CHAPTER V

CONCLUSION AND DISCUSSION

1. Truncated peptides design

The amino acid sequence of Tat exon I truncated peptides was designed base on the sequences of viruses from 12 HIV-1 infected Thais, HIV-1 CFR01_AE (CM240), and the consensus sequence from the databases. The representative sequences of viruses from 12 patients are shown in 2 patterns. The first representative sequence from six out of 12 amino acid sequences is shown as pattern 1 and the rest is shown as pattern 2

Pattern 1: The representative sequence of Tat exon I from 6 HIV-1 infected patients. (67 amino acids)

MELVDPNLEPWNHPGSQPTTACSKCYCKKCCWHCQLCFLKKGLGISYGRKKRKHRRGTPQSSKDHQN

Pattern 2: The representative sequence of Tat exon I from 6 HIV-1 infected patients. (67 amino acids)

MEPVDPNLEPWNHPGSQPTSACSKCYCKKCCWHCQLCFLKKGLGISYGRKKRKHRRGTPQSSKDHQN

The amino acid sequence of pattern 1 is different from pattern 2 in 2 residues (residue 3 and 20). However, the consensus sequence of previously reported Tat exon I from13 HIV-1 CRF01_AE isolates from Thailand during 1995-1996 was used to compare with the amino acid sequences of these 12 patients to find the suitable amino acids at residue 3 and 20.

Based on the consensus sequence of 13 HIV-1 CRF01_AE Tat exon I isolates from Thailand during 1995-1996. (67 amino acids). There is high homology to pattern 1 at reresidue 3 and 20 the amino acid sequence is L and T respectively.

ME**L**VDPNLEPWNHPGSQPT**T**ACSKCYCKICCWHCQLCFLKKGLGISYGRKKRKHRRGTPQSSKDHQY

Interestingly our final amino acid sequence of Tat exon I (67 amino acids) is 100% homology compare to the amino acid sequence of Tat exon I CRF01_AE CM240 assession number U54771 isolated from Thailand in 1990. So, this consensus amino acid sequence was used for the Tat exon I truncated peptide residues 1-67, as well as for residues 68-72.

The consensus amino acid sequence from 42 Tat exon II HIV-1 CRF_AE isolated from Thailand during 1990-1995 were used for Tat exon II truncated peptide design. The consensus amino acid sequence of Tat exon II was different from Tat exon II of HIV-1 CRF01_AE CM240 assession number U54771 isolated from Thailand in 1990 in only 2 residues. (Residue 79 and 99)

Consensus sequence: PLPIIR**G**NPTDPKESKKEVASKAETD**P**CD (29 amino acids)
CRF01_AE CM240: PLPIIR**R**NPTDPKESKKEVASKAETD**Q**CD (29 amino acids)

The amino acid sequence of Tat used in this study was different from CM240 (1990) in only 2 amino acids in Tat exon II region (98% homology). It could be concluded that Tat has been conserved since 1990 up until now. Thus, Tat may be one of the appropriate target for vaccine design.

2. Tat-specific IFN-γ-Elispot responses

In this study, 10 out of 20 (50%) patients showed IFN-γ-Elispot positive to pooled Tat peptides. Patients who showed positive results were then further identified for Tat specific epitope. Seven out of 10 (70%) patients showed IFN-γ-Elispot positive to individual Tat peptides but 3 showed negative result although their Elispot

response was positive for pooled peptides. The possible explanation may be because the screening of pooled Tat peptides were done in whole blood samples that collected from the first visit but the epitope mapping was performed by the samples of the second visit which was approximately 3 months apart. Three patients who showed IFN-γ-Elispot negative to individual Tat peptides had the magnitude of responses to the pooled peptide at screening lower than those patients who showed IFN-γ-Elispot positive to individual Tat peptides (table 4).

Table 4: The magnitude of responses in patients who showed IFN-γ-Elispot positive to pooled Tat peptides.

	The magnitude of responses (SFU/ 10^6 PBMCs)	
	Range	median
Patients who showed IFN-γ-Elispot positive to individual Tat peptides	320-912	704
Patients who showed IFN-γ-Elispot negative to individual Tat peptides	260-316	308

This observation suggests that patients who showed IFN-γ-Elispot negative to individual Tat peptides may have caused by the lower number of CTL clone in the second visit.

