## THE ROLE OF THE TAT GENE IN THE PATHOGENESIS OF HIV INFECTION

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Abstract. The human immunodeficiency virus Tat regulatory protein is essential for virus replication and for the efficient transcription of HIV-1 provirus, and in the pathogenesis of AIDS. The role of the tat gene was investigated in 300 samples. It was found that 71.7% were subtype CRF\_01AE, 9.3% were subtype B, while 11.7 and 7.3% of them were cross-reactive and non-typeable, respectively. Moreover the results from peptide ELISA also showed that a low CD4 cell count was related to a low anti-Tat antibody (p<0.05), which may be due to the progression of HIV-1, which can be found predominantly in AIDS patients. The results of nested PCR showed that the second Tat exon might also play a role in T-cell activation. Reverse transcription polymerase chain reaction (RT-PCR) was used to measure HIV-1 mRNA expression in PBMC. RT-PCR negative results were found mostly in the asymptomatic HIV-seropositive group (88%). HIV-1 mRNA expression was found to correlate with current immunologic status. The differences in Tat protein sequences from DNA sequencing between the patients who had anti-Tat antibody positive and anti-Tat antibody negative, were not significant (p>0.05). These results suggested that the Tat amino acid sequences were conserved among each group of samples and did not change significantly compared with the consensus sequence in previous studies. Several factors make Tat an attractive target for vaccine design.

### INTRODUCTION

The tat gene encodes a transregulatory protein with important functions in the expression of HIV-1 genes (Gaynor, 1995). Tat is required for efficient viral gene expression and functions by enhancing transcription elongation through interactions with the cis-acting transactivation response element (TAR) (Laspia *et al*, 1989). Studies reveal that tat is sufficient to cause at least some of the features of AIDS. Tat may be involved in the initiation of reverse transcription prior to the subsequent switch to elongation (Isel *et al*, 1995). It should be mentioned that reverse transcription can occur in the absence of a functional tat gene, but the accumulation of proviral DNA intermediates is greatly reduced (Kashanchi et al, 1994). These results suggest that in the absence of tat, an optimal reverse transcription complex cannot be formed. It is possible that tat may be directly involved in these early steps and tat may interact with a cellular factor(s) during initiation to enhance the process of HIV-1 reverse transcription (Trono, 1992; Kashanchi et al, 1994). Tat may also function during viral assembly by either recruiting a cellular factor or modifying an existing viral protein. Although mutations in the tat gene reduce viral replication several thousand fold, they only partially offset the severe defects in viral replication and cytopathicity (Cullen, 1991). This result suggests that tat may function in the steps of the viral life cycle other than increasing transcription. HIV-1 proviruses that lack tat can be complemented by the expression of a functional tat gene in reverse transcription have also been seen in en-

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dogenous reverse transcription assays (Cullen, 1992). Thus, HIV-1 tat is required at early stages of reverse transcription (Peterlin et al, 1993). Most of the previous work in HIV-1 studied the tat gene subtype B, which can be found mostly in Europe and North America, while CRF01\_AE can be found mostly in Southeast Asia. In this study, the antibody against the Tat protein subtype B and CRF01\_AE in different groups of patients in Thailand was detected, and the efficiency of tat gene was analyzed, which led to an understanding of the pathogenesis of HIV-1 infection in both subtypes. This knowledge can facilitate the development of strategies for vaccination and specific immunotherapy, especially in CRF01\_AE, which can be found mostly in Southeast Asia.

### MATERIALS AND METHODS

### Subjects

The subjects were divided into three groups. The first group comprised of 100 volunteer asymptomatic HIV seropositive adults (Group I) from the Vaccine Trial Center, Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok. Their ages varied from 20-40 years. The second group comprised of 100 AIDS patients from the Bumrasnaradura Institute, Nonthaburi (Group II). The ages of this group varied between 20-70 years. The third group comprised of 100 pediatric AIDS patients (Group III) who attended the out patients unit at Queen Sirikit National Institute of Child Health, Bangkok. Their ages varied from 1.5 to 10 years.

### Specimen collection and preparation

Ten milliliters of blood was collected from each subject and divided into two tubes. The first tube contained 5 ml of EDTA blood for PCR, DNA sequencing and RT-PCR assay. The second tube contained 5 ml of clotted blood, and the sera were separated and used for the detection of anti-Tat antibody by peptide ELISA.

