Detection, cloning, molecular characterization and phylogenic analyses of a new primate T-Cell lymphotropic virus type I in olive baboon

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Abstract

Infection with Human T-cell Lymphotropic Virus Type I (HTLV-I) is a global health problem, affecting 10 to 20 million people around the world, including north-east of Iran. It has been recognized to be the etiologic agent of adult T-cell Leukemia and HTLV-I-associated Myelopathy. In both cases, the HTLV-I transactivator protein (Tax), plays crucial role. Monkeys are suitable host for a related virus called STLV, which together with HTLV are called Primate T-cell Lymphotropic Viruses (PTLVs). Primates are the only known hosts, in addition to human, to be able to develop malignant changes in the natural course of infection with PTLV-I and therefore, could be a valuable animal model for studies on this virus. In the present study, we report PCR-based detection and cloning of 1.8 kb pX region of a new PTLV in olive baboon (Papio anubis). Sequence alignments and phylogenic studies on nucleotide sequence of this region and amino acids of conceptually translated Tax protein showed that monkeys are infected with a PTLV much closer to HTLV-I sub-types a and b, rather than STLV-I. Moreover, analyses of its Tax protein suggest that it might have the same function as HTLV-I Tax proteins. Results of our study indicate the possibility of exploiting these baboons as an animal model of choice for evaluating a tax-based DNA vaccine to decrease the viral load of HTLV-I in carriers , in order to prevent the outcomes, as well as they may be utilized for other HTLV-I physiopathologic or therapeutic studies.

Keywords *: Primate T-lymphotropic virus 1, Human T-lymphotropic virus 1, pX genes, Phylogeny, Animal models, Papio.*

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INTRODUCTION

Primate T lymphotropic Virus (PTLV) is a general name for a group of delta retro viruses which infect T lymphocytes of different primates (Mahieux *et al*., 1998; Saksena *et al*., 1994; Meertens *et al*., 2001 and Goubau *et al*., 1994). Human T Lymphotropic virus type I (HTLV-I), firstly described in 1980 (Poiesz *et al*.), may be the most famous member of this family. This virus is a well known etiologic agent for Adult T cell Leukemia (ATL) and HTLV-I associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) (Gessain *et al*., 1985 and Barmak *et al.*, 2003). It is estimated that some 15-20 million people are infected with this virus around the world (Slattery, 1999). A known endemic focus is Khorasan, the north-eastern province of Iran, with 0.76-3% seropositivity in Mashhad and even higher percentage in Neishabour (Safai *et al.*, 1996 and Abbaszadegan *et al*., 2003). HTLV-II, another member of this family, has been also linked to some cases of hairy cell leukemia, but its importance and prevalence is much less than HTLV-I (Lewis *et al.*, 2002). There are other viruses in this family, Simian T lymphotropic Viruses (STLVs), whose primary hosts are monkeys (Slattery, 1999 and Salemi, 2000). These viruses share both common features and pathogenic properties of their human counterparts in non human primates (Vincent, 1996).

Among animals, primates are the closest neighbors of humans in the evolutionary tree. They are divided into different species. Moreover, monkeys who live in Old World (Asia/Africa continents) show higher similarity to humans than New World Monkey (America) (for further information about primates: http://www.primate.wisc.edu/pin/factsheets.html). Different monkeys have been studied for the presence

of HTLV/STLV viruses, and seropositive animals have been identified both in their natural environment and in captivity with prevalence rates as high as 60% (Mahieux *et al.,* 1998; Saksena *et al.,* 1994; Meertens *et al.,* 2001 and Allan *et al.,* 2001). In addition, different diagnostic tests for detecting HTLVs, like ELISA, Southern and Western Blotting, and PCR have been successfully applied for these animals (Komuro *et al.,* 1984;Voevodin *et al.,* 1985; Traina-Dorge *et al*., 1992 and Voevodin *et al.,* 1996). Besides, in some baboons (Papio species) some ATL-like lymphocytic changes have been reported (Saksena *et al.,* 1994; McCarty *et al.,* 1990; Hubard *et al.,* 1993 and Voevodin *et al*., 1996). Although Tax, transactivator protein of HTLV -I, has been shown to be able to induce malignant changes in non primate animal cells (Matsumoto *et al.,* 1997 and Liang *et al.,* 2001) but Monkeys are the only known hosts, in addition to human, who have developed such a malignant change in the natural course of infection (Voevodin *et al.,* 1985; Voevodin *et al.,* 1996; McCarhty *et al.,* 1990; Hubard *et al.,* 1993 and Allan *et al.,* 2001).

