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Genetic Differences Accounting for Evolution and Pathogenicity of Simian Immunodeficiency Virus from a Sooty Mangabey Monkey after Cross-Species Transmission to a Pig-Tailed Macaque

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We determined the nucleotide sequences of two related isolates of simian immunodeficiency virus from the sooty mangabey monkey (SIVsmm) that exhibit dramatic differences in virulence. These isolates are separated by one experimental cross-species transmission, from sooty mangabey to pig-tailed macaque. The parental virus (SIVsmm9), nonpathogenic in the original host (sooty mangabeys), causes a chronic AIDS-like disease in macaques. In contrast, the variant virus (SIVsmmPBj14) induces an acute lethal disease in various macaque species and is also pathogenic for sooty mangabeys. The combination of necessary and sufficient mutations that determined the acutely lethal phenotype on the SIVsmm9 genetic background is included within a maximal set of 57 point mutations, plus two insertions located in the long terminal repeat (22 bp spanning an NF-κB-like enhancer element) and in the surface envelope glycoprotein (5 amino acids). Comparisons of synonymous and nonsynonymous nucleotide substitutions in the genome of SIVsmm indicated that selective pressures, probably due to the host immune response, favored amino acid changes in the envelope. This immunoevolutionary mechanism could explain the increase in diversity and the apparition of new virulent phenotypes after cross-species transmission.

In murine or feline retroviral infections, enhanced pathogenicity has been linked to major genetic changes, such as the generation of replication-defective viral genomes (for a review, see reference 30), and/or a limited number of point mutations, which were located mostly in the long terminal repeat (LTR) or envelope gene (25, 26, 28). For human immunodeficiency virus (HIV), sequence variations throughout the genome have been extensively documented (16). Mutations are generated by the high error rate of RNA-dependent polymerases involved in retrovirus replication (19, 21). While limited changes have been shown to induce significant changes in HIV tropism or biological properties in vitro (5, 23, 29), little is known about the consequences of observed genetic variability on the pathogenicity in vivo. In addition, the nature of selective pressures that might fix or eliminate particular mutations in the viral population is still unclear.

SIVsmmPBj14, an acutely lethal variant of simian immunodeficiency virus (SIV), provides an attractive model to study the interplay between primate lentivirus variability and pathogenicity in vivo. SIVsmmPBj14 was obtained after experimental transmission to a pig-tailed macaque (*Macaca nemestrina*) of SIVsmm9, which was isolated from a naturally infected asymptomatic sooty mangabey monkey (*Cercocebus atys*) (10, 11). The parental isolate (SIVsmm9), nonpathogenic in the original host (sooty mangabeys), causes a chronic AIDS-like disease in various macaque species. In contrast, the variant isolate (SIVsmmPBj14) induces a profuse mucoid diarrhea and death within 2 weeks after infection in macaques. The variant is also pathogenic for seronegative sooty mangabeys (11).

Thus, these two closely related SIV strains, separated by only one cross-species transmission in vivo, constitute a unique model for analyzing genetic changes responsible for distinct pathogenic effects. Infectious molecular clones were previously derived from SIVsmmPBj14 (7). Some of these reproduce in pig-tailed macaques the same acute disease as that induced by the original isolate (7). Sequence comparisons between SIVsmmPBj14 molecular clones and unrelated isolates from the SIVsmm-SIVmac-HIV type 2 (HIV-2) subgroup suggest that the acutely lethal phenotype could be associated with a duplication encompassing an NF-κB-like enhancer element in the LTR (7). However, no sequence data or molecular clones from the parental SIVsmm9 were available for sequence comparisons and genetic analysis.

In the present work, we analyzed nucleotide sequences of viral DNA present in cells infected with the parental virus (SIVsmm9) or the lethal variant (SIVsmmPBj14). These sequences, representing the equivalent of five complete proviral genomes, were compared to each other and to previously reported sequences of molecular clones of SIVsmmPBj14 that exhibited the lethal phenotype (7). These comparisons provided an appraisal of the number of mutations and selective pressures that accompanied the interspecies transmission: divergence between these two SIV strains was driven by positive selection for change, especially in *env*. In addition, we observed that a maximum of 57 point mutations on the SIVsmm9 genetic background, plus two insertions (22 bp in the LTR and 15 bp in *env*), were sufficient to explain the lethal SIVsmmPBj14 phenotype.

