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A Distinct African Lentivirus from Sykes' Monkeys

VANESSA M. HIRSCH,[1,2]* GEORGE A. DAPOLITO,[1] SIMOY GOLDSTEIN,[1] HAROLD McCLURE,[2] PETER EMAU,[2] PATRICIA N. FULTZ,[3] MOHAMED ISAHAKIA,[4] RHOSHEL LENROOT,[5] GERALD MYERS,[5] AND PHILIP R. JOHNSON[2,6]

Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Rockville, Maryland 20852[1]; Department of Pathobiology, Yerkes Primate Research Center, and Department of Pathology, Emory University, Atlanta, Georgia 30322[2]; Department of Microbiology, University of Alabama Medical Center, Birmingham, Alabama 35294[3]; Institute of Primate Research, National Museums of Kenya, Nairobi, Kenya[4]; Theoretical Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87544[5]; and Department of Pediatrics, The Ohio State University, Columbus, Ohio 43210[6]

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Asymptomatic infection with simian immunodeficiency virus (SIV) has been demonstrated in African Sykes' monkeys (*Cercopithecus mitis albogularis*), and virus isolation confirmed infection with a novel SIV from Sykes' monkeys (SIVsyk). Macaques inoculated with SIVsyk became persistently infected but remained clinically healthy. We utilized polymerase chain reaction amplification to generate a full-length, infectious molecular clone of SIVsyk. The genome organization of SIVsyk is similar to that of the other primate lentiviruses, consisting of *gag*, *pol*, *vif*, *vpr*, *tat*, *rev*, *env*, and *nef*. A unique feature is the absence of the highly conserved NF-κB binding site in the long terminal repeat. SIVsyk is genetically equidistant from other primate lentiviruses. Thus, SIVsyk represents a new group that is distinct from the four previously recognized primate lentivirus groups: human immunodeficiency virus type 1 (HIV-1), SIV from sooty mangabeys (SIVsmm) and HIV-2, SIV from African green monkeys (SIVagm), and SIV from mandrills (SIVmnd). The genetic differences between SIVsyk and SIVagm, isolates derived from monkeys of the same genus, underscore the potential for other distinct SIVs which have yet to be isolated and characterized.

The etiologic agents of human AIDS, the human immunodeficiency viruses (human immunodeficiency virus type 1 [HIV-1] and HIV-2), are members of a large family of primate lentiviruses that includes simian immunodeficiency viruses (SIV), which infect nonhuman primates. There is still considerable controversy regarding the origin of the AIDS epidemic (5, 6, 15, 41). However, circumstantial evidence suggests that the human viruses arose following zoonotic transmission from naturally infected nonhuman primates indigenous to Africa (13, 18, 19, 25, 33). The concept of cross-species transmission from monkeys to humans may be plausible because of natural, apparently asymptomatic, infection of African primates with related lentiviruses (SIV). The most conclusive evidence for such a link is the close relationship between HIV-2 infecting West African individuals and SIV from sooty mangabeys (SIVsmm) (18, 19); these viruses share approximately 80% amino acid identity in their Gag proteins, evidence of a close genetic relationship. The natural habitat of sooty mangabeys (*Cercocebus atys*) overlaps the range of the HIV-2 epidemic in West Africa, and SIV infection can be demonstrated in feral populations of these monkeys (33). These data suggest that the HIV-2 epidemic may have been the result of transmission of SIVsmm from sooty mangabeys to humans and subsequent evolution as HIV-2. The genetic similarity of SIVsmm to SIV isolated from captive macaques (SIVmac) in North American primate centers also is consistent with cross-species transmission between these two species while in captivity (18, 19). A similar link between HIV-1 and SIV isolated from a wild-born chimpanzee (SIVcpz [21]) is more tenuous. SIVcpz is genetically similar to HIV-1; however, definitive proof of infection in wild chimpanzees has not been demonstrated. Thus, the origins of the HIV-1 epidemic are obscure, and the search for novel simian counterparts remains of great interest.

Currently two strains, in addition to SIVcpz and SIVsmm, have been molecularly characterized; these are various isolates of SIV from African green monkeys (*Cercopithecus aethiops*) (SIVagm) (2, 3, 10, 11, 22, 31, 39) and from a mandrill monkey (*Papio sphinx*) (SIVmnd) (44, 45). SIVagm and SIVmnd are only distantly related to HIV-1 and HIV-2. Thus, presently characterized primate lentiviruses can be classified into one of four distinct genetic groups: (i) HIV-1 and SIVcpz, (ii) SIVsmm and HIV-2, (iii) SIVagm, and (iv) SIVmnd. The groups are equidistant genetically and share approximately 50% amino acid similarity in Gag and Pol proteins (25). These strains form the basis for our present understanding of the phylogeny and evolution of primate lentiviruses.

There may be many more SIV strains than those listed above. Evidence for a large family of related viruses comes from serologic surveys of primates of African origin. On the basis of serologic cross-reactivity with SIV or HIV, at least 10 additional primate species harbor related lentiviruses. Those species for which serologic evidence of SIV infection has been demonstrated include colobus monkeys (32), yellow baboons (27), talapoins (32), Hamlyn's monkeys (36), DeBrazza's monkeys (39), l'hoesti monkeys (36), Diana monkeys (32), Kolb's guenon (32), Dent's monkeys (39), redtail monkeys (unpublished data), and Sykes' monkeys (7). The last seven monkeys belong to the genus *Cercopithecus*, as do African green monkeys. Limited availability of blood samples from these species has hampered attempts to isolate and characterize these SIV strains. However, SIV was recently isolated from wild-caught Sykes' monkeys (*Cercopithecus mitis albogularis*) that had been imported from Kenya (7). Characterization of the biologic and sero-

* Corresponding author.

logic properties of this virus suggested that this virus, SIVsyk, represented a novel primate lentivirus.

In this report, we describe the molecular characterization of SIV isolated from one of these Sykes' monkeys and initial studies to assess the pathogenicity of SIVsyk for several macaque species. Infectious molecular clones of SIVsyk were generated, and the sequence was compared to those of other primate lentiviruses. The genome structure of SIVsyk is similar to that of SIVmnd; both lack *vpx* and *vpu*. A unique feature of SIVsyk is the absence of the highly conserved NF-κB binding site in the long terminal repeat (LTR). Although Sykes' monkeys and African green monkeys are more closely related than the other known SIV hosts, SIVsyk and SIVagm are as divergent from one another as from the other primate lentiviruses. Therefore, SIVsyk is phylogenetically distinct from previously described lentiviruses and represents a new primate lentivirus group.

MATERIALS AND METHODS

Cell lines and virus isolation. Peripheral blood mononuclear cells (PBMC) from seronegative (cm 260) and seropositive (cm 173) Sykes' monkeys were cocultivated in RPMI 1640 supplemented with 1% interleukin-2 and phytohemagglutinin (10 μg/ml). When virus was detected by reverse transcriptase (RT) activity in the culture supernatant, PBMC were then cocultivated with CEM × 174 cells to produce a persistently infected cell line. Neither cell death nor syncytium formation was noted in either the PBMC or CEM × 174 cells. Genomic DNA was prepared from persistently infected cells harvested at peak RT activity and used as a template for all subsequent polymerase chain reaction (PCR) amplifications and Southern blot analysis.

Cloning strategy. The strategy used to generate the SIVsyk molecular clones is shown in Fig. 1. The SIVsyk genome was derived by PCR amplification and cloning of 5′ and 3′ genome halves and reconstruction to generate a full-length virus. Prior to these half-genome amplifications, we used PCR under reduced stringency, inverse PCR (38), and Southern blot analysis to determine the sequence of the LTR, the sequence of the central region of the genome, and an approximate restriction map of the viral genome. We used conserved PCR primers (22) to amplify and clone the *gag* region of SIVsyk (primers shown below). The *gag* clone was sequenced and used as a probe to hybridize to Southern blots of various restriction enzyme digestions of SIVsyk-infected CEM × 174 cell DNA. *Sal*I, *Xho*I, and *Aat*II were identified as enzymes that did not cleave within the SIVsyk genome, and *Sau*I was determined to be cutting within the LTR and 3′ to the *gag* probe. Since amplification of the LTR was unsuccessful, inverse PCR was utilized on *Sau*I-digested and ligated DNA to amplify a large portion of the 5′ LTR and a portion of the polymerase gene. This technique, described in detail previously (38), uses a core of known sequence (in this case, *gag*) that is flanked by unknown sequences. The DNA is digested with a restriction enzyme that cleaves the DNA in the uncharacterized flanking region 5′ and 3′ to the core sequence, and the DNA is then ligated to form a circular molecule. PCR amplification is then performed to amplify from the core sequence into the uncharacterized sequence around the circular molecule as shown in Fig. 1. Sequences of the LTR and *pol* were generated from the *Sau*I inverse PCR, while that of the central region of the genome was obtained by PCR amplification from the 3′ end of the inverse PCR fragment to a conserved region in *pol*. PCR primers were then designed to amplify the 5′ and 3′ halves of the genome. Since a convenient site was not found in the middle of the genome for ligation of the two genome halves, an *Aat*II site was introduced in this region by a single base substitution in the PCR primers. The PCR products were digested with the appropriate restriction enzymes (*Aat*II and *Xho*I for the 3′ end and *Aat*II and *Sal*I for the 5′ end) and cloned into the vector pGEM-7Zf (Pharmacia). One 5′ end clone was used as a vector after digestion with *Aat*II and *Xho*I, and each of the 4.5-kb fragments was ligated in to generate two full-length clones.