Olive baboon is an old world monkey. In nature, these animals are inhabitants of central Africa and Ethiopia (Mahieux *et al.,* 1998; Goubau *et al.,* 1994; Slattery, 1999 and Row, 1996), however several baboons have been captured and kept in colonies around the world (Voevodin *et al.,* 1996 and Allan *et al.,* 2001). Recently, it is shown that *P. anubis* is natural carrier of an STLV-1 strain which shows much higher homology in LTR and *env* regions to HTLV1 than other STLV1 strains (Mahieux *et al.,* 1998), indicating that this animal may be a proper choice for srudies on HTLV-I associated diseases.

Herein, we report detection, cloning, characterization and phylogenic analysis of a new PTLV-I *pX* region and its conceptually translated Tax protein in two *Papio anubis* (Olive Baboon). This information might particularly be beneficial for establishing a suitable animal model for HTLV-I vaccine studies based on Tax protein.

MATERIALS AND METHODS

Animals and preparation of peripheral blood mononuclear cells (PBMCs): Baboons are kept in a colony in south of France, (Centre de Recherches du Service de Sante des Armes (CRSSA, La Tronche, France) under the French ethical regulations for animal care. PBMCs were separated from EDTA-treated blood samples of animals whose sera had been positive in HTLV-I western blot tests. The cells were isolated by sucrose density centrifugation (Ficoll-Paque gradient), washed twice in RPMI 1640 medium (Gibco-BRL, USA) and cultured for 24 h at 37°C with 5% CO_2 in RPMI 1640 medium containing foetal calf serum (20%), L-glutamine (2 mmol/l), penicillin (50 IU/ml) and streptomycin (50 µg/ml). Subsequently the cells were cultivated in the presence of phytohaemagglutinin (10 µg/ml) and recombinant IL-2 (Boehringer-Mannheim, Germany) at 25 U/ml and freezed in FCS plus 10% DMSO and stored in liquid Nitrogen Tank until use. Samples of two different animals were exploited for PTLV genes isolation.

DNA extraction: PBMC pellets were resuspended in 530 µl TE buffer (pH 7.5), then 60 µl SDS 10% and 12 µl proteinase K (10 mg/ml) and 67 µl NaCl (5 M) were added to it , mixed gently and incubated at 42°C overnight. DNA was extracted by conventional phenol/chloroform extraction method and ethanol precipitation (Ausubel *et al.,* 1999). Finally DNA pellet was dissolved in 200 µl of distilled water and used as template for amplification of PTLV sequences.

Isolation of PTLV-1 by PCR: The *pX* region was amplified in 2 fragments by a nested and a modified semi-nested approach. Tax was first amplified by QTAX1-KALTRAS then by TAXATG-TAXSTP pair of primers. TAXATG primer, which overlaps with 5´ terminus of QTAX1, adds the 4 early nucleotides in the cDNA which are located in the second exon of *tax* while the rest is coded by the third exon (Koralnik *et. al.,* 1992). The upper *pX* region was amplified separately by KA1-KA2AS and then KA3-PRIM2 primers (sequence of primers and their locations are listed in table 1).

Table 1. Sequence of primers used for PCR amplification and their positions according to ATK1 sequence.

Primer	Sequence	Position
QTAX1	5'-TTCCCAGGGTTTGGACAGAGT-3'	7330-7350
KALTRAS	5'-GAGACGTCAGAGCCTTAGTCT-3'	8417-8397
TAXATG	5'-ATGGCCCACTTCCCAGGGTTTGG-3'	7325-7343
TAXSTP	5'-GAAACAGAAGTCTGA-3'	8368-8382
KA1	5'-GGCCTTAACTGGGACCTTGG-3'	6468-6503
KA2AS	5'-AGGAGGGTGGAATGTTGGG-3'	7633-7615
KA3	5'-TCTTGTTATCCTTGCAGG-3'	6564-6581
PRIM2	5'-GTAAGGACCTTGAGGGTC-3'	7592-7575

All PCR reactions were carried out as: Five minutes of denaturation at 94°C followed by 35 cycles of denaturation (95°C, 1 min), annealing (55°C, 1 min except for TAXATG-TAXSTP for which temprature was adjusted to 45°C) and elongation (72°C, 1 min) and then 5 minutes at 72°C . The reactions were performed in a GeneAmp™ PCR System 9700 thermocycler (Applied Biosystems, MA. USA) using Taq DNA polymerase (Promega).

