

## Characterization and Comparison of Recombinant Simian Immunodeficiency Virus from Drill (*Mandrillus leucophaeus*) and Mandrill (*Mandrillus sphinx*) Isolates

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Since simian immunodeficiency virus (SIV) was found to be the source of the human AIDS pandemic, a major goal has been to characterize the diversity of SIV strains in the wild and to assess their potential for crossover into humans. In the present study, SIV was isolated from a seropositive drill (*Mandrillus leucophaeus*) and three seropositive mandrills (*Mandrillus sphinx*) by using macaque peripheral blood mononuclear cells (PBMC). Full-length sequences were obtained from a drill and mandrill and designated SIVdr11FAO and SIVmnd5440, respectively. A 182-bp fragment of the *pol* genes of the two remaining mandrill SIV isolates was also analyzed. Phylogenetic analyses demonstrated that SIVdr11FAO formed a monophyletic clade with SIVmnd5440 and SIVmndM14, recently designated SIVmnd type 2. Both the SIVdr1 and SIVmnd type 2 genomes carried a *vpx* gene and appeared to share a common ancestor with SIVrcm in the 5' region of the genome and with SIVmndGB1 (type 1) in the 3' region of the genome. A statistically significant recombination breakpoint was detected at the beginning of envelope, suggesting that the viruses were descendents of the same recombinant. Phylogenetic analysis of *vpx* and *vpr* genes demonstrated that the *vpx* genes formed a monophyletic cluster that grouped with *vpr* from SIVagm. In addition, both SIVdr11FAO and SIVmnd5440 replicated in human PBMC and therefore could pose a risk of transmission to the human population.

Since human immunodeficiency virus type 1 (HIV-1) and HIV-2 have been shown to originate from simian immunodeficiency virus (SIV) in African primates (9, 17, 40, 41), characterizing these viruses has become a major goal of AIDS research. To date, more than 20 species of SIV-infected African nonhuman primates have been detected (4, 18, 31, 37, 39); 34 complete sequences of these viruses have been published (30). SIV and HIV are classified as primate lentiviruses (PLVs), and at the present time six lineages, based upon phylogenetic relationships of full-length genomes, are recognized (5, 12). These six designated lineages are (i) SIVcpz from chimpanzees (*Pan troglodytes*), including HIV-1 (17, 25, 26, 41, 55); (ii) SIVsm from sooty mangabeys (*Cercocebus atys*), including HIV-2 and SIVmac from captive macaques (*Macaca* spp.) (21, 24, 36); (iii) SIVagm from African green monkeys (members of the *Chlorocebus aethiops* superspecies) (1, 2, 14–16, 23, 27, 29, 35); (iv) SIVsyk from Sykes' monkeys (*Cercopithecus albogularis*); (v) SIVlhoest from L'Hoest monkeys (*Cercopithecus lhoesti*), including SIVsun from sun-tailed monkeys (*Cercopithecus solatus*) and SIVmnd type 1 (GB1) from mandrills (*Mandrillus sphinx*) (3, 4, 22, 54); and (vi) SIVcol from Colobus monkeys (*Colobus guereza*) (12). In addition,

another novel recombinant SIV from greater spot-nosed monkeys (*Cercopithecus nictans*) was recently described (13). SIVgsn shares the *vpu* gene of HIV-1 and SIVcpz and is most closely related to these viruses in the envelope but clusters with SIVsyk in Gag and part of Pol. Some SIV strains, such as SIVdr1 from drills (*Mandrillus leucophaeus*), SIVtal from talapoin monkeys (*Miopithecus talapoin*), SIVmus from mustached guenons (*Cercopithecus cephus*), SIVmon from Mona monkeys (*Cercopithecus mona*), and SIVdeb from DeBrazza monkeys (*Cercopithecus neglectus*) have only been partially characterized (11, 38, 39). Sequencing of the entire genome is necessary to classify those viruses into a particular lineage or to designate them as members of a novel lineage.

Phylogenetic studies of nonhuman PLVs have suggested that the divergence of viral lineages reflected in some cases the divergence of their host lineages, suggesting host-dependent evolution (1, 4, 44, 45). However, in many cases the differences between phylogenetic relationships of some viruses and primate phylogeny indicated that there have been multiple cross-species transmissions in the past (7, 28, 51). Cross-species transmission of PLVs can have a variety of outcomes in the new host. For example, if the recipient of the transmitted virus is already infected by a PLV, superinfection by other PLVs has the potential for generating recombinant viruses that could have a complex mosaic genome. Thus, some SIV isolates could not be classified into a particular lentivirus lineage because their genome was the result of one or more recombination events between viruses from different lineages. For example,

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TABLE 1. Nested primer pairs and their genomic locations

Primer	Sequence (5' to 3')	Specificity	Amplified fragment (bp)	Gene	Location (nt)		
mpolF1	ATTATAGAACAGGTTAGAGATCAGGCA	SIV	233	<i>pol</i> (outer set)	4272–4297 <sup>a</sup>		
mpolR1	GGTCCTTTCCACTGTTGATCCCTT				4480–4503 <sup>a</sup>		
mpolF2	TTAGAGACAGCAGTTCAAATGGCAGTA				182	<i>pol</i> (inner set)	4304–4330 <sup>a</sup>
mpolR2	TCCCTTCCTTCTCTGTAATAAACCCGA						4459–4485 <sup>a</sup>
drIF1	TGGCGCCCGAACAGGGACTTGA	SIVdr1FAO	4480	PBS	1–22		
mpolR1	GGTCCTTTCCACTGTTGATTCCTT				<i>pol</i>	4480–4503	
drIF2	GCATACCACAGGAGTTCCTATAA				5935	<i>pol</i>	4204–4227
drIR2	GTTCTTTCGCTACCCAGACAAGAT						<i>gag</i>
mnd5440F1	TGGCGCCCGAACAGGGACTTGA	SIVmnd5440	4485	PBS	1–22		
mnd5440R1	TCCCTTCCTTCTCTGTAATAAACCCGA				<i>pol</i>	4459–4485	
mnd5440F2	GGCTAGAAAACAGCAGTGCAAATGG				5457	<i>pol</i>	4302–4325
mnd5440R2	GTCCCTTACAGGTGGGTGCGACCTGAA						PBS

<sup>a</sup> The primer location is in SIVmnd5440.

SIVagm from sabaeus monkeys was the result of a recombination event between a SIVagm ancestor and another virus potentially from the SIVsm lineage (27). SIVrcm has been shown to cluster with different lentivirus lineages in different regions of the genome, but it is still uncertain whether SIVrcm or one of the potential parental viruses is the recombinant (6, 18). The most recently discovered mosaic virus was SIVmndM14, which was found to be recombinant between SIVrcm and SIVmndGB1 (48).

Based on the commonly accepted hypothesis that HIV-1 and HIV-2 were the result of multiple cross-species transmissions to humans from chimpanzees and sooty mangabeys, respectively (9, 10, 17, 40, 41), it is important to consider the possibility of ongoing zoonotic transfer. People in west central Africa are still routinely exposed to a large variety of genetically divergent SIV stains (20, 39). Indeed, epidemiological surveys identified a human serum sample from Cameroon that showed a high reactivity directed solely and strongly against the SIVmnd V3 loop, raising the possibility that mandrills may represent a viral reservoir for humans similar to sooty mangabeys in western Africa and chimpanzees in central Africa (48). Moreover, a variety of SIV strains are able to replicate in human peripheral blood mononuclear cells (PBMC) and macrophages in vitro, indicating the possibility for another cross-over of SIV into the human population (6, 19).

In the present study, we isolated SIV from blood samples from one drill and three mandrills housed in North American and European zoos. The highly endangered drill is a large, short-tailed monkey restricted in the wild to several blocks of forest between the Cross river in southeastern Nigeria, the Sanaga river in southwestern Cameroon, and the island of Bioko (Equatorial Guinea) (11). The related mandrill is also a large semiterrestrial primate that lives in the tropical rain forests of Cameroon and Gabon (48). Our goal was to isolate and molecularly characterize SIV from a drill and mandrill and evaluate their in vitro host ranges and coreceptor usages. For this purpose, we characterized the full-length genomes of the SIV isolates from the drill (no. 1FAO) and from one of the mandrills (no. 5440). We also determined the host range of SIVdr1FAO and SIVmnd5440 in permanent cell lines and in

primary PBMC and evaluated the coreceptor usage of SIVdr1FAO.

#### MATERIALS AND METHODS

**Primate samples.** Fresh blood specimens were obtained from a drill (1FAO) and three mandrills (5440, Pearl, and Gunnite) housed at North American or European zoological parks in accordance with the guidelines of the animal care and use committees at their institutions. The single male drill was in an exhibit that was exposed to a wild-born drill with an unknown SIV serostatus. Mandrill 5440 [female] was captive born to African-born parents with unknown SIV serostatus. The countries of origin of the wild-born animals are not known. Mandrill Gunnite [male] and Pearl [female] were born in captivity to captive-born parents of which the mother was SIV seropositive in both cases. From the four animals, PBMC and plasma were obtained by standard Ficoll-Hypaque density gradient centrifugation and stored in liquid nitrogen until use.

**Virus isolation.** After being thawed, drill and mandrill PBMC were washed twice with Hanks' buffered salt solution and stimulated with 2  $\mu$ g of phytohemagglutinin (PHA) (Sigma, St. Louis, Mo.) per ml for 3 days. PBMC from SIV-negative pig-tailed macaques were separated by Ficoll-Hypaque density gradient centrifugation of whole blood and also stimulated with 2  $\mu$ g of PHA per ml for 3 days. PBMC blasts from the SIV-positive drill and three SIV-positive mandrills (5440, Pearl, and Gunnite) were cocultivated with  $2 \times 10^7$  PBMC blasts from pig-tailed macaques and maintained in complete RPMI (RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 U of penicillin per ml, 100  $\mu$ g of streptomycin per ml, and 10 mM HEPES) supplemented with 5 half-maximal units of human interleukin-2 (Advanced Biotechnologies, Columbia, Md.) per ml. Supernatants were sampled every three days and subjected to reverse transcriptase (RT) assay. Supernatants were harvested at the peak of RT activity, filtered through a 0.45- $\mu$ m-pore-size filter, and cryopreserved as virus stock. Virus stocks were designated SIVdr1FAO, SIVmnd5440, SIVmndPea, and SIVmndGun.

