Comparative Analysis of *Macaca Arctoides* STLV-1 *env* Gene Sequenced Fragment Coding for the gp46 Immunodominant Site with HTLV-1 (ATK) and STLV-1 of Different Primates

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Comparative analysis of the results of sequencing of the immunodominant site of STLV-1 *env* gene of *Macaca arctoides* (amino acid residues 176-256) with analogous sites of STLV-1 of different primates and with HTLV-1 showed that the majority of nucleotide replacements at this site of STLV-1 (81.8%) represent silent translation push mutations of the transition type. These replacements of STLV-1 amino acids possess hydrophobic and chemical properties similar to those of amino acid residues at the same positions of HTLV-1 and slightly affect spatial organization of the polypeptide carcass of ENV protein.

Key Words: *STLV-1; HTLV-1; immunodominant site*

STLV-1, simian T-lymphotropic type I retrovirus, possesses many common structural and biological properties with human T-lymphotropic virus HTLV-1 and is associated with T-cell lymphomas in monkeys [8,16]. Antibodies cross-reacting with HTLV-1/STLV-1 were detected in different monkey species in the Old World [6]. Analysis of sera from HTLV-1-infected humans [2,13] and STLV-1-infected monkeys [9] showed that the majority of sera contain neutralizing antibodies to the C-terminal immunodominant gp46 site localized between the 176th and 256th amino acid residues (Fig. 1, *a*) [3]; this site plays an important role in the HTLV-1 vital cycle [5]. Analysis of mutations at the immunodominant site of HTLV-1 *env* gene showed their functional significance; they can decrease the maturation rate of ENV protein precursor and impair formation of the infected cell syncytium [5]. The functional significance of mutation at this gene site and the phylogenetic similarity of STLV-1 and HTLV-1 prompted us to compare the fragments of STLV-1 *env* genes of different primates and the primary amino acid residues and their secondary structures derived from these fragments with a similar site of HTLV-1 (ATK) [11] in order to assess the significance of the detected amino acid replacements.

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MATERIALS AND METHODS

Total cell DNA was extracted by the phenol-chloroform method [10] from STLV-1 Ma (*Macaca arctoides*) producing lymphoid MAL-1 cells [1].

For polymerase chain reaction (PCR) and sequencing, we used oligonucleotide primers to the HTLV-1/STLV-1 conservative *env* gene site (positions 5684-5708) 5'-CTCCCTTCTAGTCGACGC TCCAGG-3'; (positions 6127-6151) 5'-CTCCCGCC GAGCGGTACCGGTGGC-3', amplifying the gene site coding for the immunodominant ENV protein fragment of amino acid residues position 176-256. Primers were synthesized in an Applied Biosystems-381A synthesizer and then purified routinely. STLV-1 fragments were amplified according to the Gene