Cloning and sequencing: All PCR fragments were cloned into pCR4-TOPO vectors and transformed into *E. coli* TOP10 competent cells according to manufacturer's instructions (Invitrogen), and subsequently were sent for sequencing (ESGS, Evry, France). 3 to 6 clones for each fragment were sequenced to ensure the authenticity of the consensus sequences.

Phylogenetic studies: Twenty complete sequences of PTLVs and a BLV sequence were downloaded from NCBI genbank. These sequences are representative of different PTLV viruses (Salemi *et al.*, 2000). For comparing *pX* regions, all PTLV and Bovine Leukemia Virus (BLV) sequences were manually edited in BioEdit (Hall, 1999) and aligned in ClustalX softwares (Thompson *et al.*, 1997) with the following preset parameters: Gap opening score: 10, Gap extension: 0.20, DNA transition weight: 0.50 and IUB DNA weight matrix. Then the sequences were manually trimmed to remove the extra neighbouring sequences, and re-aligned by ClustalX with the same parameters. The aligned files were imported into MEGA2 version 2.1 software (Kumar *et al.*, 2001). Phylogenetic trees were calculated and drawn by Neighbor-Joining and UPGMA methods (Mount, 2001), applying Kimura 2 parameter model and bootstrapped with 1000 replications. BLV sequence was used to root the PTLV tree. Tax proteins were conceptually translated from genomic DNA sequence, by adding 4 early nucleotides (ATGG) to the *tax* splicing donor site of *pX* region (Koralnik *et al.*, 1992). Phylogenic tree for Tax amino acid sequence was constructed by p-distance method and bootstrapped with 1000 replications. DNA and Protein divergence tables were also calculated by Pair wise Distance method.

Protein structure analysis: Sequences of Tax proteins of MT2 cell line, M32 mutant of pCTax (Rimsky *et al.*, 1988 and Smith and Greene, 1990) and the newly found PTLV were analyzed by Kyte-Dollitle Hydropathy graph using BioEdit software (Hall, 1999). The window size for calculations was set to 9.

RESULTS

***pX* region sequence:** *pX* region of the PTLV virus was amplified in two 1061 bp and 1028 bp fragments, with an overlapping region of 267 bps. In order to have a complete *tax* cDNA, 4 early nucleotides of this cDNA were added to the forward primer of the second PCR of the last exon of *tax* (TAXATG) (Koralnik *et al.*, 1992). This cDNA which is cloned in pCR4 could be used for subcloning into expression vectors for further functional studies. The upstream part of *pX* region was amplified separately to cover the end of *env* gene and a short fragment of *tax*.

The *tax* gene isolated from our baboons showed a high homology with prototypic HTLV-I sequences both in 1.8 bp fragment of the *pX* region, *tax* region, and conceptually translated Tax protein (Table 2). This relationship was stronger between this newly found sequence and HTLV-1 strains rather than STLV-1 or HTLV/STLV II strains, as it is indicated by lower nucleotide and amino acid divergence in table 2. The baboon Tax differs from MT2 Tax only in 9 amino acids (Fig. 1). Another interesting difference with all other analyzed sequences was observed in the part of *pX* region upstream to the last exon of *tax*, which was a unique 21 bp deletion in all 4 sequences derived from the both baboons in this region (Fig. 2).

Phylogenetic Analysis: studies on *pX* region nucleotide sequence, *tax* nucleotide and amino acid sequences show that our new PTLV lies between HTLV-1 a and b strains (Fig. 3). This is indicated by very high bootstrap values for clusters of HTLV-Ia and b that contain our PTLV both in *pX* region and Tax protein (100 and 99, respectively) and short distances between different branches of these clusters.

Secondary structure prediction studies: The mutation site which is located in a known inactivating site within Tax protein (Smith and Greene, 1990) was further analysed for structural change due to the amino acid difference between the newly found PTLV Tax and the wild type HTLV-I Tax. The hydrophaty plot for residues 180 to 220 of 3 Tax variants , wild type MT2, M32 mutant and baboon PTLV , are shown in figure 4. Hydropathy indices for Histidine (H), Tyrosine (Y) and Alanin (A) are -3.2, -1.3 and 1.8, respectively, which reflex hydrophilic nature of Histidine and Tyrosine in contrast to hydrophobic property of Alanin (Kyte and Doolittle, 1982).