# Serological determination of HIV-1 Tat protein subtype by peptide ELISA

Peptide EIA was performed using the peptide EIA method described by Balachandra *et al* (2002). The panels of Tat protein peptides were as follow: SubtypeB: MEPVDPRLEPWK HPGSQPKTAC, CRF01\_AE: MELVDPNLE PWNHPGSQPTTAC.

### DNA sequencing of Tat protein gene

Isolation of DNA and nested-PCR. Peripheral blood leukocytes were isolated from 5 ml of EDTA-treated venous blood by density centrifugation and washed twice in RPMI medium (Gibco, USA). The genomic DNA extraction from PBMC using the InstaGene matrix (Bio-Rad, USA) was performed according to the manufacturer's instructions. The genomic DNA extracted from PBMC was used as the template for the PCR reaction. Amplification was performed using a DNA thermal cycler (PCR system 9700, USA). The amplification protocol consisted of an initial denaturation step at 95°C for 9 minutes followed by 34 cycles of: denaturation at 95°C for 1 minute, annealing at 53°C for 2 minutes, extension at 72°C for 1 minute and one final extension step at 72°C for 7 minutes. Four pair of primers were synthesized by PROLIGO Primers & Probes Company, USA (Table 1). Ten microliters of PCR products were separated on 2% agarose gel (Bio Whittaker Molecular application, USA) and stained with ethidium bromide. The PCR product band was visualized on an ultraviolet transilluminator.

PCR cycle sequencing. The PCR cycle sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc, USA). The thermal cycling reactions were an initial denaturation step at 96°C for 1 minute followed by 25 cycles of: 96°C for 10 seconds, 50°C for 10 seconds, and 60°C for 4 minutes.

DNA sequencing of the HIV-1 tat gene. The dried samples were resuspended in the loading buffer [formamide and 25 mM EDTA (5:1)]. The samples were heated at 95°C for 2 minutes and loaded onto a polyacrylamide gel before DNA sequencing with the automated DNA sequencer ABI PRISM377 (Perkin-Elmer, California, USA). The data were analyzed and translated into nucleotide or amino acid sequences by the DNASIS program.

**Isolation of cellular mRNA and RT-PCR.** Total RNA were extracted from the PBMC using a

single-step method with TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. The RNA was stored as a precipitate under ethanol at -20°C; this was achieved by adding 1/10 volume of 3M sodium acetate pH 5.2 and 2.5 volume of absolute ethanol.

Detection of the tat RNA by RT-PCR. A 21-mer oligonucleotide (5'-TTGGGAAGGGGTTGAA GCTAT-3') and a 20-mer oligonucleotide (5'-GTCCCAGAAGTTCCACAGTC-3') were used as primers to amplify the sequence of tat RNA. The RNA extract used as template was reverse transcribed at 48°C for 45 minutes using the Access Quick one-step RT-PCR (Promega, USA). The conditions of amplification were 40 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 1 minute, extension at 68°C for 1 minute and one final extension step at 68°C for 10 minutes. The PCR product was used as a template for the nested PCR, which amplified a 324-bp product. A 28-mer oligonucleotide (5'-GGAATTCACCATGGAGCTGGTAGATCCT-3') was used as a forward primer and a 27-mer oligonucleotide (5'-CGGGATCCCTAATCGCA CGGATCTGT-3') was used as a reverse primer. The PCR amplification was performed including an initial denaturation step at 95°C for 9 minutes followed by 34 cycles of: denaturation at 95°C for 1 minute, annealing at 53°C for 2 minutes, extension at 72°C for 1 minute and one final extension step at 72°C for 7 minutes. The nested-PCR product was separated on a 2.0% agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator.

### Data analysis

The chi-square test was used to assess the relationship between the CD4 lymphocyte count and the optical density from the peptide ELISA method. The computation was done with SPSS software (SPSS 8.0; SPSS Inc, Chicago, IL) with 95% confidence intervals (CIs). The association between anti-Tat antibodies and sequence similarity was performed by the Student's *t* test.