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These data will help to identify more precisely the set of genetic modifications that were necessary and sufficient to determine the acutely lethal phenotype of SIVsmmPBj14.

MATERIALS AND METHODS

Cell cultures and DNA samples. As a starting material, we used total cellular DNA from human or pig-tailed macaque peripheral blood mononuclear cells (PBMC) infected with either SIVsmm9 or SIVsmmPBj14 virions. DNA was extracted within the first week postinfection to minimize in vitro selection. SIVsmm9 virions were originally isolated from PBMC of a naturally infected asymptomatic sooty mangabey monkey in Yerkes Regional Primate Research Center (10). SIVsmmPBj14 virions were originally obtained from necropsy samples from pig-tailed macaque PBj or from a biological clone (no. 3) obtained from a limiting dilution of the original isolate that also induced the acute lethal disease in vivo (7, 11).

PCR. The polymerase chain reaction (PCR) was carried out for 40 cycles with 1 μg of total genomic DNA, 20 pmol of each primer, and 40 nmol of each deoxynucleoside triphosphate in 10 mM Tris–50 mM KCl–2 mM $MgCl_2$ (total volume, 100 μl). The cycling conditions were 94°C denaturation (1 min), 55°C annealing (1 min), and 72°C extension (1 or 2 min). All experiments included a negative control without DNA. To control PCR artifacts, each fragment was obtained from a minimum of two independent amplifications. In similar amplification conditions, *Taq* polymerase errors have been estimated to occur at a frequency of less than 10^{-5} per cycle (8). Amplified products were separated by electrophoresis and further analyzed by Southern blot hybridization with an SIVmac probe.

Primer sequences and positions (the latter as described by Chakrabarti et al. [4]) were as follows: LTR1, GGTTCTC TCCAGCACTAGCAGG (40 to 62); POL2, CCAAAGAG AGAATTGAGGTGCAGC (2035 to 2058); POL5, GCTA TACATGGGCAAGTAAATGC (4158 to 4181); P1, CAGA AATAGGGATACTTGGGGAAC (6227 to 6251); P2, GCCT GAATAATTGGTATCATTACA (6825 to 6848); P7, ATCA GCTGGCGGATCAGGAAATG (8406 to 8428); and LTR2, GGTCCTAACAGACCAGGGTC (240 to 259). The following pairs of primers were used for amplification to cover the complete genome: LTR1-POL2, POL2-POL5, POL5-P1, P1-P2, P2-P7, and P7-LTR2.

Cloning and sequencing. For each region, the products from two or more different amplifications were blunt-end ligated into *Sma*I-digested and phosphatase-treated M13mp18 vector and transformed into competent *Escherichia coli* DH5αF′. The recombinant clones were selected by in situ hybridization with ^{32}P-labelled, amplified fragments. The single-stranded DNA from recombinant phages was sequenced by using dideoxy nucleotides with T7 DNA polymerase (Sequenase) primed by specific internal sequences or universal sequencing oligonucleotides.

Direct sequencing of PCR products was used to confirm major genotypes by a modification of a published procedure (17). Amplified DNA was extracted from low-melting-point agarose, and 2 μl of annealing buffer (40 mM Tris, 20 mM $MgCl_2$, 50 mM NaCl) and 10 pmol of one of the PCR primers were then added. This mixture was denatured at 100°C for 5 min and then immediately transferred to a dry ice-ethanol bath for 1 min before sequencing. Sequencing reaction mixtures were incubated at 37°C.

Nucleotide sequence accession numbers. The nucleotide sequence data of SIVsmmPBj14 and SIVsmm9 proviral DNA, including indications of variable positions within each strain, have been submitted to the EMBL nucleotide sequence data base and have been assigned the accession numbers M80193 and M80194, respectively.

RESULTS

The complete proviral sequences of SIVsmm9 (10,637 bp) and SIVsmmPBj14 (10,674 bp) were determined. Each nucleotide position was sequenced several times from different cloned PCR fragments. Using this method, we obtained an average of three different complete proviral sequences for SIVsmmPBj14 (for LTR, 4 sequences per position [s/p]; for *gag*, 2.8 s/p; for *pol* and central region, 3.3 s/p; for *env* and *nef*, 3.5 s/p) and an average of two different complete proviral sequences for SIVsmm9 (for LTR, 2.7 s/p; for *gag*, 1 s/p; for *pol* and central region, 1.6 s/p; for *env* and *nef*, 3.3 s/p).