Table 2. PTLV1- sequences: Nucleotide and amino acid divergence of pX region, tax gene and Tax proteins between the newly found PTLV and other strains of reported PTLVs.

Strain	Accession No.	Nucleotide divergence of pX region (%)	Nucleotide divergence of tax (%)	Amino acid divergence (%)
HTLV- Ia(MT2)	L03561.2	0.0499	0.0388	0.0259
HTLV-Ia	L36905.1	0.0505	0.0397	0.0259
HTLV-Ia	AF042071.1	0.0505	0.0388	0.0259
HTLV-Ia	M86840.1	0.0531	0.0406	0.0317
HTLV-Ia(ATK1)	J02029.1	0.0524	0. 0388	0.0259
HTLV-Ia	U19949.1	0.0531	0.0406	0.0317
HTLV-Ia	D13784.1	0.0473	0.0406	0.0288
HTLV-Ib	M67514.1	0.0512	0.0388	0.0317
HTLV-Ic	L02534.1	0.1113	0.0945	0.0807
STLV-I	Z46900.1	0.1272	0.0936	0.0749
HTLV-IIa	M10060.1	0.3568	0.275	0.2622
HTLV-IIb	L20734.1	0.3504	0.2694	0.2565
HTLV-IIb	Y13051.1	0.3485	0.2684	0.2565
HTLV-IIb	L11456.1	0.3491	0.2684	0.2536
HTLV-IId	Y14365.1	0.3529	0.2713	0.2565
STLV-II	U90557.1	0.3574	0.3261	0.317
STLV-II	Y14570.1	0.36	0.2873	0.2305
STLV-L(PH969)	Y07616.1	0.3785	0.2722	0.245
BLV	K02120.1	0.5978	-	-

DISCUSSION

There is no doubt about the importance of animal models for studying the effects of viral pathogens and specially developing vaccines against them for a final goal of utilization in human being (Kazanji *et al.*, 2001). Although handling monkeys is not as easy as small laboratory animals like mice or rabbits, and much more expensive than them, their striking similarity with man has made them the most reliable animal model for human pathogens (Kaup, 2002). This picture is more striking in the case of HTLV, because monkeys are the only known non-human animal model so far, in which the virus replicates and leads to pathogenisity (Kazanji *et al*., 2000; Morteux *et al*., 2001; Allan *et al*., 2001).

To our knowledge, this is the first report of a new PTLV-*pX* region sequence in *P. anubis* which is very close to HTLV-I while is somehow different from other reported PTLV sequences. There is a unique 21 bp deletion in the noncoding region of the provirus between *env* and the second coding exon of *tax* (Fig. 2). Although this part of genome does not code the essential proteins of HTLV-I (ORF-I and II) (Koralnik, 1992), this variation is a good index that the sequence isolated from these baboons is not a close relative of any previously reported sequences, as no similar 21 bp sequence could be found by Blast tool in nr bank of NCBI Genbank. We have sequenced about 1.8 kb which encompasses about 20% of the 9 kb genome of PTLV-I. Phylogenic studies based on DNA and protein sequences both classify the newly found virus among HTLV-I strains and closely related to Ia and Ib subtypes, but not exactly in either group (Fig. 3). This is in accordance with a previous report of an HTLV-I related virus which was classified as Ib based on a 514 bp fragment of its LTR region, but Ia based on its 523 bp *env* fragment, in another baboon from Kenya (Mahieux *et al.*, 1998). Finding a PTLV which is closer to HTLV rather than STLV in a monkey might not be a rare event, because the evolution of this family of viruses seems to have stronger correlation with geographical distribution rather than species of the primate host (Goubau *et al.*, 1994 and Slattery, 1999). This similarity reflects several past exchanges between different hosts during the evolutionary history of these viruses (Slattery, 1999 and Salemi, 2000).

Tax is the transactivator protein of HTLV-I and plays the most important role in pathogenesis of different HTLV-I associated diseases (Matsumoto *et al.*, 1997;

Neuveut and Jeang, 2000; Grant *et al.,* 2002 and Barmak *et al.,* 2003). The similarity of *tax* gene and protein of this new sequence and other HTLV-1 sequences (Fig. 1), suggests that the newly found virus in our baboons might have similar pathogenic effects in this monkey as it is in the case for human. This similarity also supports the previous reports of developing ATL like diseases in baboons (McCarthy *et al.,* 1990 and Hubard *et al.,* 1993 and Voevodin *et al.,* 1996).