### RESULTS

# Detection of HIV-1 Tat protein subtypes B and E by peptide ELISA

Three hundred specimens were evaluated

for HIV-1 Tat protein subtypes by using peptide ELISA. The seroreactivity of the 300 serum samples showed 215 (71.7%) as CRF01 AE, 28 (9.3%) as subtype B, 35 (11.7%) as cross-reactive between both subtypes and 22 (7.3%) as non-typeable. The proportion of CRF01\_AE was highest in the AIDS patients, while subtype B and the cross-reactivity between subtypes was found mostly in the asymptomatic HIV seropositive groups. The non-typeable specimens were higher in the AIDS group than in any other groups (Table 2). The correlation between the CD4 lymphocytes count and optical density from the peptide ELISA in this study population are shown in Table 3. Twenty-two (22.0%) of the asymptomatic HIV seropositive group had a CD4 count less than 200 cells/mm<sup>3</sup> and 78 (78.0%) of the asymptomatic group had a CD4 count more than 200 cells/mm<sup>3</sup>. In the adult AIDS patients, 81 (81.0%) of them had a CD4 count less than 200 cells/mm<sup>3</sup> and 19 (19.0%) had a CD4 count more than 200 cells/mm<sup>3</sup>. In the pediatric AIDS patients, 60 (60.0%) of them had a CD4 count less than 200 cells/mm<sup>3</sup> and 40 (40.0%) had a CD4 count more than 200 cells/mm<sup>3</sup>.

### Amplification of the tat gene exon 1 and exon 2 from PBMC blood samples using a nested PCR method in asymptomatic HIV seropositive, pediatric and adult AIDS patients

The tat gene specific primers generated a single band of 366-bp PCR product from the tat gene exon 1 and a 266-bp PCR product from the tat gene exon 2. The results of PCR products of the tat gene from both exons are shown in Table 4. The positive PCR products from only exon 1 in asymptomatic HIV seropositive, adults with AIDS and pediatric AIDS patients were 63.8, 11.2, and 25%, respectively while the positive PCR products from only exon 2 were 25% both in the asymptomatic HIV seropositive and pediatric AIDS patients and 50% in the adult AIDS patients. The positive PCR products from both exon 1 and 2 in asymptomatic HIV seropositive, adults with AIDS and pediatric AIDS patients were 28.9, 37.2, and 33.9%, respectively. In contrast, the negative results from both exons 1 and 2 in asymptomatic HIV seropositive, adults with AIDS and pediatric AIDS patients were 33.3, 22.2, and 44.5%, respectively.

Sequence	Size	PCR product (Base pair)
5'-TTGGGAAGGGGTTGAAGCTAT-3'	21	
5'-CTTATTCTCTTAACTAACCTGTC-3'	23	488
5'-TAAATGGGCAAGTCTGTGGAAT-3'	22	
5'-GTCCCAGAAGTTCCACAGTC-3'	20	368
5'-GTTCATTTCAGAATTGGGTGTC-3'	22	
5'-ACACTACTATTGCTAAGATTAGC-3'	23	366
5'-GTAGGAGGTTTAATAGGTTTAAG-3'	23	
5'-AGATGAAGTCTCTCAAGCGGT-3'	21	266
	Sequence 5'-TTGGGAAGGGGTTGAAGCTAT-3' 5'-CTTATTCTCTTAACTAACCTGTC-3' 5'-TAAATGGGCAAGTCTGTGGAAT-3' 5'-GTCCCAGAAGTTCCACAGTC-3' 5'-GTTCATTTCAGAATTGGGTGTC-3' 5'-ACACTACTATTGCTAAGATTAGC-3' 5'-GTAGGAGGTTTAATAGGTTTAAG-3' 5'-AGATGAAGTCTCTCAAGCGGT-3'	SequenceSize5'-TTGGGAAGGGGTTGAAGCTAT-3'215'-CTTATTCTCTTAACTAACCTGTC-3'235'-TAAATGGGCAAGTCTGTGGAAT-3'225'-GTCCCAGAAGTTCCACAGTC-3'205'-GTCCATTTCAGAATTGGGTGTC-3'225'-ACACTACTATTGCTAAGATTAGC-3'235'-GTAGGAGGTTTAATAGGTTTAAG-3'235'-AGATGAAGTCTCTCAAGCGGT-3'21

Table 1 Outer and inner primers for tat exons 1 and 2.

