11-1989

Significance of Premature Stop Codons in *env* of Simian Immunodeficiency Virus

Toshiaki Kodama

Dawn P. Wooley
Wright State University - Main Campus, dawn.wooley@wright.edu

Yathirajulu M. Naidu

Harry W. Kestler III

Muthiah D. Daniel

See next page for additional authors

JOURNAL OF VIROLOGY, Nov. 1989, p. 4709–4714
0022-538X/89/114709-06$02.00/0

Vol. 63, No. 11

Significance of Premature Stop Codons in *env* of Simian Immunodeficiency Virus

TOSHIAKI KODAMA, DAWN P. WOOLEY, YATHIRAJULU M. NAIDU, HARRY W. KESTLER III, MUTHIAH D. DANIEL, YEN LI, AND RONALD C. DESROSIERS*

New England Regional Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, Massachusetts 01772

Received 12 May 1989/Accepted 24 July 1989

The location of the translational termination codon for the transmembrane protein (TMP) varies in three infectious molecular clones of simian immunodeficiency virus from macaques (SIV_{mac}). The SIV_{mac}251 and SIV_{mac}142 infectious clones have premature stop signals that differ in location by one codon; transfection of these DNAs into human HUT-78 cells yielded virus with a truncated TMP (28 to 30 kilodaltons [kDa]). The SIV_{mac}239 infectious clone does not have a premature stop codon in its TMP-coding region. Transfection of HUT-78 cells with this clone initially yielded virus with a full-length TMP (41 kDa). At 20 to 30 days posttransfection, SIV_{mac}239 virus with a 41-kDa TMP gradually disappeared coincident with the emergence of a virus with a 28-kDa TMP. Virus production dramatically increased in parallel with the emergence of a virus with a 28-kDa TMP. Sequence analysis of viral DNAs from these cultures showed that premature stop codons arising by point mutation were responsible for the change in size of the TMP with time. A similar selective pressure for truncated forms of TMP was observed when the SIV_{mac}239 clone was transfected into human peripheral blood lymphocytes (PBL). In contrast, no such selective pressure was observed in macaque PBL. When the SIV_{mac}239 clone was transfected into macaque PBL and the resultant virus was serially passaged in macaque PBL, the virus replicated very well and maintained a 41-kDa TMP for 80 days in culture. Macaque monkeys were infected with SIV_{mac}239 having a 28-kDa TMP; virus subsequently recovered from T4-enriched lymphocytes of peripheral blood showed only the 41-kDa form of TMP. These results indicate that the natural form of TMP in SIV_{mac} is the full-length 41-kDa TMP, just as in human immunodeficiency virus type 1. Viruses with truncated forms of TMP appear to result from mutation and selection during propagation in unnatural human cells.

Two related but distinct human lentiviruses, human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), have been identified (1, 5, 27). The simian immunodeficiency viruses (SIVs) are a diverse group of nonhuman primate lentiviruses that are the closest known relatives of the HIVs. To date, SIV has been isolated from macaques (3, 6, 8, 24), African green monkeys (9, 26), sooty mangabey monkeys (12, 22, 24), and mandrills (31). The SIVs share extensive similarity with their human counterparts in both genetic and biological properties. Both SIV and HIV are tropic for cells bearing the CD4 molecule, and both can be cytopathic for these cells (6, 19, 26). SIV, like HIV and other lentiviruses, can induce chronic debilitating disease following long-term persistent infection. The pathologic features of SIV- and HIV-induced diseases are quite similar (2, 3, 6, 7, 21, 28). Both exhibit the unique lentivirus virion morphology. Their genomic organizations, represented by long terminal repeat-*gag-pol-vip*-central region-*env-nef*-long terminal repeat, are very similar, with extensive gene-for-gene homology along the genome (4, 11). The extensive similarity of SIV to HIV and the ability to induce acquired immunodeficiency syndrome in common rhesus monkeys (*Macaca mulatta*) make SIV ideally suited for study of the pathogenesis of acquired immunodeficiency syndrome and approaches to vaccination and therapy.

One feature of SIV that has been found to differ from HIV-1 is the length of the transmembrane protein (TMP) of the envelope. A premature stop codon in the envelope reading frame results in truncated forms of SIV TMP (4, 11, 16). Some isolates of HIV-2 also contain a truncation of the TMP (14, 15, 33). These findings have led to speculation as to whether SIV and HIV-1 differ in the utilization of these sequences, particularly in the sequences downstream of the premature stop codon. Since the reading frame in *env* beyond the stop codon has been conserved, questions arise as to whether these sequences may be expressed in alternate fashion, perhaps through RNA splicing.

Here we report that truncated forms of TMP in SIV from macaques (SIV_{mac}) result from mutation and selection during propagation in unnatural human cell lines and that the natural form of the TMP in SIV_{mac} is the full-length 41 kilodaltons (kDa), just as in HIV-1.

MATERIALS AND METHODS

SIV_{mac} infectious clone. Full-length molecular clones containing integrated proviral DNA from SIV_{mac}142, SIV_{mac}239, and SIV_{mac}251 in bacteriophage lambda EMBL4 were used. All three of these cloned DNAs were infectious upon transfection into HUT-78 cells (25).