It is note worthy that the first 93 amino acids of Tax are highly conserved among all PTLV subtypes, including our newly found virus (Fig. 1). This is in concordance with critical role of Tax N-terminal in interactions with CREB and CBP proteins that lead to transactivating the promoter of PTLV ,which is located in its LTR (Grant *et al.,* 2002 and Yin *et al.,* 1995; Goren *et al.,* 1995 and Barmak *et al.* ,2003). This function of Tax might be more important for the replication cycle of the virus than its activity up on other cellular promoters which are related to other pathogenic effects of Tax (Smith and Greene, 1990; Matsumoto *et al.,* 1997 and Grant *et al.,* 2002).

The most detailed study on effects of different Tax mutations on its function has been reported by Smith

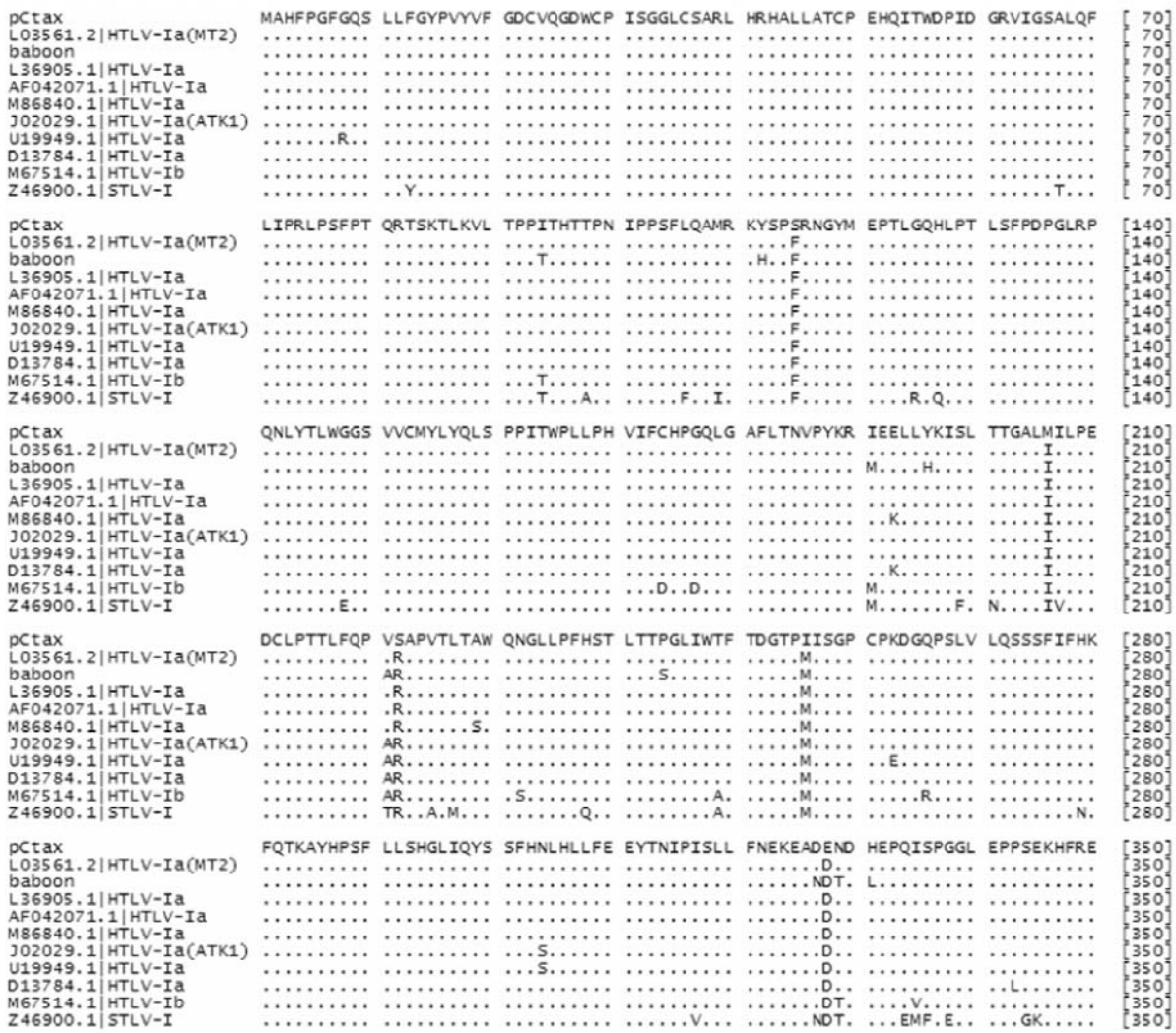

Figure 1. Amino acid alignment of different Tax proteins (1 to 350). The last 3 amino acids (351-3) are TEV in all strains. pCtax is the tax expression vector was described by Rimsky (Rimsky *et al.,*1988) and later employed by Smith and Greene and M1-M52 mutants are its derivatives (Smith and Greene, 1990). Dots (.) represent the same amino acid as the ones present in pCTax.

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baboon-px           AACTCCTGCA TTTTTTCTTT C---------- ---------- ---TTCTCAG CCCCTTGT-- -------CTC  [ 450]
L03561.2|HTLV-Ia(MT2) .......... .......... .C-TAGCACT ATGCTGTTTC GCC..... ........-- -------...  [ 450]
L36905.1|HTLV-Ia    .......... .......... .C-TAGCACT ATGCTGTTTC GCC..... ........-- -------...  [ 450]
AF042071.1|HTLV-Ia  .......... .......... .C-TAGCACT ATGCTGTTTC GCC..... ........-- -------...  [ 450]
M86840.1|HTLV-Ia    .......... .......... .C-TAGCACT ATGCTGTTTC GCC..... ........-- -------...  [ 450]
J02029.1|HTLV-Ia(ATK1) .......... .......... .C-TAGCACT ATGCTGTTTC GCC..... ........-- -------...  [ 450]
