CHAPTER VI

DISCUSSION

HIV-specific CTL play an important role in the control of the viral replication (92, 93). Analysis of immune responses in individuals infected with HIV-1 indicated that the CTL were critical in controlling the initial viremia following acute infection and in suppressing viral replication during the chronic phase of infection (4, 5). Moreover, HIV-1-specific CTL activity in exposed uninfected individuals and in long-term nonprogressors further supported their protective role (8-13). Insights into the HIV-specific responses in Thai patients may provide essential information for vaccine development.

In this study, seventeen HLA-A11-restricted CD8+ T cell epitopes were tested. We focused on HLA-A11-restricted HIV-specific CD8+ T cell responses in HIV-1-infected Thai patients who were asymptomatic and antiretroviral treatment naïve. Median CD4+ T lymphocyte was 463 cells/mm^3 (range 325-730 cells/mm^3). Median plasma HIV-1 RNA was 10,875 copies/ml with a range of range 13,118-67,000 copies/ml. Majority of patients whose HIV subtype was identified were A/E subtype. This data was consistent with the several reports previously shown that majority of Thai patients were infected with this subtype (126).

Seventeen HLA-A11-restricted epitopes located in Gag, Pol, Env, and Nef were tested in this study. For control group, CD8+ T cell responses of HIV-seronegative with HLA-A11 could not be stimulated with these epitope peptides and detected by peptide-based ELISpot assay. This result confirmed that HIV-seronegative individuals even with HLA-A11 were not able to recognise HLA-A11-restricted HIV-specific epitopes. For T cell responses of HLA-A11 negative HIV-infected patients, we found that only two patients, VP and SY, had response to QVPLRPMTYK and ITVGPGQVFY peptides, respectively. In the patient VP, HLA alleles were HLA-A24, -B15, -B35. It was likely that this patient responses to HLA-B35 restricted epitope, VPLRPMTY, which lied within QVPLRPMTYK peptide. Indeed, this patient was later demonstrated that she had the CD8+ T cell responses against VPLRPMTY epitope by ELISpot assay (data not shown). For the patient SY, this patient had HLA-A33 which belonged to the same A3 supertypes as HLA-A11. It has been shown that these HLA molecules share similar binding motif. Moreover, in the previous study, Buseyne

et al (127) identified an HLA-A33-restricted HIV-specific CTL epitope (D**T**GHSNQVSQN**Y**) the anchor residues (threonine at Position (P) 2 and tyrosine at C-terminal) of which were similar to those of our epitope (I**T**VGPGQVF**Y**). Therefore, it was possible that the cross-reactive responses in the patient SY might be mediated through HLA-33 restriction.

In this study, HIV-1-specific CD8+ T cell responses to each HIV protein were demonstrated. The result showed that Nef appeared to be the most immunodominant protein, whereby all patients had responses against this protein, and followed by Pol, Gag, and Env. Several groups also reported immunodominance of Nef protein in HIV-infected individuals (128, 129). Frahm et al (128), studied HIV-specific T cell responses against overlapping peptides spanning Pol, Gag, Nef, Vpu, Vpr, Rev, and Tat in 150 HIV-1 subtype B-infected donors with various ethnic backgrounds and showed that Nef-specific responses dominated the total responses and followed by Gag, Pol and Env-specific responses. However, in contrast to our study, all persons in this study were included regardless of CD4 count, viral load, or treatment status. In addition, Dalod et al (129) used ELISpot assay to characterise the HLA-restricted CD8+ T cell responses against a large variety of synthetic HIV peptides derived from the Env, Gag, Pol, and Nef and confirmed the immunodominance of Nef. On the other hand, some reports did not confirm the immunodominance of Nef. Larsson et al (130) studied HIV-1-specific CD8+ T cell responses using recombinant vaccinia vectors expressing various HIV-1 proteins (Gag, Pol, Env, and Nef) by ELISpot assay in 19 HIV-infected individuals regardless of treatment status. The results showed that Pol was the most immunodominant protein and followed by Gag, Nef and Env, respectively. However, some patients in this study were treated with antiretroviral drugs. Similarly, Novistsky et al (131) described HIV-specific CD8+ T cell responses against overlapping peptide spanning HIV-1 proteins (Gag, Pol, Nef, Rev, Tat, Vpu, Vpr, Env, and Vif) by ELISpot assay in 105 donors infected with HIV-1 subtype C. In that study, they found that Gag was the most immunodominant protein followed by Nef, Pol, and Env. Moreover, Musey et al (132) tested HIV-specific CTL responses against target cells expressing various HIV-1 gene products (Gag, Pol, and Env) by cytotoxicity assay in 33 persons who enrolled at the primary HIV infection. The most common gene product to be recognised was HIV-1 Env; by contrast, HIV-1 Gag and Pol were recognised less frequently.