			Table 2			
HIV-1	Tat pr	otein	subtype	by	peptide	ELISA

Study populations (Group)	Subtype					
	CRF01_AE <sup>m</sup>	B <sup>m</sup>	B & CRF01_AE <sup>c</sup>	Non-typeable <sup>n</sup>		
Asymptomatic HIV seropositive (I) patients	s 67	12	17	4		
Adult AIDS patients (II)	71	9	5	15		
Pediatric AIDS patients (III)	77	7	13	3		
Total (%)	215 (71.7%)	28 (9.3%)	35 (11.7%)	22 (7.3%)		

<sup>m</sup> : Monoreactive

<sup>c</sup> : Cross-reactive between both subtypes B & CRF01\_AE

<sup>n</sup> : Non-reactive by both subtype B & CRF01\_AE

Table 3 Correlation between the CD4 lymphocyte count and the optical density from the peptide ELISA in the study population.

				Study	populatio	n			
lymphocytes count (cells/mm <sup>3</sup> )	OD value of asymptomatic HIV seropositive		OD value of AIDS patients			OD value of pediatric AIDS patients			
	0.0-2.0	>2.0	Total	0.0-2.0	>2.0	Total	0.0-2.0	>2.0	Total
<200	15	7	22	67	14	81	50	10	60
≥200	15	63	78	11	8	19	8	32	40
Total	30	70	100	78	22	100	58	42	100

# The expression of tat mRNA in peripheral blood cells by RT-PCR assays

The RT-PCR assay was used to determine the tat mRNA expression in peripheral blood cells samples. The results of the RT-PCR in the three groups of samples are shown in Table 5. Among these 3 groups of the study population, the expression of tat mRNA was found predominantly in the adults with AIDS patient group (74%). In the pediatric AIDS patients, 38% of them gave positive results, while in the asymptomatic HIV seropositive patients, positive results were found in only 12%. The RT-PCR negative results were found mostly in the asymptomatic HIV sero-

	-			-	-	
Study population	PCR Results					
	Exon 1 positive (%)	Exon 2 positive (%)	Exon 1and 2 positive (%)	Exon 1and 2 negative (%)	Total	
Asymptomatic HIV seropositive patients	25/38 (65.8)	1/4 (25)	68/240 (28.3)	6/18 (33.3)	100	
Adult AIDS patients	4/38 (10.5)	2/4 (50)	90/240 (37.5)	4/18 (22.2)	100	
Pediatric AIDS patients Total	9/8 (23.7) 38/300 (12.0)	1/4 (25) 4/300 (1.3)	82/240 (34.2) 240/300 (81.0)	8/18 (44.4) 18/300 (6)	100 300	

Table 4 Numbers of positive and negative results for Tat exon 1 and exen 2 by nested-PCR assay.

#### Table 5

Numbers of positive and negative results of tat mRNA expression in peripheral blood cells by RT-PCR assays in study population.

Study population	RT-PCF	Total	
5	Positive	Negative	
Asymptomatic HIV seropositive patients	12	88	100
Adult AIDS patients	74	26	100
Pediatric AIDS patients	38	62	100
Total	124	176	300

positive patient group (88%). In the adults with AIDS and pediatric AIDS patients, 26 and 62% of them gave negative results, respectively.

#### Sequence analysis

DNA sequences were edited and translated. The amino acid sequences and phylogenetic analysis were aligned with DNAMANN for Windows sequence analysis software. The alignments of the translated amino acid sequences for amino acids 1 to 20 (neutralization antibody epitope of Tat protein), which test for the anti-Tat antibody, are shown in Fig 1. The top line of the alignment is the sequence of the peptide that was used for peptide ELISA (CRF01\_AE). Twenty-four of them had the same sequence as CRF01\_AE. Twenty-six had one amino acid substitution and 40 of them had amino acid changes in more than two positions. The phylogenetic trees were constructed from the samples to determine the relationship of the consensus sequence to other CRF01\_AE sequences, such as 95TNIH022, 95THIH047, 93TH065, 92TH057, and 93TH253. The results show that the consensus sequence in this study is closer to 93TH065 than the others in the previous study (Fig 2). Therefore, in order to determine the relationship between the sequences in this study and the previous sequence, we used HXB2 as subtype B and 93TH065 as CRF01\_AE reference strains, respectively. The results showed that the sequences clustered distinctively between HXB2 and CRF01\_AE and there was a relationship between the samples who had the same OD value (Fig 3). For the alignments, 101 amino acids of the Tat protein were used, gapped and stripped (Fig 4). The statistical robustness of the neighbor-joining tree and the reliability of the branching pattern were confirmed with the bootstrap test (using 500 replications), which was included in DNAMANN. The results show that there was high variability in both exons 1 and 2 of the Tat protein, and the amino acid alignments of each group of patients were undistinguishable. The average of these Tat sequences did not significantly differ between the anti-Tat antibody positive (78) and negative (12) patients. The average similarities were 93.88 and 83.50% in individu-