Two patients who were infected with subtype B' showed IFN-γ-Elispot negative to pooled Tat peptides. This could be resulted from the un-recognition of the peptides that were designed base on HIV-1 CRF_AE (Table5).

Table 5: Tat-specific IFN-γ-Elispot responses (N=20) in different HIV-1 subtype infection.

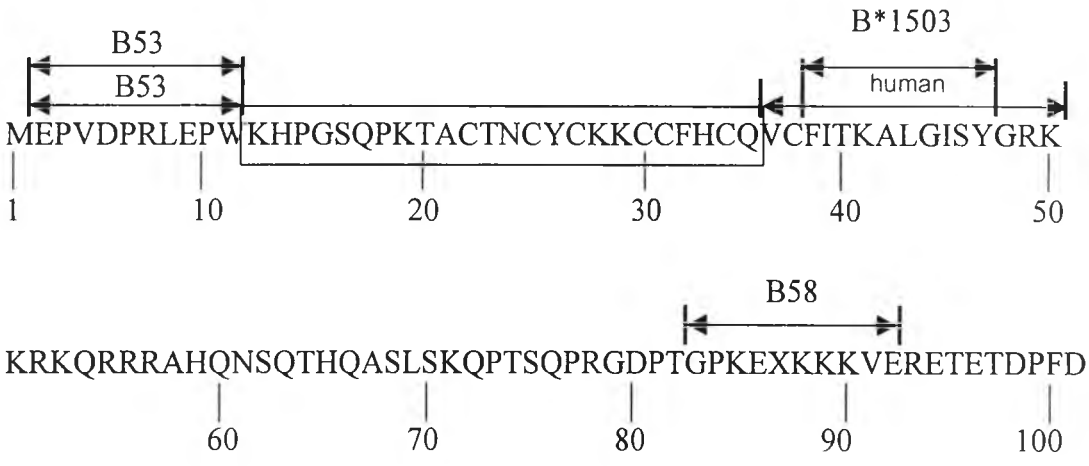
Subtype	N	IFN-γ-Elispot positive to pooled peptides	IFN-γ-Elispot positive to individual peptides
B	2	0	0
A/E	14	7/14	6/14
ND	4	3/4	1/4

The results from this study were correlated with the study of V. Novitsky *et al.*[102] HIV-1C-specific Elispot-based CTL responses against Tat peptide were done in 48 HIV-1 infected blood donors in Botswana. Seventeen tested subjects (35.4%) showed CTL responses to the 15-mer peptide at residues 36-50. The immunodominant region in our study at residues 30-49 covered the core region of Tat, which has been shown in previous studies that it was crucial for activation of HIV-1 transcription.

3. HIV-1 CRF01_AE Tat epitope mapping

Six HIV-1 CRF01_AE Tat epitopes of 17-21 amino acid in length were identified in 7 HIV-1 infected patients, as shown in figure 12. However, some of them were overlapping. Therefore, a total of epitope found in this study might less than 6. The immunodominant epitope (Tat 30-49: CCWHCQLCFLKKGLGISYGR) have been found to be the same as the immunodominant region in HIV-1 subtype C (Tat 36-50: VCFQTKGLGISYGRK). Of interest, the amino acid sequence of Tat residues 12-35 has not yet been reported in the Los Alamos database or other recent publications. They might be novel Tat epitopes of which further characterizations are needed.

Figure 12. Tat CTL epitope map (Los Alamos database)



The block represents the new region that might be the novel Tat CTL epitope.

Furthermore, patients who showed Tat-specific IFN-γ-Elispot responses to the epitopes reported in the Los Alamos database had HLA class I genotypes that difference from these previous reports. Therefore, these patients may have different HLA-restrictions and warranted for further elaboration.

The HIV-1 regulatory protein Tat has been targeted recently in a vaccine strategy to confine virus replication and transmission and to block or retard progression to AIDS. However, this study found that only one-third (35%) of HIV-1 Thai individual showed HIV-1 Tat-specific IFN-γ responses. Wheras other studies had shown that gag- and pol-specific CTL responses were much higher that was 100% and 84%, respectively.[103] Thus, Tat may be a good candidate to be included in HIV vaccine design rather to be an isolated Tat vaccine.