CHAPTER V

DISCUSSION

The rate of disease progression and outcome of HIV-1 infection are highly variable among different individuals. Most individuals infected with HIV-1 develop AIDS within 8 to 10 years (typical progressors) and about 5% remain relatively healthy for 8 to 10 years or more (long-term nonprogressors), while others 5% to 10% progress to AIDS within the first 2 to 3 years after infection (rapid progressors). However, some individuals remain uninfected by HIV-1 despite repeated exposure to the virus (highly exposed persistently seronegative; HEPS). Factors that influence resistibility to HIV-1 infection in HEPS individuals and effect to rate of disease progression in HIV-1 infected individuals are multi-factors composed of viral factors, host factors, and environmental determinations. Since the discovery that CCR5 serves as a major co-receptor along with CD4 for primary macrophage (M)-tropic HIV-1 strains (Dragic *et al.*, 1996; Deng *et al.*, 1996; Choe *et al.*, 1996; Doranz *et al.*, 1996; Alkhatib *et al.*, 1996), at present the CCR5 gene has become a central theme of studies regarding host genetic influence on HIV-1 infection and disease progression. Because the growing numbers of genetic variants within coding region and promoter region of CCR5 gene have been identified, several of them have functional consequences for HIV-1 infection and pathogenesis. The role of controlling HIV-1 infection and disease progression of CCR5 needs more studying in each population. To understand the influence of the CCR5 on resistibility to HIV-1 infection in HEPS individuals, we recruited 20 HIV-1 serodiscordant couples and 10 HIV-1 non infected healthy individuals who lived in Chiang Mai province and conducted study for the sequence variations in the coding and the promoter region of CCR5 gene and the expression of the CCR5 molecule on the surface of HIV-1 target cells.

Both CCR5Δ32 and CCR5-m303 were not detected in all samples. This finding was not surprised because those alleles had low allele frequencies in non-Caucasian population. The CCR5Δ32 was common in Caucasians especially in northern European populations

(approximately 1-3% of homozygous individuals and 14% of heterozygous individuals), less common in other American racial groups and rare to absent in African and Asian populations (approximately 6% of African-Americans, 7% of Hispanics, 13% of Native Americans, and less than 1% of Asians) (McNicholl *et al.*, 1997). The CCR5Δ32 variation does not produce a functional CCR5 protein on the surface of cells (Liu *et al.*, 1996) explaining the complete protection against HIV-1 infection in individuals homozygous for this alleles. However, a rare cases of HIV-1 infection in absence of CCR5 molecules have been reported (Biti *et al.*, 1997; Theodorou *et al.*, 1997), this suggests that X4 HIV-1 isolates can sometimes initiate the infection. The CCR5-m303 variation was found in compound heterozygote for the CCR5Δ32 allele, which results in a non-functional and truncated protein as the CCR5-Δ32 allele (Quillent *et al.*, 1998). The CCR5 expression was detected in all study subjects of this study that confirmed the absence of the CCR5Δ32 and CCR5-m303 alleles in these subjects. However, the CCR5 density on the surface of HIV-1 target cells of each individuals had highly variable. The low expression of CCR5 correlates with reduced infection of T cells by R5 HIV-1 isolates of HIV-1 *in vitro* (Wu *et al.*, 1997). Explaining by the gene dosage effect, the CCR5Δ32/wild-type heterocomplex causes CCR5 to be retained in the endoplasmic reticulum resulting in reduced cell surface expression of the wild-type molecule (Benkirane *et al.*, 1997). Only the CCR5Δ32 allele or CCR5-m303 allele can not explain the appearance of the level of CCR5 expression variation in each individual. As in this and other studies (Wu *et al.*, 1997; Paxton *et al.*, 1998; Ostrowski *et al.*, 1998) showed that there were a highly variation of the expression of the CCR5 molecule among the homozygous wild-type individuals. Other factors are likely to be involved. It is possible that additional alleles of the CCR5 coding region (Ansari-Lari *et al.*, 1997; Carrington *et al.*, 1997; Carrington *et al.*, 1999) may play a role in HIV-1 infection and pathogenesis controlling via the level of CCR5 expression. One of the alleles (893delC or CCR5-893(-)) causes premature termination of transition and was found to be an Asian-specific variation (allelic frequency of approximately 4% in Japanese and Chinese populations) (Ansari-Lari et al., 1997). However, the CCR5-893(-) allele was also absent form 22 HIV-1 discordant Thai couples (Louisirirotchanakul *et al.*, 2002). Thus, it could be suggested that the CCR5Δ32 and CCR5-m303 alleles did not affect HIV-1 transmission in this HEPS group. However, the further study in the other variations in the CCR5 coding region is needed.

