CHAPTER V

DISCUSSION

Prevalence of HIV-1 gag and pol specific CTL responses

In this study, twenty-five HIV-1 infected Thais who were asymptomatic and antiretroviral treatment naïve were enrolled. Mean CD4+ T lymphocytes was 540±179 cells/cu.mm. (range 307-978). Median plasma HIV-1 RNA was 6936 copies/ml (range 886-57022). The HIV-1 genotyping by automated DNA sequencing analysis of V3 and gag regions revealed 23 were infected with subtype CRF A/E, 2 were infected with B (B'; GPGQ). In classical recombinant HIV-1 gag and pol, Cr51 release CTL are analysed, all of them (100 %) showed HIV-1 specific CTL killing against gag A. Whereas, twenty-one of 25 (84%) showed specific CTL against HIV-1 pol A. In 1998, Lynch JA *et al.*, found 8 of 9 or 89% HIV-1 infected patients showed HIV-1 gag E CTL response, 2 of 9 or 22% in HIV-1 A/E-pol-RT, 3 of 12 or 25% in E-nef and E-env of CTL killing[12]. Deniz D *et al.* (1998) have reported CTLs from eight patients reacted with several clade A proteins. Three of eight or 37.5% reacted with all four proteins tested (env, gag, pol, nef) and 2 of 8 or 25% reacted with two proteins. Eight patients showed CTL recognition of HIV-1 pol and gag. Seven of eight or 87.5% showed CTL recognition of HIV-1 nef. But CTLs against HIV-1 env were found in only 3 of 8 or 37.5%. In clade B proteins, only one patient showed CTL recognition in all four proteins tested[143]. Our results confirmed that HIV-1 gag CTL is the most common CTL recognition among HIV-1 infected patients.

HIV-1 cross-clade CTL results

In this study of 23 clade A/E infected patients, 14 or 61 % showed HIV-1 cross-clade CTL activities against gag A versus B. Cross-clade CTL against pol A versus B was much lower in percentage which is only 5 of 23 or 22 %. Deniz D *et al.* had reported a study in African patients. They found that reactivity against env was rare, only 1 of 7, who produced CTLs specific for both gp120 and gp41 of the LAI isolate. However, broad cross-reactivities were detected with pol and gag, as p24 and p18 from the LAI isolate were recognized by CTLs from 4 of 6 clade A-infected patients: RT was recognized by CTLs from 2 of 7, protease was recognized by CTLs from only one of 7. Huyen Cao *et al.* had evaluated the CTL cross-recognition in individuals infected with non-B clade HIV-1. Fourteen patients were evaluated, ten were infected with clade A virus, three were infected with clade G virus and one was infected with clade C virus.

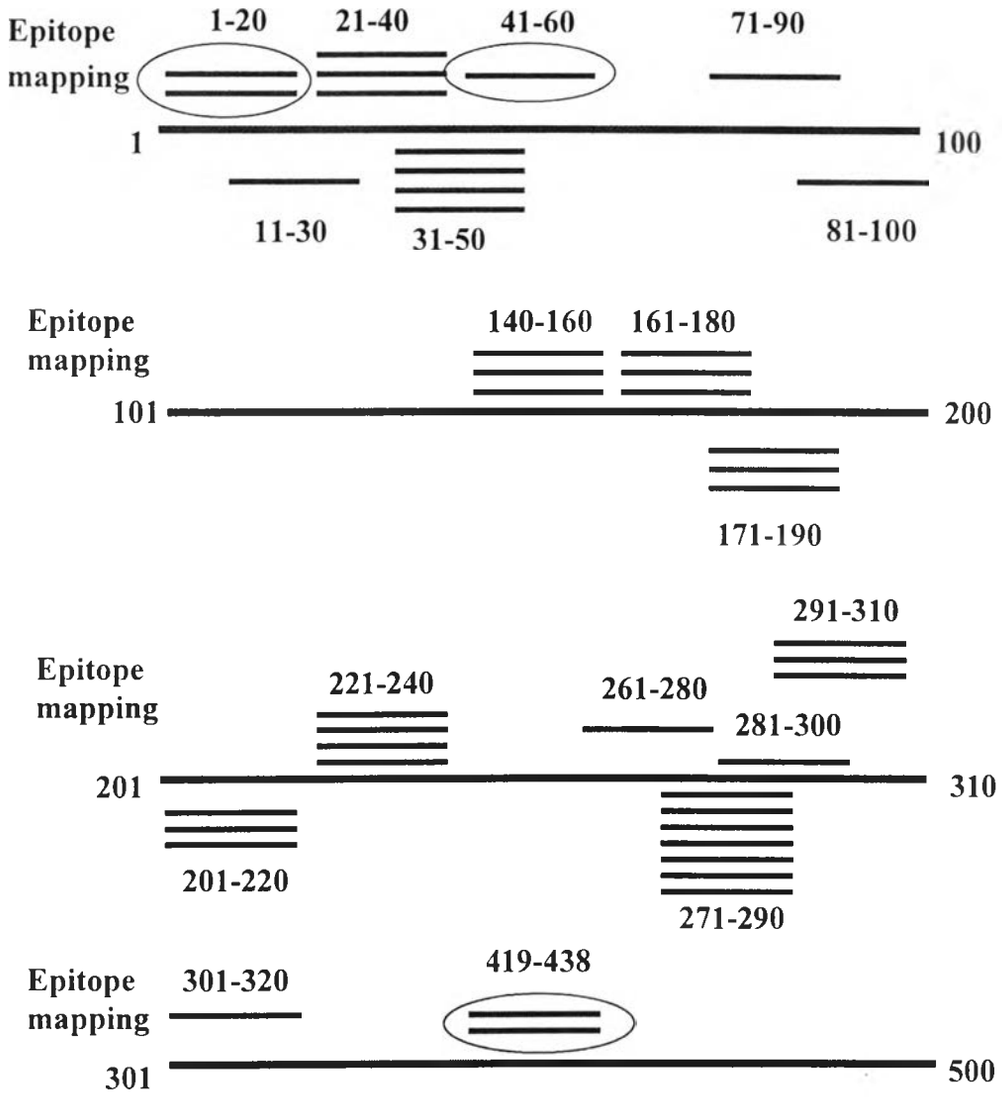
All subjects were recognized at least one of the clade B vaccinia virus constructs (gag, env, RT and nef)[15]. In 1997, Betts MR *et al.* had examined cross-clade HIV-specific CTL activity in peripheral blood of eight Zambian individuals infected with non-B-clade HIV-1. They found that six of eight C-clade HIV-infected individuals elicited CTL activity specific for rVV-infected autologous targets expressing HIV *gag-pol-env* derived from B-clade HIV-1 (IIIB). These data demonstrate that HIV clade C-infected individuals can mount vigorous HIV clade B-reactive CTL responses[13]. In 1998, McAdam S *et al.* had evaluated cross-clade recognition of p55 antigen by CTL in persons infected with diverse clades of HIV-1. The result shown that Uganda patients tested mounted cross-reactive CTL responses that recognized gag proteins from clades A of HIV-1 other than those with which they were infected[14]. In the same year, Lynch JA *et al.* had reported a cross-clade CTL response to HIV-1 proteins among HLA disparate North Americans and Thais. These studies suggest that gag epitopes are common cross a wide range of HLA alleles and sufficiently conserved among several HIV-1 clades to make gag an important component antigen in a broadly immunogenic vaccine[12]. Our results confirmed previous studies that HIV-1 gag is the most common target of CTL recognition in chronic HIV-1 infected patients and with the highest cross-clade CTL responses, in this case between clade A/E and B.