U19949.1|HTLV-Ia    .......... .......... .C-TAGCACT ATGCTGTTTC GCC..... ........-- -------...  [ 450]
D13784.1|HTLV-Ia    .......... .......... .C-TAGCACT ATGCTGTTTC GCC..... ........-- -------...  [ 450]
M67514.1|HTLV-Ib    .......... .......... .C-DDDDDDD DDDDTGTTTC GCC..... ........-- -------...  [ 450]
L02534.1|HTLV-Ic    ......GT.. .......C.. TC-TAGCACT TCGCTGTTTT GCTCCT.GTT .TGTC...-- -------...  [ 450]
Z46900.1|STLV-I     ......CCT. .....C..C. .C-TAGCACC ACGCGGCTTT GCC....G.. ...T....TC TGCCTCT...  [ 450]
M10060.1|HTLV-IIa   ...C..C..T CACA...C.C .CATAGGA-- ---------- -CC....TTT ......C--- ----------  [ 450]
L20734.1|HTLV-IIb   ...C..C..T CACA...C.C .CATAGGG-- ---------- -CC..T.TTT ..T...CC-- ----------  [ 450]
Y13051.1|HTLV-IIb   ...C..C..T CACA...C.C .CATAGGG-- ---------- -CC..T.TTT ..T...CC-- ----------  [ 450]
L11456.1|HTLV-IIb   ...C..C..T CACA...C.C .CATAGGG-- ---------- -CC..T.TTT ..T...CC-- ----------  [ 450]
Y14365.1|HTLV-IId   ...C.....T CACA...C.C .CATAGGA-- ---------- -CC....TTT ..T.C.CC-- ----------  [ 450]
U90557.1|STLV-II    ......C.TT CGCA...CCC .CACAAAC-- ---------- -ACG..CGCT ......CC-- ----------  [ 450]
Y14570.1|STLV-II    .........T CACA...C.C .CATAAAC-- ---------- -ACG..CGCT ....C.CC-- ----------  [ 450]
Y07616.1|STLV-L(PH969) .G..G...GT ...CCCGC.C .AAGAAATCT A---TGTAGC CCCC.AGG.A TT.ACA.AAC CCCTCAAAC.  [ 450]
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Figure 2. Nucleotide alignment of different PTLV sequences in a region upstream to the last exon of tax .The sequence alignment corresponds to 6828-6887 region in ATK1 strain (J02029.1). The 21 bp deletion is shown by dashes in the newly found PTLV (first row). A shorter deletion in PTLV-II sequences is present in the same region, but is absent in all PTLV-I sequences. Dots (.) represent the same nucleotide as the ones in our PTLV sequence and dashes (-) represent missing nucleotides.

