K. Mori, D. L. Panicali, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers.** 1991. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell **65**:651–662.
51. **Koller, C. A., G. W. King, P. E. Hurtubise, A. L. Sagone, and A. F. LoBuglio.** 1973. Characterization of glass adherent human mononuclear cells. J. Immunol. **111**:1610–1612.
52. **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.
53. **Kusumi, K., B. Conway, S. Cunningham, A. Berson, C. Evans, A. K. N. Iversen, D. Colvin, M. V. Gallo, S. Coutre, E. G. Shpaer, D. V. Faulkner, A. de Ronde, S. Volkman, C. Williams, M. S. Hirsch, and J. I. Mullins.** 1992. Human immunodeficiency virus type 1 envelope gene structure and diversity in vivo and after cocultivation in vitro. J. Virol. **66**:875–885.
54. **Larder, B. A., G. Darby, and D. D. Richman.** 1989. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science **243**:1731–1734.
55. **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.
56. **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.
57. **Lever, A., H. Gottlinger, W. Haseltine, and J. Sodroski.** 1989. Identification of a sequence required for efficient packaging of human immunodeficiency virus type 1 RNA into virions. J. Virol. **63**:4085–4087.
58. **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.
59. **Li, W.-H., C.-I. Wu, and C.-C. Luo.** 1985. A new method for estimating synonymous and nonsynonymous rates of nucleotide substitution considering the relative likelihood of nucleotide and codon changes. Mol. Biol. Evol. **2**:150–174.
60. **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.

61. **McKinney, R. E., M. A. Maha, and E. M. Connor.** 1991. A multicenter trial of oral zidovudine in children with advanced human immunodeficiency virus disease. N. Engl. J. Med. **324**:1018–1025.
62. **Merrill, J. E., Y. Koyanagi, J. Zack, L. Thomas, F. Martin, and I. S. Y. Chen.** 1992. Induction of interleukin-1 and tumor necrosis factor alpha in brain cultures by human immunodeficiency virus type 1. J. Virol. **66**:2217–2225.
63. **Mervis, R. J., N. Ahmad, E. P. Lillehoj, M. G. Raum, F. H. R. Salazar, H. W. Chan, and S. Venkatesan.** 1988. The *gag* gene products of human immunodeficiency virus type 1: alignment within the *gag* open reading frame, identification of posttranslational modifications, and evidence of alternative *gag* precursors. J. Virol. **62**:3993–4002.
64. **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.
65. **Michael, N. L., M. Vahey, D. Burke, and R. R. Redfield.** 1992. Viral DNA and mRNA expression correlate with the stage of human immunodeficiency virus (HIV) type 1 infection in humans: evidence for viral replication in all stages of HIV disease. J. Virol. **66**:310–316.
66. **Michaels, J., R. W. Price, and M. K. Rosenblum.** 1988. Microglia in the giant cell encephalitis of acquired immune deficiency syndrome: proliferation, infection and fusion. Acta Neuropathol. **76**:373–379.
67. **Miedema, F., M. Tersmette, and R. A. W. van Lier.** 1990. AIDS pathogenesis: a dynamic interaction between HIV and the immune system. Immunol. Today **11**:293–297.
68. **Moore, J. P., J. A. McKeating, Y. Huang, A. Ashkenazi, and D. D. Ho.** 1992. Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. J. Virol. **66**:235–243.
69. **Muesing, M. A., D. M. Smith, C. D. Cabradilla, C. V. Benton, L. A. Lasky, and D. J. Capon.** 1985. Nucleic acid structure and expression of the human AIDS/lymphadenopathy retroviruses. Nature (London) **313**:450–458.
70. **Myers, G., J. A. Berzofsky, B. Korber, R. F. Smith, and G. N. Pavlakis.** 1991. Human retroviruses and AIDS 1991. Los Alamos National Laboratory, Los Alamos, N. Mex.
71. **Nabel, G., and D. Baltimore.** 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) **326**:711–713.
72. **Nara, P. L., L. Smit, 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.
73. **O'Brien, W. A., Y. Koyanagi, A. Namazie, J.-Q. 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.