Small sample size of subtype B infected patients (2/25) may limit the data analysis in this study.

HIV-1 gag A CTL epitope mapping results

Eighteen HIV-1 gag A epitopes of 20 amino acid in length were identified in 19 HIV-1 infected clade A/E patients who were able to be epitope mapped, as shown in Figure 6. Seven are p17 epitopes, whereas 10 are p24 and 1 is p6 epitopes. However, some of them were overlapping, it is therefore in fact a total of epitope found in this cohort may no more than 10. Almost all of these epitopes were located within the previously reported immunodominant regions have been found in subtype B infected western cohorts (Figure 6). Nine (47%) patients showed CTL recognition within HIV-1 gag A residue 261-300 (p24), 9 showed CTL activities against gag A 140-190, another 8 recognized gag A 11-50. Of interest, three HIV-1 gag CTL potentially novel epitopes were identified: peptide gag A residue 1-20 in p17 region, peptide gag 41-60 in p17 region, and gag 419-438 in p6 region, as shown in Figure 15. Further characterization of these epitopes are needed.

Fig. 15 **CTL epitope mapping in HIV-1 A/E infection (N = 19). Three potential novel HIV-1 gag CTL epitopes were identified.**



Circle line represents the new CTL epitopes that have not been reported in the Los Alamos database.

In four HIV-1 infected clade A/E patients (DK, PC, AJ, JM) were unable to identify any dominant peptide despite gag A recognition by classical Cr-51 release assay. One possible explanation is that 20 amino acid in length peptides may be longer than the optimal epitope to generate high affinity binding to HLA class I molecule in those patients[144].

HLA class I genotyping results

HLA class I alleles, found in this cohort, are similar to those of early surveys in general Thai population (Figure 7). The three most common A alleles are A11, A24 and A33 (44%, 39%, and 24%, respectively). Three most common B alleles are B58, B15 and B46 (39%,28%,22%), and 3 most common C alleles are C3, Cw1, C7 (39%,33%,28%). Based on HIV-1 immunology database of Los Alamos 1999 and most of the recent literatures, very few HLA C allele-CTL epitope restriction have been identified. In HIV-1 gag region, there were 95 CTL epitopes have been reported, among those 31 are HLA A allele-restricted, 61 are HLA B allele-restricted, and 3 are HLA C allele-restricted[145].

HLA class I restriction results

The preliminary HLA restriction experiments were tested in three patients, who showed high CTL killing against HIV-1 gag A and also showed cross-clade CTL against HIV-1 gag clade B. A HLA A2 restricted epitope: p17 region at residues gag 140-160: GQMIHQSLSPRTLNAWVKVIE was found (patient AP; Table 6 and Figure 11). MFSALSEGATPQDLN MMLNI of HIV-1 gag residue 171-190 located in p24 is the epitope restricted by HLA B7 (patient KM; Table 7 and Figure 13). These two HLA-restricted epitopes, however, are corresponding to those have previous been reported in the database. Interestingly, two of the 3 patients recognized the epitope "NKIVRMYSPVSILDIKQGPK" of HIV-1 gag residue 271-290 located in p24, which has been known as B52 restricted peptide. Nevertheless, our results showed that this epitope is actually presented and restricted by Cw1(patient PU; Table 5 and Figure 9, patient KM; Table 8 and Figure 14). It is likely that this is the second Cw1 restricted HIV-1 epitope even been reported. The first one is VIPMFSAL of HIV-1 gag residue 36-43 LAI located in p24 by Golder in 1997c. More interestingly, this peptide "NKIVRMYSPVSILDIKQGPK" of gag 271-290 in p24 region is also the most common gag CTL epitope recognized by 7 of 19 patients (37%) (2 more patients recognized a overlapping epitope of 261-280 and 281-290).