Cell culture and DNA transfection. The continuously growing $CD4^+$ human T-cell lines HUT-78 (13) and MT-4 (23) were maintained in RPMI 1640 medium with 10% fetal bovine serum. Peripheral blood lymphocytes (PBL) of healthy macaques and humans were separated by Ficoll-Hypaque, stimulated with 10 μg of phytohemagglutinin per ml for 48 h at 37°C, and maintained in RPMI 1640 medium with 20% fetal bovine serum containing 10% human interleukin 2.

Infectious cloned DNAs were transfected into HUT-78 cells and phytohemagglutinin-stimulated macaque and hu-

* Corresponding author.

man PBL with a DEAE-dextran procedure (25). At 7 to 14 days posttransfection or postinfection of PBL, culture supernatants were used to infect fresh phytohemagglutinin-stimulated PBL. This procedure was repeated for serial passage.

Analysis of virus replication and viral products. Virus replication was analyzed by measurement of reverse transcriptase activity in the culture supernatant (6). For analysis of viral proteins by immunoblotting, virus particles were concentrated (1,000×) from culture supernatants by being pelleted in an SW27 rotor (Beckman Instruments, Inc.) through a 20% sucrose cushion at 25,000 rpm for 2 h. The crude viral proteins were electrophoresed through sodium dodecyl sulfate–12% polyacrylamide gels and transferred to hydrophobic Durapore membranes (Millipore Corp.) by electroblotting. After the blocking of nonspecific reactions, the membranes were treated with 1:100 diluted SIV_{mac}-positive macaque sera for 1 h at 37°C. The membranes were treated with biotinylated anti-human immunoglobulin (Amersham Corp.) and then with a biotinylated peroxidase-streptavidin complex (Amersham). Peroxidase activities were detected by using 0.5 mg of diaminobenzidine per ml with 0.02% H_2O_2 in phosphate-buffered saline.

To assist virus recovery from a macaque persistently infected with cloned $SIV_{mac}239$, $CD8^+$ lymphocytes were depleted by panning (18). This macaque had been inoculated 58 weeks previously with cloned $SIV_{mac}239$ produced in HUT-78 cells (25).

Nucleotide sequencing. Hirt supernatant DNA (17) was prepared from $SIV_{mac}239$-infected HUT-78 cells. DNA sequences between nucleotides 8111 and 8613 (the numbers correspond to those of $SIV_{mac}142$ [4]) were specifically amplified by using the polymerase chain reaction (29). Polymerase chain reaction amplifications were performed on an automated DNA thermal cycler (The Perkin-Elmer Corp.) in the presence of two synthetic oligonucleotide primers corresponding to 8082 to 8111 and 8613 to 8642. An *Eco*RI restriction site was engineered in primer 8082 to 8111 by replacing adenine with cytosine at nucleotide position 8105. By using the *Eco*RI and *Bgl*II sites in the synthetic primers, the 560-base-pair amplified DNA fragment was cloned into vector pGEM-4Z (Promega Biotec). Nucleotide sequences in amplified, cloned DNA and in a TMP subclone of the original infectious $SIV_{mac}239$ clone were determined by the primer-directed dideoxy-chain termination method (30). Sequenase and synthetic oligonucleotide primers were used to sequence double-stranded DNA.

RESULTS

The translational stop codon in the $SIV_{mac}142$ infectious clone was described previously as part of its entire nucleotide sequence (4). A similar stop codon was found in the infectious clone of $SIV_{mac}251$ but not in that of $SIV_{mac}239$ by partial sequencing of the TMP region (25). $SIV_{mac}142$ and $SIV_{mac}251$ have stop signals that differ in location by one codon; no such stop codon was found in this region of $SIV_{mac}239$ (Fig. 1A). We confirmed the absence of a premature stop codon in $SIV_{mac}239$ by determining the nucleotide sequence of the entire TMP region (Fig. 1B).

Comparative analysis of the TMP regions of $SIV_{mac}239$ and $SIV_{mac}142$ showed an overall nucleotide identity of 95%. However, the homology decreased from 97% before the stop codon of $SIV_{mac}142$ to 93% after it; the predicted amino acid sequence homology decreased from 93% before the stop codon to 80% after it. The N-terminal region, which is a putative fusogenic domain, was well conserved. Despite