**PCR amplification of the viral genome.** PCRs were performed with DNA extracted from PBMC by using phenol-chloroform or from cell lysates as described previously (49). To generate a small fragment of 194 bp in the RT portion of the *pol* gene from cell lysate of drill, degenerate primers DR1, DR2, DR4, and DR5 were used as previously described (11). Amplification was performed with an ExTaq kit (Takara, Otsu, Shiga, Japan) with an initial denaturation at 94°C for 2 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension of 72°C for 10 min. Primer DR1 was used at 0.8 pmol per  $\mu$ l, and DR2, DR4, and DR5 were used at 2.5 pmol per  $\mu$ l. A second fragment of 182 bp located in the integrase part of the *pol* gene was generated by nested PCR with primers mpolF1, mpolR1, mpolF2, and mpolR2 (Table 1). The conditions for PCR amplification were the same as described above.

To amplify the entire SIVdr1FAO or SIVmnd5440 genomes, genomic DNA was extracted from infected Molt4clone8 cells by using phenol-chloroform. Two overlapping fragments, from the primer binding site (PBS) to integrase (4.5 kb) and from integrase to the PBS-*gag* gene (5.5 and 5.9 kb, respectively), were

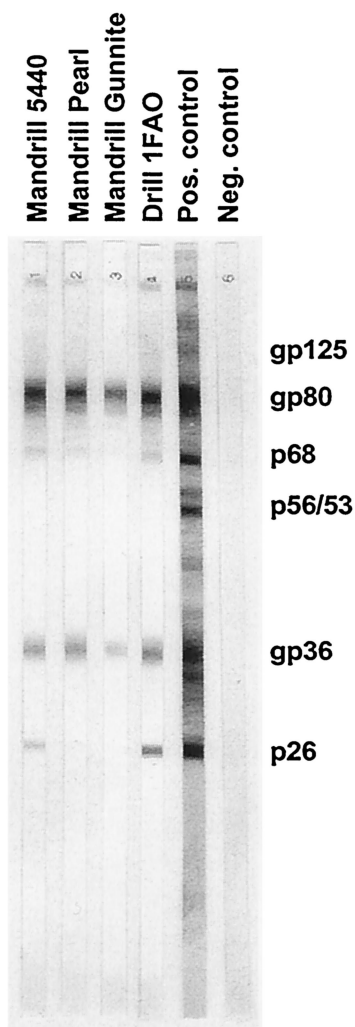


FIG. 1. Western blot analysis (HIV-2 blot1.2; Genelabs) of drill and mandrill sera. Sera from mandrills 5440, Pearl, and Gunnite and drill 1FAO showed cross-reactive antibodies against HIV-2 proteins Env gp36 and gp80. Mandrill 5440 and drill 1FAO sera also reacted with Gag p26.

amplified by targeting the integrated genomic as well as unintegrated circular DNA. The primer sequences and location of the expected amplified product are described in Table 1. PCRs were performed in a volume of 50 µl with the Expand Long Template High Fidelity PCR system (Roche Molecular Biochemicals) under the following conditions: a hot start at 94°C for 3 min; 10 cycles of denaturation at 94°C for 20 s, annealing at 58°C for 30 s, and extension at 68°C for 5 to 7 min; and 20 cycles with extension at 68°C for 5 to 7 min with an increment of 20 s per cycle. Amplification was completed by a final extension at 72°C for 15 min. The entire 50-µl PCR product was separated on a 0.9% agarose gel. Bands of the correct size were excised, cloned into pCRII-TOPO vector (TOPO TA Cloning kit; Invitrogen, Carlsbad, Calif.) and sequenced by automated fluorescent sequencing (*Taq* amplification and termination; Perkin-Elmer Applied Biosystems, Warrington, United Kingdom).

**Sequence analysis.** The sequences of SIVdr11FAO and SIVmnd5440 were aligned and compared to representatives of the major lentivirus lineages, including some of those in the HIV sequence database at [http://hiv-web.lanl.gov/ALIGN\\_CURRENT/ALIGN-INDEX.html](http://hiv-web.lanl.gov/ALIGN_CURRENT/ALIGN-INDEX.html) ("other SIV complete genome DNA" alignment). For some of the phylogenetic analyses, third-codon positions were removed. Columns in the alignment, in which gaps had been inserted to maintain the alignment through regions with insertions and deletions, were stripped prior to the analyses. Protein identities were calculated from the gap-stripped alignment by using BioEdit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). The gap-stripped alignment was further analyzed with the SIMPLOT

TABLE 2. Protein sequence identities between SIVdr11FAO and other PLVs

Virus (accession no.)	% Identity with SIVdr11FAO in:								
	Gag	Pol	Vif	Vpx	Vpr	Tat <sup>a</sup>	Rev <sup>b</sup>	Env	Nef
SIVdr1/Clewley (AJ310481)	92 <sup>c</sup>	93 <sup>d</sup>							
SIVmnd5440 (AY159322)	83	81	69	70	78	52	57	67	66
SIVmndM14 (AF328295)	81	81	70	68	80	52	61	72	63
SIVrcm (AF349680)	73	77	51	57	63	46	39	38	41
SIVcpz (X52154)	59	67	42		49	37	28	36	40
SIVsm (L09212)	64	61	44	42	63	39	48	40	43
SIVagmSab (U04005)	72	72	49		42	39	39	37	48
SIVagmVer (L40990)	67	66	46		42	44	43	38	44 <sup>e</sup>
SIVlhoest (AF075269)	50	58	35		44	37	50	55	45
SIVsyk (L06042)	60	55	41		25	50	30	38	39
SIVcol (AF301156)	47	51	22		28	36	30	29	31
SIVmndGB1 (M27470)	54	58	36		40	44	37	63	55

<sup>a</sup> Based on a 55-amino-acid alignment.  
<sup>b</sup> Based on a 46-amino-acid alignment.  
<sup>c</sup> Based on a 316-amino-acid alignment.  
<sup>d</sup> Based on a 967-amino-acid alignment.  
<sup>e</sup> Comparison with SIVagmVer155 (M29975).

program from Stuart Ray (<http://www.med.jhu.edu/deptmed/sray/download>), both as nucleotide and as translated amino acid sequences, in order to determine the regions of the alignment to be analyzed separately by phylogenetic methods. A neighbor-joining phylogenetic tree was built for each genomic region, using the PHYLIP, DNADIST, and NEIGHBOR programs (<http://evolution.genetics.washington.edu/phylip.html>) with the F84 model of evolution. The resulting tree was used as input, along with the alignment, to Gary Olsen's DNARates

TABLE 3. Protein sequence identities between SIVmnd5440 and other PLVs

Virus (accession no.)	% Identity with SIVmnd5440 in:								
	Gag	Pol	Vif	Vpx	Vpr	Tat <sup>a</sup>	Rev <sup>b</sup>	Env	Nef
SIVmndM14 (AF328295)	93	91	75	83	85	74	76	80	78
SIVdr11FAO (AY159321)	83	81	69	70	78	52	57	67	66
SIVrcm (AF349680)	70	77	49	63	66	54	30	36	46
SIVcpz (X52154)	56	67	40		52	37	30	35	40
SIVsm (L09212)	65	59	40	39	64	43	39	38	48
SIVagmSab (U04005)	71	70	47		42	41	43	36	48
SIVagmVer (L40990)	65	65	47		40	54	41	38	43 <sup>c</sup>
SIVlhoest (AF075269)	51	58	34		44	44	50	49	45
SIVsyk (L06042)	60	54	40		25	48	30	35	38
SIVcol (AF301156)	47	50	25		27	33	28	30	30
SIVmndGB1 (M27470)	53	61	36		44	39	46	58	54

<sup>a</sup> Based on a 55-amino-acid alignment.  
<sup>b</sup> Based on a 46-amino-acid alignment.  
<sup>c</sup> Comparison with SIVagmVer155 (M29975).

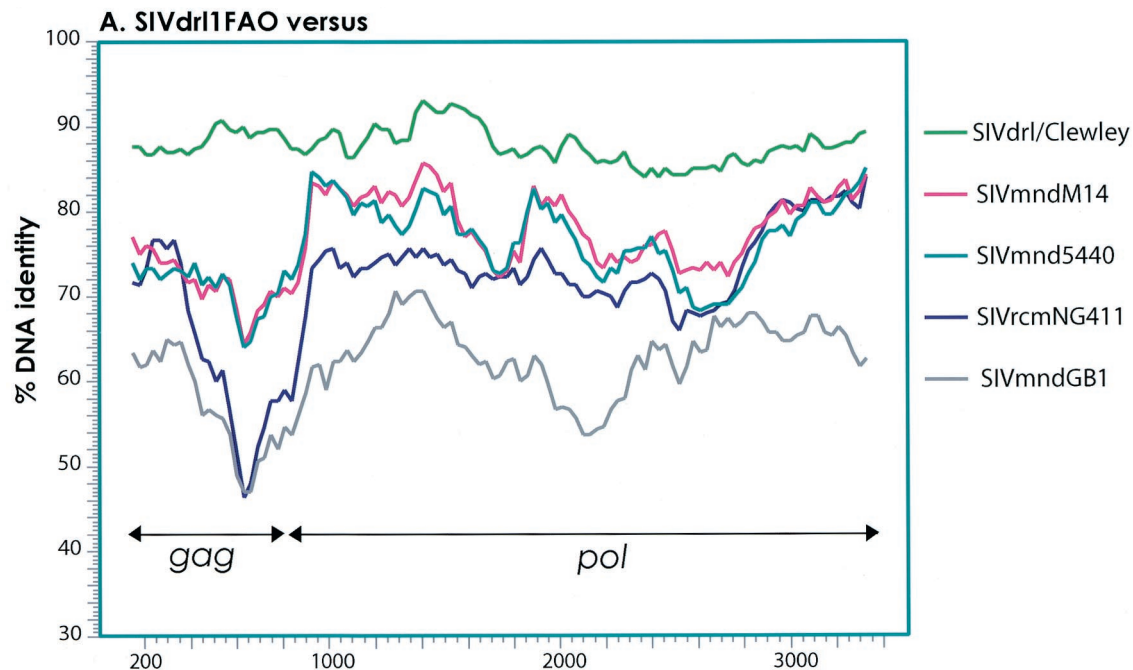


FIG. 2. Similarity plots comparing a 3,450-bp gap-stripped partial *gag-pol* alignment of SIVdr11FAO with other SIV isolates from drill and mandrill (A), comparing the full-length genome of SIVdr11FAO with SIVmnd5440, SIVmndM14, SIVrcm, and representatives of the other major PLV lineages (B), and comparing the full-length genome of SIVmnd5440 with SIVdr11FAO, SIVmndM14, SIVrcm, and representatives of the other major PLV lineages (C). The gene boundaries are indicated by arrows. In the Simplots for panels B and C, third-codon positions of the alignment have been removed. The fractional nucleotide sequence difference was calculated for a window size of 300 bp (A) or 400 bp (B and C) moved in steps of 30 bp (A) or 40 bp (B and C) with Hamming distances.

program (<http://geta.life.uiuc.edu/~gary/programs/DNArates.html>) to compute site-specific rates of evolution. These rates were then included in the input to a modified version of fastDNAmI written by Tanmoy Bhattacharya (<http://www.santafe.edu/~btk/science-paper/bette.html>), which computes maximum-likelihood trees incorporating site-specific rates of evolution. Bootstrap support (100 replicates) for each of the trees was calculated by using the PHYLIP SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE programs. Although the bootstrap trees were computed via the neighbor-joining method, rather than by maximum likelihood with site-specific rates, the topologies were often identical although branch lengths differed significantly. Trees were edited in TreeTool (<ftp://ftp.cme.msu.edu/pub/RDP/programs/TreeTool/>).