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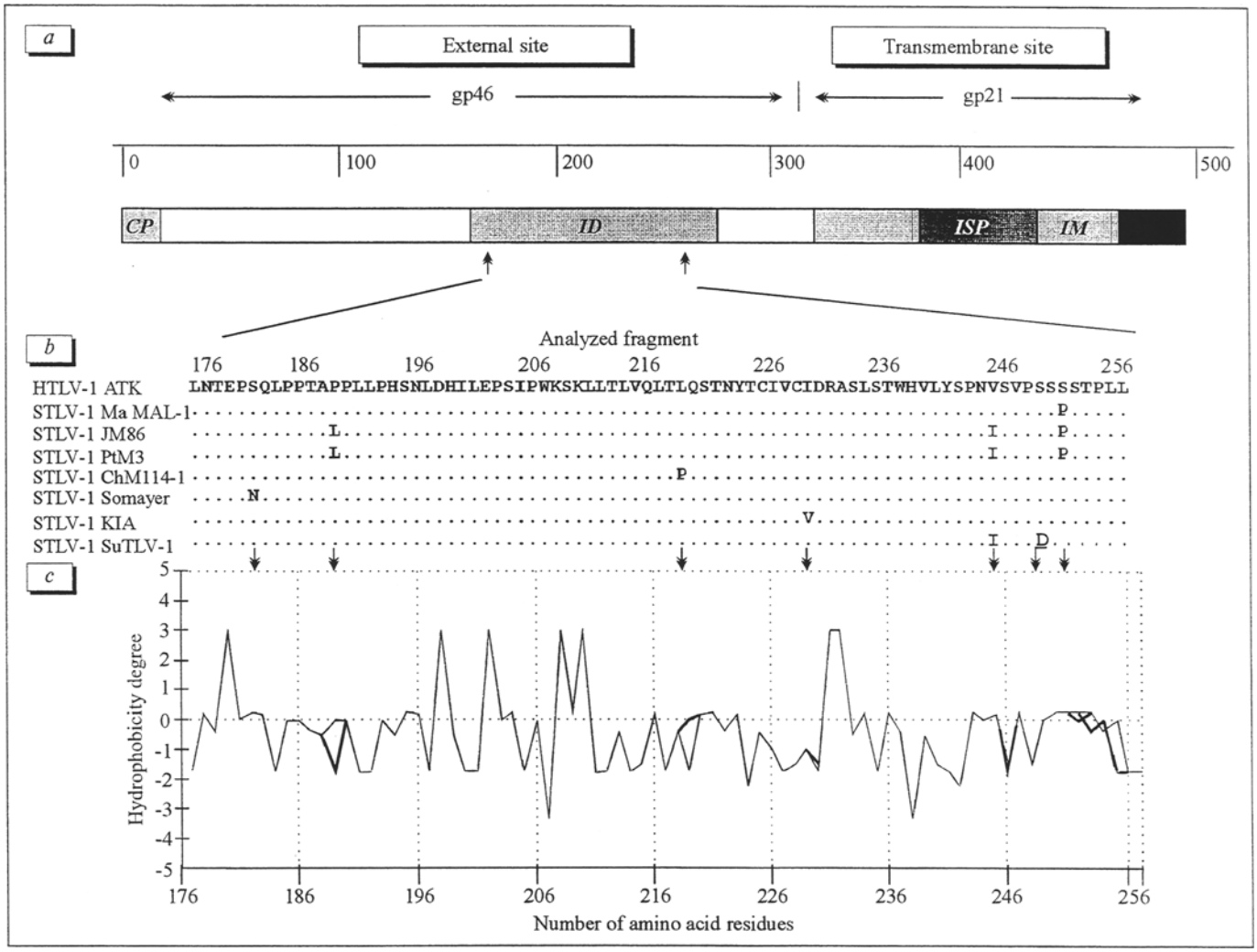

Fig. 1. Diagram of HTLV-1 ENV protein (*a*); comparison of the primary amino acid sequences of ENV protein fragments of STLV-1 from different species of primates with HTLV-1 (*b*) and comparative analysis of hydrophobicity of amino acid residues of ENV protein fragments of STLV-1 and HTLV-a (*c*). *a*) *SP*: signal peptide; *ID*: immunodominant site; *ISP*: immunosuppressor peptide; *IM*: intramembrane site. *b*) amino acids on ENV protein fragment identical to those of HTLV-1 are shown with dots. Amino acid residues are denoted routinely, with one letter: A — Ala; R — Arg; N — Asn; D — Asp; C — Cys; Q — Gln; E — Glu; G — Gly; H — His; I — Ile; L — Leu; K — Lys; M — Met; F — Phe; P — Pro; S — Ser; T — Thr; W — Trp; Y — Tyr; V — Val. D: deletion. *c*) The curve was plotted by successive overlapping of the curves reflecting hydrophobicity of amino acid fragments of ENV proteins of STLV-1 on HTLV-1. Hydrophobic profiles of ENV protein of HTLV-1 (thin line) and STLV-1 (dark line). Positive values: hydrophobicity, negative: hydrophilicity of amino acids.