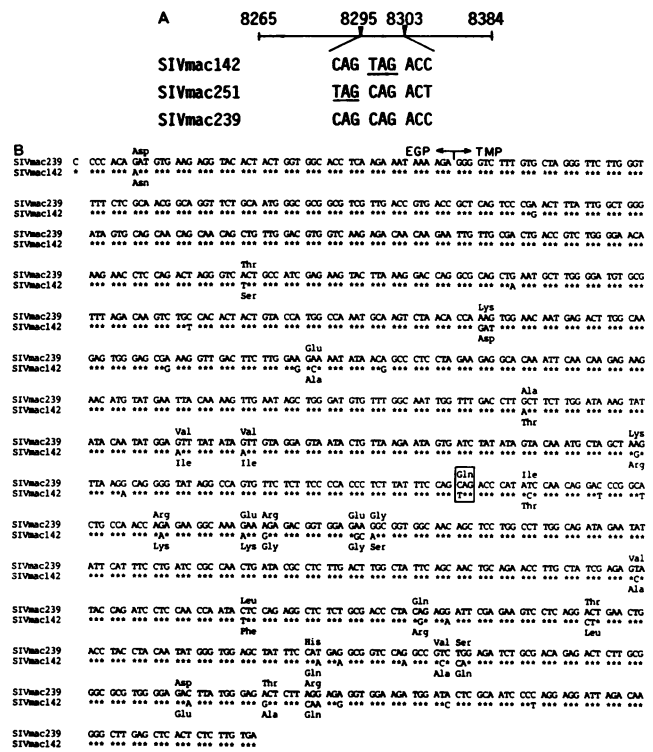

FIG. 1. (A) Variability in location of the stop codon for TMP. $SIV_{mac}142$, $SIV_{mac}251$, and $SIV_{mac}239$ are infectious molecular clones from different SIV isolates (25). The premature stop codons in $SIV_{mac}142$ and $SIV_{mac}251$ are underlined. (B) Comparison of nucleotide and predicted amino acid sequences from the TMP regions of $SIV_{mac}239$ and $SIV_{mac}142$. The cleavage site between the external glycoprotein (EGP) and transmembrane protein (TMP) is indicated (32). The identical nucleotide sequences (*) and the predicted amino acid sequences that differ are shown. A change of C to T (boxed) results in a stop codon rather than a glutamine at the indicated position. Nucleotide numbers correspond to those published previously for $SIV_{mac}142$ (4).

decreasing homology after the stop codon, the C-terminal 22 amino acids were also well conserved (Fig. 1B).

After transfection of infectious $SIV_{mac}251$ and $SIV_{mac}142$ cloned DNAs into HUT-78 cells, the kinetics of virus replication and the viral products were analyzed. Cloned $SIV_{mac}142$ and $SIV_{mac}251$ replicated quite well in these HUT-78 cells and yielded high levels of reverse transcriptase activity during the course of infection. The size of the TMP of $SIV_{mac}142$ was 28 kDa, and that of $SIV_{mac}251$ was 30 kDa; the size of the TMP was stable in culture over time (Fig. 2).

Consistent with the DNA sequence, transfection of HUT-78 cells with $SIV_{mac}239$ cloned DNA initially yielded a virus with a full-length 41-kDa TMP (Fig. 3). However, during the first 20 to 30 days posttransfection, virus replication was very low compared with that of $SIV_{mac}142$ and $SIV_{mac}251$ (Fig. 3). Upon continued passage of these $SIV_{mac}239$-transfected cultures, the virus with a 41-kDa TMP gradually decreased below detectable levels, coincident with the emergence of the virus with a 28-kDa TMP (Fig. 3). Virus replication increased dramatically concomitant with the emergence of the virus with a 28-kDa TMP (Fig. 3). Selection in HUT-78 cells for SIV_{mac} with increased replicative potential and with truncated forms of TMP was reproduced in separate experiments (Fig. 3).

Hirt supernatant DNA was prepared from HUT-78 cells

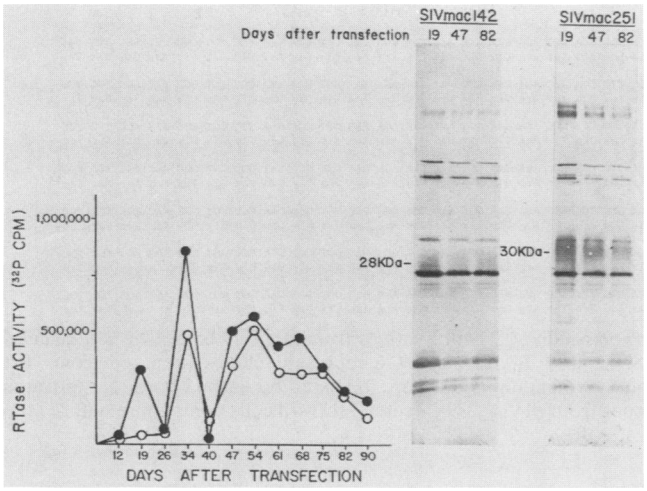

FIG. 2. Growth kinetics and virion proteins of $SIV_{mac}142$ and $SIV_{mac}251$ in HUT-78 cells. Infectious cloned DNAs for $SIV_{mac}142$ and $SIV_{mac}251$ were transfected into HUT-78 cells. Virus production was monitored by reverse transcriptase activity in culture supernatants. Viral proteins were concentrated from culture supernatants and analyzed by Western blotting (immunoblotting). The proteins from 19, 47, and 82 days posttransfection are shown. The TMP for each is indicated. Closed symbols, $SIV_{mac}142$; open symbols, $SIV_{mac}251$.