**Infection of chimpanzee PBMC, human PBMC, and human CD4<sup>+</sup>-T-cell lines with SIVdr11FAO or SIVmnd5440.** To test the in vitro host range of SIVdr11FAO and SIVmnd5440, human and chimpanzee PBMC and a panel of CD4<sup>+</sup> human cells were used. PBMC were separated from heparinized whole blood of HIV-negative humans and SIV- and HIV-negative chimpanzees by Ficoll-Hypaque density gradient centrifugation. PBMC were stimulated with 2  $\mu$ g of PHA (Sigma) per ml in complete RPMI 1640 with 10% fetal calf serum, supplemented with 5 half-maximal units of human interleukin-2 per ml, for 3 days before viral inoculation. The human CD4<sup>+</sup> cell lines CEMx174, CEM-SS, C8166, H9, Hut78, MT-2, MT-4, Molt4clone8, PM1, SupT1, and U937 were maintained in RPMI complete medium. Five million PHA-stimulated PBMC or 2 million human CD4<sup>+</sup> T cells were pelleted, and the cell pellet was incubated with 1 ml of SIVdr11FAO or SIVmnd5440 (approximately 500,000 cpm of RT activity) for 2 h with agitation every 30 min. The cells were washed once with medium, 5 ml of complete RPMI was added, and the cells were transferred to T25 flasks (Nalge Nunc International, Rochester, N.Y.). Cell culture supernatants were collected every 3 days, and the infection of the cells was monitored for up to 6 weeks.

**GHOST cell assay.** The coreceptor usages of SIVdr11FAO and SIVmnd5440 were determined by using the GHOST cell assay (34). The GHOST cell panel was obtained through the AIDS Reagent and Reference Program, National Institutes of Health (Rockville, Md.). Cells ( $2 \times 10^4$ ) from each of the cell lines were seeded in 24-well plates (Costar, Coring, N.Y.) and cultivated for 2 days prior to infection. One milliliter of virus stock (corresponding to approximately 500,000 cpm of RT activity) was gently added to the cells, and after 4 h of incubation at 37°C, the cells were washed once with medium, followed by the addition of 1 ml of fresh medium. Every other day the cells were split 1:2 and 0.5

ml of culture supernatant was collected. The production of SIV Gag p27 antigen was measured by using the RETRO-TEK SIV-1 p27 antigen enzyme-linked immunosorbent assay (ZeptoMetrix Corp., Buffalo, N.Y.) according to the manufacturer's instructions.

**Nucleotide sequence accession numbers.** The complete genomes of SIVdr11FAO and SIVmnd5440 have been submitted to GenBank under accession numbers AY159321 and AY159322.

## RESULTS

**Identification and isolation of SIVdr1 and SIVmnd.** Antibodies to SIV were detected using a commercially available HIV-2 Western blot kit (HIV-2 blot1.2; Genelabs, Singapore). Sera of mandrill 5440 and drill FAO cross-reacted with Env gp36, Env gp80, and Gag p26 proteins of HIV-2 (Fig. 1). Virus was isolated from PBMC of the seropositive drill and from all three seropositive mandrills (Gunnite, Pearl, and 5440) by cocultivation with PHA-stimulated pig-tailed macaque PBMC. Virus was isolated from the SIV-positive mandrills after 5 weeks of coculture, with peak RT activity by 6 weeks postinfection. Cytopathic effects were not observed during the entire period of coculture. A similar strategy was used for isolation of SIVdr11FAO, except that isolation only required 1 week of coculture.

**Genomic organizations of SIVdr1 and SIVmnd.** Partial *pol* sequences were amplified from three SIV-positive mandrills and one SIV-positive drill by using degenerate primers as detailed in Materials and Methods. Subsequently, the complete genomes of SIVdr11FAO and SIVmnd5440 were amplified in two fragments (4.5 kb and 5.5 to 5.9 kb) by targeting the integrated genomic as well as unintegrated circular DNA. The

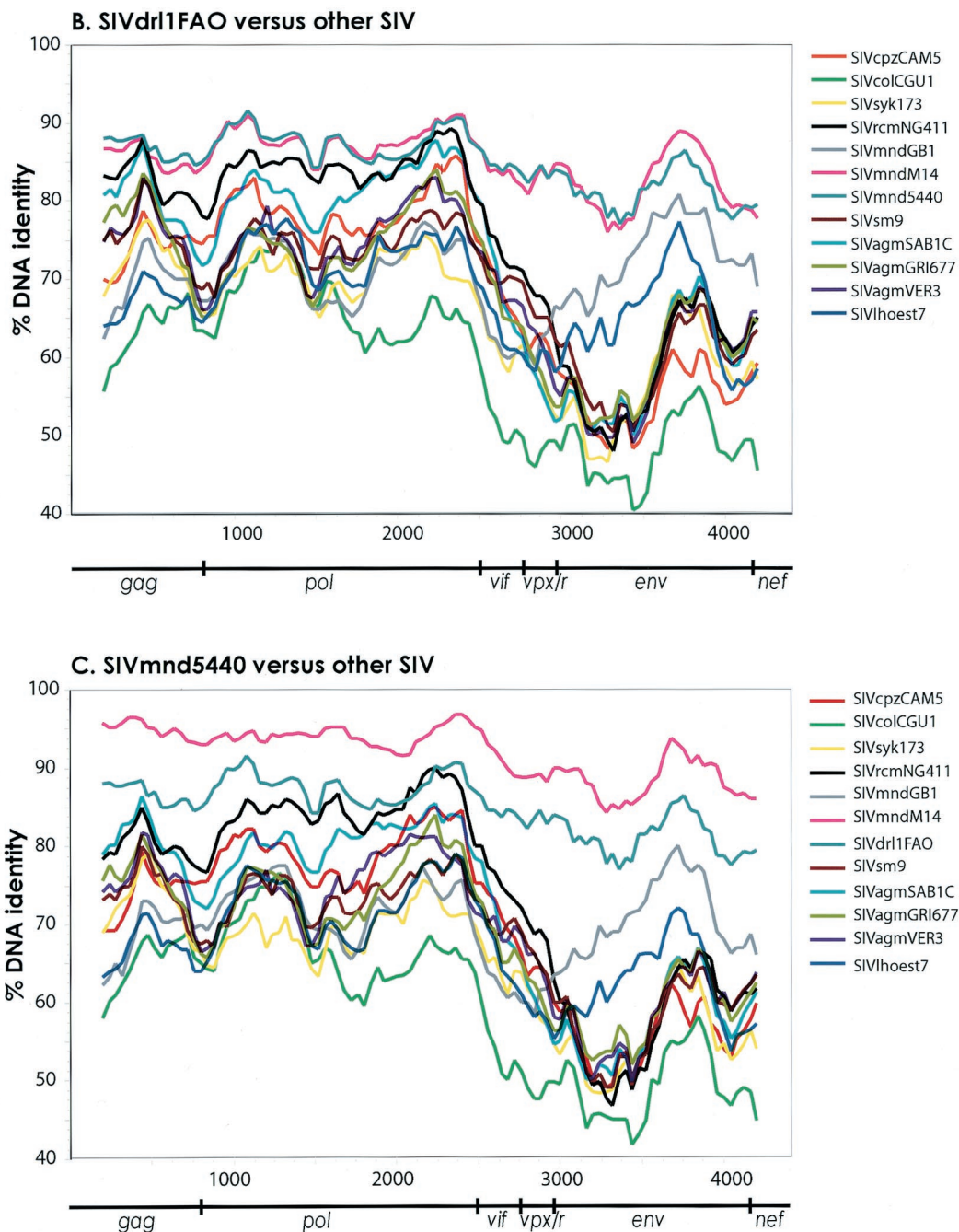


FIG. 2—Continued.

PCR fragments were cloned by using the TOPO cloning system (Invitrogen) and sequenced, and the sequences assembled to obtain the full-length genomes, which were compared to those of other PLVs. The proviral genomes of SIVdr11FAO and SIVmnd5440 shared their genomic organization with SIVmndM14, SIVrcm, SIVsm, SIVmac, and HIV-2. These viruses possess a *vpx* gene in addition to *vpr* but lack a *vpu* gene which is present in the SIVcpz/HIV-1 lineage and in SIV from greater spot-nosed and mona monkeys (13; J. Clewley, 9th International Workshop on HIV Dynamics and Evolution, abstr. 5, 2002).