All sequences from both isolates were aligned; this multiple alignment defined variable and conserved positions. It also allowed the visualization of inter- and intraisolate variations. Intraisolate nucleotide variations in SIVsmm9 or SIVsmmPBj14 averaged 1%. Nucleotide divergence between the two isolates averaged 1.6%.

The observed variations fell into four categories: variations present in clones derived from the isolate SIVsmm PBj14 and never observed in clones derived from SIVsmm9 were designated "PBj specific"; likewise, those present only in smm9 were designated "smm specific"; positions at which we found both smm-specific and PBj-specific variations were designated "completely segregated" (or PBj plus smm specific); and variations present in both isolates were designated "nonspecific" (Fig. 1).

Fifty-seven PBj-specific mutations were identified, representing 0.74% of mutated base pairs in proviral DNA. It is important to note that all the PBj-specific mutations described here were also present in an infectious clone carrying the lethal phenotype, previously obtained from the same biologically cloned isolate (7). Thirty-six of these mutations led to amino acid changes; i.e., 1.08% of the amino acids in the viral proteins were changed. Two insertions due to sequence duplications in SIVsmmPBj14-derived PCR fragments were observed. These insertions were also present in lethal SIVsmmPBj14 infectious clones and absent from all the clones derived from the parental minimally pathogenic isolate. They included a 22-bp duplication in the U3 domain of the LTR, encompassing a NF-κB-like enhancer element and a 15-bp insertion in the SUgp120-coding region (Fig. 2). Direct sequencing of PCR products from both isolates confirmed that viral genomes with these two insertions formed the major (if not the only) genotype in SIVsmmPBj14, while those without these insertions constituted the major (if not the only) genotype in SIVsmm9.

In addition, we noted a 36-bp duplication in *tat* of both SIVsmmPBj14 and SIVsmm9, absent from other known SIVs and HIVs. This duplication maps to the first domain of *tat*, which is not essential for transactivating activity for HIV-1. As is frequently observed for most SIV isolates (3, 15), the in-frame stop codon that prematurely truncates TMgp41 was observed for clones derived from SIVsmm PBj14 cultivated on human peripheral blood lymphocytes but not for SIVsmmPBj14 cultivated on macaque peripheral blood lymphocytes. No other truncation was observed, suggesting a low frequency of defective viruses in the analyzed samples.