PCR and PCR conditions. Genomic DNA prepared from SIVsyk-infected CEM × 174 cells was used as the template for PCR amplification. Briefly, a PCR mixture (100 μl) contained genomic DNA (500 ng); dGTP, dATP, TTP, and dCTP (200 mM each; United States Biochemicals, Cleveland, Ohio); KCl (50 nM); Tris-hydrochloride, pH 8.3 (10 mM); $MgCl_2$ (1.5 mM); gelatin (0.001%, wt/vol); and *Taq* polymerase (2.5 U; Perkin Elmer Cetus, Norwalk, Conn.) and consisted of 30 rounds of the following program cycle: 94°C, 1 min; 55°C, 1.5 min; 72°C, 1 to 5 min. The extension time depended on the size of the desired product, with the 5-min extension being reserved for the 5′ and 3′ end amplifications. Combinations of three forward and three reverse primers were necessary to obtain a primer pair sufficiently efficient to amplify the 5′ and 3′ half genomes. The annealing temperature for PCR amplification of *gag* was lowered to 45°C to allow for mismatch in the primer sequences. Inverse PCR required an additional 30 cycles with an inner set (nested) of primers; for this reaction, 10 μl from the first-round reaction mixture was transferred to a new tube with fresh reagents. To confirm infection of macaques inoculated with SIVsyk, inner and outer PCR primer pairs were designed in the LTR (LTRF1-LTRR1 and LTRF2-LTRR2) on the basis of the sequence of the full-length clone and nested PCR was performed on DNA extracted from PBMC. Primers were synthesized on an Applied Biosystems 380B DNA synthesizer and contained restriction sites to facilitate cloning (double underlined). For each of the primers that follow, the underlined bases represent SIV-specific sequences: *gag*R444, 5′-ATAGCGGCCGCTTATTGGTCTTCTCCAAAGA-3′; *gag*F445, 5′-TGCAGTCGACGGGCGCCCGAACAGGGACTTG-3′; *Sau*IFinv (outer), 5′-TACACTCGAGAGCCAACAAGCACAAGCGAAGGCA-3′; *Sau*IRinv (outer), 5′-ATTGAAGCTTACCTCGTCTCGTGTGTACACCACG-3′; *Sau*IFinv (inner), 5′-TACACTCGAGTAGGAAGGAGGAGACCAAGAGTCT-3′; *Sau*IRinv (inner), 5′-ATTGAAGCTTTCCCTTGACTGGCGTCACCTCCTC-3′; Sy5′F 1646, 5′-CATGGTCGACAGGAACCCGGTACCCGTTAA-3′; Sy5′R 1650, 5′-CCACAGCTGTTTGCAAATGTGTGACGTCGT-3′; Sy3′F 1628, 5′-ACAGATTAGAAGACGACGTCACACATTTGC-3′; Sys3′R 1631, 5′-ATGCCTCGAGCACCTTCGGGAGGTGACCCA-3′; SypolF 1857, 5′-ATGACTCGAGCATTACAGTTAGGCAATATA-3′; SypolR 1586, 5′-TTGACTCGAGCCTCCCCTTTCTTTTAAAATT-3′; LTRF1 8918, 5′-TGCAGGGCATGAATTATTGTGAA-3′; LTRR1 9220, 5′-ACTGCAAGCGGGCCATCTCCCAA-3′; LTRF2 8960, 5′-TTCATCTCTACCTACAGAATGAG-3′; and LTRR2 9170, 5′-AGTTCCACATGAGGTTCTCCCAT-3′.

Transfection. The two full-length clones were transfected into CEM × 174 cells by DEAE-dextran transfection (14), and culture supernatants were monitored for RT activity. RT activity was detected by day 4 after transfection with one clone (pSIVsyk 1.2) and peaked by day 14 without evidence of cytopathology. However, the peak RT activity was ap-