infected with cloned $SIV_{mac}239$ after the emergence of truncated forms of TMP. DNA corresponding to the TMP region was amplified by a polymerase chain reaction. Amplified DNA was cloned, and nucleotide sequences from six independent clones were analyzed (Fig. 4). Four of the six clones (T_02 to T_05) were identical to each other but differed at one nucleotide (nucleotide 8295) compared with the parental $SIV_{mac}239$ clone; a C-to-T change converted a glutamine codon to a stop codon. The position of this change corresponded to the position of the premature stop codon in clone $SIV_{mac}251$. The analyzed clones otherwise matched the $SIV_{mac}239$ sequence perfectly and differed from the $SIV_{mac}251$ and $SIV_{mac}142$ sequences at several locations. A fifth clone (T_06) had a stop codon acquired by point mutation (A→T) six codons downstream (nucleotide 8313) from its location in T_02 to T_05. A sixth clone (T_01) had no premature stop codon in this region (Fig. 4).

A virus stock with predominantly a 41-kDa TMP ($SIV_{mac}239$/TM41K obtained 27 days posttransfection) and a virus stock with predominantly a 28-kDa TMP ($SIV_{mac}239$/TM28K obtained 83 days posttransfection) were studied further. Both were infectious for HUT-78 and MT-4 cells, both induced syncytia in HUT-78 cells, and both had killing activity in the MT-4 cell-killing assay. Replication of the virus with a 28-kDa TMP predominated in both human cell lines upon infection with $SIV_{mac}239$/TM28K. When $SIV_{mac}239$/TM41K was used to infect cells, selection for the virus with a truncated 28-kDa TMP occurred in MT-4 and HUT-78 cells (data not shown). Quite different results were obtained when $SIV_{mac}239$/41K and $SIV_{mac}239$/28K were used to infect phytohemagglutinin-stimulated macaque PBL

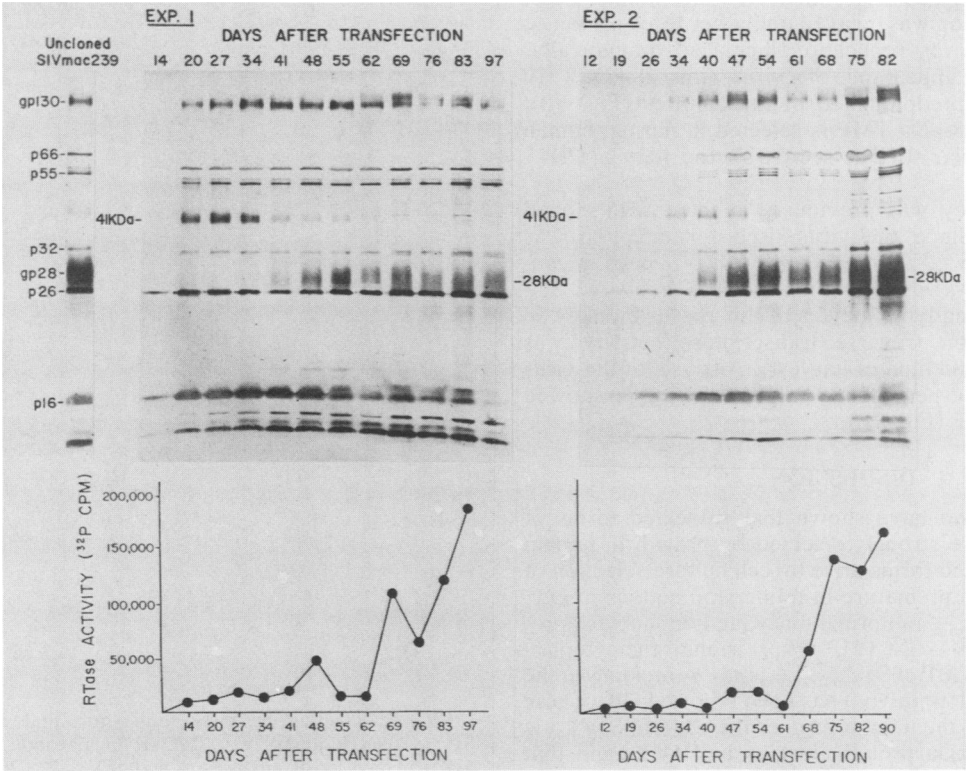

FIG. 3. Selection of $SIV_{mac}239$ with a truncated TMP in HUT-78 cells. Major viral proteins of uncloned $SIV_{mac}239$, which was isolated and maintained in HUT-78 cells, are indicated on the left; this virus has predominantly a truncated form of TMP (28 kDa). The infectious molecular clone of $SIV_{mac}239$ was transfected into HUT-78 cells. The left and right panels show independent experiments (experiments [Exp.] 1 and 2). The correlation between the kinetics of viral replication and the selection of a virus with a truncated TMP is shown. The untruncated (41-kDa) and truncated (28-kDa) forms of TMP are indicated. RTase, Reverse transcriptase.