**SIVdr11FAO and SIVmnd5440 share the same mosaic structure.** We compared protein identities of SIVdr11FAO and SIVmnd5440 to published full-length sequences of SIV in order to estimate their relationship to other PLVs. SIVdr11FAO was most closely related to SIVdr1/Clewley (unpublished; GenBank accession no. AJ310481), with identities of 92 and 93% in partial fragments of Gag and Pol, respectively (Table 2). Next, SIVdr11FAO was most closely related throughout the entire genome to SIVmnd5440 and SIVmndM14, with a protein identity of 81 to 83% in Gag and Pol. In comparison to the other PLVs, SIVdr11FAO was most closely related in the Gag, Pol,

## A. Pol Fragment

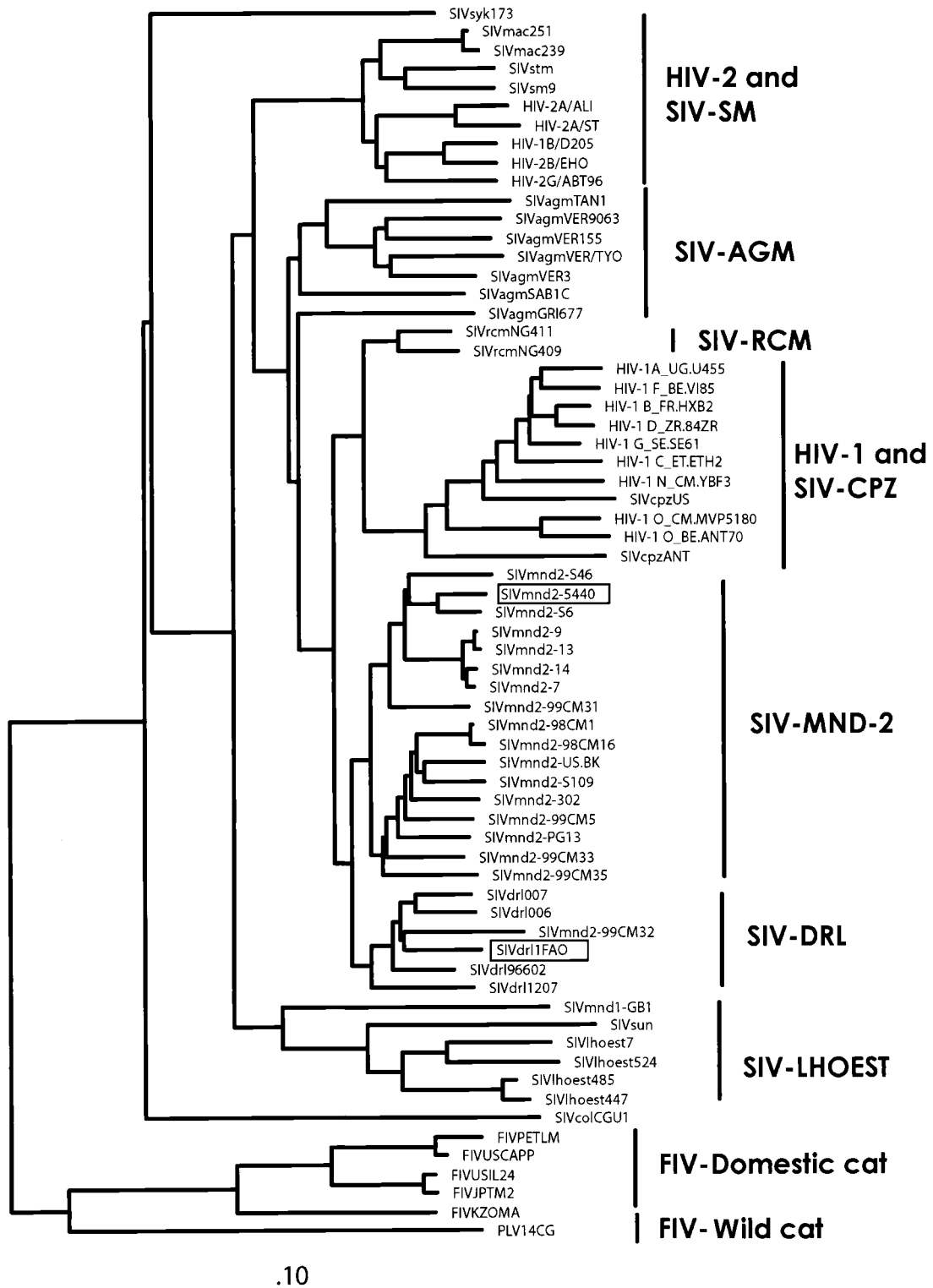


FIG. 3. Phylogenetic trees of PLV full-length genomes. (A) Unrooted phylogenetic tree (maximum likelihood with site-specific substitution rates) of a conserved fragment of the *pol* gene, including many of the available SIVmnd and SIVdrl sequences. The alignment was 397 nucleotides after gap stripping. SIVdrl1FAO and SIVmnd5440 are boxed. (B and C) The primate lentiviral genomes were aligned as described in Materials and Methods, third-codon positions were removed, and columns containing gaps were stripped from the alignment. (B) Representative tree (maximum likelihood with site-specific substitution rates) from the 5' part of the genome, spanning bases 651 to 1600 of the alignment. (C) Representative tree (maximum likelihood with site-specific substitution rates) from the 3' part of the genome, spanning bases 3001 to 4001 of the alignment. The sequences used are all available at <http://hiv-web.lanl.gov> by using either the common names or accession numbers (see Materials and Methods). The tree is unrooted. The scale shows the number of substitutions per site.



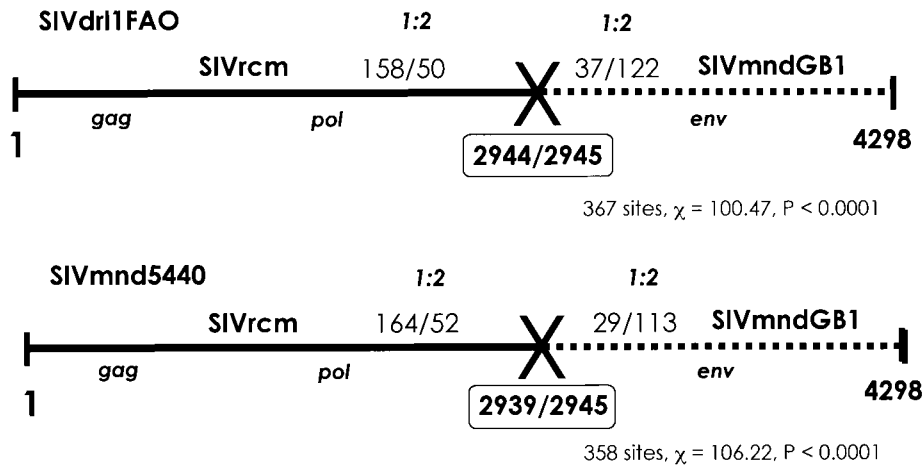


FIG. 4. Informative-site analysis. The recombination breakpoint in SIVdr11FAO and SIVmnd5440 at which the closest relationship changed from SIVrcm to SIVmndGB1 was determined by informative-site analysis of first- and second-codon positions of a nucleotide alignment of SIVdr11FAO or SIVmnd5440, SIVrcm, SIVmndGB1, and SIVsyk (outgroup) by surveying the informative sites supporting either of the two rooted phylogenies and maximizing a chi-square value to locate the optimal breakpoint location (33, 42). Statistical significance was assessed by performing 10,000 permutations and for each maximizing a chi-square value in order to again locate the optimal breakpoint. The *P* value reflects the proportion of permuted informative sites with chi-square values greater than the observed value. Total of 367 and 358 informative sites in SIVdr11FAO and SIVmnd5440, respectively, were considered. A highly significant breakpoint was found between nt 2944 and 2945 for SIVdr11FAO and between nt 2939 and 2945 for SIVmnd5440. Both breakpoints were located at the beginning of the envelope.

to SIVrcm in the 5' part of the genome and to SIVmndGB1 in the 3' part of the genome. The relationship of SIVdr11FAO and SIVmnd5440 to SIVrcm from the Gag to the Tat protein and to SIVmndGB1 in Env and Nef and the overall identity to SIVmndM14 across the entire genome indicated that both novel SIV isolates might have been generated by recombination.

In order to more thoroughly investigate the relationship of SIVdr11FAO and SIVmnd5440 to other PLVs and to rule out evidence of recombination, similarity plots were constructed. As shown in Fig. 2A, SIVdr11FAO was compared to partial *gag-pol* sequences of SIVdr1/Clewley, SIVmnd5440, SIVmndM14, SIVmndGB1, and SIVrcm. As expected from the amino acid identities, SIVdr11FAO was most closely related to SIVdr1/Clewley in the *gag-pol* region. The relationship between the two SIVdr1 strains appeared to be similar to the relationship between SIVmnd5440 and SIVmndM14.

We also constructed similarity plots to compare SIVdr11FAO and SIVmnd5440 to full-length genomes of SIV. As shown in Fig. 2B, SIVdr11FAO was most closely related to SIVmnd5440 and SIVmndM14 across the entire genome, indicating a common origin of both viruses. As already indicated by the protein identities, SIVdr11FAO and SIVmnd5440 were most closely related to SIVrcm in *gag*, *pol*, *vif*, *vpx*, and *vpr*, whereas in *env* they were most closely related to SIVmndGB1 (Fig. 2B and C). This observation confirmed that the genomes of SIVdr11FAO and SIVmnd5440 were recombinant.

A phylogenetic tree of a small *pol* fragment (Fig. 3A) demonstrated that SIVmnd type 2 and SIVdr1 sequences formed monophyletic clusters, with the exception of SIVmnd2-99CM32 which fell in the SIVdr1 cluster. Since the host of SIVmnd2-99CM32 was a wild-born pet monkey genetically confirmed to be a mandrill by mitochondrial sequencing (50), some cross-species transmission of SIVdr1 to a mandrill must have been occurred in the past. This is particularly interesting,

because the habitats of drills and mandrills are located north and south of the Sanaga River, respectively, and do not overlap. Therefore, transmission of SIV between drill and mandrill is an unexpected finding.

In order to examine individual regions of the entire genome, phylogenetic trees were constructed by using the maximum-likelihood method (Fig. 3B and C). The third-codon positions of the alignment were removed, and the alignment was gap stripped. Since phylogenetic trees spanning recombinant sequences are prohibited, the 4,411-bp remaining nucleotide alignment was divided into seven regions that did not contain any obvious recombination breakpoints in any of the aligned sequences (6). Two representative trees, one from the 5' part and one from the 3' part of the genome, are shown in Fig. 3B and C, respectively. SIVdr11FAO, SIVmnd5440, and SIVmndM14 clustered together in all the phylogenetic trees built, and this relationship was supported in 100% of bootstrap replicates. As indicated by the short branch lengths, SIVmnd5440 and SIVmndM14 were extremely closely related and SIVdr11FAO was more distantly related. In the 5' tree, SIVmnd5440, SIVmndM14, and SIVdr11FAO formed a cluster with SIVrcm. In the 3' tree, however, SIVmnd5440, SIVmndM14, and SIVdr11FAO grouped together with SIVmndGB1 and therefore clustered with the SIVIhoest lineage. This discordant topology was again indicative of recombination between an ancestor of SIVrcm and of SIVmndGB1 (type 1). Phylogenetic trees including third-codon positions in the alignment showed the same topology as trees without third-codon positions.

