CHAPTER V

DISCUSSION

I. Peptide serotyping immunoassay

Several techniques have been used to identify HIV-1 subtypes such as DNA sequencing analysis, peptide immunoassay, PCR-based genotyping, and heteroduplex assays.[37,40,41,42,43,45] For epidemiological surveillance or other large scale clinical study, a simple, rapid and economic technique is sufficient. Peptide immunoassay is therefore, the most appropriate method in this circumstance.

Previous reports have used different length of synthetic V3-peptide from different HIV-1 subtypes immunoassay. For instance , a 15 amino acid length (including an extra-amino acid to facilitate adherence to the solid phase) was used by Ou and colleages[138] , while a 26 mers peptide was used for their serotyping by Okuda and colleages.[45]

In this study, when the 26 a.a. peptide, i.e., PND-A (subtype E : TRPSNNTRTSITIGPGQVFYRTGDII) and PND-B (subtype B : TRPNNNTRKSIHLGPGQAWYTTGQII) were used for the immunoassay, a cross reactivity was observed (TableV). These may due to the 2 common epitopes both at N-terminal (8 a.a, namely, TRPSNNTR of the PND-A peptide vs TRPNNNTR of the PND-B peptide) and at the C-terminal (2 a.a., namely II). When these common epitopes were excluded by generating a shorter synthetic peptide which contained only 16 a.a. in length, the cross reactivity between PND-E and PND-B_{Thai} was eliminated (Table V).

After the assay was validated by the use of 5 known HIV-1 subtype sera which were previously documented by DNA sequencing analysis, the serum samples from 60 patients with different risks behaviors were analysed for the prevalence of subtype E and B_{Thai}. The results (Table VI) confirm the previous observations[36,37] that HIV-1 subtypes in Thailand are segregated by mode of transmission. Subtype E is most prevalent in heterosexually acquired persons (70%) while B_{Thai} is more common in IVDUs (70%). Of interest, 60% of homo/bisexuals, 10% of IVDUs and 10% of heterosexuals in this study were not typable by the peptide immunoassay. As there was no information of HIV-1 subtypes in homo/bisexually acquired persons in Thailand and the North American originated subtype B was also not found to be associated with any specific risk groups in Thailand, the nontypable serum samples were then tested with PND-B_{MN} peptide. Interestingly, 50% of homo/bisexual samples were reactive with PND-B_{MN}. This indicated that the majority of homo/bisexually acquired Thai patients were infected with subtype B_{MN} (the North American originated strain). This B_{MN} subtype was also found in a minor group of heterosexual (6%) and IVDUs (10%) patients. Nevertheless, a significant number of samples in all risk groups still remained untypable with the three PND peptides used in this study.

The PCR-based genotyping analysis was developed using seminested PCR technique [141] (Figure 1). Stored PBMC of 10 patients randomly selected from 41 patients with known serotypes were analysed by newly developed method to assess the sensitivity and specificity of the immunoassay. It appeared that all the serotypable results were confirmed by PCR-based genotyping (Table VIII). This supports the reliability or specificity of the immunoassay. However, 9 of 18 (50%) non-typable samples were shown to be genotype E by PCR analysis, indicating that immunoassay was less sensitive

than the genotyping method. In addition, for the dual-reactive sample, PCR-based genotyping revealed that it was of the B_{Thai} genotype. Interestingly, this patient belonged to the IVDU group.

Our results confirm that peptide immunoassay is the most appropriate method to be used for large-scale subtyping analysis. Samples that are nontypable by immunoassay should be subjected for further analysis by PCR-based genotyping. DNA sequencing analysis or heteroduplex mobility assay (HMA) should then be required for the remaining untypable samples.

II. Natural history study

The retrospective cohort of 94 patients from different risk groups who had been followed for more than 3 years and with CD4 cell count > 200 cells/cu.mm. at baseline collecting from 1989 to 1994 was analysed (Table X). Interestingly, the mean CD4 cell counts of the heterosexual group was significantly lower than that of the homo/bixual and IVDU groups (Table XII and figure 4). The possible explanations could be either that on the average, the heterosexual group had been infected earlier than the others, or that HIV-1 subtype E which is the predominant subtype in the heterosexual group behaved more aggressively than the subtype B_{Thai} and B_{MN}, which predominates in the other risk groups.

There was no difference in CD4 declining rate or in HIV-disease progression among the three risk groups up to 3 years of follow up, except in the second year, where CD4 declining rate of the homo/bisexual group was significantly greater than that of the heterosexual group ($p< 0.05$) .

When the group, consisted of 64 heterosexually-acquired patients with known HIV-1 subtypes was analysed, it was found that there was a significantly higher rate of disease progression in the group with HIV-1 subtype E as compared to the group with subtype Non-E at the third year of follow-up ($p=0.028$) but not at year 1 or year 2. (Table XVI and figure 8). However, if only progression to AIDS was considered , no difference was found between subtype E and Non-E heterosexual patients. For the rate of CD4 decline, there was no significant difference between subtype E and subtype Non-E throughout the 3 years of follow up, eventhough there was a trend that on the second year subtype Non-E might have a higher rate of CD4 decline than subtype E. However, CD4 cell count is only a surrogate marker, the natural course of the disease should be evaluated primarily on the clinical ground.

Our results suggest that HIV-1 subtype E migth be more aggressive than the other HIV-1 subtypes whereas different risk behaviors per se did not have any significant affects on disease progression. However, larger studies of the prospective cohort type are warranted to confirm this observation.

CONCLUSIONS :

1. A peptide serotyping method was utilized for the investigation of HIV-1 subtypes in Thailand. This method is simple, inexpensive and highly specific.

2. Genotyping by seminested PCR using selective specific primers was utilized to validate peptide serotyping method and was found highly sensitive.

3. From this study, peptide serotyping is recommended to be used as a first line screening for HIV-1 subtypes and the genotyping method to be used as a subsequent test for sero-nontypable and dual-reactive samples.

4. HIV-1 subtypes in the patients at Chulalongkorn Hospital during 1989-1994 were segregated by mode of transmission. Subtype E was a major subtype in heterosexual group, whereas subtype B_{Thai} was found predominantly in IVDUs and subtype B_{MN} was more prevalent in homo/bisexuals.

5. Baseline CD4 cell counts among heterosexuals were significantly lower than the non-heterosexuals whereas no significant difference between subtype E and Non-E in the heterosexual group.

6. Rate of CD4 decline among various risk groups was not significantly different, same for subtype E and Non-E. However, subtype Non-E had a higher rate of CD4 decline than subtype E in the second year of follow up.

7. Annual CD4 declining rate in heterosexual group was between 50 to 100 cells/cu.mm.

8. There was no significant difference in disease progression among risk groups. However, among the heterosexuals, subtype E was found to have a significantly higher rate of disease progression than subtype Non-E after 2 years of follow-up.