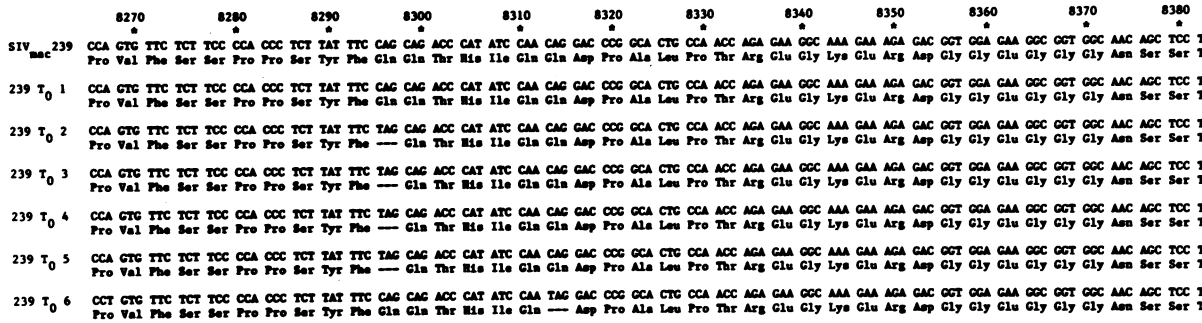

FIG. 4. Nucleotide and predicted amino acid sequences of the TMP region of $SIV_{mac}239$ derivatives from HUT-78 cells. The sequence of $SIV_{mac}239$ is that of the parental clone (Fig. 1B). The sequences of $SIV_{mac}239$ $T_0$1 through $T_0$6 represent clones prepared from Hirt supernatant DNA of cloned $SIV_{mac}239$-infected HUT-78 cells. Translational termination codons are indicated by dashed lines. No in-frame stop codon is found in the $SIV_{mac}239$ infectious clone, while five of six clones from $SIV_{mac}239$-infected HUT-78 cells contain premature stop codons.

(Fig. 5). In these macaque lymphocytes growing in interleukin 2, replication of SIV with a 41-kDa TMP predominated. Infection of macaque lymphocytes with $SIV_{mac}239/41K$ yielded a virus with a 41-kDa TMP that persisted upon serial passage (Fig. 5A), while $SIV_{mac}239/28K$ eventually yielded a virus with significant levels of a 41-kDa TMP (Fig. 5B). These results suggested that truncated forms of the TMP of $SIV_{mac}239$ might result from selective growth in human cells.

To further investigate this possibility, cloned $SIV_{mac}239$ DNA was transfected directly into primary macaque and human PBL cultures. In transfected macaque lymphocytes, $SIV_{mac}239$ replicated very well and maintained a 41-kDa TMP over 80 days of serial passage (Fig. 6A). When $SIV_{mac}239$ cloned DNA was transfected into human PBL, significant replication was delayed until after three passages. After this time, virus replication increased dramatically, coincident with the appearance of a virus with a 28-kDa TMP which eventually predominated in the cultures (Fig. 6B). Thus, virus with 28-kDa TMP is selected in normal human PBL cultures similar to that seen with the human $CD4^+$ tumor cell lines.

A rhesus monkey was previously infected with cloned $SIV_{mac}239$ (27). Since the virus used for infection was harvested from $SIV_{mac}239$-transfected HUT-78 cells, it contained mainly a 28-kDa TMP (Fig. 7A). We recovered SIV from $CD4^+$-enriched lymphocytes of this rhesus monkey 58 weeks postinfection (reverse transcriptase activity was 81,000 cpm/ml at the time of harvest). Analysis of the virus recovered from the persistently infected macaque showed only a 41-kDa TMP (Fig. 7B).

DISCUSSION

In this report, we have shown that truncated forms of TMP of SIV_{mac} are strongly selected by growth in human cell types. Truncated forms arise by cell culture selection of point mutants with premature in-frame stop codons in *env*. Replication of SIV_{mac} in normal macaque lymphocytes preferred a full-length 41-kDa TMP. Expression of the cytoplasmic domain of the TMP of SIV_{mac} (i.e., the *env* region past the premature stop codon) in vivo has been reported (10). These results suggest that the natural form of the TMP of SIV_{mac} is the full-length 41-kDa protein, just as in HIV-1, and that truncated forms of SIV appear to result from mutation and selection during propagation in human cell lines.

In addition to SIV_{mac}, SIV isolates from African green monkeys, sooty mangabeys, and mandrills have exhibited truncation of the TMP (12, 24, 26, 31). For these studies, SIV was grown in human $CD4^+$ T-cell lines. Several SIV isolates from African green monkeys showed heterogeneity in the sizes of their TMPs (20). After 3 months of culture of $SIV_{agm}385$ in MOLT-4/C18 cells (9), only a 41-kDa TMP was detected in $SIV_{agm}385$ preparations, but by 11 months a truncated 34-kDa form predominated (T. Kodama and R.