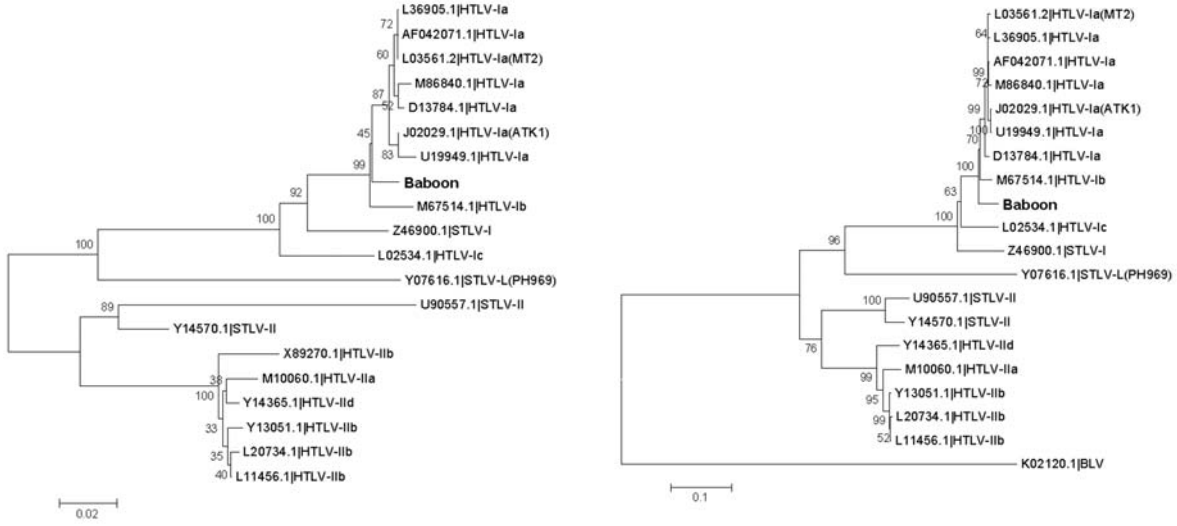

Figure 3. Phylogenetic trees based on amino acid sequence of Tax proteins of different PTLV strains (a) and DNA sequence of pX region (b). Different sequences are represented by their accession numbers in NCBI genbank and their subtype. Only Neighbour-joining trees are shown here, however UPGMA method led to similar results. Bootstrap values are mentioned at each node. The PTLV of our baboons is indicated in bold. Both dendrograms, with minor differences, show clustering of the newly found PTLV with HTLV-Ia and b strains rather than STLVs.

and Greene (1990) who have introduced 52 different point mutations in a Tax expression plasmid (pCTax) (Rimsky *et al.,* 1988). Their terminology in naming Tax mutants, M1 to M52, has been adapted by several other authors. As it is depicted in figure 1, the newly found Tax differs in 14 amino acids from pCTax (Rimsky *et al.*, 1988). However, some of these differences are common in other reported HTLV/STLV-I sequences (Fig. 1) which may be interpreted as these differences are not functionally critical. Using Smith and Greene terminology, the only mutation which has occurred in a previously studied locus is Y196H which lies in M32 position (Y196A, K197S). M32 mutant has shown almost no trans-activation potential upon CREB and NFkB controlled promoters and abrogates normal function of Tax (Smith and Greene, 1990). However, the point mutations in M32 changes the positive Kyte-Doolitle hydrophilicity index to negative