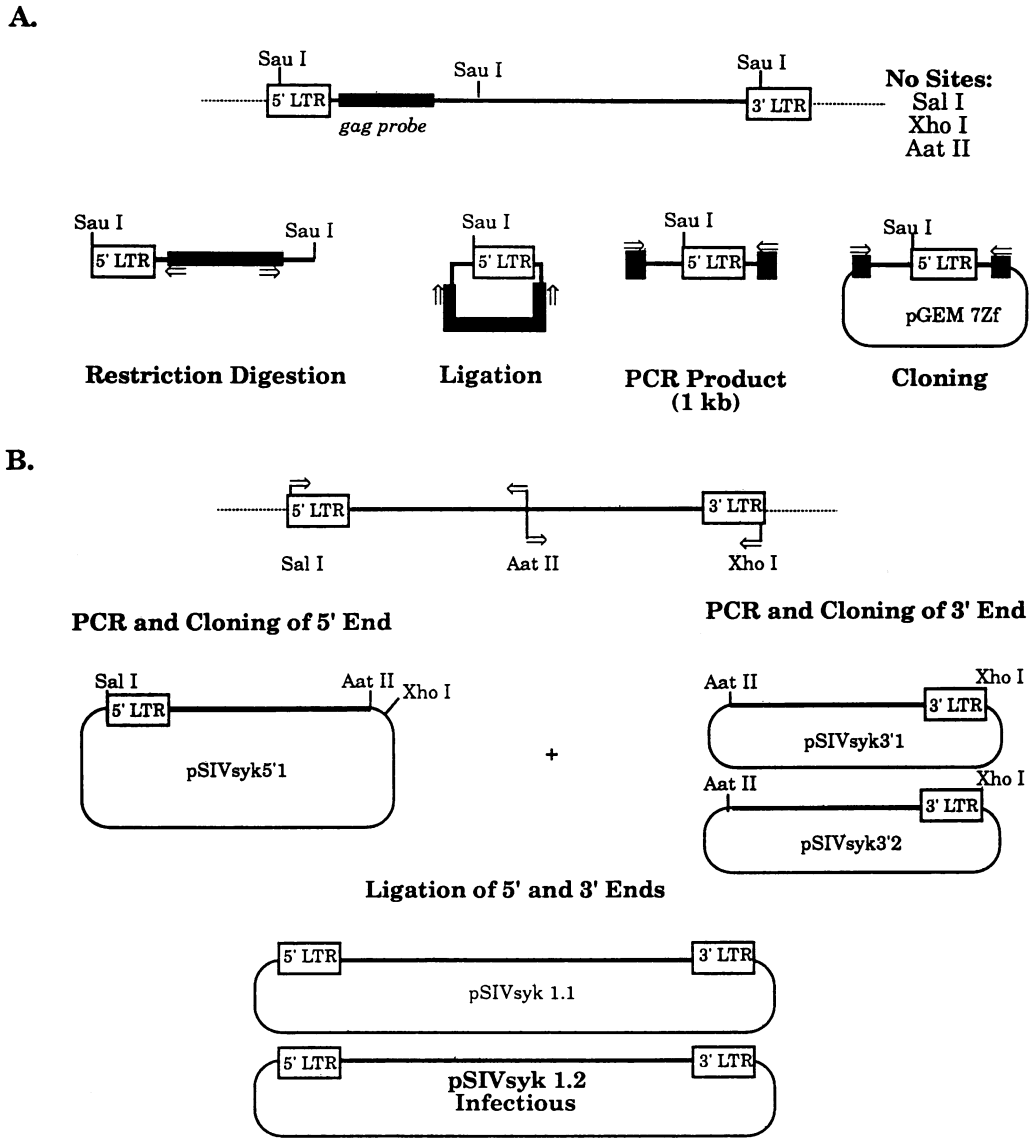

FIG. 1. Strategy for cloning the SIVsyk genome. (A) Strategy for inverse PCR. The strategy used to generate the sequence of the LTR by inverse PCR is shown schematically. The approximate positions of relevant restriction sites in the SIVsyk genome are also shown schematically. The *gag* clone used as a probe is shown within the genome as an open box. Dotted lines indicate flanking human genomic DNA. The first step demonstrates the relevant 3-kb *Sau*I fragment; the *gag* region (known sequence) is shown as a black box. The position of the *Sau*I site within the LTR is shown in Fig. 3. Step 2 (ligation) demonstrates the predicted product after self-ligation; the black box indicates the known *gag* sequence, and the PCR primers are shown as arrows. Step 3 (PCR product) shows the predicted form of the product of PCR amplification, and step 4 (cloning) shows the cloned PCR product. (B) PCR amplification and cloning of full-length SIVsyk genomes. A schematic diagram of the SIVsyk genome and PCR primers (arrows) is shown at the top. The resulting 5′ end clone (pSIVsyk5′1) and two 3′ end clones (pSIVsyk3′1 and pSIVsyk3′2) are shown below, and the two full-length clones formed by ligation of the 3′ end inserts to the 5′ end clone are shown at the bottom.

proximately fivefold less than that observed after transfection of infectious SIVsmm or SIVagm clones. Therefore, to confirm infectivity of this clone, cell-free culture supernatants from the transfected cells were used to infect PBMC from an uninfected Sykes' monkey in parallel with the uncloned virus isolate. These supernatants were infectious for Sykes' monkey PBMC as indicated by a rise in RT activity in the infected cultures (data not shown).

Infection of macaques. A total of 12 macaques were inoculated intravenously with 1 ml of filtered supernatant from SIVsyk-infected CEM × 174 cells. Three species of macaque were represented: four rhesus macaques (*Macaca mulatta*), four pig-tailed macaques (*Macaca nemestrina*), and four cynomolgus macaques (*Macaca fascicularis*). Animals were monitored for evidence of infection and disease over a 10-month period postinoculation. Infection was classified on the basis of various criteria, including isolation of virus from PBMC, Western blot (immunoblot) reactivity of plasma, and PCR amplification (primers LTRF1, LTRR1,

LTRF2, and LTRR2) of SIVsyk-specific sequences from PBMC-derived DNA.

Sequence analysis. Components of the infectious clone were sequenced (i.e., pSIVsyk5'1 and pSIVsyk3'2) in both strands by the dideoxy chain termination method with T7 DNA polymerase (United States Biochemicals). Nucleotide and amino acid sequence alignments were performed with the programs Genalign (Intelligenetics, Mountain View, Calif.) and MASE (Los Alamos National Laboratory HIV data base, Los Alamos, N.Mex.). Phylogenetic tree analysis was performed by using PAUP (41a), which infers a probable evolutionary lineage among a group of sequences by using a principle of maximum parsimony. Bootstrap analysis was performed by using the DNABOOT program in PHYLIP (9). The PLSEARCH program, supplied by R. F. Smith and T. F. Smith (MBCRR, Harvard University, Cambridge, Mass.) was used to search the SWISS-PROT protein sequence data bank for protein similarities.

Nucleotide sequence accession number. The nucleotide sequence of the full-length infectious clone, SIVsyk173/1.2, described in this paper has been submitted to GenBank under accession number L06042.

TABLE 1. Percent amino acid identities between SIVsyk and primate lentivirus proteins

SIV isolates being compared	% Identity[a] for:								
	Gag	Pol	Vif	Vpx	Vpr	Tat	Rev	Env	Nef
SIVsyk173/1.2 and:									
SIVsmm/H-4	50	53	38	**13**	**18**	32	36	38	34
SIVagm/155-4	51	52	36	**24**	—[b]	38	36	36	35
SIVmnd/GB-1	48	51	25	—	**22**	34	30	25	34
HIV-1/HXB2	46	50	30	—	**30**	33	30	36	37
SIVsmm/H-4 and:									
SIVagm/155-4	59	58	33	41	—	35	46	46	43
SIVmnd/GB-1	45	56	13	—	21	26	21	26	33
HIV-1/HXB2	55	58	28	—	43	26	32	36	36
SIVmac/251	90	92	84	95	90	72	70	81	77
HIV-2/ROD	84	84	69	80	70	65	62	72	64

[a] Sequences were obtained from the Los Alamos sequence data base. Alignments were performed with the program Genalign (Intelligenetics, Mountain View, Calif.) by using default parameters. The percent identity between two sequences was calculated with the inclusion of deletions and insertions. Values in boldface type represent comparisons of the SIVsyk Vpx and Vpr proteins with other primate lentiviral Vpx and Vpr proteins.

[b] —, a comparison was not performed for the following reasons: SIVsyk, HIV-1, and SIVmnd lack Vpx, and SIVagm lacks Vpr.

RESULTS

Genomic DNA isolated from SIVsyk-infected CEM × 174 cells was used as a template for PCR amplification to derive full-length molecular clones of SIVsyk. As described in Materials and Methods and schematically in Fig. 1, we used PCR amplification of the *gag* region, Southern blot analysis, and inverse PCR to generate sequences to design oligonucleotide primers for PCR amplification of 5' and 3' half genomes. Subsequently, two full-length clones, which differed in the 3' half of the genome, were generated; one clone (SIVsyk173/1.2) was infectious following transfection of CEM × 174 cells. Cell-free virus derived by transfection of SIVsyk173/1.2 was infectious for Sykes' monkey PBMC. We determined the complete nucleotide sequence (9,597 bp) of this biologically active clone. This clone lacks a portion of both LTRs, since it was generated by a forward primer placed at base 99 within U3 of the 5' LTR and a reverse primer 20 bp internal to the end of the 3' LTR. Thus, the predicted length of the integrated SIVsyk genome is 9,716 bp, which is similar to the size of the SIVagm and SIVmnd genomes. The predicted gene products of SIVsyk173/1.2 were compared to those of other primate lentiviruses, as shown in Table 1 (amino acid similarities) and Fig. 2 (genome structure). Like SIVagm and SIVmnd, SIVsyk encodes eight gene products. Seven of these gene products are clearly identifiable on the basis of amino acid similarities as *gag*, *pol*, *vif*, *tat*, *rev*, *env*, and *nef* products. The product of the eighth gene in a central region of the genome, adjacent to *vif* and *tat*, has borderline similarity to both Vpx and Vpr proteins. The protein identified in Table 1 as being most similar to this SIVsyk protein was the HIV-1 *vpr* product, and other comparisons, detailed below, suggest that this gene is the *vpr* homolog.

The LTR of SIVsyk lacks an NF-κB motif. The LTR of SIVsyk was 702 bp in length (Fig. 3). On the basis of a predicted mRNA start site, the U3, R, and U5 regions measure approximately 460, 170, and 70 bp, respectively, similar to lengths calculated for those of other SIV and HIV LTR sequences. The sequences of the LTRs of SIVsyk and other primate lentiviruses were divergent, with the highest degree of similarity occurring within a region that encompassed the TATA element. This element and the polyadenylation signal (AATAAA) are conserved in the SIVsyk LTR.

The U3 region of retroviral LTRs contains both core promoter (TATA box) and modulatory elements, which bind cellular transcription factors. The U3 region of the SIVsyk LTR was of a length similar to that of the LTRs of other SIV and HIV clones (455 to 490 bp). At least six defined elements that bind cellular transcription factors have been identified in the HIV-1 LTR (for a review, see reference 14). The SIVsyk LTR sequence contains identifiable binding sites for three cellular proteins: Sp-1, UBP-2 (CCTGGGAGC), and CTF/NFI (Fig. 3). However, surprisingly, the 10-bp NF-κB motif (GGGACTTTCCA), which is the most conserved motif of these binding elements, was absent from the SIVsyk LTR. This motif is usually found immediately 5' to the potential Sp-1 binding sites. All previously characterized SIV and HIV LTRs contain at least one copy; some, such as HIV-1, SIVagm clones from vervets (22, 34), and the acutely lethal SIVsmm/PBj variant (4, 37), have a duplicated copy. NF-κB motifs are critical for regulating HIV-1 expression in T lymphocytes, at least in vitro. However, the absence of this element in an infectious SIV clone is not implausible; although deletion of NF-κB from the HIV-1 LTR reduces transcriptional activity of LTR-CAT constructs, it has minimal effects upon biologic activity of a complete provirus (14, 29, 40). Thus, the NF-κB site does not appear to be absolutely required for transcriptional activity and virus infectivity.