There are several factors that could influence the observed CTL epitope frequencies and immunodominance. In this assay, we used only the previously described optimal HLA-A11-restricted CD8+ T cell epitopes which did not cover entire proteins. Therefore, we might have underestimated CD8+ T cell responses against some particular protein, such as Gag (only one peptide was included in this study). It was likely that within an infected individual more epitopes could have been targeted. Moreover, the immunodominant of HIV-specific T cell responses might be affected by HLA type of the donors. In our study, we focused on the HLA-A11-restricted HIV-specific T cell responses and showed that Nef was immunodominant epitope, whilst Goulder et al (133) focused on HLA-A*3002-restricted HIV-specific CD8+ T cell response and showed that the strongest response was Gag epitope. In addition, the antiretroviral therapy and CD4 counts might be the factor to modify the pattern of immunodominance. Larsson et al (130) studied HIV-1-specific CD8+ T cell responses in three untreated, six long-term treated patients and 10 patients who had samples taken 4 and 10 weeks following the initiation of HAART. The CD4 counts of donor ranged from 73 to 925 cells/mm^3. In this study, the results showed that Pol was the most immunodominant protein. This result might have significant influence from antiretroviral therapy or low CD4 counts. Moreover, technique to analyse HIV-specific T cell responses might have an effect on the pattern of immunodominance as seen in a study by Musey et al (132). They analysed HIV-specific T cell responses by cytotoxicity assay and showed that Env was the immunodominant protein which differ from our study.

From our results, HIV-specific CD8+ T cell responses against QVPLRPMTYK peptide seemed to be the most commonly-recognised epitope, whereby 16 out of 18 patients had responses to this epitope. This observation was consistent with Frahm et al (128). They studied T cell responses against overlapping peptides spanning Pol, Gag, Nef, Vpu, Vpr, Rev, and Tat. The results showed that the overlapping peptide, which QVPLRPMTYK lied within, was the most frequently targeted epitope. Moreover, our study showed that the other nef epitope, GAFDLSFFLK peptide, was recognised by most patients. For the HIV-specific CD8+ T cell responses in longitudinal analysis, we found some peptides which were not recognised by any patients at 1^{st} time point but their responses were detected by ELISpot at 2^{nd} or 3^{rd} time point. Except for the patient PS, all patients had persistent Nef-specific T cell responses throughout the study.

From the data of this study, the relationship between viral load and CD8+ T cell responses was still controversial. We therefore would like to determine the correlation between CD8+ T cell responses and viral load. In this study, we focused on Nef-specific CD8+ T cell responses which seemed to be the immunodominant epitopes. We have demonstrated that there were no correlations between Nef-specific T cell responses and plasma RNA viral load in cross-sectional study (Figure 5). Similarly, QVPL-or GAFD-specific T cell responses had no correlations with viral load. Moreover, when we then looked for a relationship between CD8+ T cell response and viral load in longitudinal study, we failed to show the correlation between the Nef-specific CD8+ T cell responses and plasma HIV-1 RNA in most patients (Figure 6-7, 10). However, we found that there are no reports that study the correlation between viral load and Nef-specific CD8+ T cell responses, whilst there are the previous studies that focused on other proteins which showed the no correlation (100, 129). For example, Gray et al (100) determined the relationship between viral load and the frequency of Gag-and Pol-specific CD8+ T cells detected by tetramer staining in a cross-sectional study from 18 HLA-A*0201 patients. There were no correlations between the frequency of T cells and viral load. In addition, the study of Migueles and Connors (134) also supported our result. They failed to show the correlation between plasma viral load and the frequencies of HIV-specific CD8+ T cells detected by ICS assay in the HIV-infected patients.

On the other hand, in our study, the inverse or positive correlation was observed in some patients when we focused in longitudinal study from each patient (Figure 8-9, 11-12). There were previous studies showed the inverse or positive correlation between T cell responses and viral load (6, 132, 135). For example, Ogg, et al (6) demonstrated an inverse correlation between Gag-and Pol-specific CD8+ T cell frequencies and plasma RNA viral load for a cross-sectional analysis from 14 of HLA-A*0201-positive individuals at different stages of infection. In addition, Musey et al (132) showed the inverse correlation between viral load and Env-specific CTL responses analysed by cytotoxicity assay in 33 patients with primary infection On the other hand, Propato et al (135) demonstrated the positive correlation between HIV RNA and the CD8+ T cell frequencies, as detected by ELISpot or tetramer assay from each patient in longitudinal study.

However, there was difference in each study. One possible explanation of these controversies might be due to the methodological difference in different studies. In our study,

we analysed the IFN-γ secretion of CD8+ T cell by ELISpot assay, whilst the other reports used the different assays (6, 132). Moreover, different studies analysed CD8+ T cell response against different proteins. In our study, we focused on the CD8+ T cell responses against Nef epitopes, whilst the other studies focused on the other epitopes or the other proteins (6, 132). Therefore, the different proteins that were used for each analysis might be one possibility of these discrepancies.