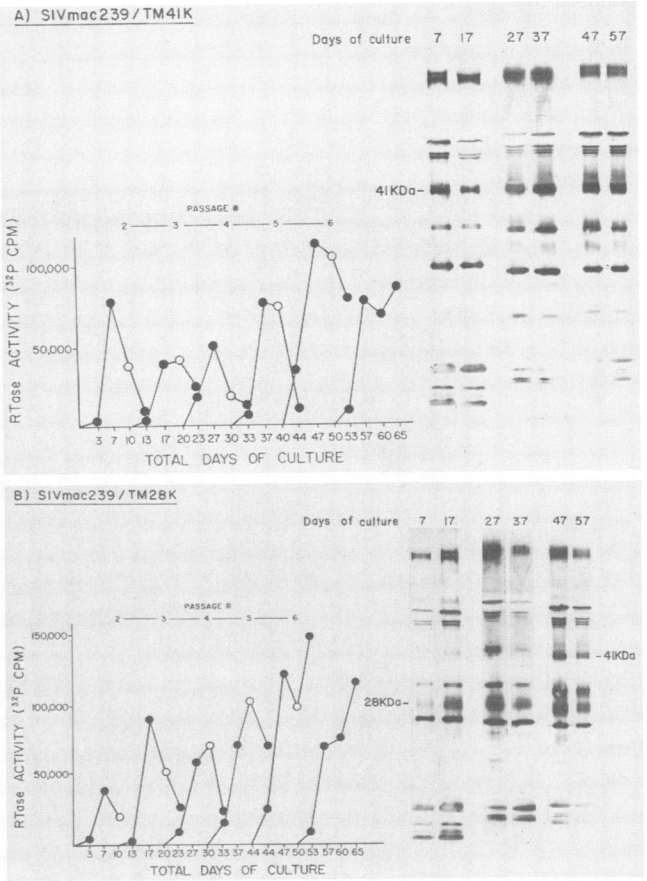

FIG. 5. Kinetics of viral replication and viral proteins of $SIV_{mac}239/41K$ and $SIV_{mac}239/28K$ in macaque PBL. $SIV_{mac}239/41K$ (27 days posttransfection) and $SIV_{mac}239/28K$ (82 days posttransfection) were harvested from culture supernatants of HUT-78 cells that contained predominantly 41- and 28-kDa TMPs, respectively (Fig. 3, experiment 1). The supernatant from each culture used for inoculation of fresh cultures is indicated by open circles. RTase, Reverse transcriptase.

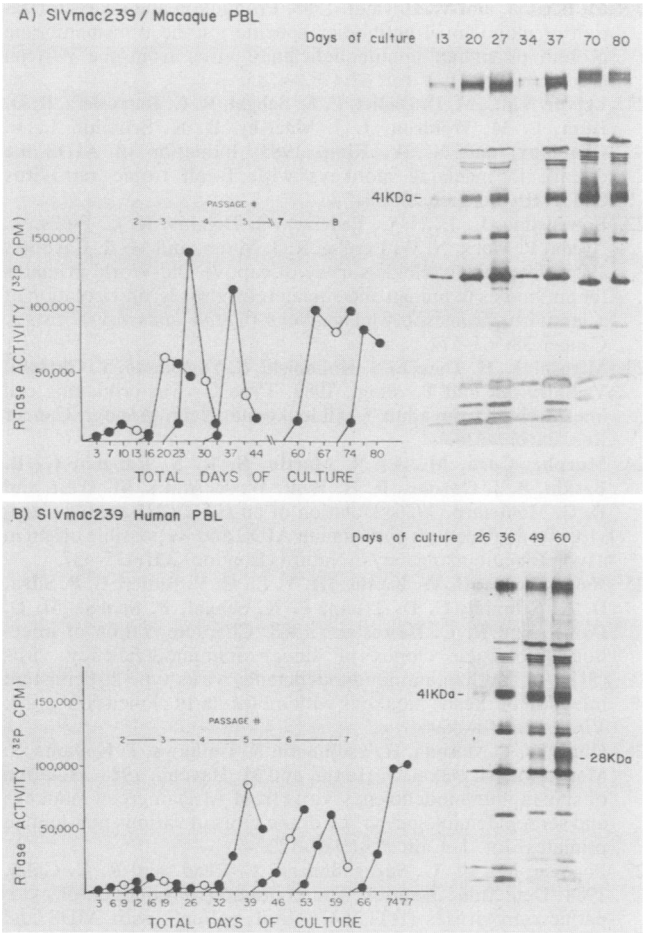

FIG. 6. Transfection of $SIV_{mac}239$ into macaque (A) and human (B) PBL. Viral replication was relatively delayed in human cells compared with that of macaque cells until passage 3. The macaque PBL continued to yield virus with 41-kDa TMP; however, the human PBL selectively replicated a virus with a 28-kDa TMP. Open symbols, culture supernatants used for passage; RTase, reverse transcriptase.

Desrosiers, unpublished data). The selective pressure for shorter versions of SIV TMP in human cells may vary with the particular isolate, the species of origin, and the particular human cells used for growth of the virus. In all likelihood, however, use of human cells has contributed to the appearance of shorter versions of the TMP with these different viruses. Since adaptation of SIV_{mac} to growth in human cell types has been shown to select variants which are antigenically, genetically, and biologically distinguishable from the original SIV_{mac}, the use of human cell lines for isolation and propagation of SIV should be avoided for many applications.