Although the CCR5Δ32 and CCR5-m303 alleles were not detected in all study subjects, but the regulation of CCR5 expression is likely to be complex. We found here that the density of CCR5 molecules on the cell surface was highly variable in those who were homozygous for the normal allele of the gene (without CCR5Δ32 and CCR5-m303 alleles). These findings are concordant with Wu *et al.* (1997), Paxton *et al.* (1998) and Ostrowski *et al.* (1998) described a highly variable aspect of CCR5 surface expression in EU, HIV-1 infected and normal control individuals. Moreover, Paxton *et al.* (1998) found that the level of CCR5 expression in homozygote CCR5 normal alleles in EU individuals correlates with the infectability by M-tropic HIV-1 strains *in vitro*. This implies that more variation in CCR5 gene especially in the promoter region, needed to identified. Thus, the nucleotide polymorphisms in the promoter region of CCR5 gene were focused in this study. The mechanism behind the variable CCR5 cell surface expression in wild-type homozygous CCR5 individuals may depend upon the variation within the promoter region of CCR5 gene which regulates the transcription in the CCR5 mRNA level. Liu *et al.* (1998) reported that the Pd promoter region means to the transcription of CCR5 gene. The polymorphism in this region could potentially disrupt transcription factor binding motifs, accounting for some of the heterogeneity in CCR5 expression among individuals (Carrington *et al.*, 1999). In this study, the nucleotide polymorphisms in the CCR5 promoter region of HEPS individuals and their HIV-1 seropositive spouses were determined by using the nucleotide sequencing technique. According to Martin *et al.* (1998)'s nomenclature of CCR5 promoter haplotype, 18 out of 19 HEPS and all 16 HIV-1 seropositive individuals were classified as CCR5P4 haplotype, while only 1 out of 19 HEPS individuals was the CCR5P2 haplotype. Both CCR5P2 and CCR5P4 alleles are common promoter haplotypes identified in Caucasian and African American samples. (Martin *et al.* 1998) The other six haplotypes called CCR5P5-CCR5P10 are the rare promoter haplotypes identified in the some cohort. The CCR5P1 was found association with an accelerated rate of disease progression of AIDS (Martin *et al.*, 1998; An *et al.*, 2000) and the CCR5P1 was found independent to the protective alleles, CCR5Δ32 and CCR2-64I. (Martin *et al.*, 1998; Carrington *et al.*, 1999) The survival analysis in individuals with homozygous CCR5P1 showed that the rate of progression to AIDS was more rapid than those individuals with CCR5P2-P4. (Martin *et al.*, 1998) The other polymorphisms with single

nucleotide changes in the CCR5 promoter region have been described at the position 59029, 59353, 59356. The homozygous CCR5-59029A/A genotype was known to have an association with disease acceleration. The one perinatal transmission cohort showed that HIV-1 infected children with the CCR5P1/P1 genotype have the CCR5-59029A/A. This finding suggested a linkage disequilibrium between the P1 haplotype and the 59029A and might explains the previous report an association between 59029A/A genotype and rapid disease progression. However, we found here that all except one of our study subjects in both HEPS and their seropositive spouses have the CCR5P4/P4 with CCR5-59029G/G genotypes. The CCR5-59029G haplotype was known to associate with the delay of disease progression in adults. The study of promoter activity in vitro was consisted with the finding above that the CCR5 promoter with 59029G

haplotype has lower activity than those with the 59029A. This suggests that the polymorphism at 59029 could directly affect CCR5 expression. However, one of HEPS (H07) who carried the CCR5P2 haplotype has homozygous CCR5-59029A. To date, the role of CCR5P2 haplotype in clinical outcome of HIV-1 infection is not yet known. The other polymorphisms or factors may help to clarify this finding. However, when comparing the polymorphic sites in the promoter region of the HEPS-H07 with other HEPS individuals, the HEPS-H07 has more polymorphic site than those observe in other HEPS. Those polymorphic sites may at least have the counter affect to the 59029A haplotype. The CCR5-59353C/T polymorphism is one of 10 single nucleotide polymorphisms reported by Martin *et al.* The CCR5-59353C/C with CCR5-59029A/A genotype has been reported to accelerate progression to AIDS more rapidly than the CCR5-59353T/T with CCR5-59029G/G genotype. (McDermott *et al.*, 1998; An *et al.*, 2000). The Australian long-term non-progressor study reported the high frequency of linkage between genotype CCR5-59353T/T and CCR5-59029G/G in those HIV-1 long-term non-progressors when compared to the HIV-1 progressor (41.2% vs. 17.5%, p=0.005). This type of linkage was also observed in our study, but however, in both HEPS and seropositive groups. It might be suggested that the linkage between CCR5-59353T/T and 59029G/G may not have a protective role but only delay disease progression. Moreover, the CCR5-59029A/A genotype was found in association with an increased number of CD4+ T cells expressing CCR5 (Shieh *et al.*, 2000). Taken together, the percentages of cells containing CCR5 and CCR5 protein density on surface of CD4+ T lymphocytes from individuals who carry CCR5-59029A/A were