When the different viral proteins were considered, PBj-

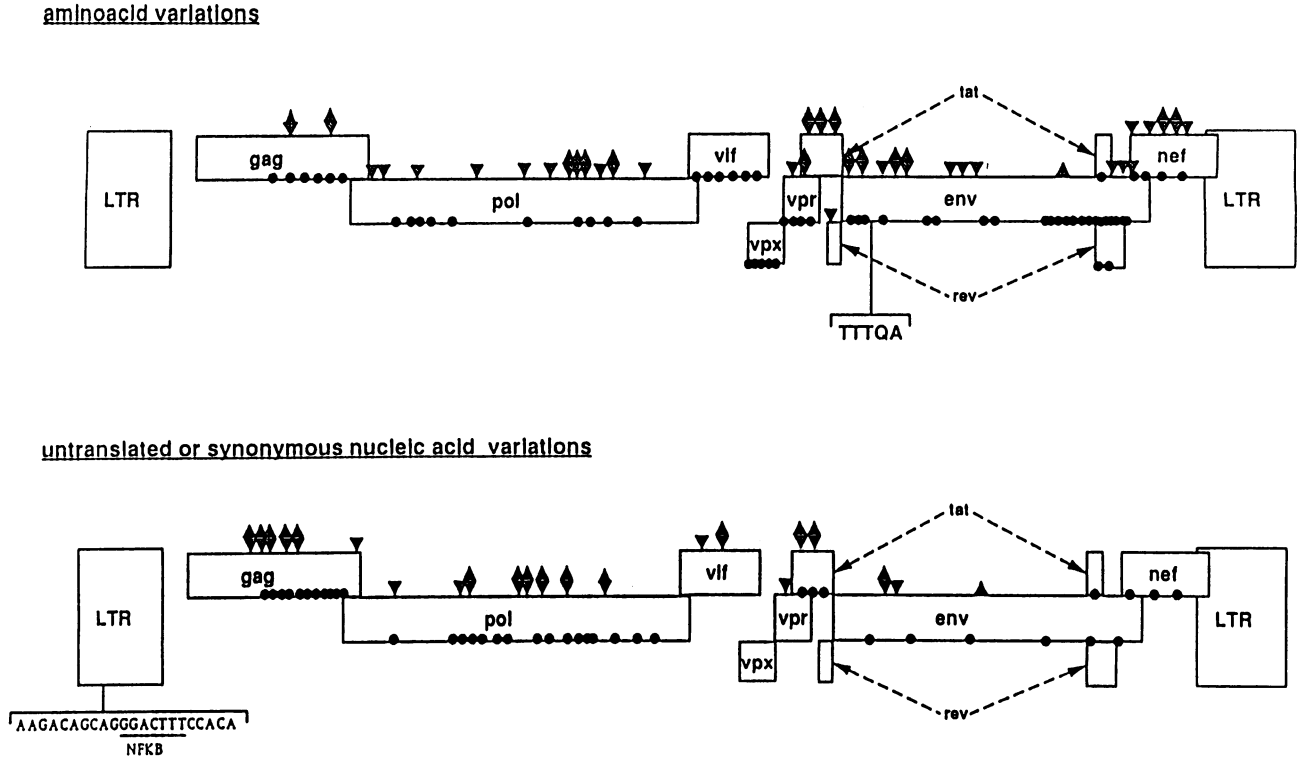

FIG. 1. Schematic representation of the genomic maps of SIVsmmPBj14 and SIVsmm9, with the corresponding nucleic acid variations. Top panel, mutations leading to amino acid changes; bottom panel, untranslated or synonymous mutations. The two insertions, in the LTR (NFKB site) and in the *env* gene, are shown. Symbols for mutations are as follows: ▲, smm specific; ▼, PBj specific; ●, nonspecific; ◆, PBj plus smm specific (see Results for explanation of designations).

specific amino acid changes varied from 0% (Vif and Vpx) to 2.3% (Tat) (Table 1). Although we sequenced fewer *gag* fragments from SIVsmm9, only two mutations led to PBj-specific amino acid changes, one in CAp25 and the other in MAp16. In Pol, two, four, and six PBj-specific amino acid changes were observed for the protease, reverse transcriptase, and integrase/endonuclease domains, respectively. In Env, eight and three PBj-specific amino acid changes were observed for SUgp120 and TMgp41, respectively.

Apart from point mutations in SUgp120, a unique cluster of five nucleotide changes at positions 7205 to 7211 was observed that led to three amino acid changes: amino acids 378 to 380 are Asp-Ile-Lys in SIVsmm9 PCR fragments, Asp-Thr-Lys in a previously sequenced SIVsmmPBj14 molecular clone that did not carry the lethal phenotype (7), and Lys-Thr-Glu in SIVsmmPBj14 PCR fragments and another previously sequenced infectious clone that carried the lethal phenotype (7). The domain encompassing this hypervariable motif does not align with previously identified HIV or SIV epitopes or functional domains.

Three of the 31 cysteines present in Env of SIVsmm9 (at positions 312, 460, and 742) were changed to tyrosines in all SIVsmmPBj14 sequences. However, these three cysteines were also mutated to tyrosines in SIVsmmH4, an indepen-

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          6473                                                    6506
SIVSMM9     ACAACAACACAAACA.............TCAACAACACCACCATCACCAATAATAGCAAAG
SIVPBJ14    ---------------ACAACAACACAAGCA----------A----------C-------
SIVSMMH4    ---------GC--T-.............A--------G--A--C--AGTG--GC--A---T
SIVmac251   -T-------GC-G--.............C-----T--G-----GT-T--GA--A-AT-G-C
SIVmac142   GC-T-----AC----.............A--------G--AA----GT-GAG-C-AG-G-C


SIVSMM9     ThrThrThrGlnThr.............SerThrThrProProSerProIleIleAlaLys
SIVPBJ14    ---------------ThrThrThrGlnAla----------Thr---------Thr------
SIVSMMH4    ---------AlaIle.............Thr------AlaThrProSerValAlaGluAsn
SIVmac251   Ile------AlaAla.............Pro---SerAla---ValSerGluLysIleAsp
SIVmac142   AlaSer---Thr---.............Thr------AlaLys---ValGluThrArgAsp
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FIG. 2. Comparison of nucleic acid and amino acid sequences of the *env* gene of different SIV isolates. A short insertion (TTTQA) is present only in SIVsmmPBj14 isolate. Numbers (top of figure) are positions in SIVsmm9 isolate.