74. **Olshevsky, U., E. Helseth, C. Furman, J. Li, W. Haseltine, and J. Sodroski.** 1990. Identification of individual human immunodeficiency virus type 1 gp120 amino acids important for CD4 receptor binding. J. Virol. **64**:5701–5707.
75. **Overbaugh, J., P. R. Donahue, S. L. Quackenbush, E. A. Hoover, and J. I. Mullins.** 1988. Molecular cloning of a feline leukemia virus that induces fatal immunodeficiency disease in cats. Science **239**:906–910.
76. **Pang, S., H. V. Vinters, T. Akashi, W. A. O'Brien, and I. S. Y. Chen.** 1991. HIV-1 *env* sequence variation in brain tissue of patients with AIDS-related neurologic disease. J. Acquired Immune Defic. Syndr. **4**:1082–1092.
77. **Pang, S., Y. Koyanagi, S. Miles, C. Wiley, H. V. Vinters, and I. S. Y. Chen.** 1990. High levels of unintegrated HIV-1 DNA in brain tissue of AIDS dementia patients. Nature (London) **343**:85–89.
78. **Pantaleo, G., C. Graziosi, L. Butini, P. A. Pizzo, S. M. Schnittman, D. P. Kotler, and A. S. Fauci.** 1991. Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **88**:9838–9842.
79. **Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. M. Bangham, C. R. Rizza, and A. J. McMichael.** 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature (London) **354**:453–459.
80. **Pizzo, P. A., J. Eddy, and J. Falloon.** 1988. Effect of continuous intravenous infusion of zidovudine (AZT) in children with symptomatic HIV infection. N. Engl. J. Med. **319**:889–896.
81. **Price, R. W., and J. J. Sidtis.** 1990. Early HIV infection and the AIDS dementia complex. Neurology **40**:323–326.
82. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, et al.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature (London) **313**:277–284.
83. **Rosen, C. A., J. G. Sodroski, and W. A. Haseltine.** 1985. The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. Cell **41**:813–823.
84. **Saag, M. S., M. J. Crain, W. D. Decker, S. Campbell-Hill, S. Robinson, W. E. Brown, M. Leuther, R. J. Whitley, B. H. Hahn, and G. M. Shaw.** 1991. High-level viremia in adults and children infected with human immunodeficiency virus: relation to disease stage and CD4+ lymphocyte levels. J. Infect. Dis. **164**:72–80.
85. **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.
86. **Saitou, N., and T. Imanishi.** 1989. Relative efficiencies of the Fitch-Margoliash, maximum-parsimony, maximum likelihood, minimum-evolution, and neighbor-joining methods of phylogenetic tree construction in obtaining the correct tree. Mol. Biol. Evol. **6**:514–525.
87. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.
88. **Sanchez-Pescador, R., M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, S. L. Brown-Shimer, W. W. Gee, A. Renard, A. Randolph, J. A. Levy, D. Dina, and P. A. Luciw.** 1985. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). Science **227**:484–492.
89. **Schnittman, S. M., J. J. Greenhouse, M. C. Psallidopoulos, M. Baseler, N. P. Salzman, A. S. Fauci, and H. C. Lane.** 1990. Increasing viral burden in CD4+ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. Ann. Intern. Med. **113**:438–443.
90. **Schnittman, S. M., M. C. Psallidopoulos, H. C. Lane, L. Thompson, M. Baseler, F. Massari, C. H. Fox, N. P. Salzman, and A. S. Fauci.** 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. Science **245**:305–308.
91. **Schuitemaker, H., M. Koot, N. A. Kootstra, M. W. Dercksen, R. E. Y. de Goede, R. P. van Steenwijk, J. M. A. Lange, J. K. M. E. Schattenkerk, F. Miedema, and M. Tersmette.** 1992. Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus populations. J. Virol. **66**:1354–1360.
92. **Schuitemaker, H., N. A. Kootstra, R. E. Y. de Goede, F. de Wolf, F. Miedema, and M. Tersmette.** 1991. Monocytotropic human immunodeficiency virus type 1 (HIV-1) variants detectable in all stages of HIV-1 infection lack T-cell line tropism and syncytium-inducing ability in primary T-cell culture. J. Virol. **65**:356–363.