VC3 (439)PK VC4 (388)PK VC5 (449)PK VC41 (531)PK VC44 (462)PK	
VC4 (388)PK VC5 (449)PK VC41 (531)PK VC44 (462)PK	
VC5 (449)PK VC41 (531)PK VC44 (462)PK	
VC41 (531)PK VC44 (462)PK VC51 (801)PK	
VC44 (462)PKKKK	
V(C51 (901)	
VC77 (596) PKKK	
СН94 (320)РККККК	
СН43 (1,855) РККККК	
BR5 (57) PKK	
СН30 (283)КР	
VC58 (135)KP	
VC84 (149)KP	
BR16 (217) PS-R	
BR63 (43) PS-K	
CH95 (70) PLKKK	
CH28 (96) PKS	
CH29 (116)PKS	
CH46 (319)PKS	
VC2 (217)PKK	
BR37 (17)PK-K-	
BR39 (1)PK-K-	
BR56 (208)PK-K-	
СН63 (1,171) РК-К-	
VC70 (163)PK-K-	
VC15 (96)PK-S	
VC8 (204) PKL	W
CH64 (1,018) PAA	s
VC87 (64)PK-K	s
VC95 (69)PK-K	s
BR18 (116)PS-K	-E-
BR31 (1)PDK-K	s
BR69 <i>(174)</i> PDK-K	s
BR78 (239)PDK-K	s
СН69 (8)РДК-К	s
СН77 (31)РDК-К	s
CH5 (293)PS-RQ	-P-
VC88 (1)PS-KK	s

Fig 1–Multiple sequence alignment of Tat protein gene by peptide ELISA.

0.05



Fig 2–Neighbor-joining trees of consensus sequence and the other CRF01\_AE sequences. The percentage of identity is indicated on the right of the tree. als with and without anti-Tat antibodies, respectively (p>0.05).

#### DISCUSSION

The human immunodeficiency virus type 1 (HIV-1) Tat protein is required for virus replication and pathogenesis (Wu and Marsh, 2001). It can be found very early after virus entry, even prior to virus integration (Rodman et al, 2001). It also plays a key role in the virus life cycle and infectivity (Gallo, 1999). In this study, an antibody against the Tat protein subtype in asymptomatic HIV seropositive, adults with AIDS, and pediatric AIDS patients were detected using the peptide ELISA assay. Our analysis demonstrated that most of Thai patients had HIV-1 CRF01\_AE (71.7%), 9.3% had subtype B, 11.7% had crossreactivity between both subtypes and 7.3% were non-typeable (Table 2). These results are similar to a previous observation in HIV-1 subtypes by Balachandra et al in 2002, in which 80.6% were subtype E and 5.7% were subtype B; and by Subbarao et al in 2000, in which 70.7% and 20.3% were subtypes E and B, respectively. Our study also provided information relevant to the role of the anti-Tat antibody in the pathogenesis of HIV-1 infections. Most asymptomatic HIV seropositive patients had a high optical density (OD>0.2) by peptide ELISA. These results demonstrate that these patients had a higher immunoreactivity against the N-terminal region (amino acids 1-20) of the Tat protein more than the other groups, which indicates that antibody against Tat is correlated with nonprogression to AIDS (Re et al, 2001).

The results from Table 3 also show that a low CD4 cell count is related to a low Tat antibody level, which may be due to progression of the immunodefiency found predominantly in AIDS patients. In this group, many demonstrated low seroactivity to peptide subtypes (non-typeable), which may indicate that these patients are in the final stage of HIV-1 infection. Even though the CD4 lymphocyte counts in some of the pediatric AIDS patients seemed to be higher than in the adults, this is normally found in small children. The mechanism of Tat neutralization in this study was indistinguishable between adults and children in AIDS patient groups (Group II and III).



Fig 3–Neighbor-joining trees of 90 samples from the patients, HXB2 and CRF01\_AE.



Fig 4–Multiple sequence alignment of full Tat protein by peptide ELISA compare with HXB2 and CRF01\_AE (93TH065).