TABLE 1. Analysis of nucleic acid variation between SIVPBj14 and SIVsmm9 in the different viral regions

Viral region[a]	No. of nucleic acid variations[b]:			
	Associated with phenotype[c]		Not associated with phenotype[d]	
	Synonymous	Expressed	Synonymous	Expressed
Gag (507)	6	2	11	6
Pol (1,022)	8	12	16	10
Vif (214)	2	0	0	7
Vpx (112)	0	0	0	5
Vpr (101)	1	2	0	4
Tat 1 (97)	2	3	3	0
Tat 2 (31)	0	0	1	1
Rev 1 (21)	0	1	0	0
Rev 2 (79)	0	0	4	2
Env (887)	2	11	6	24
Nef (261)	0	5	3	4
Total	21	36	44	63

[a] Number of amino acids is given in parentheses.
[b] Mutations are classified according to their specificities. Mutations leading to amino acid changes and those without amino acid modification are designated expressed and synonymous, respectively.
[c] PBj-specific mutations.
[d] Nonspecific mutations.

dent isolate from an asymptomatic sooty mangabey monkey (14). SIVsmmH4 has an additional cysteine at position 21 (Tyr in both smm9 and PBj14) and lacks one cysteine residue, found at position 803 in both smm9 and PBj14, which is replaced by Tyr.

Six amino acid variations that have never been observed with other SIVs and HIVs (16) were identified in SIVsmmPBj14 (three in Env, one in Pol, and two in Nef). Finally, for intra- and/or interisolate variations in SIVsmm9 and SIVsmmPBj14, a higher number of nucleotide substitutions led to amino acid changes. This is essentially due to a low ratio of synonymous to nonsynonymous mutations in the 3′ part of the genome, encompassing *env* and *nef* (Table 1). This has been also observed for HIV-1 (24) and SIVmac (2).

DISCUSSION

In this report, we provide comparative sequence information for the complete genomes of two closely related SIV isolates that diverged during an interspecies in vivo transmission and resulted in a major change in virulence.

Infectious clones of SIVsmmPBj14 that exhibited acute pathogenicity in pig-tailed macaques were previously sequenced (7). However, the closest previously sequenced relative of these infectious clones was SIVsmmH4, an independent isolate from an asymptomatic sooty mangabey monkey (14). Amino acid divergence between these previously sequenced isolates, ranging from 3% (Pol) to 23% (Nef), was too high to allow any reasonable interpretation of the molecular basis for SIVsmmPBj14 virulence. No sequence information on the parental strain SIVsmm9 was then available.

Because of the small amount of viral DNA in infected tissues and PBMC, we used a strategy based on the cloning and sequencing of PCR products to obtain sequence information on these parental and variant SIV isolates. In addition, we could examine structure-pathogenicity relationships by taking advantage of three types of information: first, the sequence of molecular clones known to exhibit the acutely lethal phenotype, which allowed us to confirm that PBj-specific mutations observed for the PCR-derived fragments were indeed linked to the lethal phenotype and were not introduced during PCR amplification; second, the absence of the acutely lethal pathogenicity of isolate SIVsmm9, which ruled out a detectable presence of the lethal genotype in this isolate; and third, the close genetic relationship between SIVsmm9 and SIVsmmPBj14, which allowed informative sequence comparisons.

In that context, the following interpretation was attributed to the different classes of variations defined in Results. (i) Nonspecific variations cannot be sufficient by themselves to explain the phenotypic differences between the two isolates. They define the common genetic background of both phenotypes. This background, in conjunction with additional isolate-specific variations, is necessary for encoding both phenotypes. (ii) While each isolate-specific variation could be sufficient by itself to determine the phenotype, a combination of two or more might be necessary, with the others being neutral. The identification of phenotypically neutral or necessary changes among the set of isolate-specific changes described here will require future studies involving directed mutagenesis and the construction of chimeric infectious clones.