The R region is variable in size among the primate lentiviruses because of variability in the length of the transactivation response element (TAR). The TAR region of HIV-1 has been the region most extensively characterized with regard to secondary structure and function; the sequence appears to form a stable stem-loop structure (G = −37 kcal/mol [1 cal = 4.184 J]) that confers transactivation responsiveness of HIV-1 to the Tat protein (8, 14). The TAR element of the SIVsmm/HIV-2 group of viruses is duplicated in length (8) and is predicted to form a similar but more complex stem-loop structure; this duplication has sequence similarity to immunoglobulin heavy chain genes (34). Inter-

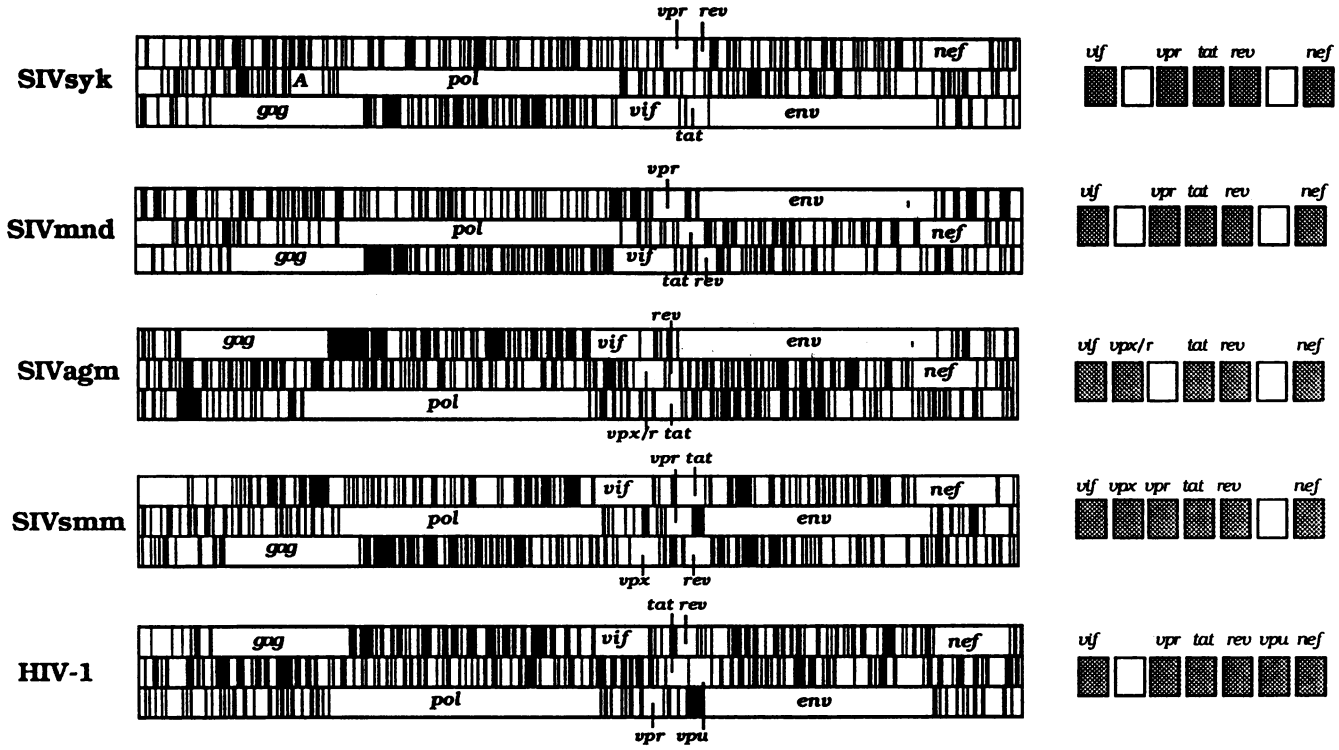

FIG. 2. Genome organization of the primate lentiviruses. A schematic representation of the ORFs of representative viruses from each of the five primate lentivirus groups is shown on the left. Each reading frame in the plus strand is represented by an open bar, stop codons are shown as vertical lines, and the designations of known genes are shown. On the right is a diagram of the regulatory genes of each of the viruses, with shaded boxes representing genes and open boxes indicating the absence of those genes. Sequences used to generate these ORF maps were obtained from the Los Alamos HIV sequence data base (34).

estingly, the putative SIVsyk TAR is of a length similar to that of the SIVsmm/HIV-2 group of viruses; however, the putative TAR of SIVsyk does not have a sequence similar to these LTR sequences or to that of the immunoglobulin heavy chain. The SIVsyk TAR is capable of folding into a stable stem-loop structure ($G = -83$ kcal/mol [data not shown]) and contains the highly conserved CUGGGA motif (Fig. 3) that is essential for HIV-1 transactivation responsiveness.

Similarities between proteins of SIVsyk and other primate lentiviruses. (i) Comparisons of Gag and Pol proteins. SIVsyk is related only distantly to any of the previously described primate lentiviruses. Gag and Pol were the most highly conserved proteins (46 to 53% amino acid identity with analogous SIV and HIV proteins). This is similar to the genetic distance between the previously defined primate lentivirus groups (represented by the similarities between SIVsmm and other viruses shown in Table 1) (for a review, see reference 25). None of the previously characterized primate lentiviruses was significantly more closely related on the basis of sequence identity. The Gag protein of SIVsyk contained a small insertion near the amino terminus (data not shown) within the putative p17 protein. As a result, the *gag* PCR product of SIVsyk is slightly larger than that of the positive control SIVagm. The insertion falls within a highly variable region of the Gag protein of the primate lentiviruses, prior to the putative cleavage site for the major core antigen, p24.

(ii) Regulatory genes of SIVsyk and the designation of *vpx* and *vpr* genes. Other genes of SIVsyk, including the regulatory genes *vif*, *tat*, *rev*, and *nef* and the envelope gene, were highly divergent (18 to 38% similarity). However, sequences shown previously to be conserved among other SIV and HIV clones such as the cysteine-rich metal-binding motif in the first coding exon of Tat and the arginine-rich putative nuclear localization signal in Rev were highly conserved, emphasizing the importance of these domains to the function of these viruses. One of the regulatory genes of SIVsyk presented a dilemma with regard to identification because of limited similarity to other lentiviral gene products. The putative 113-amino-acid protein encoded by this gene, juxtaposed between *vif* and the first codon exon of *tat*, demonstrated weak similarity to Vpx or Vpr of other SIV or HIV clones. The absence of the proline-rich carboxy terminus seen in the Vpx protein of SIVsmm, SIVmac, and HIV-2 suggested that this SIVsyk protein might be the Vpr homolog. However, the criteria for designation of *vpx* genes were unclear since the putative SIVagm Vpx protein also lacks this proline-rich terminus. Indeed, previous analyses have suggested that *vpr* would be a more accurate designation of the putative SIVagm gene and that *vpx* may be unique to the SIVsmm/HIV-2 group of viruses (35, 42, 43). To further examine this situation, we utilized PLSEARCH, a protein similarity program that scores conservative amino acid substitutions as well as identity, to compare the SIVsyk and SIVagm analogs to a library of 450 lentiviral proteins. As is shown in Fig. 4, this search identified Vpr proteins as being most similar to the SIVsyk protein; however, these closet relatives possessed only borderline identity (18%)

A.