Amply Kit protocol in a Perkin-Elmar Cetus Instruments automated amplifier. DNA extracted from MT-2 HTLV-1-positive culture was used as a positive control in the PCR. The negative control was a sample including all components of the reaction mixture without DNA. STLV-1 DNA fragments amplified in the PCR were eluted from gel using Geneclean II Kit BIO-101 and sequenced in two directions using the Promega fmol™ DNA Sequencing System Kit. Electrophoresis of sequenced specimens, gel fixation, and radioautography were carried out routinely [10].

For comparative computer-aided analysis, data of sequencing of STLV-1 *env* gene fragments of the following primates were used: Somayer (*Cercocebus atys*), ChM114-1 (*Pan troglodytes*), KIA (*Papio cynocephalus*), JM86 (*Macaca fuscata*) [14], SuTLV-1 (*Papio hamadryas*) [12], and PtM3 (*Macaca nemestrina*) [15].

Hydrophobic profiles and secondary structures of analyzed fragments were estimated using a Hitachi HIBIO Prosis System (protein input and analysis software system) [4,7].

RESULTS

Table 1 shows that the majority (88.8%) of nucleotide replacements on STLV-1 *env* gene fragments of different primates are point mutations of the transition type (C ↔ T, 72.8%; G ↔ A, 16%). Transversion replacements took place in 11.2% of cases (A ↔ C, 9%, A ↔ T, 1.7%,: G ↔ C, 0.5%). 81.8%

of nucleotide replacements are detected in the third codone base, 2.8% in the second, and 15.4% in the first codone base (Table 1). Since the majority of replacements are observed in the silent sites of the third codone base, the homologies of amino acid residues of ENV protein fragments in all STLV-1 are higher than the nucleotide homologies (Table 2).

Comparison of primary amino acid sequences (Fig. 1, *b*) showed replacements of amino acid residues on STLV-1 gp46 ENV fragments in positions 188 (P → L), 245 (V → I), 251 (S → P) in STLV-1 (JM86, PtM3), 218 (L → P) STLV-1 (ChM114-1), 181 (S → N) STLV-1 Somayer, 229 (I → V) STLV-1 (KIA), 245 (V → I) STLV-1 (SuTLV-1), and 251 (S → P) STLV-1 Ma MAL-1. Due to the tendency towards similarity of codones for amino acids of the same type, nucleotide replacements on STLV-1 *env* gene led to replacements for amino acids with hydrophobic and chemical properties similar to those of amino acids in the same positions on HTLV-1 ENV protein. Computer hydrophobic analysis (Fig. 1, *c*) showed that hydrophobicity indices differing from the relevant amino acid residues in HTLV-1 ENV detect amino acids in positions 188 (P → L) (STLV-1, JM86, PtM3), 218 (L → P) (STLV-1, ChM114-1). The HTLV-1 ENV protein site contains the T-helper (194-210 amino acids) and linear B-cell epitopes (187-193, 191-196, 193-199 amino acids) [2]; in the STLV-1 from monkeys of different species this site was highly conservative (Fig. 1, *b*).

The capacity for a certain conformation is critical for this protein. Therefore, in order to detect the effects of replacements in primary amino acid sequence, we compared the tentative secondary structures of HTLV-1 and STLV-1 ENV protein fragment [4] (Fig. 2).

Analysis of secondary structures showed that the conformation changes add replacements 218 (P → L) (STLV-1 ChM114-1) (abolition of α-spiral conformation) and 188 (L → P) (STLV-1, JM86, PtM3) (addition of extra β-plicae and tetrapeptide b-wave). At the same time, replacements of 245 (V → I) (STLV-1, JM86, PtM3, SuTLV-1), 181 (S → N)

TABLE 1. Analysis of Sequenced Fragments of the External Site of *env* Gene Coding for 5'-Terminal of STLV-1 gp46 (Positions 5727-5969 [11])