It is important to note that, if only one sequence had been obtained for each isolate, all differences between these two sequences would have had to be considered isolate specific. In fact, the definition of isolate specificity stricto sensu requires the sequencing of a large number of fragments. In the present study, the equivalents of three and two complete proviruses were sequenced for SIVsmmPBj14 and SIVsmm9, respectively. Previous sequences of lethal molecular clones of SIVsmmPBj14 were also included in our comparisons. However, with further sequencing data, some variations designated here as isolate specific could be identified as nonspecific. Therefore, the number of PBj-specific changes identified here represents an overestimation of the number of changes responsible for the phenotypic differences.

The phenotypic influence of rare variants might be missed by our approach. However, most infectious clones derived from PCR fragments carry the PBj phenotype (7), indicating that the acute pathogenicity is indeed carried by a genotype frequently present in biological clone 3 and is not due to the combined action of multiple genotypes. Thus, the minimal number of necessary and sufficient changes that determined the acutely lethal phenotype is included within the set of isolate-specific changes described here.

Therefore, starting from the SIVsmm9 genetic background, the combination of necessary and sufficient mutations that determined the PBj14 phenotype is included within a maximal set of 57 mutations (36 if synonymous mutations in known open reading frames are excluded) plus two insertions (22 bp in the LTR and 15 bp in *env*).

From these results, it is tempting to speculate on the genetic events possibly involved in the acute pathogenicity of SIVsmmPBj14. Previous work (7) suggested that the duplication of an NF-κB-like enhancer site in the U3 domain of the LTR may be necessary to determine the acutely lethal phenotype. In murine retroviruses, limited modifications of the viral LTRs lead to significant changes in the specificity of the virus-induced pathology as well as in the length of the latent period before the onset of disease (12, 26). For HIV or SIV LTR, several studies have shown that the deletion of enhancer sites can delay replication (18, 20, 22). However, the link between replication kinetics in vitro and pathoge-

nicity in vivo is not clearly demonstrated. Additional enhancer elements have also been found in nonpathogenic SIVagm and SIVmnd isolates (16), and other molecular changes are likely to participate in SIVsmmPBj14 pathogenicity.

Apart from the NF-κB duplication in the LTR, most changes in these otherwise remarkably conserved isolates are located in *env*: a 15-bp duplication, a hypervariable motif, and several point mutations. From the present data and data from similar models of other pathogenic retroviral infections (for example, see references 1 and 25), the candidates that could best explain the SIVsmmPBj14 phenotype by a combined effect are *env* and LTR. For HIV-1, limited modifications in Env can be associated with marked differences of the viral tropism (5, 23, 29) and of direct cytopathic effects (9). Other experiments based on the reconstruction of recombinant viral hybrids between cytopathic and noncytopathic isolates suggested that different parts of the viral genome are required to reconstitute a given virulent phenotype (13, 27).

The sequence data presented here also provide information on the pattern of nucleotide and amino acid substitutions in SIVsmm. Comparison of synonymous and nonsynonymous mutations within and between these two closely related SIV isolates reflected selective pressures in different parts of the genome. For Pol (1,022 amino acids), we observed a total of 24 synonymous versus 22 expressed mutations; for Gag (507 amino acids), we observed 17 synonymous versus 8 expressed mutations; and for Env (887 amino acids), we observed only 8 synonymous versus 35 expressed mutations (Table 1). A low rate of fixation of synonymous mutations in *env* suggested the existence of strong selective pressures favoring envelope protein variations. Similarly, with closely related isolates of HIV-1 from patients infected from the same source (24) or with SIV in which mutations are monitored over time (2), positive selection for change is observed: the fixation of a replacement mutation in *env* is two to four times as likely as the fixation of a synonymous mutation. These differences likely reflect the adaptation of the viral envelope protein to the host immune response. In our study, this interpretation is supported by previous work showing that SIVsmmPBj14 escapes neutralization by neutralizing antibodies to SIVsmm9, detected in serum from pig-tailed macaque PBj (11).