Some of the patients in this study were crossreactive or non-typeable, and some of them were subtype B by peptide ELISA, but were CRF01\_AE by DNA sequencing. These results suggest that it may be necessary to use or include alternative methods, such as heteroduplex mobility assays or peptide competition assays, for a more definitive subtype determination (Balachandra *et al*, 2002).

In order to characterize the functions of the tat gene exons 1 and 2, the nested PCR of these exons was carried out. In this study, both exons 1 and 2 were found predominantly in the adults with AIDS and in the pediatric AIDS patients (groups II and III). These results were similar to a previous study by Ross and Hewitt (2002), which showed that monkeys or humans who had only exon 1 remained healthy. Therefore, it can be concluded that exon 2 plays an important role in HIV infection even though exon1 sequence is enough for a functional domain of Tat protine.

RT-PCR was used in this study to measure HIV-1 mRNA expression in PBMC (Table 5). The RT-PCR positive results were found mostly in the adults with AIDS patient group (74%). In the pediatric AIDS group (Group III), the RT-PCR positive results were less than in the adult AIDS patients (Group II). These results may be due to the amount of blood collected from each child. which was less than the amount of blood from the adult patients. Therefore, the RNA copy number from the PBMC in some children would not be enough for RT-PCR amplification. HIV-1 mRNA expression was found to correlate with the current immunologic status. The low level of viral mRNA expression observed in the blood cells of asymptomatic individuals was due to a particular effect of the anti-HIV immune response. Some previous studies also reported that no evidence of viral replication was detected in PBMCs from asymptomatic individuals who had normal levels of CD4 for at least 5 years (Saksela et al, 1993).

Alignment of translated amino acid sequences of amino acids 1 to 20 (neutralization antibody epitope of Tat protein), which was tested for the anti-Tat antibody, is shown in Fig 1. The top line of the alignment is the sequence of the peptide used for peptide ELISA (CRF01\_AE). Interestingly, among these patterns, the substitution of leucine (L) at amino acid position 3 by proline (P) and asparagine (N) at amino acid position 12 by lysine (K) might suppress the function of the Tat protein. Since most of the CD4 lymphocytes of these patients were quite high, the mutated protein might reduce Tat activity.

Many previous studies have discussed the basic region of the Tat protein (amino acids 49-59), which is rich in both arginine and lysine, is strongly basic and contains the strictly conserved motif GRKKRRQRR throughout all the subtypes of HIV. It plays a critical role in the transactivation of the LTR of HIV. Mutation in this area causes a loss of transactivating activity (Hauber et al, 1989). Conversely, the basic region of the consensus sequence from this study was GRKKR KHRR (Fig 2), which can be found in both the asymptomatic and AIDS groups. This suggests that amino acid substitution at the basic region of the Tat protein may not lead to a reduction in the activity of Tat in CRF01\_AE. Moreover, there is some evidence that the second exon of the Tat protein from subtype B, which contains a conserved RGD motif, is important for the uptake of extracellular Tat (Howcraft et al, 1993). According to the data presented in this study, most of the samples were CRF01\_AE, and all of them had an RGN motif instead of RGD, but the Tat protein still functioned, especially in patients who had a progression to AIDS. This data indicates that the RGN motif in CRF01\_AE has the same function as the RGD motif in subtype B.

The phylogenetic trees were constructed from 90 patient samples to determine the relationship of the consensus sequence to other CRF01\_AE sequences, such as 95TNIH022, 95THIH047, 93TH065, 92TH057, and 93TH253. The results show that the whole amino acid sequence of Tat is conserved among each of the groups of samples and did not significantly change when compared to the previous study (Fig 2). In order to determine the relationship between the sequences of the 90 patient samples of this study and the previous sequences, we used HXB2 and 93TH065 as CRF01 AE. The results show that the sequences clustered distinctively between HXB2 and CRF01\_AE and there was a relationship between

the samples who had the similar OD value in peptide ELISA. Moreover, the sequence from the patients with a low optical density appeared to be far more diverse than patients with a high optical density (Fig 3). This observation suggests that there may be some mutation in the patients who have a lower immunity to the Tat protein when compared to the patients who had a high level of immunity.

This study indicates that the antibody against the Tat protein has protective effects in controlling the progression of disease in AIDS patients. The role of the tat gene should be further studied by using the peptide microarray and tat protein cloning for detecting serum antibody responses to the complete 101 amino acid sequence Tat protein and to examine the Tat protein expression.

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