Finally, in order to accurately delineate the recombination point where the relative relationship of SIVdr11FAO or SIVmnd5440 to SIVrcm and SIVmndGB1 changed, we performed informative-site analysis and permutation tests (Fig. 4) (42). An informative site is one at which, in a four-sequence



Consensus	<b>Mae?r--er? P??p?Gag?v -e???WL?r? le?iNreA?l HfhpEfiFrl W?tc?ehwHD</b>
SIVmac251	.SDPR--ERI .PGNS.EETI GEAFE..NRT VEEI.RE.VN .LPR.LI.QV .QRSWEYW..
HIV2/Ben	.TDPR--ERV .PGNS.EETI GEAFE..ERT IEAL.RE.VN .LPR.LI.QV .QRSWRVW..
SIVsmPBj6.6	.SDPR--ERI .PGNS.EETI GEAFD..DRT VEEI.RA.VN .LPR.LI.QV .RRSWEYW..
SIVrcmNG411	.AEGR--ERV .EAPT.AGVD -EFAP..HRM LTEV.LE.RL .FHP.FI.RL .RTCVEHW..
SIVmndM14	.AER-----A .EAPQ.AGEV -GLEQ..ERS LEQV.RE.QL .FHP.FL.RL .NTCVEHW..
SIVmnd5440	.AER-----A .EAPE.AGEV -GLEQ..ETS LERI.RE.RL .FHP.FL.RL .NTCVEHW..
SIVdr11FAO	.AERQSVERA .AEPM.AGEV -ELEE..QRS LLRI.QE.RL .FHP.FL.RL .NTCMEHY.. 60
Consensus	??g?s??Y?k <b>YRYLlLmqKA</b> mf?H?q?GC? c??g???--- ??G?r?--p pppppPglA-
SIVmac251	EQGMSQS.VK .....C.MQ.. LFM.CKK..R CLGEGHG--- -AG.WRP-GP PPPPP.GLAX
HIV2/Ben	EQGMSAS.TK .....C.MQ.. IFT.FKR..T CWGEDMG--- -RE.LEDQGP PPPPP.GLVX
SIVsmPBj6.6	EMGMSVS.TK .....C.IQ.. MFM.CKK..R CLGGEHG--- -AG.WRP-GP PPPPP.GLA-
SIVrcmNG411	RLGRSLE.AG .....L.MQ.. LFI.CQS..S QRHGQG--QA REA.ER----- -IQIL.GM--
SIVmndM14	RLRRTL.N.AK .....L.MQ.. MFV.MQQ..P CRSG----- HSR.----- --PPP.GLA-
SIVmnd5440	RHQRSLD.AK .....L.MH.. MYT.MQQ..P CRNG----- RPR.----- --PPP.GMA-
SIVdr11FAO	ALQLSFT.SK .....L.LQ.. MFM.FQQ..S CLQGRHPPPL RPA.DRL--P PPPPP.-LVX 120

FIG. 5. Alignment of Vpx proteins from SIVmac251, HIV2/Ben, SIVsmPBj6.6, SIVrcmNG411, SIVmndM14, SIVmnd5440, and SIVdr11FAO. The consensus is shown at the top. Dots indicate amino acid identity, and dashes indicate gaps introduced to optimize the alignment. Twenty-six conserved amino acids in the consensus sequence are in boldface and underlined.

alignment, two sequences share one residue and the two others share another residue. The four-sequence alignment consisted of the potentially recombinant sequence (SIVdr11FAO or SIVmnd5440, respectively), the two parental representative sequences (SIVrcm and SIVmndGB1), and an outgroup (SIVsyk). We considered only informative sites of first- and second-codon positions, since the majority of changes in the third positions are usually saturated in divergent sequences and do not contribute any reliable phylogenetic information. A total of 367 informative sites in SIVdr11FAO and 358 sites in SIVmnd5440 were considered, and statistically significant breakpoints at the beginning of the envelope were observed for both SIVdr11FAO (between nucleotides [nt] 2944 and 2945 of the alignment) and SIVmnd5440 (between nt 2939 and 2945). These data suggest with high probability that SIVdr11FAO and SIVmnd5440 are descendents of the same recombinant.

**Phylogenetic relationship and origin of the vpx gene.** There are now several highly divergent SIV and HIV-2 strains that contain a vpx gene in addition to vpr. Previous studies have shown that the vpx and vpr genes of HIV-2, SIVsm, and SIVmac are phylogenetically related (46, 52). Recently, we described a novel PLV, SIVrcm, with a highly divergent vpx gene; it was only 42% identical to the amino acid level to SIVsm Vpx. As shown in Table 2, SIVdr11FAO Vpx was 70% related to SIVmnd5440 and SIVmndM14 Vpx and 57% related to SIVrcm Vpx. Figure 5 depicts an alignment of the major representatives of Vpx protein sequences, with the consensus sequence shown on top. Although the majority of the amino acids differ from each other, there was a highly conserved tyrosine motif in the central part of Vpx (Y-x-x-Y-R-Y-L-x-L). In addition, as already reported previously (32), most of the Vpx sequences, except that of SIVrcm, have a proline stretch at the C terminus.

In order to determine the phylogenetic relationships, we included all of the vpx sequences in a maximum-likelihood tree with published representatives of vpr sequences to investigate the origins of the vpx genes (Fig. 6). Despite being highly divergent, the vpx sequences formed a monophyletic cluster relative to vpr sequences. This supports a common origin of

vpx genes found in the different vpx-bearing SIV strains and HIV-2, rather than the alternative that multiple vpr genes ultimately gave rise to the diverse forms of vpx. The SIVdr11FAO/SIVmnd5440 vpx was more closely related to SIVrcm vpx than to SIVsm/HIV-2 vpx. As previously reported (46), the vpx gene cluster was phylogenetically most closely related to SIVagm vpr and did not closely group with SIVsm/HIV-2 vpr or with SIVrcm, SIVmnd, or SIVdr11FAO vpr.

**SIVdr11FAO and SIVmnd5440 replicate in human PBMC and human T-cell lines but not in chimpanzee PBMC.** The replication potentials of SIVdr11FAO and SIVmnd5440 were tested in a panel of human T-cell lines and human or chimpanzee PBMC. The human CD4<sup>+</sup> cell lines included CEM-SS, CEMx174, C8166, H9, Hut-78, MT-2, MT-4, Molt4clone8, PM1, SupT1, and U937. The in vitro host ranges of SIVdr11FAO and SIVmnd5440 are shown in Table 4. Human PBMC supported replication of both SIVdr11FAO and SIVmnd5440 with similar replication kinetics (Fig. 7A; Table 4). However, SIVdr11FAO and SIVmnd5440, like SIVrcm, did

TABLE 4. Host ranges of SIVdr11FAO and SIVmnd5440 in different cell lines

PBMC or cell line	RT activity of:	
	SIVdr11FAO	SIVmnd5440
CEMx174	+	+
CEMss	+	-
C8166	-	-
H9	-	-
Hut78	-	-
MT-2	-	-
MT-4	+	-
Molt4 clone8	+	+
PM1	-	-
SupT1	+	+
U937	-	-
PBMC		
Pig-tailed macaque	+	+
Chimpanzee	-	-
Human	+	+