In Yerkes Regional Primate Research Center, SIVsmm chronically infects a large proportion of sooty mangabey monkeys, which do not show any sign of AIDS-like disease (10). Thus, the apparent phenotypic stability of SIVsmm in the original host is in contrast to the virulence change that occurred following the experimental cross-species transmission. Although the acute lethality of SIVsmmPBj14 is unusual, the origin of SIVmac subacute pathogenicity has also been attributed to a recent cross-species transmission from sooty mangabeys to rhesus macaques (for a review, see reference 6). The respective roles of virus and host factors remain unclear. However, these observations could find some immunoevolutionary explanation if we assume the hypothesis that positive selection for change in SIVsmm Env, also observed for SIVmac (2) and HIV-1 (24), is due to immunological pressures that in turn depend on viral immunogenicity.

Long-term adaptation of persistent viruses (and other parasites) tends to reduce their immunogenicity. The evolutionary achievement of a minimal level of viral immunogenicity should be possible if the virus is kept in a stable immunological context, i.e., if it persists for a long period of time in a group of genetically related hosts and their descendants. A low immunogenicity should in turn reduce immunological selection for change. It could even result in negative selection of further changes: most new virus variants could be more immunogenic and thus counterselected. On the other hand, following cross-species transmission into a new immunological environment, the virus could be more immunogenic. The resulting increase in immunological pressures would restore positive selection for change. Thus, a sudden modification of the immunological environment of the virus could occasion a shift from negative to positive selection for virus genetic change that boosts virus diversity. This could explain the outbreak of new phenotypes or changes in virulence that occur after the transmission of viruses from the original host to a genetically unrelated organism.

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REFERENCES

1. **Brown, D. W., B. P. Blais, and H. L. Robinson.** 1988. Long terminal repeat (LTR) sequences, *env*, and a region near the 5′ LTR influence the pathogenic potential of recombinants between Rous-associated virus types 0 and 1. J. Virol. **62**:3431–3437.
2. **Burns, D. P. W., and R. C. Desrosiers.** 1991. Selection of genetic variants of simian immunodeficiency virus in persistently infected rhesus monkeys. J. Virol. **65**:1843–1854.
3. **Chakrabarti, L., M. Emerman, P. Tiollais, and P. Sonigo.** 1989. Genetic analysis of the C-terminal domain of the transmembrane envelope protein. J. Virol. **63**:4395–4403.
4. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo.** 1987. Molecular cloning and nucleotide sequence of simian immunodeficiency virus from macaques. Nature (London) **328**:543–547.
5. **Cordonnier, A., L. Montagnier, and M. Emerman.** 1989. Single amino-acid changes in HIV envelope affect viral tropism and receptor binding. Nature (London) **340**:571–574.
6. **Desrosiers, R. C.** 1990. The simian immunodeficiency viruses. Annu. Rev. Immunol. **8**:557–578.
7. **Dewhurst, S., J. E. Embretson, D. C. Anderson, J. I. Mullins, and P. N. Fultz.** 1990. Sequence analysis and acute pathogenicity of molecularly cloned SIVsmm-pbj14. Nature (London) **345**:636–639.
8. **Erlich, H. A., D. Gelfand, and J. J. Sninsky.** 1991. Recent advances in the polymerase chain reaction. Science **252**:1643–1651.
9. **Fenyö, E. M., L. Morfeldt-Månson, F. Chiodi, B. Lind, A. V. Gegerfelt, J. Albert, E. Olausson, and B. Asjö.** 1988. Distinct replicative and cytopathic characteristics of human immunodeficiency virus isolates. J. Virol. **62**:4414–4419.
10. **Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan.** 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (Cercocebus atys). Proc. Natl. Acad. Sci. USA **83**:5286–5290.
11. **Fultz, P. N., H. M. McClure, D. C. Anderson, and W. M. Switzer.** 1989. Identification and biologic characterization of an acutely lethal variant of SIV from sooty mangabeys (SIV/SMM). AIDS Res. Hum. Retroviruses **5**:397–409.
12. **Golemis, E., Y. Li, T. N. Frederickson, J. W. Hartley, and N. Hopkins.** 1989. Distinct segments within the enhancer region collaborate to specify the type of leukemia induced by nonde-