The premature stop codons are located in the cytoplasmic region of the TMP, just after the membrane-spanning domain. Human and macaque cells apparently differ in their requirements for cytoplasmic domain sequences to support SIV_{mac} replication. The cytoplasmic domain sequences are likely to interact with cellular molecules in the process of virus entry and in the assembly-and-budding process.

In addition to the TMP region, changes in the long terminal repeat or other regions are likely to contribute to selective growth of SIV_{mac} in human cells. This may be especially true for the HUT-78-adapted $SIV_{mac}142$ infectious clone, which is not able to replicate significantly in macaque lymphocytes or to infect macaque monkeys (25).

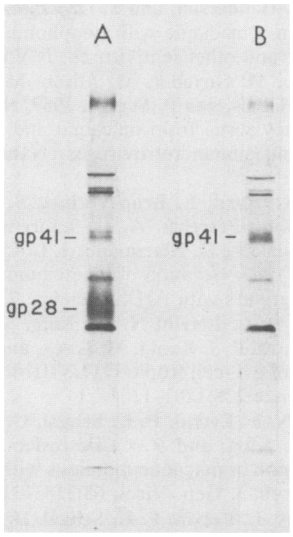

FIG. 7. Selection of $SIV_{mac}239$ with a 41-kDa TMP in vivo. Viral proteins present in derivatives of cloned $SIV_{mac}239$ which were harvested from a culture supernatant of HUT-78 cells for inoculation into a macaque (A) and those of viruses recovered from a T4-enriched PBL culture from a persistently infected macaque monkey 58 weeks after inoculation (B) are shown.

The determinants of this inability to replicate in macaque lymphocytes are likely to reside in regions other than that of the TMP stop codon.

The location of the stop signal in *env* of $SIV_{mac}142$ differed from its location in $SIV_{mac}251$ by only one codon. The calculated molecular weights of the unglycosylated forms of their TMPs are 23.8 kDa ($SIV_{mac}142$) and 23.9 kDa ($SIV_{mac}251$), and each has three potential sites for N-linked glycosylation. Unexpectedly, the TMP of the $SIV_{mac}251$ virus was 2 kDa larger than that of $SIV_{mac}142$ (30 versus 28 kDa) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The individual amino acid composition of the TMP could possibly influence the rate at which it proceeds through the Golgi or the nature or extent of glycosylation.

One possible origin of the HIVs is from monkeys via cross-species transmission. If interspecies transmission of SIV to humans did occur, we would expect adaptive mutation to select variants better suited to replication and spread in the human population.

ACKNOWLEDGMENTS

We thank Diane Schmidt and Cindi Troup for technical assistance, Beverly Blake for editorial assistance, and Nancy Adams and Joanne Newton for preparation of the manuscript.

This work was supported by Public Health Service grants AI25328, AI26463, AI25108, and RR00168 from the National Institutes of Health.

LITERATURE CITED

1. **Barre-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**:868–871.
2. **Baskin, G. B., M. Murphey-Corb, E. A. Watson, and L. N. Martin.** 1988. Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/Delta. Vet. Pathol. **25**:456–467.
3. **Benveniste, R. E., L. O. Arthur, C.-C. Tsai, R. Sowder, T. D.**

Copeland, L. E. Henderson, and S. Oroszlan. 1986. Isolation of a lentivirus from a macaque with lymphoma: comparison with HTLV-III/LAV and other lentiviruses. J. Virol. **60**:483–490.

4. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo.** 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature (London) **328**: 543–547.
5. **Clavel, F., D. Guetard, F. Brun-Vezinet, S. Chamaret, M.-A. Rey, M. O. Santos-Ferreira, A. G. Laurent, C. Dauguet, C. Katlama, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier.** 1986. Isolation of a new human retrovirus from West African patients with AIDS. Science **233**:343–346.
6. **Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers.** 1985. Isolation of a T-cell tropic HTLV-III-like retrovirus from macaques. Science **228**:1201–1204.
7. **Daniel, M. D., N. L. Letvin, P. K. Sehgal, G. Hunsmann, D. K. Schmidt, N. W. King, and R. C. Desrosiers.** 1987. Long-term persistent infection of macaque monkeys with the simian immunodeficiency virus. J. Gen. Virol. **68**:3183–3189.
8. **Daniel, M. D., N. L. Letvin, P. K. Sehgal, D. K. Schmidt, D. P. Silva, K. R. Solomon, F. S. Hodi, Jr., D. J. Ringler, R. D. Hunt, N. W. King, and R. C. Desrosiers.** 1988. Prevalence of antibodies to 3 retroviruses in a captive colony of macaque monkeys. Int. J. Cancer **41**:601–608.
9. **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 III, P. K. Sehgal, N. W. King, Y. Ohta, M. Hayami, and R. C. Desrosiers.** 1988. Simian immunodeficiency virus from African green monkeys. J. Virol. **62**:4123–4128.
10. **Franchini, G., P. J. Kanki, M. L. Bosch, K. Fargnoli, and F. Wong-Staal.** 1988. The simian immunodeficiency virus envelope open reading frame located after the termination codon is expressed in vivo in infected animals. AIDS Res. Hum. Retroviruses **4**:251–258.
11. **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 (London) **333**: 457–461.
12. **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.
13. **Getchell, J. P., J. L. Heath, D. R. Hicks, C. Sporborg, C. R. McGrath, and V. S. Kalyanaraman.** 1986. Continuous production of a cytopathic human T-lymphotropic virus in a permissive neoplastic T-cell line. J. Clin. Microbiol. **23**:737–742.
14. **Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) **236**:662–669.
15. **Hahn, B. H., L. I. Kong, S.-W. Lee, P. Kumar, M. E. Taylor, S. K. Arya, and G. M. Shaw.** 1987. Relation of HTLV-4 to simian and human immunodeficiency-associated viruses. Nature (London) **330**:184–186.
16. **Hirsch, V., N. Riedel, and J. I. Mullins.** 1987. The genome organization of STLV-3 is similar to that of the AIDS virus except for a truncated transmembrane protein. Cell **49**:307–319.
17. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
18. **Kannagi, M., L. V. Chalifoux, C. I. Lord, and N. L. Letvin.** 1988. Suppression of simian immunodeficiency virus replication in vitro by $CD8^+$ lymphocytes. J. Immunol. **140**:2237–2242.
19. **Kannagi, M., J. M. Yetz, and N. L. Letvin.** 1985. *In vitro* growth characteristics of simian T-lymphotropic virus type III. Proc. Natl. Acad. Sci. USA **82**:7053–7057.
20. **Kodama, T., Y. Ohta, T. Masuda, K.-I. Ishikawa, H. Tsujimoto, M. Ishakia, and M. Hayami.** 1988. Production and characterization of monoclonal antibodies specific for the transmembrane protein of simian immunodeficiency virus from the African green monkey. J. Virol. **62**:4782–4785.
21. **Letvin, N. L., M. D. Daniel, P. K. Sehgal, R. C. Desrosiers, R. D. Hunt, L. M. Waldron, J. J. MacKey, D. K. Schmidt, L. V. Chalifoux, and N. W. King.** 1985. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science **230**:71–73.
22. **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.
23. **Miyoshi, I., H. Taguchi, I. Kubonishi, S. Yoshimoto, Y. Ohtsuki, Y. Shiraishi, and T. Akagi.** 1982. Type C virus-producing cell lines derived from adult T cell leukemia. Gann Monogr. Cancer Res. **28**:219–228.
24. **Murphey-Corb, M., L. N. Martin, S. R. S. Rangan, G. B. Baskin, B. J. Gormus, R. H. Wolf, W. A. Andes, M. West, and R. C. Montelaro.** 1986. Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. Nature (London) **321**:435–437.
25. **Naidu, Y. M., H. W. Kestler III, Y. Li, C. V. Butler, D. P. Silva, D. K. Schmidt, C. D. Troup, P. K. Sehgal, P. Sonigo, M. D. Daniel, and R. C. Desrosiers.** 1988. Characterization of infectious molecular clones of simian immunodeficiency virus (SIV_{mac}) and human immunodeficiency virus type 2: persistent infection of rhesus monkeys with molecularly cloned SIV_{mac}. J. Virol. **62**:4691–4696.
26. **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.
27. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
28. **Ringler, D. J., M. S. Wyand, D. G. Walsh, J. J. MacKey, L. V. Chailfoux, M. Popovic, A. A. Minassian, P. K. Sehgal, M. D. Daniel, R. C. Desrosiers, and N. W. King.** 1989. Cellular localization of simian immunodeficiency virus in lymphoid tissues. I. Immunohistochemistry and electron microscopy. Am. J. Pathol. **134**:373–383.
29. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
31. **Tsujimoto, H., R. W. Cooper, T. Kodama, M. Fukasawa, T. Miura, Y. Ohta, K.-I. Ishikawa, M. Nakai, E. Frost, G. E. Roelants, J. Roffi, and M. Hayami.** 1988. Isolation and characterization of simian immunodeficiency virus from mandrills in Africa and its relationship to other human and simian immunodeficiency viruses. J. Virol. **62**:4044–4050.
32. **Veronese, F. D., B. Joseph, T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan.** 1989. Identification of simian immunodeficiency virus SIV_{MAC} *env* gene products. J. Virol. **63**:1416–1419.
33. **Zagury, J. F., G. Franchini, M. Reitz, E. Collalti, B. Starcich, L. Hall, K. Fargnoli, L. Jagodzinski, H.-G. Guo, F. Laure, S. K. Arya, S. Josephs, D. Zagury, F. Wong-Staal, and R. C. Gallo.** 1988. Genetic variability between isolates of human immunodeficiency virus (HIV) type 2 is comparable to the variability among HIV type 1. Proc. Natl. Acad. Sci. USA **85**:5941–5945.