	5727	5807
HTLV-1 ATK	CTTAATACCGAACCCAGCCAACTGCCTCCCACCGCCCCTCCTCTACTCCCCCACTCTAACCTAGACCACATCCTCGAGCCC	
STLV-1 Ma MAL-1	.....	
STLV-1 JM86	.....C..T.....T.....C.....T....T.....T..C...T.G.....T.....	
STLV-1 PtM3	.....C.....T.....C.....T....T.....T..C...T.G.....T	
STLV-1 Somayer	.....C.....T.A.....C.....T.....	
STLV-1 ChM114-1	..C..C.....T.....C.....T.....	
STLV-1 KIA	..C..C.....T.....C.....T.....T.....A..G	
STLV-1 SuTLV-1	.....C..T.....C.....T.....T..C...T.G.....A..T	

	5808	5888
HTLV-1 ATK	TCTATACCATGGAAATCAAAACTCCTGACCCTTGTCCAGTTAACCCTACAAAGCACTAATTATACTTGCATTGTCTGTATC	
STLV-1 Ma MAL-1	.....T.....T.....AC.....G.....C.....C..A	
STLV-1 JM86	..C.....G.....A..T.....AC....T.....C..C.....T.....C..A	
STLV-1 PtM3	..C.....G.....A..T.....A.....T.....C..C.....T.....C..A	
STLV-1 Somayer	.....T....T.....AC.....G.....C.....A	
STLV-1 ChM114-1	.....T....T.....C.....C.....A	
STLV-1 KIA	.....T....T..C.....C.....C.....G.A	
STLV-1 SuTLV-1	..C.....T..T..A.....G..G..T.....C..C.....C.....A	

	5889	5969
HTLV-1 ATK	GATCGTGCCAGCCTCTCCACTTGGCACGTCCTATACTCTCCCAACGTCTCTGTTCCATCCTCTTCTTCTACCCCCCTCCTT	
STLV-1 Ma MAL-1	.....A.....G.....C.....	
STLV-1 JM86	.....T.A..T.....T....T.....A.....CC.C.....T.....	
STLV-1 PtM3	.....T.A..T.....T.....A.....CC.C.....T.....	
STLV-1 ChM114-1	.....A.....G.....	
STLV-1 Somayer	.....A.....G.....C.....	
STLV-1 KIA	.....A.....G.....A.....	
STLV-1 SuTLV-1	..C.....T..TT.A..T..C.....T..T.....T...A.....C...TCC..C..C..C..T.....A	