fective Friend and Moloney viruses. J. Virol. **63**:328–337.
13. **Hirsch, I., B. Spire, Y. Tsunetsugu-Yokota, C. Neuveut, J. Sire, and J. C. Chermann.** 1990. Differences in replication and cytopathogenicity of human immunodeficiency virus type 1 (HIV-1) are not determined by long terminal repeats (LTR). Virology **177**:759–763.
14. **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 (London) **339**:389–392.
15. **Kodama, T., D. P. Wooley, Y. M. Naidu, H. W. I. Kestler, M. D. Daniel, Y. Li, and R. C. Desrosiers.** 1989. Significance of premature stop codons in *env* of simian immunodeficiency virus. J. Virol. **63**:4709–4714.
16. **Myers, G., A. B. Rabson, J. A. Berzofsky, T. F. Smith, and F. Wong-Staal.** 1990. Human retroviruses and AIDS. Los Alamos National Laboratory, Los Alamos, N. Mex.
17. **Newton, C. R., N. Kalsheker, A. Graham, S. Powell, A. Gammacka, J. Riley, and A. F. Markham.** 1988. Diagnosis of alpha-1-antitrypsin deficiency by enzymatic amplification of human genomic DNA and direct sequencing of polymerase chain reaction products. Nucleic Acids Res. **16**:8233–8243.
18. **Parrott, C., T. Seidner, E. Duh, J. Leonard, T. S. Theodore, A. Buckler-White, M. A. Martin, and A. B. Rabson.** 1991. Variable role of the long terminal repeat Sp1-binding sites in human immunodeficiency virus replication in T lymphocytes. J. Virol. **65**:1414–1419.
19. **Preston, B. D., B. J. Poiesz, and L. A. Loeb.** 1988. Fidelity of HIV-1 reverse transcriptase. Science **242**:1168–1170.
20. **Renjifo, B., N. A. Speck, S. Winandy, N. Hopkins, and Y. Li.** 1990. *cis*-acting elements in the U3 region of a simian immunodeficiency virus. J. Virol. **64**:3130–3134.
21. **Roberts, J. D., K. Bebenek, and T. A. Kunkel.** 1988. The accuracy of reverse transcriptase from HIV-1. Science **242**: 1171–1173.
22. **Ross, E. K., A. J. Buckler-White, A. B. Rabson, G. Englund, and M. A. Martin.** 1991. Contribution of NF-κB and Sp1 binding motifs to the replicative capacity of human immunodeficiency virus type 1: distinct patterns of viral growth are determined by T-cell types. J. Virol. **65**:4350–4358.
23. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp 120 gene. Nature (London) **349**:167–169.
24. **Simmonds, P., P. Balfe, C. A. Ludlam, J. O. Bishop, and A. J. L. Brown.** 1990. Analysis of sequence diversity in hypervariable regions of the external glycoprotein of human immunodeficiency virus type 1. J. Virol. **64**:5840–5850.
25. **Sitbon, M., L. D'Auriol, H. Ellerbrok, C. André, J. Nishio, S. Perryman, F. Pozo, S. F. Hayes, K. Wehrly, P. Tambourin, F. Galibert, and B. Chesebro.** 1991. Substitution of leucine for isoleucine in a sequence highly conserved among retroviral envelope surface glycoproteins attenuates the lytic effect of the Friend murine leukemia virus. Proc. Natl. Acad. Sci. USA **88**:5932–5936.
26. **Speck, N. A., B. Renjifo, E. Golemis, T. N. Frederickson, J. W. Hartley, and N. Hopkins.** 1990. Mutation of the core or adjacent LVb elements of the Moloney murine leukemia virus enhancer alters disease specificity. Genes Dev. **4**:233–242.
27. **Spire, B., I. Hirsch, C. Neuveut, J. Sire, and J. C. Chermann.** 1990. The env gene variability is not directly related to the high cytopathogenicity of an HIV1 variant. Virology **177**:756–758.
28. **Szurek, P. F., E. Floyd, P. H. Yuen, and P. K. Y. Wong.** 1990. Site-directed mutagenesis of the codon for Ile-25 in $gPr80^{env}$ alters the neurovirulence of *ts*1, a mutant of Moloney murine leukemia virus TB. J. Virol. **64**:5241–5249.
29. **Takeuchi, Y., M. Akutsu, K. Murayama, N. Shimizu, and H. Hoshino.** 1991. Host range mutant of human immunodeficiency virus type 1: modification of cell tropism by a single point mutation at the neutralization epitope in the *env* gene. J. Virol. **65**:1710–1718.
30. **Teich, N., J. Wyke, and P. Kaplan.** 1985. Pathogenesis of retrovirus-induced disease, p. 187–248. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses: molecular biology of tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.