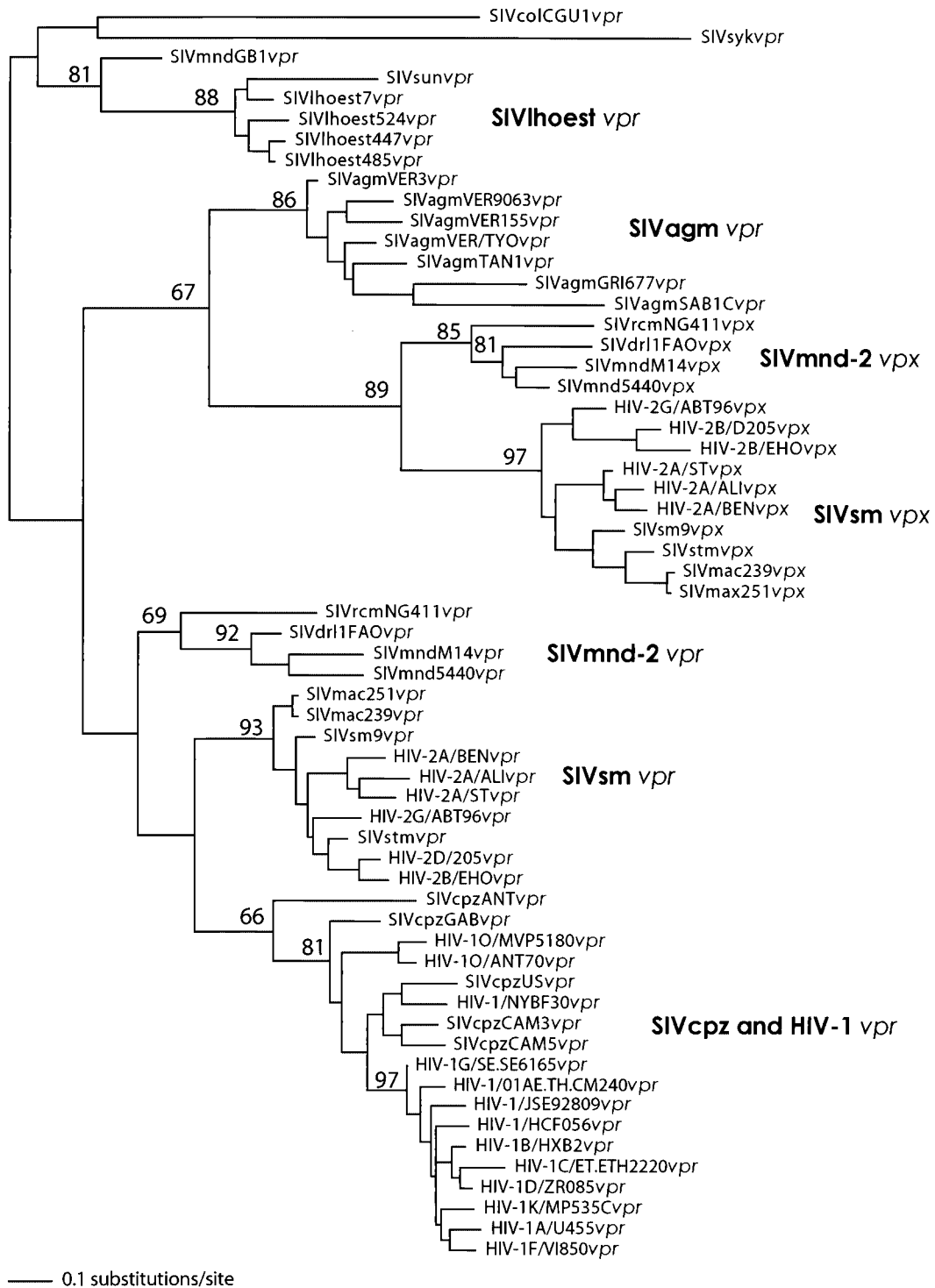


FIG. 6. Phylogenetic analysis of *vpx* and *vpr* genes. A maximum-likelihood tree (Hasegawa-Kishino-Yano 1985 nucleotide substitution model [20a], codon position site-specific rates, tree bisection-reconnection branch swapping) was built from a *vpx* and *vpr* codon-based sequence alignment of 179 nucleotides (after excluding gaps and ambiguous nucleotides). The sequences used are all available from <http://hiv-web.lanl.gov>. Bootstrap support (100 replicates) was calculated by using the PHYLIP SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE programs. The tree is unrooted. The different lineages are annotated in boldface.

**A. Human PBMC**

**B. Chimpanzee PBMC**

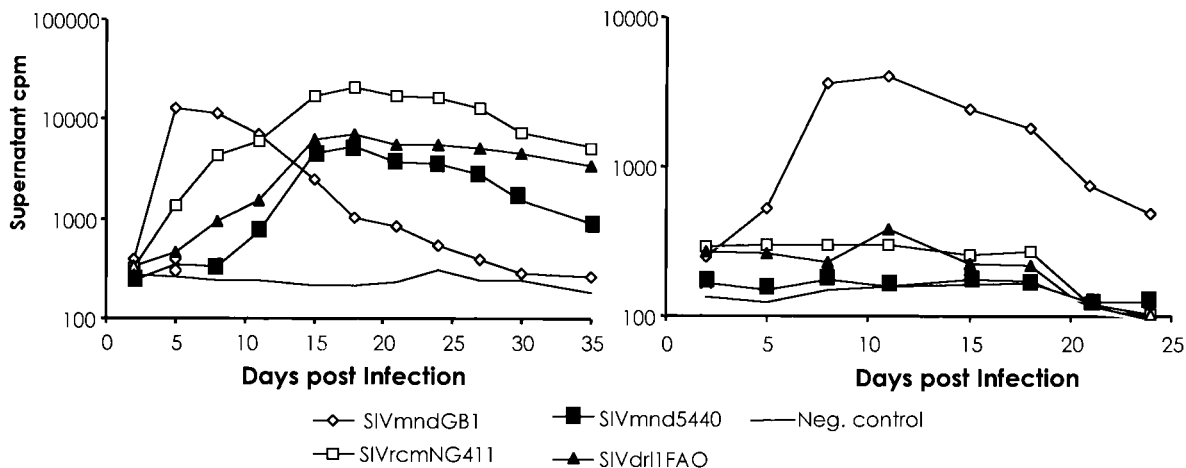


FIG. 7. Comparative replication kinetics of SIVdr11FAO and SIVmnd5440 in human and chimpanzee PBMC. PHA-stimulated human PBMC (A) and chimpanzee PBMC (B) were infected with equivalent amounts of virus (measured by RT as described in Material and Methods). Cell supernatants were collected at 3-day intervals and tested for RT activity. SIVrcmNG411 and SIVmndGB1 were used as positive controls for the replication in human and chimpanzee PBMC, respectively.

not replicate in chimpanzee PBMC (Fig. 7B). SIVmndGB1 served as positive control, since it has been shown previously to replicate to high titers in chimpanzee PBMC (6). SIVdr11FAO also replicated in the CEMx174, CEM-SS, MT-4, Molt4clone8, and SupT1 cell lines whereas SIVmnd5440 replicated in CEMx174, Molt4clone8, and SupT1 cells (Table 4). No cytopathic effect was observed following infection of the susceptible cell lines or PBMC.

**The coreceptor usage of SIVdr11FAO differs from those of SIVrcm and SIVmndGB1.** The coreceptor usage of SIVdr11FAO was determined by using the GHOST cell assay. The GHOST cell lines constitute of a HIV-1-HIV-2-SIV in-

dicator cell panel whose individual lines express a specific viral coreceptor molecule in conjunction with human CD4 (34). The coreceptor molecules included CCR1, CCR2B, CCR3, CCR4, CCR5, CCR8, CXCR4, GPR15 (BOB), and CXCR6 (STRL33, Bonzo). The production of SIV Gag p27 antigen in the supernatants collected at different time points post infection was measured. Figure 8 illustrates the time course of production of p27 antigen in the panel of GHOST cell lines. SIVdr11FAO used CCR5, CXCR6 (STRL33, Bonzo), and GPR15 (BOB) as coreceptors for viral entry. This contrasted to the coreceptor usage of CCR2B and CXCR6 (STRL33, Bonzo) of SIVrcm and CXCR4 of SIVmndGB1 (6, 8, 43).

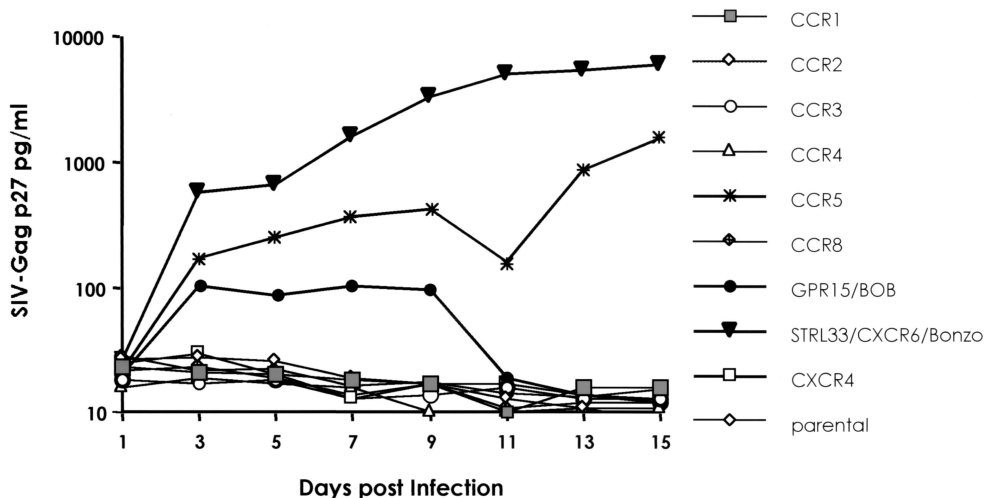


FIG. 8. Evaluation of coreceptor usage of SIVdr11FAO in the GHOST cell assay. Human osteosarcoma cells transfected with HIV-2 long terminal repeat-green fluorescent protein, CD4, and different HIV coreceptors were infected with SIV or HIV in the presence of Polybrene as indicated. The course of infection of GHOST cell lines was monitored by SIV Gag p27 antigen measurements in culture supernatants with the RETRO-TEK SIV-1 p27 antigen enzyme-linked immunosorbent assay (ZeptoMetrix Corp.) according to the manufacturer's instructions.

## DISCUSSION

Recent studies (48, 50) have demonstrated that there are two different forms of SIV circulating in wild mandrills in Africa: SIVmnd type 1, represented by SIVmndGB1, and SIVmnd type 2, represented by SIVmndM14. SIVmnd type 1 clustered with SIVlhoest and SIVsun, whereas SIVmnd type 2 was related to SIVrcm in *gag* and *pol* and to SIVlhoest/sun in *env* and *nef* and carried a *vpx* gene in addition to *vpr*. In the present study, we showed that SIV from drill was most closely related to the recently described SIVmnd type 2 and also carried a *vpx* gene. In addition, we found a novel SIV from mandrill that was more closely related to SIVmndM14 than SIVdr11FAO and therefore represented another SIVmnd type 2 isolate. Partial sequencing of a small portion of *pol* of two other mandrill isolates indicated that these isolates were also representatives of SIVmnd type 2.

Earlier phylogenetic analyses of SIVmndM14 were suggestive of recombination between SIVrcm and SIVmnd type 2, with the breakpoint estimated to be located in the *vpr-tat* region (48). Since our full-length sequence of SIVdr11FAO was related to SIVmndM14, we wished to determine whether (i) SIVdr1 was also recombinant between SIVrcm and SIVmnd type 1 and (ii) SIVdr1 and SIVmnd type 2 were generated by the same recombination event. By applying informative-site analysis, a highly significant breakpoint was observed at the same location of the 5' part of the envelope in SIVdr11FAO and SIVmnd5440 (Fig. 4). These results suggested that SIVdr11FAO and SIVmnd5440 are descendents of the same recombinant. Either drills or mandrills may have been the original host for this type of virus, with the virus subsequently crossing the species barrier at some time in the past. Alternatively, the recombinant form could have been present in an ancestor of drill and mandrill. This scenario is highly unlikely, since SIVmnd type 2 is present only in mandrills in their northern habitat (south of the Sanaga river in Cameroon and north of the Ogooue river in Gabon) and not in mandrills in their southern habitat (south of the Ogooue river in Gabon). Interestingly, the habitat of SIVmnd type 2-infected mandrills is adjacent to but not overlapping with the drill habitat. An isolated population of drills also lives offshore on Bioko island (for drill and mandrill geographic distributions, see Fig. 1 in reference 48). The relationship between SIVdr11FAO from a captive drill and previously reported SIVdr1 sequences from wild-caught drills (11, 48) indicated that SIVdr1 occurs naturally in drill populations in the wild. Since the habitats of drill and mandrill are not sympatric, it is not clear how the cross-species transmission between mandrills and drills occurred. However, the present distribution of primates in Africa is likely to differ from that at the time when the recombination event occurred. The presence of recombinant forms in different primate species indicates not only that cross-species transmission occurs in wild populations but also that these events are not necessarily dead ends, since the transmitted forms can spread within the new host population. In addition, the unexpected finding of a SIVdr1 like sequence (SIVmnd2-99CM32) in a mandrill supports the possibility of viral transmission between both species.