It was not clear from our study as to why individuals of the same HLA type did not target the same epitopes. Several factors likely contribute to immunodominance, including efficiency of processing of peptides, binding of peptides to HLA class I molecules, affinity of T cell receptors for peptide-HLA complexs, and development of immune escape (67, 120, 121, 136, 137). Although immune escape alone cannot account for lack of recognition, sequencing of autologous virus was performed to answer the question as whether lack of response to a particular epitope was due to sequence variation in our study.

In our study, QVPL-and GAFD-specific CD8+ T cell responses seem to be two most immunodominant epitopes. However, some patients had fluctuation or absence of responses against these epitopes. For the lack of responses, it might be resulted from mutation within epitope or mutation outside of epitope (flanking resion). For the patient UP who was GAFD-non responder, the point mutation (G83E) was found within the epitope region. This mutation might have an influence on preventing the binding of the corresponding peptide to HLA molecules or abrogation recognition by the TCR (120, 137). However, it is likely that mutation at position 1 of this epitope might abrogate the binding of peptide to HLA molecule (138).

On the other hand, some patients in our study who had no HIV-specific responses, but the relevant sequences within the epitope were conserved. There were two patients who lacked two most immunodominant responses; the patient PN had no responses against GAFDLSFFLK peptide, whilst the patient WY had no responses against QVPLRPMTYK peptide. The sequence analysis revealed that the epitopes remained unchanged but the amino acid in the flanking region mutated. These amino acid mutations in the flanking region of epitope might lead to inefficient epitope processing resulting in abrogation of the presentation on the HLA-class I molecule and hence escape of CTL in our study. The mutation in flanking region of these epitopes might affect at steps of antigen processing in

the cleavage of proteins either by proteasomal processing (137) or by NH2-terminal trimming (67). Yokomoko et al (137) observed that the amino acid mutation in the flanking region of epitope, which was only two amino acid adjacent to the N-terminus of the A24-restricted epitope. This mutation might shift the optimal proteosome cleavage site, resulting in the generation of a larger peptide, which had a lower affinity to the HLA class I molecule. Moreover, Draenert, et al (67) found that the amino acid mutation in the flanking region of epitope leaded to inability of ERAP I to cleave the variant N-extend peptide to the optimal size. Failure of ERAPI to trim the NH2-terminal flanking residues may therefore contribute substantially to the processing escape mechanism.

Surprisingly, in one patient (PS) who the QVPL responses in the 1st and 2nd time points, the amino acid mutations neither within the epitope nor in the flanking region was found. We thought at that time there might be some synonymous mutations and leaded to ineffective translation of antigen. Our idea was indeed followed works done by MeintJies and Rodrigo (139) who proposed that the codon bias may allow the expression of viral proteins to be suppressed in order to minimise the antigenic profile. Unexpectedly, we found the same synonymous mutation was found epitope region of both non-responding and responding strains and therefore could not explain these discrepancies of responses. However, this might be from the fact that we sample only 5 clones for DNA sequencing and hence failing to detect minority and perhaps non synonymous sequences.

Even though Nef epitopes were immunodominant, and most residues in these epitope were surprisingly conserved. Despite of the strong selection pressure from T cell responses against immunodominant Nef epitopes, these epitopes were relatively conserved. This observation might be due to the amino acid within Nef epitopes (QVPLRPMTYK at position 73-82 and GAFDLSFFLK at position 83-92) are important for interaction with SH3 domain. This kinase association of Nef is necessary for efficient proviral DNA synthesis and for promotion of virion infectivity of viruses (39). These data suggested that the conserved Nef epitopes regions might be relevant biological functions and might have required for maintaining the virus to be replication competent throughout the course of infection *in vivo*, and that these function may be required for the viral pathogenesis (40, 42, 140-142).

In conclusion, our results showed that the HIV-infected patients with HLA-A11 had a broad HIV specific response against several epitopes. Nef protein (QVPLRPMTYK and

GAFDLSFFLK peptides) seemed to be the most immunodominant epitopes followed by AIFQSSMTK QIYAGIKVK and QIYQEPFKNLK peptides of Pol protein. In contrast to our hypothesis, we failed to show the correlation between viral load and the CD8+ T cell responses against immunodominant epitope in most patients. Interestingly, whilst most patients who lacked Nef-specific responses had mutation either within epitope or in the flanking region only one patient had evidence of neither mutations. This interesting observation may require further investigation. Taken together, our study provided basic information HIV-specific HLA-A11-restricted T cell responses in Thai patients. This information is particularly useful for understanding HIV pathogenesis and development of HIV vaccine. The possible implication of these finding is that Nef may be an attractive target for vaccines or immunotherapies. These epitopes might be used for epitope-based vaccine development in the future.