Note. *Identical nucleotides are shown with dots.*

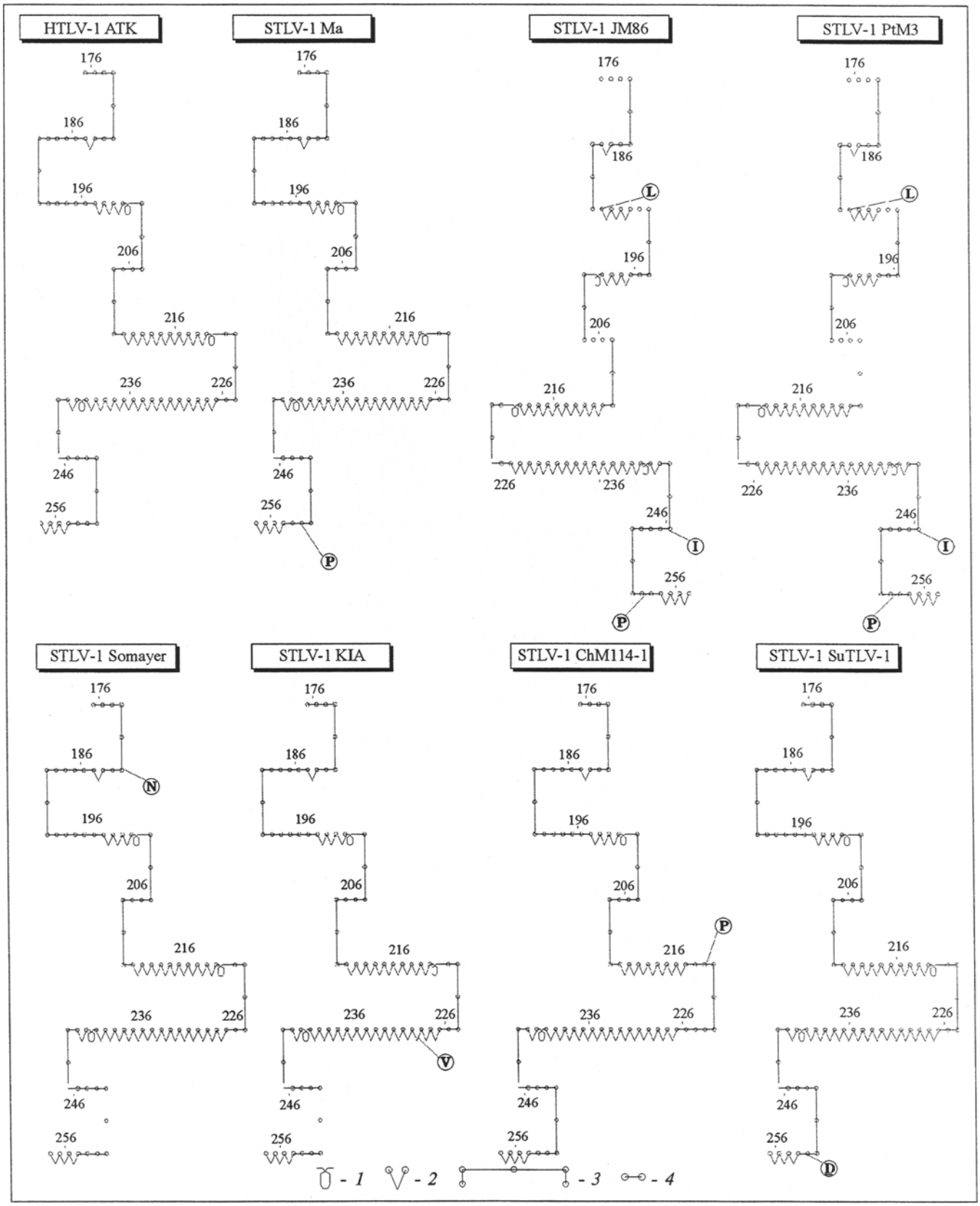

Fig. 2. Probable secondary structures of sequences of ENV protein amino acid residue fragments of STLV-1 from different species of primates with HTLV-1. Amino acid residues different from HTLV-1 in corresponding positions are shown with letters. Two-dimensional structure: *1*) α-spiral; *2*) β-plica; *3*) tetrapeptide b-wave; *4*) curve. D - deletion.

TABLE 2. Homology of Sequences of Nucleotides and Amino Acids of HTLV-1 (ATK) [11] and STLV-1 from Different Species of Primates [12,14,15]

Virus/isolate/species	Nucleotide homology of *env* gene fragments 243 b.p. (5727-5969)							
	HTLV-1 ATK	STLV-1 Ma	STLV-1 JM86	STLV-1 PtM3	STLV-1 Somayer	STLV-1 ChM114-1	STLV-1 KIA	STLV-1 SuTLV-1
HTLV-1 ATK (*Homo sapiens*)		95.5	86.4	87.8	93.8	95.5	92.6	84.1
STLV-1 Ma MAL-1 (*Macaca arctoides*)	98.7		88.5	88.9	95.9	94.7	92.2	83.7
STLV-1 JM86 (*Macaca fuscata*)	97.5	97.5		97.9	88.5	88.1	86.4	87.2
STLV-1 PtM3 (*Macaca nemestrina*)	97.5	97.5	100		88.9	88.5	87.2	88.4
STLV-1 Somayer (*Cercocebus atys*)	98.7	97.5	95.1	95.1		97.1	95.1	85.4
STLV-1 ChM114-1 (*Papio troglodytes*)	98.7	97.5	95.1	95.1	97.5		96.7	84.1
STLV-1 KIA (*Papio cynocephalus*)	97.5	97.5	95.1	95.1	97.5	97.5		84.5
STLV-1Ph (SuTLV-1) (*P. hamadryas*)	97.5	97.5	97.5	97.5	96.3	96.3	96.3	
	Homology of amino acid residues of ENV protein fragments (amino acids 176-256)							

