CHAPTER VI

DISCUSSTION

HIV-specific CTL responses appear to play a critical role in the containment of HIV-1 infection (1-5, 88). However, the study in Highly Exposed but Persistently Seronegative (HEPS) African sex workers showed that antigenic specificity of $CD8^+$ T lymphocyte might differ in the control of HIV-1 infection (6). Thus, it is very interesting to analyse the HIV-specific T cell specificities in HIV-1-infected person with different level of plasma HIV-RNA.

Most studies investigating HIV-1-specific $CD8^+$ T lymphocyte responses focused either on single peptide (4), restricted panels of optimal CTL epitopes (90), or a limited selection of HIV proteins (89), the responses might not be accurately represent the total responses. Recently, a comprehensive study analysing T cell responses against all HIV proteins demonstrated that the breadth and the magnitude of responses differed significantly among individuals in different stages of HIV infection (8). We were therefore interested to study $CD8^+$ T lymphocyte responses against two immunogenic proteins, Nef and Gag, in HIV-1-infected Thais who had different level of plasma viral load and in high risk HIV-1-seronegative Thais using peptide-based IFN-γ ELISpot assay.

Similar to what has been reported (8, 9), no associations between the breadth or the magnitude of HIV-1-specific $CD8^+$ T lymphocyte responses and plasma viral load were shown in our study. Moreover, the magnitude and breadth of responses were not significantly different among groups of individuals who had different viral load. However, the positive correlation (98) or negative correlation (89) was reported in other studies using other T cell analysis techniques. These results indicated that analysis of T cell responses using different techniques might not measure the same population of T cells. Previous studies showed that cytotoxic function was likely to correlate with protective role of the T cells. Thus, it is possible that the IFN-γ-secreting T cells population (which were analysed in this study) do not play protective role in containment of HIV infection.

Surprisingly, all Nef peptides were not recognised in five subjects (NKP, PMH, TKH, VMK, and VPT) whose plasma viral load were ranged from < 50 to 11,392 copies/ml. Since

Nef has been demonstrated to be immunodominant and most frequently recognised in other studies (8, 9, 98), we hypothesised that Nef might have mutated to escape immune response in these non-responders. The generation of many HLA class I-restricted peptides is profoundly influenced by amino acid variation in and around epitope. The escape mutation within epitope, especially in the anchor residue may result in the inhibition of epitope presenting and recognition. Moreover, the mutation in the flanking region may result in the inhibition of epitope processing (99-101).

We were able to show that the Nef amino acid sequences of nonresponders had mutations either inside or around the predicted epitope regions. The non response phenomenon could be simply explained by intra-epitope mutations whereby the changed amino acid residues might affect either binding affinity to the HLA molecule (anchor residue) or T cell receptor (T cell receptor residue). On the other hand, the mutation outside epitope (flanking region) might affect the antigen processing as previously described (99-101).

In this study, there are a fifth and a sixth acidic residue insertion in an acidic cluster ($EEEE_{(66)}$) of all autologous isolates of the subject NKP (viral load, 2,180 copies/ml) ($DE_{(68)}$) and subject PMH (viral load, 477 copies/ml) ($EE_{(68)}$). In addition, there were E71G and E68D substitution in all autologous isolates of the subject NKP and TKH (viral load, 11,392 copies/ml), respectively. Previous study demonstrated that this region (W61 through T180) involved in the conformation of $PxxP_4$ loop (102). It is possible that the variations occur at the positions which has an effect on either inhibiting of T cell receptor signaling (103) and in MHC class I downregulation (104, 105). In addition, there is P137T substitution mutation in the GPG sequence in 1/5 clone of subject PMH. This sequence was noted previously to be conserved and similar to that in V3 loop of HIV-1 envelope and predicted to form a beta-turn (106). Thus, the mutation in the GPG sequence may disrupt Nef structure.

In case of the subject TKH, there were amino acid insertions within N terminal arm of Nef protein in all autologous isolates but the function of these regions has not been reported. In addition, there is W61R substitution mutation in 2/5 clones of the Nef amino acid sequences of this subject. This position is the HIV-1 protease cleavage site ($WL_{(61)}$) and determines the modular organization of Nef (107). The cleavage releases the core domain

from the myristoylated membrane anchor domain. Analogous to other HIV proteins, cleavage of Nef could be crucial for correct biological function (107).