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Polypurine tract | U3
AAAAACAAGGGAGGACTGCAGGGCATGAATTATTGTGAAAAGAGAGATGAAATTCTTCATCTCTACCTACAGAATGAGCATGGAAT- 70

                    Sau I   Sy5'F:Primer 1646->
CATAGACAGGATCAATTACACCTCAGGGCCAGGAACCCGGTACCCGTTAATCTTTGGATGGTTATGGGAACTAGTTCCAAATGAGA- 156

TAGAGGGATGCTTGGAGTATGAGGAGAATACACTCCTCCTCCATCCAGCCTCTGGGACAGGGGAGTTCGAACATGGGGAGAACCTC- 242

ATGTGGAACTTCAACCCCCACCTGGCCTATACACCAGGCTGGGAGATGGCCCGCTTGCAGTTAGAGAGACAAACAGGAAAACCACA- 328

AGAGCTGCAGTCAGCACTTAGTAAGAACATCTCCTAGGAGACTCCATGGTGACAAGCTCGGCCCAGGGGAGGGACCTGGGCGGGGG- 414
                                                  Sp-1    Sp-1    Sp-1    Sp-1

AAGGCTCACCCTGCTGCATAAAAGCCGATGCTCTGTAGCTATCCGCGGAGTTGCAACTAGCTCTCTCCAGCGGCCTGGGAGCCCTG- 500
                TATA                U3 / R                          UBP-2
                                                  ----------------------------------------

AGAGACTGGTAGAGCGGCCCGGGAGCCCTTGCCAGTAAAGGCTAGTGAGGTCCGGGTTTGGCCGCCCGGTCTTCGGCAGCTCTCCG- 586
                  UBP-2           CTF/NFI
--------------------------------- TAR ------------------------------------

                                                                    <- Sy3'R
TTGCTTGCTTTGCTTACGCTGATCAGCCCAAATAAAGTGTGGTAAACGCAAGTAACGTCTGCTCTCATCTGTTCTGCTGGGTCACC- 672
---------------           Poly A            R / U5

Primer 1631
TCCCGAAGGTGCTCAGGGAGCGCGCCTTCAGTGGCGCCGAACAGGGACTT
                          U5|  Primer binding site
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B.

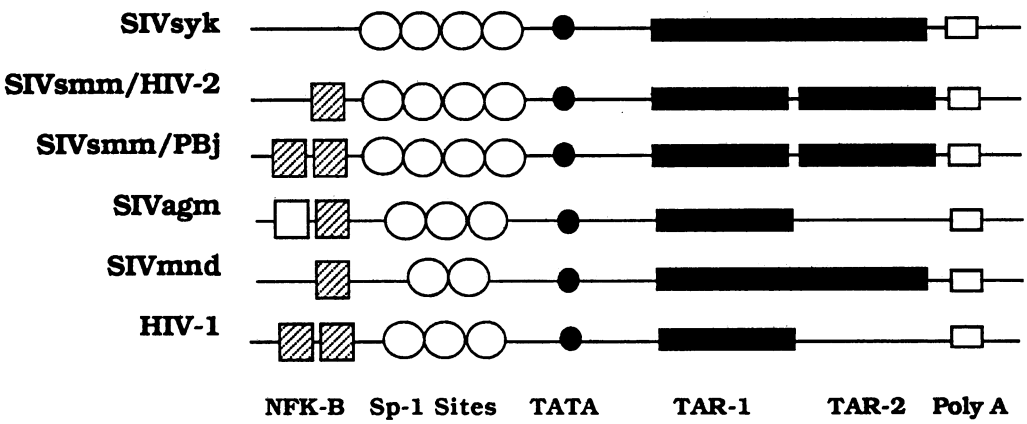


FIG. 3. Comparisons of the LTRs of the primate lentiviruses. (A) Sequence of the SIVsyk LTR. The U3, R, and U5 regions and the positions of the *Sau*I site and the primers used to generate the 5′ end (primer 1646) and 3′ end (primer 1631) of the SIVsyk genome are indicated. Regions with similarity to the HIV-1 UBP-2, Sp-1, and CTF/NF1 binding sites are shown in italics and highlighted with a line below and above the sequence. The TATA box and poly(A) signal are underlined and labelled, and the putative TAR region is shown by a dashed line below the sequence. The direct repeats at the 5′ and 3′ ends of the LTR are conserved (TG/CA); however, the next 10 bases that follow are divergent from most other primate lentiviral LTR sequences. Note the absence of an NF-κB site 5′ to the Sp-1 binding sites. (B) Schematic representation of the regulatory elements in primate lentiviral LTRs, with NF-κB sites shown as shaded boxes (an open box indicates variability among sequences in this group), potential Sp-1 binding sites shown as open circles, the TATA box shown as a black circle, TAR regions shown as black rectangles, and polyadenylation sites (Poly A) shown as an open box. These schematic diagrams were generated from sequences in the Los Alamos HIV data base (34).

with the SIVsyk gene. The analysis of the SIVagm analog was similar; Vpx and Vpr proteins of other primate lentiviruses were identified as possessing borderline identity. In addition, the Vpx and Vpr proteins of other SIVagm isolates were clearly identified as close relatives. The most plausible explanation of these data is that all five of the SIV and HIV groups encode a Vpr analog; the Vpx protein is probably unique to the SIVsmm/HIV-2 group.

Many of the lentivirus genomes contain additional short open reading frames (ORFs) that could encode novel proteins, but in many situations these ORFs have not been adequately examined for function. We noted that the SIVsyk

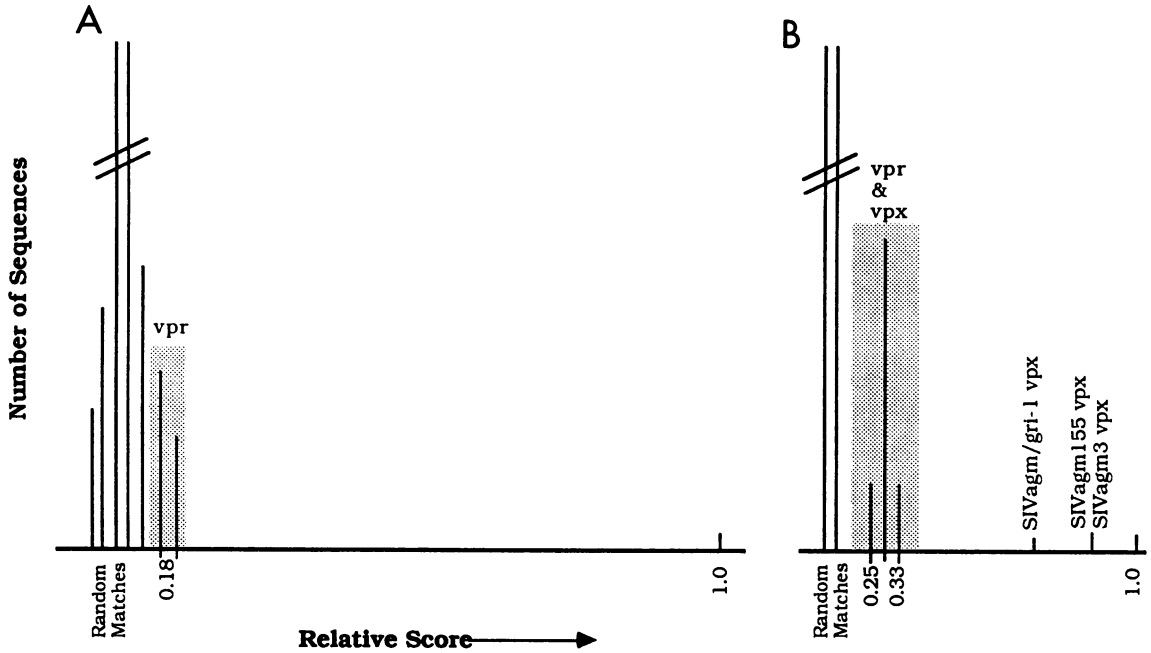


FIG. 4. Protein similarity profiles. Similarity profiles of SIVsyk Vpr protein (A) and SIVagm Vpx protein (B) sequences resulting from comparison to a library of approximately 450 lentiviral protein sequences. These are direct readouts of the PLSEARCH program. Perfectly homologous matches are assigned a score of 1.0. The borderline matches of the respective sequences are highlighted, with similarity scores shown below. For SIVsyk Vpr, the best borderline scores (0.18) are with HIV and SIV Vpr sequences. For SIVagm Vpx, the best matches are with other SIVagm Vpx proteins; the best borderline scores are with HIV and SIV Vpr and Vpx proteins (0.25 to 0.33). The height of the cluster is proportional to that number of query sequences, except for those containing the random matches, which are represented by a broken line. The abscissa is linear in standard deviation units (not shown) rather than with percent similarities (numbers below). This figure can be read as an elution profile, with the weakly matching sequences eluting first, followed by borderline matches, then definitive matches, and finally perfect matches.

genome contained two such short ORFs. The first, in an alternative reading frame to the *gag* gene (labelled as A in Fig. 2), has an initiating methionine and the potential to encode a 122-amino-acid protein. The second is in an alternative reading frame to the first coding exons of *tat* and *rev* and terminates immediately prior to the splice acceptor site for these two proteins (35 amino acids). Neither putative protein encoded by these ORFs has significant similarity to any known proteins, including lentiviral proteins. Thus, their biologic significance is presently unknown.