93. **Sharpless, N. E., W. A. O'Brien, E. Verdin, C. V. Kufta, I. S. Y. Chen, and M. Dubois-Dalcq.** 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.
94. **Shaw, G. M., B. H. Hahn, S. A. Arya, J. E. Groopman, R. C. Gallo, and F. Wong-Staal.** 1984. Molecular characterization of human T-cell leukemia (lymphotropic) virus type III in the acquired immune deficiency syndrome. Science **226**:1165–1171.
95. **Shaw, G. M., M. E. Harper, B. H. Hahn, L. G. Epstein, D. C. Gajdusek, R. W. Price, B. A. Navia, C. K. Petito, C. J. O'Hara, E. S. Cho, J. M. Oleske, F. Wong-Staal, and R. C. Gallo.** 1985. HTLV-III infection in brains of children and adults with AIDS encephalopathy. Science **227**:177–182.
96. **Shaw, G. M., P. C. Levy, and A. F. LoBuglio.** 1978. Human lymphocyte, monocyte, and neutrophil antibody-dependent cell-mediated cytotoxicity toward human erythrocytes. Cell Immunol. **41**:122–133.
97. **Shaw, G. M., P. C. Levy, and A. F. LoBuglio.** 1978. Human monocyte antibody-dependent cell-mediated cytotoxicity to tumor cells. J. Clin. Invest. **62**:1172–1180.
98. **Spencer, D. C., and R. W. Price.** 1992. Human immunodeficiency virus and the central nervous system. Annu. Rev. Microbiol. **46**:655–693.
99. **Staden, R.** 1980. A new computer method for the storage and manipulation of DNA gel reading data. Nucleic Acids Res. **8**:3673–3694.
100. **Starcich, B. R., B. H. Hahn, G. M. Shaw, P. D. McNeely, S. Modrow, H. Wolf, E. S. Parks, W. P. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal.** 1986. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell **45**:637–648.
101. **Stein, D. S., J. A. Korvick, and S. H. Vermund.** 1992. CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: a review. J. Infect. Dis. **165**:352–363.
102. **Strebel, K., T. Klimkait, and M. A. Martin.** 1988. A novel gene of HIV-1, vpu, and its 16-kilodalton product. Science **241**: 1221–1223.
103. **Tersmette, M., R. E. Y. de Goede, B. J. M. Al, I. N. Winkel, R. A. Gruters, H. T. Cuypers, H. G. Huisman, and F. Miedema.** 1988. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. J. Virol. **62**:2026–2032.
104. **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.
105. **Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, J. Li, and J. Sodroski.** 1991. Effects of changes in gp120-CD4 binding affinity on human immunodeficiency virus type 1 envelope glycoprotein function and soluble CD4 sensitivity. J. Virol. **65**:5007–5012.
106. **Vaishnav, Y. N., and F. Wong-Staal.** 1991. The biochemistry of AIDS. Annu. Rev. Biochem. **60**:577–630.
107. **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.
108. **Veronese, F., T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan.** 1988. Biochemical and immunological analysis of human immunodeficiency virus *gag* gene products p17 and p24. J. Virol. **62**:795–801.
109. **Volberding, P. A., S. W. Lagakos, M. A. Koch, et al.** 1990. Zidovudine in asymptomatic human immunodeficiency virus infection: a controlled trial in persons with fewer than 500 CD4-positive cells per cubic millimeter. N. Engl. J. Med. **322**:941–949.
110. **Wain-Hobson, S., P. Sonigo, O. Danos, S. Cole, and M. Alizon.** 1985. Nucleotide sequence of the AIDS virus, LAV. Cell **40**:9–17.
111. **Westervelt, P., D. B. Trowbridge, L. G. Epstein, B. M. Blumberg, Y. Li, B. H. Hahn, G. M. Shaw, R. W. Price, and L. Ratner.** 1992. Macrophage tropism determinants of human immunodeficiency virus type 1 in vivo. J. Virol. **66**:2577–2582.
112. **Wong-Staal, F., G. M. Shaw, B. H. Hahn, S. Z. Salahuddin, M. Popovic, P. Markham, R. Redfield, and R. C. Gallo.** 1985. Genomic diversity of human T-lymphotropic virus type III (HTLV-III). Science **229**:759–762.