Unexpectedly, Gag peptides were recognised at low frequencies in all study groups the finding of which was contrast to what have been described in other comprehensive studies (8, 9). It may be due to the variation of autologous virus which differs in sequences from that of the synthetic peptide tested.

There were L490P and L493S substitution mutations in all autologous sequences of subject VMK and subject NKP, respectively. These mutations occur in the N-terminal p6 leucine rich domain ($[Lxx]_4$ motif). This $(Lxx)_4$ domain was required for Vpr incorporation into virus particle (108, 109). Interestingly, the termination codon was demonstrated in the C-terminus of p6 in 2/5 clones of subject VMK and all 5 clones of subject NKP. These results may help explain why these two subjects had low level of plasma viral load.

Improved understanding of the factors that determine as to why someone who remained uninfected despite repeated exposure to HIV infection may provide the information about the immunological correlates of protection. Although most high risk HIV-1 seronegative individuals in previous studies had low level of HIV-1-specific $CD8^+$ T lymphocyte responses (91, 110), one high risk HIV-1 seronegative subject (subject ASP) in our study mediated high magnitude of responses. The responses were directed against Nef 7 (AQEEEEVGFPVRPQVPLRPM) (1,114 SFU/million PBMC), Nef 8 (VRPQVPLRPMTYKGAFDLSF) (334 SFU/million PBMC), and Nef 9 (TYKGAFDLSFFLKEKGGL) (1,852 SFU/million PBMC). The strong responses against these peptides suggested that HIV must have replicated in the subject sufficiently to prime a cell-mediated immune response that was able to protect against HIV infection, by containing virus replication and spread. This finding in our study further support an important role of HIV-specific $CD8^+$ T cells in controlling of HIV infection.

Interestingly, the HIV-1-seropositive partner of the subject ASP (subject ABM) responded to Nef 9 (TYKGAFDLSFFLKEKGGL) (602 SFU/million PBMC), Nef 14 (WQNYTPGPGIRYPLCFGWCF) (288 SFU/million PBMC), and Nef 15 (RYPLCFGWCFKLVPVDPREV) (306 SFU/million PBMC). The difference in specificity of T cell responses in subject ASP (HEPS) and subject ABM (HIV-infected partner) might imply that specificities of HIV-specific T cell responses were qualitatively different in a

controlling and protecting HIV infection. Indeed, the responses against Nef 7 and Nef 8 (which were exclusively recognised by the HEPS donor) may be more critical in controlling of HIV infection. These two peptides are located in the highly conserved region of Nef protein. Nef 7 is located in an acidic region which is essential in protease recognition and cleavage. Nef 8 is located in a $PxxP_4$ motif which is important in cell activation, inhibiting of T cell receptor signaling and in MHC class I downregulation. We proposed that two peptides (Nef 7 and Nef 8) or relevant epitopes should be included in the HIV vaccine construct.

Surprisingly, these responses in subject ASP were detectable only at a single time point. This may suggest that the ongoing antigenic exposure may be necessary to maintain a protective T cell response. It is possible that the low HIV-specific T cell frequencies in HEPS donors may be due to either duration of exposure or absence time of unprotected sex before enrolment into the study. Furthermore, the other protective mechanisms including a 32-bp deletion in the gene coding for CCR5 coreceptor (111) and humoral responses at the mucosal surface (112-115) may play a resistance to HIV infection in these subjects.

In conclusion, this study demonstrated the Gag and Nef-specific $CD8^+$ T cell responses in HIV-infected subjects with different level of HIV-RNA, and in HIV serodiscordant couples. However, we failed to show the inverse relationship between level or breadth of HIV-specific $CD8^+$ T cell response in the donors. This does not simply mean the T cells do not play in the protection against HIV infection. Instead, it is likely that the HIV-specific T cell population which secreting IFN-γ may not be essential for HIV control. The future study should also analyse other HIV-specific T cell populations such as TNF-secreting or IL-2 secreting T cells. We also show that one of our HEPS donors mediated strong HIV-specific immune responses in the level comparable to HIV-infected donors. And indeed the HIV-specific T cells of this subject directed against different peptides compared to those of his HIV-infected partner. Detailed study such as fine mapping of the epitopes and HLA-restriction study are warranted.