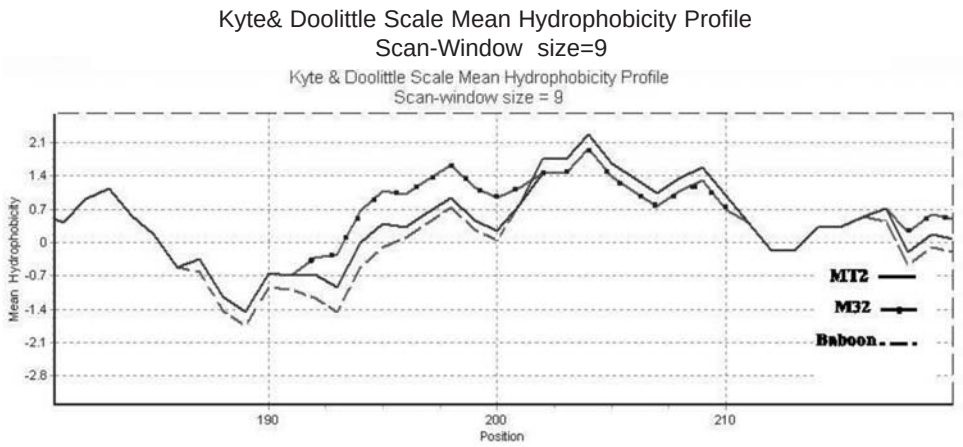

Figure 4. Kyte-Doolittle hydrophobicity graph. The graph is prepared for full length proteins but here only amino acids 180 to 220 are shown. MT2 Tax is wild type , M32 mutant differs from MT2 in positions Y196A and K197S while its other residues are like the protein produced by pCTax .These two latter Tax proteins also differ from MT2 in position 206 (M->I), however both MT2 and pCTax produce active Tax proteins . Baboon PTLV Tax differs from MT2 in I191M and Y196H . This graphs shows that the hydrophobicity of baboon Tax parallels MT2 Tax more than M32 mutant, so its structure might be less affected by the mutation in residue 196.

(from -1.3 to 1.8), while in baboon Tax the amino acid change increases this index (-1.8 to -3.2). For a given region of protein, this scale reflexes the probability of its appearance in the surface of protein (for a globular protein) or intramembrane domains (of a transmembrane molecule) (Kyte and Doolittle, 1982). As it can be seen in figure 4, the hydropathy pattern of baboon PTLV Tax parallels that of MT2 both in sign and amplitude while the gap between M32 and MT2 is wider. So, this new mutation may not affect the tertiary structure of Tax protein but M32 does. There is another mutation in the proximity of this site, I191M that lies between M29 and M30 mutation sites (Smith and Greene, 1990). However, since similar amino acid is observed in HTLV-Ib, Ic and STLV-I sequences (Fig. 1), this could not be an important difference. It is interesting to notice that the original pCTax is different from all other PTLV-Is studied in this report for M35 position (I206V).

Although we are not sure whether these two baboons had been infected by this virus in wild environment or in captivity, a previously reported wild caught baboon (which is kept in the same colony) has been shown to carry a virus with high homology in its LTR and *env* regions to HTLV1 (Mahieux *et al.,* 1998). This could be of value for epidemiologic studies on these baboons in their natural environment (Mahieux *et al.,* 1998; Saksena *et al.,* 1994 and Meertens *et al.,* 2001). However, regardless of source of infection, it is important that they are already infected with an HTLV-I like PTLV, which provides a valuable animal model for studies on physiopathology of the infection, and as a model to test the efficacy of a *tax*-based DNA vaccine against HTLV-I to decrease the viral load and the chance of ATL development (Daenke *et al.,* 1996; and Grant *et al.,* 2002). (Such a research is under progress by this group).

Tax is also the protein which induces the strongest anti HTLV-I cell mediated immunity in human (Parker *et al.,* 1994). This CTL response has been proposed to be a major mechanism to control the infected cell population in vivo and prevent the progress of infection toward ATL (Daenke *et al.,* 1996). On the other hand, the strong anti Tax CTL response contributes to nervous system destruction in HAM/TSP patients (Grant *et al.,* 2002). Availability of a primate model with a PTLV-I infection that has very similar Tax protein to HTLV-I might provide an invaluable tool for studies on immunologic mechanisms of both diseases. There are further studies in progress on this baboon model to clone and characterise its different cytokines which will provide tools for immunologic studies (Azadmanesh *et al.,* unpublished data).

Although it is about 2 decades since HTLV related viruses in primates attracted the researchers (Komuro *et al.,* 1984 and Voevodin *et al.,* 1985), it is still a very interesting field of research. During the preparation of this manuscript another PTLV-3 virus was reported to be isolated in gelada baboons (Van Dooren *et al.,* 2004), which high lights the diversity of this family of

viruses within primates, while another group reported the presence of an HTLV-I subtype b in gorilla and chimpanzee (Nerrienet *et al.,* 2004), which emphasizes the importance of primates as very close models for HTLV-I associated human diseases.

In summary, in the present study we described identification and characterization of a new PTLV-I closely resembling HTLV-I specially in the *pX* region in Olive baboons , and further described the importance of our finding for exploiting this baboon as an excellent animal model for studies regarding evaluation of vaccines against HTLV-I and the pathogenesis of this virus. We also constructed complete coding Tax cDNA which could be used in future functional studies of this protein by heterologous expression.

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