(iii) Structural similarities between SIVsyk and HIV-1 envelope proteins. Finally, the envelope of SIVsyk was distantly related to the envelope proteins of other primate lentiviruses. SIVsyk encoded a full-length envelope gene uninterrupted by premature transmembrane termination codons observed in many SIV molecular clones (Fig. 2). A total of 19 cysteine residues are strictly conserved among all primate lentiviral envelope proteins; some of these residues form disulfide bonds to generate the principal neutralizing domain of HIV-1 or its analog in SIV and HIV-2 (V3 loop) and the CD4 binding domain (17, 28). Regions of the protein that are conserved among other members of this family were also conserved in SIVsyk. We used a modification of the PLSEARCH program to align cysteine residues and potential N-linked glycosylation sites within gp120 of SIV and HIV (Fig. 5). gp120 of SIVsmm, SIVagm, and SIVmnd contains three or four more cysteine residues than HIV-1 gp120. These cysteines lie in the regions surrounding and including the first variable domain (labelled #1, #2, #3, and #4 in Fig. 5); secondary structure predictions of these proteins differ significantly from the structure of HIV-1 gp120 (17). The SIVsyk envelope protein lacks these additional cysteine residues. In addition, the envelope of SIVsyk is heavily glycosylated, like gp120 of HIV-1. There are approximately 1.24 and 1.28 glycosylation sites per cysteine residue in SIVsyk gp120 and HIV-1 gp120, respectively, compared with 0.78 to 0.95 for SIVagm, SIVmnd, SIVsmm, SIVmac, and HIV-2 (35). Therefore, the envelope proteins of SIVsyk and HIV-1 are skeletally similar, although their primary amino acid sequences are highly divergent.

Phylogenetic relationships to other lentiviruses. To more clearly define the evolutionary relationships of SIVsyk to other primate lentiviruses, phylogenetic tree analysis was performed by using *gag*, *pol*, or *gag-pol* nucleotide sequences by the methods of minimal parsimony and bootstrap analysis. All of these phylogenetic trees were congruent. A tree based on nucleotide substitutions in relatively conserved regions of *pol* shows the relationship of this new SIV strain to other lentiviruses (Fig. 6). The primate lentiviruses cluster separately from lentiviruses such as feline immunodeficiency virus, visna virus of sheep, equine infectious anemia virus, and bovine immunodeficiency virus. SIVsyk showed a tendency to cluster with SIVsmm and HIV-2 in this and other trees that were constructed. However, the Sykes' monkey SIV strain is clearly genetically distinct from other primate lentiviruses and represents a fifth primate lentivirus. Thus, five distinct groups of primate lentiviruses can be identified: (i) SIVsmm and HIV-2 (SIVmac not shown), (ii) SIVcpz and HIV-1 (represented by HIV-1/MN), (iii) SIVmnd, (iv) SIVagm isolates, and (v) SIVsyk. Although these groups are approximately equidistant genetically, as shown in Table 1, a perfect five-point radiation of

TABLE 2. Infection of macaques with the SIVsyk isolate[a]

Species of macaque (common name)	Macaque no.	Virus isolation at wk postinoculation:							Virus isolation as determined by:	
		3	6	12	18	26	34	42	Western blot (6 and 42 wk)	PCR (26 wk)
M. mulatta (rhesus macaque)	RBy2	Y[b]	Y	Y	–	–	Y	–	Y	Y
	ROa3	Y	Y	Y	Y	–	Y	–	Y	Y
	RCt2	Y	Y	Y	–	Y	–	–	Y	Y
	RCm2	–	–	–	–	–	–	–	–	–
M. nemestrina (pig-tailed macaque)	PAs	Y	Y	Y	–	–	–	–	Y	Y
	PIr	–	–	–	–	–	–	–	Y	–
	PLs	–	–	–	–	–	–	–	Y	–
	PRr	Y	Y	Y	Y	–	–	–	Y	Y
M. fascicularis (cynomolgus macaque)	MF400	Y	Y	–	–	–	–	–	Y	Y
	MF402	Y	–	–	–	–	–	–	Y	Y
	MF404	Y	Y	Y	–	Y	–	–	Y	Y
	MF406	Y	–	–	–	–	–	Y	Y	Y

[a] Virus isolation was attempted with 5×10^6 PBMC by cocultivation with 2×10^6 CEM × 174 cells following standard isolation techniques at each of the time points shown. Virus isolation was also attempted by cocultivation with PBMC of a seronegative Sykes' monkey following CD8 depletion of infected macaque PBMC and from monocyte/macrophages with no success.

[b] –, virus was not isolated; Y, virus was isolated.

the distinct groups from a common ancestor was not achieved in this or other published trees (21, 35). This ambiguity in the older branching events is probably attributable to the conspicuous differences among the primate lentiviruses (26, 35) and should be resolved as other lentiviruses are analyzed, particularly viruses that cluster with SIVsyk or SIVmnd. However, phylogenetic analysis and amino acid identity (Table 1) demonstrate that SIVsyk is roughly genetically equidistant, to a first approximation, from other primate lentiviruses.

Infectivity and pathogenicity of SIVsyk in macaques. Infection of macaques with either SIVsmm or SIVmac has proven to be the best available animal model for AIDS pathogenesis studies. SIVsmm infects its natural host, the sooty mangabey, without producing evidence of immune dysfunction (13, 33). However, experimental infection of macaques results in a syndrome remarkably similar to human AIDS (1, 24, 30, 46). Asymptomatic infection of naturally infected African primates appears to be the general rule, since SIVmnd-infected mandrills (44), SIVagm-infected African green monkeys (1), and SIVsyk-infected Sykes' monkeys (7) remain healthy. However, unlike SIVsmm infection of macaques, experimental inoculation of macaques with SIVagm does not generally induce AIDS (1, 3, 16, 20, 23), and infection of macaques with SIVmnd has not been reported. We were interested in whether macaques were infectable with SIVsyk and, if so, what the pathogenic properties of SIVsyk in macaques were. Therefore, three species of macaques, rhesus (*M. mulatta*), pig-tailed (*M. nemestrina*), and cynomolgus (*M. fascicularis*), were inoculated intravenously with cell-free virus supernatant of uncloned SIVsyk. As shown in Table 2, macaques of all three species became infected on the basis of seroconversion and virus isolation from peripheral blood. However, the number of circulating CD4 lymphocytes remained within normal limits and macaques were healthy at 10 months after inoculation. Virus isolation from these macaques became inconsistent after the first few months of infection, and SIVsyk sequences could not be amplified by PCR from PBMC of some animals. However, all except one rhesus macaque that did not become infected after inoculation developed strong Western blot antibody specific for SIVsyk. Therefore, SIVsyk infection of macaques appears to be persistent but restricted; this situation is similar to previous reports of SIVagm infection of macaques.

DISCUSSION

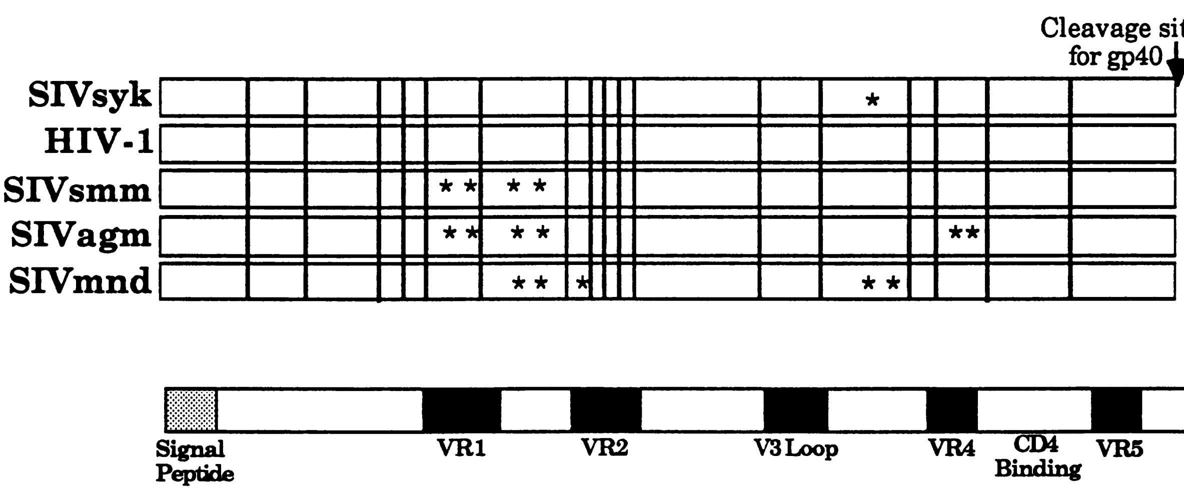
A full-length, infectious molecular clone of SIV from an African monkey, the Sykes' monkey, was derived. The molecular and biologic characterization of SIVsyk contributes valuable information to our knowledge of the phylogeny and pathogenicity of the SIV/HIV family of lentiviruses. Sequence comparisons and phylogenetic analysis demonstrated that SIVsyk represents a fifth distinct primate lentivirus group. The sequence of SIVsyk was particularly interesting since Sykes' monkeys are members of the genus *Cercopithecus*, as are African green monkeys, the source of previously characterized SIVagm molecular clones. However, although African green monkeys and Sykes' monkeys