compared with those from individuals with CCR5-59029G/G. The results showed that there were no differences found in the number of cells expressing CCR5 and the molecule density on the cell surface between individuals with CCR5-59029G/G and CCR5-2459A/A genotype. Our finding was not consisted with of Shieh *et al.* (2000). The marked different in these two study were the study subjects, the normal healthy volunteers (Shieh *et al.* 2000) and the HEPS individuals. Since the polymorphisms in the CCR5 regulatory region are more complex, the true effect of the nucleotide variant on HIV-1 disease must be carefully interpreted. However, the assay to determine the controlling of transcription and translation level is needs to be observed.

The number of CCR5 density on the surface of the HIV-1 target cells from the HEPS individuals, their HIV-1 seropositive spouses and the healthy normal individuals were determined and comparing by using direct immunofluorescent technique and flow cytometry. The results indicated that the percentage of the CCR5 expression on the surface of CD4+ T lymphocyte from HIV-1 seropositive individuals was significantly lower than those from HEPS and normal subjects. This could be explained in term of the host immune system that function in destroying the infected target cells and/or the virus itself that induce formation of the syncytium which finally leading to cell death. Moreover, the median of CCR5 density on the surface of CD4+ T lymphocytes and monocytes from HEPS and healthy normal individuals was significantly lower than those from HIV-1 seropositive individuals. The variations on the coding region including CCR5Δ32 and CCR5-m303 and also the nucleotide polymorphisms on the promoter region of the CCR5 gene seemed not to be involved with those situations. However, to understand more, the study of the function of CCR5 protein that expression on the cell surface needs to be investigated.

Recently, several groups of investigators had reported that Tat protein of HIV-1 can up-regulated expression of CCR5 molecule in peripheral blood mononuclear cells in HIV-1 infected individuals and promotes the infectivity of both M- and T-tropic HIV-1 strains in primary human leukocytes and monocytes/macrophages (Albini *et al.*, 1998; Huang *et al.*, 1998; Weiss *et al.*, 1999). This finding may explain the appearance of higher CCR5 density on the surface of CD4+ T lymphocytes and monocyte of HIV-1 infected individuals in this study. Furthermore, we also compared the CCR5 density on the cell surface from HIV-1 infected individuals who had high HIV-1 viral load (300,000 to >750,000 copies/ml) and not using any

anti-retroviral (ARV) drugs with those who had low viral load (>400 copies/ml) and using at least one regimen of the ARV. The results showed that persons with high viral load had higher CCR5 density than those with the low viral load, indicating that the virus may plays role in activation of CCR5 synthesis in the infected cells. This was consistent with the earlier finding by Albini *et al* who had reported the role of HIV-1 Tat protein in up-regulation of the CCR5 expression. It seems that the high HIV-1 viral load presence in some HIV-1 infected spouses had no effect on transmission of the virus to the HEPS. It is possible that the viruses that had been evolved in those infected individuals might less fit in transmission. However, this observation needs to be investigated further.

In addition, other host genetic factors including the polymophisms in other chemokine/cytokine receptor genes; CCR2, SDF-1, IL-10, IL-4 and also HLA genotypes had been shown to have some effect on the resistibility of HIV-1 infection. Moreover, the mechanisms of the immune system both CMI (cytotoxic T cells) and HMI (immunoglobulin subtypes) as well as β-chemokine production and Th-1 and Th-2 cytokines, also need to be emphasized. In the present study, we did not find the specific factors involved in resistibility of HIV-1 infection in the HEPS enrolled here, however, it provide the primary information in the host genetic factor, the CCR5 coding and regulatory regions, that involve in the resistibility to HIV-1 infection which may importance to understanding of mechanisms controlling HIV-1 infection in the future.