Previously we demonstrated that SIV from red-capped mangabeys carried a highly divergent *vpx* gene, which was only

42% related to SIVsm *vpx* at the amino acid level (6). SIVdr11FAO and SIVmnd5440 were genetically related to SIVrcm and also carried a *vpx* gene. Therefore, we were interested in investigating the phylogenetic relationships of the *vpx* and *vpr* genes. An alignment of all available Vpx protein sequences revealed a highly conserved tyrosine motif (Y-x-x-Y-R-Y-L-x-L), which is shared with the human acetyl coenzyme A carboxylase 1 (Swiss-Prot accession no. Q13085). As recently discussed (32), most Vpx sequences, except that of SIVrcm, have a proline stretch at the C terminus. Proline-rich sequences have been shown to provide binding sites for SH3 domains. However, since SIVrcm is lacking the proline stretch, it seems to be dispensable for Vpx function.

Phylogenetic analysis of *vpx* and another accessory gene, *vpr*, showed that *vpx* from SIVrcm/drl/mnd-2 and *vpx* from SIVsm/HIV-2 were phylogenetically related despite being highly divergent. The expanded tree with numerous *vpx* and *vpr* sequences also confirmed the earlier observation of Sharp et al. (46) that the *vpx* gene is phylogenetically related to SIVagm *vpr* and not to SIVsm or SIVrcm *vpr*. Therefore, the presence of the extra gene most probably originated by nonhomologous recombination rather than by gene duplication as proposed by Tristem et al. (52, 53).

In terms of biological properties, SIVdr11FAO and SIVmnd5440 replicated efficiently in human PBMC, which could allow the virus to establish persistent infections in humans. Indeed, epidemiological surveys revealed a case in Cameroon of a human infected by a virus serologically related to SIVmnd. This observation suggested that mandrills may represent a viral reservoir for humans, similar to sooty mangabeys in western Africa and chimpanzees in central Africa (48). Like SIVrcm, SIVdr11FAO and SIVmnd5440 were not able to infect chimpanzee PBMC in our studies (6). Since SIVmnd type 1 (GB1) was able to replicate in chimpanzee PBMC, SIVdr11FAO and SIVmnd5440 were more similar to SIVrcm in terms of their host range than to SIVmnd type 1, underlining the importance of the *gag* and *pol* genes in establishing productive infections (47).

In summary, we isolated SIV from one drill and three mandrills and sequenced the full-length genomes of SIVdr11FAO and SIVmnd5440. Both isolates were genetically most closely related to SIVmndM14, the prototype of SIVmnd type 2. Phylogenetic and informative-site analyses showed that both SIVdr1 and SIVmnd5440 were recombinant between ancestors of SIVrcm and SIVmnd type 1, with the recombination point located at the beginning of envelope. These data suggested with high probability that SIVdr1 and SIVmnd type 2 were generated by the same recombination event. Finally, evaluation of the biological properties revealed that SIVdr1 and SIVmnd5440 were able to replicate in human but not chimpanzee PBMC, indicating a potential risk of transmission upon contact between humans and infected primate specimens.

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## REFERENCES

- Allan, J. S., M. Short, M. E. Taylor, S. Su, V. M. Hirsch, P. R. Johnson, G. M. Shaw, and B. H. Hahn. 1991. Species-specific diversity among simian immunodeficiency viruses from African green monkeys. *J. Virol.* **65**:2816–2828.
- Baier, M., A. Werner, K. Cichutek, C. Garber, C. Mueller, G. Kraus, F. J. Ferdinand, S. Hartung, T. S. Papas, and R. Kurth. 1989. Molecularly cloned simian immunodeficiency virus SIVagm3 is highly divergent from other SIVagm isolates and is biologically active in vitro and in vivo. *J. Virol.* **63**:5119–5123.
- Beer, B. E., E. Bailes, G. Dapolito, B. J. Campbell, R. M. Goeken, M. K. Axthelm, P. D. Markham, J. Bernard, D. Zagury, G. Franchini, P. M. Sharp, and V. M. Hirsch. 2000. Patterns of genomic sequence diversity among their simian immunodeficiency viruses suggest that L'Hoest monkeys (*Cercopithecus lhoesti*) are a natural lentivirus reservoir. *J. Virol.* **74**:3892–3898.
- Beer, B. E., E. Bailes, R. Goeken, G. Dapolito, C. Coulibaly, S. G. Norley, R. Kurth, J. P. Gautier, A. Gautier-Hion, D. Vallet, P. M. Sharp, and V. M. Hirsch. 1999. Simian immunodeficiency virus (SIV) from sun-tailed monkeys (*Cercopithecus solatus*): evidence for host-dependent evolution of SIV within the *C. lhoesti* superspecies. *J. Virol.* **73**:7734–7744.
- Beer, B. E., E. Bailes, P. M. Sharp, and V. M. Hirsch. 1999. Diversity and evolution of primate lentiviruses, p. 460–474. In C. Kuiken, B. Foley, H. Hahn, P. Marx, et al. (ed.), *Human retroviruses and AIDS*. Los Alamos National Laboratory, Los Alamos, N.Mex.
- Beer, B. E., B. T. Foley, C. L. Kuiken, Z. Tooze, R. M. Goeken, C. R. Brown, J. Hu, M. St Claire, B. T. Korber, and V. M. Hirsch. 2001. Characterization of novel simian immunodeficiency viruses from red-capped mangabeys from Nigeria (SIVrcmNG409 and -NG411). *J. Virol.* **75**:12014–12027.
- Bibollet-Ruche, F., A. Galat-Luong, G. Cuny, P. Sarni-Manchado, G. Galat, J. P. Durand, X. Pourrut, and F. Veas. 1996. Simian immunodeficiency virus infection in a patas monkey (*Erythrocebus patas*): evidence for cross-species transmission from African green monkeys (*Cercopithecus aethiops sabaeus*) in the wild. *J. Gen. Virol.* **77**:773–781.
- Chen, Z., D. Kwon, Z. Jin, S. Monard, P. Telfer, M. S. Jones, C. Y. Lu, R. F. Aguilar, D. D. Ho, and P. A. Marx. 1998. Natural infection of a homozygous delta24 CCR5 red-capped mangabey with an R2b-tropic simian immunodeficiency virus. *J. Exp. Med.* **188**:2057–2065.
- Chen, Z., P. Telfer, A. Gettie, P. Reed, L. Zhang, D. D. Ho, and P. A. Marx. 1996. Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J. Virol.* **70**:3617–3627.
- Chen, Z., P. Telfer, P. Reed, L. Zhang, A. Gettie, D. D. Ho, and P. A. Marx. 1995. Isolation and characterization of the first simian immunodeficiency virus from a feral sooty mangabey (*Cercocebus atys*) in West Africa. *J. Med. Primatol.* **24**:108–115.
- Clewley, J. P., J. C. M. Lewis, D. W. G. Brown, and E. L. Gadsby. 1998. A novel simian immunodeficiency virus (SIVdr1) pol sequence from the drill monkey, *Mandrillus leucophaeus*. *J. Virol.* **72**:10305–10309.
- Courgnaud, V., X. Pourrut, F. Bibollet-Ruche, E. Mpoudi-Ngole, A. Bourgeois, E. Delaporte, and M. Peeters. 2001. Characterization of a novel simian immunodeficiency virus from Guereza Colobus monkeys (*Colobus guereza*) in Cameroon: a new lineage in the nonhuman primate lentivirus family. *J. Virol.* **75**:857–866.
- Courgnaud, V., M. Salemi, X. Pourrut, E. Mpoudi-Ngole, B. Abela, P. Auzel, F. Bibollet-Ruche, B. Hahn, A. M. Vandamme, E. Delaporte, and M. Peeters. 2002. Characterization of a novel simian immunodeficiency virus with a *vpu* gene from greater spot-nosed monkeys (*Cercopithecus nictitans*) provides new insights into simian/human immunodeficiency virus phylogeny. *J. Virol.* **76**:8298–8309.
- Daniel, M. D., Y. Li, Y. M. Naidu, P. J. Durda, D. K. Schmidt, C. D. Troup, D. P. Silva, J. J. MacKey, H. W. Kestler, P. K. Sehgal, et al. 1988. Simian immunodeficiency virus from African green monkeys. *J. Virol.* **62**:4123–4128.
- Fomsgaard, A., V. M. Hirsch, J. S. Allan, and P. R. Johnson. 1991. A highly divergent proviral DNA clone of SIV from a distinct species of African green monkey. *Virology* **182**:397–402.
- Fukasawa, M., T. Miura, A. Hasegawa, S. Morikawa, H. Tsujimoto, K. Miki, T. Kitamura, and M. Hayami. 1988. Sequence of simian immunodeficiency virus from African green monkey, a new member of the HIV/SIV group. *Nature* **333**:457–461.
- Gao, F., E. Bailes, D. L. Robertson, Y. Chen, C. M. Rodenburg, S. F. Michael, L. B. Cummins, L. O. Arthur, M. Peeters, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1999. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* **397**:436–441.
- Georges-Courbot, M. C., C. Y. Lu, M. Makuwa, P. Telfer, R. Onanga, G. Dubreuil, Z. Chen, S. M. Smith, A. Georges, F. Gao, B. H. Hahn, and P. A. Marx. 1998. Natural infection of a household pet red-capped mangabey (*Cercocebus torquatus torquatus*) with a new simian immunodeficiency virus. *J. Virol.* **72**:600–608.
- Grimm, T. A., B. E. Beer, V. M. Hirsch, and K. A. Clouse. Simian immunodeficiency viruses from multiple lineages infect human macrophages: implications for cross-species transmission. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.*, in press.
- Hahn, B. H., G. M. Shaw, K. De Cock, and P. M. Sharp. 2000. AIDS as a zoonosis: scientific and public health implications. *Science* **287**:607–614.
- Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**:160–174.
- Henderson, L. E., R. E. Benveniste, R. Sowder, T. D. Copeland, A. M. Schultz, and S. Oroszlan. 1988. Molecular characterization of Gag proteins from simian immunodeficiency virus (SIVMne). *J. Virol.* **62**:2587–2595.
- Hirsch, V. M., B. J. Campbell, E. Bailes, R. Goeken, C. Brown, W. R. Elkins, M. Axthelm, M. Murphey-Corb, and P. M. Sharp. 1999. Characterization of a novel simian immunodeficiency virus (SIV) from L'Hoest monkeys (*Cercopithecus lhoesti*): implications for the origins of SIVmnd and other primate lentiviruses. *J. Virol.* **73**:1036–1045.
- Hirsch, V. M., C. McGann, G. Dapolito, S. Goldstein, A. Ogen-Odoi, B. Biryawaho, T. Lakwo, and P. R. Johnson. 1993. Identification of a new subgroup of SIVagm in tantalus monkeys. *Virology* **197**:426–430.
- Hirsch, V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell, and P. R. Johnson. 1989. An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* **339**:389–392.
- Huet, T., R. Cheyner, A. Meyerhans, G. Roelants, and S. Wain-Hobson. 1990. Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature* **345**:356–359.
- Janssens, W., K. Franssen, M. Peeters, L. Heyndrickx, J. Motte, L. Bedjabaga, E. Delaporte, P. Piot, and G. van der Groen. 1994. Phylogenetic analysis of a new chimpanzee lentivirus SIVcpz-gab2 from a wild-captured chimpanzee from Gabon. *AIDS Res. Hum. Retroviruses* **10**:1191–1192.
- Jin, M. J., H. Hui, D. L. Robertson, M. C. Mueller, F. Barre-Sinoussi, V. M. Hirsch, J. S. Allan, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1994. Mosaic genome structure of simian immunodeficiency virus from west African green monkeys. *EMBO J.* **13**:2935–2947.
- Jin, M. J., J. Rogers, J. E. Phillips-Conroy, J. S. Allan, R. C. Desrosiers, G. M. Shaw, P. M. Sharp, and B. H. Hahn. 1994. Infection of a yellow baboon with simian immunodeficiency virus from African green monkeys: evidence for cross-species transmission in the wild. *J. Virol.* **68**:8454–8460.
- Johnson, P. R., A. Fomsgaard, J. S. Allan, M. Gravell, W. T. London, R. A. Olmsted, and V. M. Hirsch. 1990. Simian immunodeficiency viruses from African green monkeys display unusual genetic diversity. *J. Virol.* **64**:1086–1092.
- Kuiken, C. L., B. Foley, B. Hahn, B. T. Korber, F. McCutchan, P. A. Marx, J. W. Mellors, J. I. Mullins, J. Sodroski, and S. Wolinsky. 2000. A compilation and analysis of nucleic acid and amino acid sequences. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, N.Mex.
- Lowenstine, L. J., N. C. Pedersen, J. Higgins, K. C. Pallis, A. Uyeda, P. Marx, N. W. Lerche, R. J. Munn, and M. B. Gardner. 1986. Seroepidemiologic survey of captive Old-World primates for antibodies to human and simian retroviruses, and isolation of a lentivirus from sooty mangabeys (*Cercocebus atys*). *Int. J. Cancer* **38**:563–574.
- Mahalingam, S., B. Van Tine, M. L. Santiago, F. Gao, G. M. Shaw, and B. H. Hahn. 2001. Functional analysis of the simian immunodeficiency virus Vpx protein: identification of packaging determinants and a novel nuclear targeting domain. *J. Virol.* **75**:362–374.
- Maynard Smith, J. 1992. Analysing the mosaic structure of genes. *J. Mol. Evol.* **34**:126–129.
- Morner, A., A. Bjorndal, J. Albert, V. N. Kewalramani, D. R. Littman, R. Inoue, R. Thorstenson, E. M. Fenyo, and E. Bjorling. 1999. Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J. Virol.* **73**:2343–2349.
- Müller, M. C., N. K. Saksena, E. Nerrienet, C. Chappay, V. M. Herve, J. P. Durand, P. Legal-Campodónico, M. C. Lang, J. P. Digoutte, A. J. Georges, et al. 1993. Simian immunodeficiency viruses from central and western Africa: evidence for a new species-specific lentivirus in tantalus monkeys. *J. Virol.* **67**:1227–1235.
- Novembre, F. J., V. M. Hirsch, H. M. McClure, P. N. Fultz, and P. R. Johnson. 1992. SIV from stump-tailed macaques: molecular characterization of a highly transmissible primate lentivirus. *Virology* **186**:783–787.
- Ohta, Y., T. Masuda, H. Tsujimoto, K. Ishikawa, T. Kodama, S. Morikawa, M. Nakai, S. Honjo, and M. Hayami. 1988. Isolation of simian immunodeficiency virus from African green monkeys and seroepidemiologic survey of the virus in various non-human primates. *Int. J. Cancer* **41**:115–122.
- Osterhaus, A. D., N. Pedersen, G. van Amerongen, M. T. Frankenhuis, M. Marthas, E. Reay, T. M. Rose, J. Pamungkas, and M. L. Bosch. 1999. Isolation and partial characterization of a lentivirus from talapoin monkeys (*Myopithecus talapoin*). *Virology* **260**:116–124.
- Peeters, M., V. Courgnaud, B. Abela, P. Auzel, X. Pourrut, F. Bibollet-Ruche, S. Louf, F. Liegeois, C. Butel, D. Koulagna, E. Mpoudi-Ngole, G. M. Shaw, B. H. Hahn, and E. Delaporte. 2002. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. *Emerg. Infect. Dis.* **8**:451–457.