FIG. 5. Alignment of cysteine and potential N-linked glycosylation sites in the envelope proteins of SIV and HIV. (A) An alignment of gp120 of representative SIV and HIV sequences is shown. The amino acid sequence of SIVsyk gp120 is shown on the top line, with the cysteine and glycosylation site alignment of SIVsyk, HIV-1, SIVsmm, SIVagm, and SIVmnd shown below. Dots represent gaps introduced to maximize alignment; dashes represent an amino acid other than a cysteine or amino acid that participates in a potential N-linked glycosylation site. Other regions of the envelope shown are the signal peptide, the first variable region, the V3 loop, and the CD4 binding domain. Conserved cysteines are indicated by an asterisk above the sequence; there are 17 cysteines within gp120 that are strictly conserved. The region of greatest variability is within the first variable domain of gp120; in this region, most SIV envelopes contain three or four additional cysteines (labelled as #1 to #4). However, like HIV-1 and SIVcpz, SIVsyk lacks these additional cysteines. (B) Schematic representation of the cysteines in gp120 of representative viruses from each of the distinct groups of primate lentiviruses. Each bar represents a gp120 protein, a vertical line represents a conserved cysteine residue, and an asterisk represents a cysteine that is variable among sequences. Variable regions (VR1 to VR5), the V3 loop, the signal peptide, and the CD4 binding domain are shown below.

A.

```
                 __________Signal Peptide__________   |gp120 →
                                                                              *                    *                           120
SIVsyk         ........................MAAFRTYIVCL.FSLISLGFMEKQQYVTVFYGIPHWEDAYAPLFC......TTSHKGGWATKNCVP.SADQIEVRVNITGEYFPAWNSSHMIR
SIVsyk         ........................---------C-.--------------------------------C......------------C--.------VRVNITGE--PAWNSSHM--
HIV-1/MN       ...........-----------------------....--C-------------------------C-----------------C---------ELVNVTEN----..-----
SIVsmm/H4      ......................--C----------..------CC----------AWKNATIP--C......------------C---------LAINVT.E-----.-----
SIVagm/TYO     ..........................------------....------------------VWKNSSVQ--C......------------C---------VPLNIT.E------------
SIVmnd/GB1     -----------------------------------...----------------------------C......ATDNSSLW----C--------------IKENFTGL-------

                                                                                                                                240
                      *      *     *                                                  #1     #2         *                        #3
SIVsyk         QQILEDMSALFLQANRPCVKLAPMCIRMLCTLDNS........................PATSTPTTSPPTTPPNETWWGDNSTEPRFNCSFNLTGGFKDKKQQ.YRAFFYKDDLMKE
SIVsyk         ----------------C------C----C-----.........................------------TPPNETWWGDNSTEPRFNCSFNLTGG-------.-------------
HIV-1/MN       ----------------C------CVTLNCTDL-............................NTTNTNNSTANNNSNS-------EMKNCSFNITTS--------------------.
SIVsmm/H4      ----------------C------C--MRCNKTET----------.....----------------NVINESNPCIKNNSCA---------CKFNMTGL------E.YNETWY-----C-
SIVagm/TYO     ----------------C------C----CVELNST........-----------------C---------QSCNASII-------PASNCTFA-----------------------C-
SIVmnd/GB1     ----------------C------C----C----.............NVSAT----------------------VDKNNTEE-------CRYNITGLC------.-----------...
                                        ____________Variable Region 1 ________

                   #4      *      *          *                    *     *                                                       360
SIVsyk         .....EGNSSYYYLLHCNTSVISAACEKQTFQPFPIQYCAPPGYSLLKCNDTNFEG.DDVCTNVTAVSCTQEFNTLASTWFQLNGTYKAKDKVRFIKQK......DKNESVIILVPEALR
SIVsyk         .....EGNSSYY--LHCNTSVI---C------------C-------LKCNDTNF--.--VCTNVTAV-C-----------FQLNGTYK-----------......DKNESVI--------
HIV-1/MN       .....DNDSTS---ISCNTSVI---C------------C---------C-------.--SCKNVSTV-C-----------LLLNGSLA-----------......---------VHLNES
SIVsmm/H4      -..SANESES-C--HHCNTSVI---C------------C-------LRCNDSNY--FAPNCSKV----C-----------FGFNGTRAENRTYI----........KSNRTII---KYYN
SIVagm/TYO     -..KTNSTSK-C--IHCNDSVI---C------------C---------C-------YKQNCSNVSVV-C-GLMNTTVT--LLLNGSYHENRTQI-----......VNNNTVL----KHYN
SIVmnd/GB1     .....KCENNTCY-NHCNESVN---C-------C.---C----------------..--LCSNISAV-C-----------FGFNGTMH----------------------------------

                   *                          *                                                                                 480
SIVsyk         LQIICERPGNESIKNIQLAAGYFLPVIQGKLKTGRDAKRAFCRVTG..........................NCTEFFKQVHEQAT..KTWKNVTNTTWRSQP......GGDLEVRTHW
SIVsyk         ----C-RPGNESIK--------------------------C----.........................NCTEF---------..-TWKNVTNTTWR---.......----------
HIV-1/MN       VQINCTRP------------------..-----------AHCNISRA.......................KWNDTLR------..--QFKNKTIVFNQSS.......----------
SIVsmm/H4      LTMRC-RPENKTVL-------------...-----------C----........................--------------..-----TGTNDTRKINLTAP-.----------
SIVagm/TYO     LSVTC-RPGNKTVL-------------...-----------C----........................--------------..-------KGTNNTEH----------------
SIVmnd/GB1     ----C-RKGNRSVV-------------..------------C----------------NKLNDSIKVNQTCKNFTST--------..--------C-----.IDNYTTS---------
                    _________ V3 loop _______

                 *     *                          *                          *                                                  600
SIVsyk         FQCGGEFFYCNVSKLFANITN..............GNASKNNYASNLRLSCAIRQIINDWRYVRKLIYLPPTAG...HIKCTSNVTAVLTDIEYYPGSTL.....NFTPTANVEDVWRA
SIVsyk         --C----FYCNVSKLFANITN..............GNASKN---------C----------------------..---CTSNVTAV-------------.....NFTPT---------
HIV-1/MN       --C----FYCNTSPLFNSTWN...........GNNTWNNTTGSNNNITLQC----------------------..---CSSNITGL---------TDTNDTEI--------------
SIVsmm/H4      --C------C------------......----------------------C----------------------...-LTCNSTVT--------INSNET.....NITMS---------
SIVagm/TYO     --C------C-----------.---------C----------C-----------SVINDSYT-----------..---C-------------NSKNRT.....NVTLS---------
SIVmnd/GB1     --C----FFCNVTRI------..................---------C------------------------C-------------IDSNET.K----------------
                                                                        __CD4 Binding Domain _

SIVsyk         DLFNYKLIQIKPIGFAPTDQRRY..ELPNTREKR
SIVsyk         -----------------------..---------
HIV-/MN        ----------------------...--------
SIVmnd/GB1     ------------------------.---------
SIVagm/TYO     -----------------------..---------
SIVmnd/GB1     ----------------------.-----------
```

B.



are phylogenetically related, lentiviruses isolated from these monkeys were related only distantly. In fact, genetic comparisons place SIVsyk nearly equidistant from all other characterized SIV and HIV strains. Evaluation of the genetic distance between SIVagm and SIVsyk must take into consideration the fact that the SIVagm group is the most diverse of the primate lentiviruses, consisting of viruses that differ as much as 30% in the amino acid sequence of Gag proteins. The wide geographic range of African green monkeys and the existence of at least four distinct subspecies (*C. aethiops aethiops*, *C. aethiops pygerythrus*, *C. aethiops tantalus*, and *C. aethiops sabaeus*) account for the diversity of this group (2, 3, 11, 22, 31). Since Sykes' monkeys, like African green monkeys, are part of a group of related subspecies that reside in geographically distinct regions of Africa, we anticipate that other subspecies of *C. mitis*, such as golden monkeys (*C. mitis kanditi*), harbor lentiviruses that cluster genetically with SIVsyk.