(STLV-1 Somayer), 229 (I → V) (STLV-1 KIA), 218 (L → P) (STLV-1 ChM114-1), 251 (S → P) (STLV-1 Ma, JM86, PtM3), and serine deletion in STLV-1 Ph (SuTLV-1) do not affect spatial organization of the ENV protein polypeptide framework.

Thus, our data on conservative nature of the STLV-1 immunodominant site on the 176-256 fragment are an additional proof of structural and functional similarity of STLV-1/HTLV-1 viruses reflecting similar evolutional pressure exerted on this site *in vivo*. The highly conservative nature of the studied fragment of STLV-1 ENV protein containing linear B- (amino acids 194-210) and T-cell epitopes (amino acids 187-193, 191-196, and 193-199) responsible for production of virus-neutralizing antibodies and stimulation of T-cytotoxic lymphocytes in HTLV-1 [2] permits using STLV-1 infection of monkeys as a model system for testing subunit anti-HTLV-1 vaccines.

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REFERENCES

1. V. V. Timanovskaya, L. A. Yakovleva, L. V. Indzhiya, *et al.*, in: *Malignant Lymphomas and the Associated Lymphotropic Viruses* [in Russian], Moscow (1986), pp. 149-154.
2. E. Baba, M. Nakamura, K. Ohkuma, *et al.*, *J. Immunol.*, **154**, 399-412 (1995).
3. Y. M. Chen, T.-N. Lee, K. P. Samuel, *et al.*, *J. Virol.*, **63**, 4952-4957 (1989).
4. P. Y. Chou and G. D. Fasman, *Adv. Enzymol.*, **47**, 145-148 (1978).
5. L. Delamare, C. Pique, D. Pham, *et al.*, *J. Virol.*, **68**, 3544-3549 (1994).
6. M. Hayami, A. Komuro, K. Nozawa, *et al.*, *Int. J. Cancer*, **33**, 179-183 (1984).
7. T. P. Hopp and K. R. Woods, *Proc. Natl. Acad. Sci. USA*, **78**, 3824 (1981).
8. B. A. Lapin, in: *Virus Disease in Laboratory and Captive Animals*, G. Darai (Ed.), Boston - Dordrecht - Lancaster (1988), pp. 135-151.
9. D. L. Rudolph, J. Yee, T. Palker, *et al.*, *Res. Virol.*, **144**, 193-199 (1993).
10. J. Sambrook, E. F. Fritsch, and T. Maniatis, *Molecular Cloning. A Laboratory Manual*, New York (1989).
11. M. Seiki, S. Hattori, Y. Hirayama, *et al.*, *Proc. Natl. Acad. Sci. USA*, **80**, 3618-3622 (1983).
12. H. Shatzel, L. Yakovleva, B. Lapin, *et al.*, *Leukemia*, **6**, Suppl. 3, 158S-160S (1992).
13. M. P. Sherman, S. Dube, T. P. Spicer, *et al.*, *Cancer Res.*, **53**, 6067-6073 (1993).
14. K. J. Song, V. R. Nerurkar, N. Saitou, *et al.*, *Virology*, **199**, 56-66 (1994).
15. T. Watanabe, M. Seiki, Y. Hirayama, *et al.*, *Ibid.*, **148**, 385-388 (1986).
16. L. A. Yakovleva, K. Lennert, M. G. Chikobava, *et al.*, *Virchows Arch. [A]*, **422**, 109-120 (1993).