40. Peeters, M., K. Fransen, E. Delaporte, M. Vanden Haesevelde, G. M. Gershy-Damet, L. Kestens, G. van der Groen, and P. Piot. 1992. Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee. *AIDS* **6**:447–451.
41. Peeters, M., C. Honore, T. Huet, L. Bedjabaga, S. Ossari, P. Bussi, R. W. Cooper, and E. Delaporte. 1989. Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. *AIDS* **3**:625–630.
42. Robertson, D. L., P. M. Sharp, F. E. McCutchan, and B. H. Hahn. 1995. Recombination in HIV-1. *Nature* **374**:124–126.
43. Schols, D., and E. De Clercq. 1998. The simian immunodeficiency virus mnd(GB-1) strain uses CXCR4, not CCR5, as coreceptor for entry in human cells. *J. Gen. Virol.* **79**:2203–2205.
44. Sharp, P. M., E. Bailes, F. Gao, B. E. Beer, V. M. Hirsch, and B. H. Hahn. 2000. Origins and evolution of AIDS viruses: estimating the time-scale. *Biochem. Soc. Trans.* **28**:275–282.
45. Sharp, P. M., E. Bailes, D. L. Robertson, F. Gao, and B. H. Hahn. 1999. Origins and evolution of AIDS viruses. *Biol. Bull.* **196**:338–342.
46. Sharp, P. M., E. Bailes, M. Stevenson, M. Emerman, and B. H. Hahn. 1996. Gene acquisition in HIV and SIV. *Nature* **383**:586–587.
47. Shimano, R., R. Inubushi, K. Amano, T. Ogasawa, and A. Adachi. 1998. Gag-Pol region determines the tropism of SIVagm for human cells. *Virus Genes* **16**:137–139.
48. Souquiere, S., F. Bibollet-Ruche, D. L. Robertson, M. Makuwa, C. Apetrei, R. Onanga, C. Kornfeld, J. C. Plantier, F. Gao, K. Abernethy, L. J. White, W. Karesh, P. Telfer, E. J. Wickings, P. Maucelere, P. A. Marx, F. Barre-Sinoussi, B. H. Hahn, M. C. Muller-Trutwin, and F. Simon. 2001. Wild *Mandrillus sphinx* are carriers of two types of lentivirus. *J. Virol.* **75**:7086–7096.
49. Switzer, W. M., D. Pieniazek, P. Swanson, H. H. Samdal, V. Soriano, R. F. Khabbaz, J. E. Kaplan, R. B. Lal, and W. Heneine. 1995. Phylogenetic relationship and geographic distribution of multiple human T-cell lymphotropic virus type II subtypes. *J. Virol.* **69**:621–632.
50. Takehisa, J., Y. Harada, N. Ndombi, I. Mboudjeka, Y. Taniguchi, C. Ngansop, S. Kuate, L. Zekeng, K. Ibuki, T. Shimada, B. Bikandou, Y. Yamaguchi-Kabata, T. Miura, M. Ikeda, H. Ichimura, L. Kaptue, and M. Hayami. 2001. Natural infection of wild-born mandrills (*Mandrillus sphinx*) with two different types of simian immunodeficiency virus. *AIDS Res. Hum. Retroviruses* **17**:1143–1154.
51. Tomonaga, K., J. Katahira, M. Fukasawa, M. A. Hassan, M. Kawamura, H. Akari, T. Miura, T. Goto, M. Nakai, M. Suleman, et al. 1993. Isolation and characterization of simian immunodeficiency virus from African white-crowned mangabey monkeys (*Cercocebus torquatus lunulatus*). *Arch. Virol.* **129**:77–92.
52. Tristem, M., C. Marshall, A. Karpas, and F. Hill. 1992. Evolution of the primate lentiviruses: evidence from *vpx* and *vpr*. *EMBO J.* **11**:3405–3412.
53. Tristem, M., A. Purvis, and D. L. Quicke. 1998. Complex evolutionary history of primate lentiviral *vpr* genes. *Virology* **240**:232–237.
54. Tsujimoto, H., A. Hasegawa, N. Maki, M. Fukasawa, T. Miura, S. Speidel, R. W. Cooper, E. N. Moriyama, T. Gojobori, and M. Hayami. 1989. Sequence of a novel simian immunodeficiency virus from a wild-caught African mandrill. *Nature* **341**:539–541.
55. Vanden Haesevelde, M. M., M. Peeters, G. Jannes, W. Janssens, G. van der Groen, P. M. Sharp, and E. Saman. 1996. Sequence analysis of a highly divergent HIV-1-related lentivirus isolated from a wild captured chimpanzee. *Virology* **221**:346–350.