Analysis of the sequence of SIVsyk prompts a reexamination of the nomenclature and designations of genes of other primate lentiviruses. SIVsyk has a genome structure similar to that of SIV from mandrills (SIVmnd); each virus contains eight genes: *gag*, *pol*, *vif*, *vpr*, *tat*, *rev*, *env*, and *nef*. This same general organization is also observed in SIVagm clones, the exception being that the fourth gene has been designated *vpx* rather than *vpr* (11, 25 25, 42, 43). As shown in Table 1 and Figure 4, comparisons of Vpx and Vpr proteins demonstrate a limited degree of amino acid similarity. Additionally, Vpr proteins of different viruses, such as SIVmnd and SIVsmm, are only minimally conserved, similar to the level of identity between Vpx and Vpr. This and previous analyses (42, 43) suggest that SIVagm encodes a Vpr protein rather than Vpx, as had been previously suggested. Thus, all primate lentiviruses may encode an analogous protein that is best designated Vpr. The SIVsmm and HIV-1 groups encode additional gene products (Vpx for SIVsmm and Vpu for HIV-1). The limited degree of amino acid similarity between Vpr and Vpx protein sequences suggests that the *vpx* gene arose by gene duplication during evolution of the SIVsmm/HIV-2 group of viruses (42, 43).

In terms of biologic relevance, our study demonstrated that SIV isolated from Sykes' monkeys was infectious for rhesus, pig-tailed, and cynomologus macaques. However, the resulting infection appeared to be transient in some animals and did not induce immunodeficiency. Clinical, immunologic, and virologic parameters will be monitored for persistently infected macaques to determine whether these animals will develop immunodeficiency after a prolonged latent period. However, the kinetics of infection observed in this study are reminiscent of HIV-1 infection of chimpanzees (12) and SIVagm infection of macaques (1, 3, 16). SIVagm infection of macaques induces an AIDS-like syndrome only rarely (16), and chimpanzees infected with HIV-1 remain healthy for up to 8 years (12). Therefore, this particular isolate of SIVsyk appears to be minimally pathogenic for

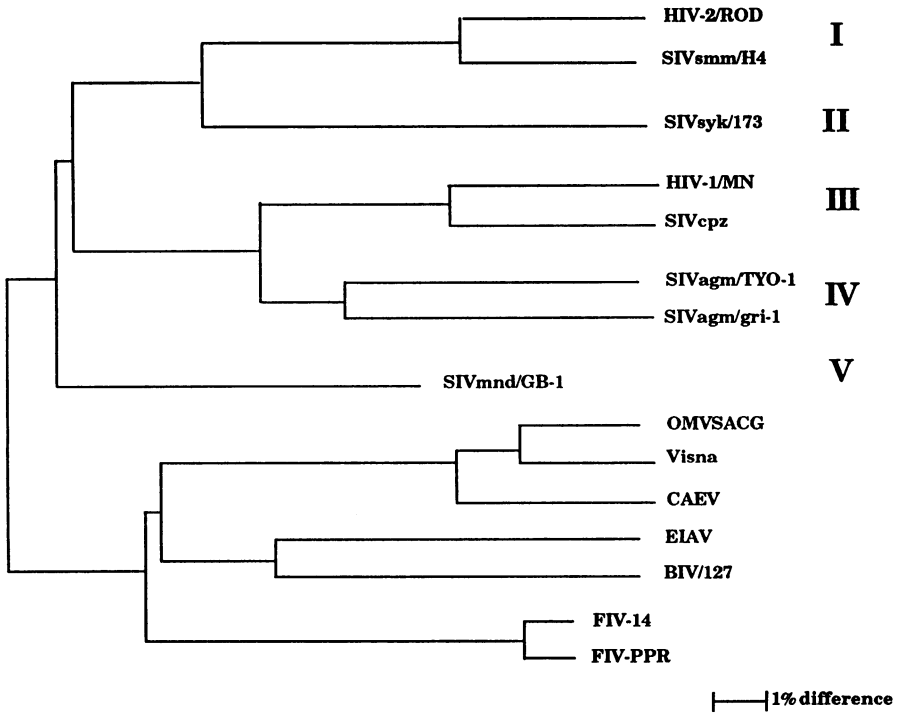


FIG. 6. Phylogenetic tree analysis comparing *pol* nucleotide sequences. Shown is a phylogenetic tree of conserved regions of *pol* in which both primate and nonprimate sequences are compared. Only representative sequences are shown for each group. OMVSACG is an ovine lentivirus isolate from South Africa with known time of divergence from visna virus (34). Five distinct groups of primate viruses that differ in nucleotide sequence of conserved regions of *pol* by approximately 15% can be distinguished: I, SIVsmm and HIV-2 (SIVmac is not shown); II, SIVsyk; III, HIV-1, represented by HIV-1/MN and SIVcpz; IV, SIVagm group; and V, SIVmnd. The tree was constructed by using the multiple parsimony algorithm (MULPARS) of PAUP, version 2.4.2. The total number of sites examined was 801, of which 617 were variable. The lengths of the horizontal lines are proportional to single-base changes and have been calibrated by assuming a similar date of isolation for each virus; these distances can be read as percent differences by using the scale bar below. Vertical lines are for clarity only. The tree was rooted on a midpoint of the greatest patristic distance. Sequences were obtained from the Los Alamos HIV data base (34).

both its naturally infected Sykes' monkey host and experimentally infected macaques.

In a complex organism such as a macaque, there are a myriad of potential reasons for transient infection. Assuming that the infectious clone was representative of the uncloned virus isolate, sequence analysis suggested two genetic factors that might contribute to transient infection of macaques. First, like SIVagm and SIVmnd, SIVsyk lacks one of the genes expressed by the pathogenic SIVsmm group. The absence of *vpr* or *vpx* in SIVagm was proposed as a potential explanation of the reduced pathogenicity of these viruses for macaques (11), although no formal test of this hypothesis has been conducted. Second, a unique feature of LTRs derived from the SIVsyk isolate (both LTRs of the infectious clone and eight additional LTR clones) was the absence of an NF-κB binding site in the LTR. This regulatory element is strictly conserved within the LTRs of other primate lentiviruses. Indeed, the LTRs of HIV-1, some SIVagm clones, and the acutely lethal SIVsmm/PBj variant contain a duplication of this motif. The role of this signal in HIV-1 and SIVsmm/PBj has been the subject of speculation; duplication of NF-κB has been hypothesized as contributing to the pathogenicity of SIVsmm/PBj (4, 37). For HIV-1, binding of cellular transactivating factors to the NF-κB motif increases viral expression from basal levels (8, 14, 29, 40). Thus, absence of this element in the SIVsyk LTR implies an inability to increase transcriptional activity upon cellular activation. In in vivo situations, in which lymphocyte activation could contribute significantly to viral replication, the absence of NF-κB might dampen virus replication and thus decrease cytopathic effects or viral dissemination. This hypothesis is highly speculative, since the in vivo significance of NF-κB deletion is unknown.

In summary, analysis of SIVsyk adds significantly to our knowledge of the genetic and biologic diversity of the primate lentivirus family. Although the first HIV or SIV sequences generated a larger volume of new information, elucidation of the sequence and biology of viruses such as SIVsyk continues to contribute additional insights into AIDS pathogenesis and evolution. First, SIVsyk defines a fifth distinct primate lentivirus group. Comparisons with SIVsyk also establish a basic genome structure shared by at least three SIV groups. This infectious SIVsyk molecular clone is the first naturally occurring SIV or HIV to lack the NF-κB motif in the LTR. The cysteine arrangement of the SIVsyk envelope also resembles most closely that of HIV-1, implying shared structural features. Finally, in terms of biologic effects, experimental inoculation of macaques with SIVsyk resulted in persistent infection but not immunodeficiency.

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