CHAPTER V

DISCUSSION

Primary HIV infection is associated with a burst of plasma viremia and an acute febrile illness, characterized by nonspecific symptomatology and spontaneous resolution. Plasma viremia then drops to relatively low level, and the individual enters a prolonged asymptomatic period known as clinical latency. Numerous factors have been proposed that may control viral replication during this period, including neutralizing antibodies, virus-specific cytotoxic T cells, cytokines, chemokines, and the availability of HIV-1 coreceptors.[74,77,82,96]

The rate of disease progression is very heterogeneous among individuals, and is affected by many factors including age, associated diseases, immune activation, nutrition status, and the viral strain. However, even when these factor are normalized, progression rates differ dramatically; in the extreme some rare individuals, known as long term non progressors, do not appear to progress at all. It is still unclear whether these subjects represent a distinct group, sharing a common biological phenomenon for such resistance to progression, or if they are only random and extremely rare exceptions to the general rule. Some investigators have proposed that the HIV-1 disease-free non-progression might be genetically controlled.[97] The first candidates were in the major histocompatibility complex gene (MHC-HLA). A combination of HLA class I (HLA-A1, HLA-A2, HLA-B14, HLA-B17, HLA-B27) and Class II antigens (HLA-DR5 and HLA-DR6) has been correlated with low rates of disease progression.[98,99] In contrast, the presence of HLA-B35, HLA-DR1, HLA-DR3, HLA-DQ1 antigens has been significantly associated with a bad prognosis and rapid progression to AIDS.[100,101]

Other genetic polymorphism determinants of HIV/AIDS resistance are located in either chemokine receptors or chemokine genes (CCR5Δ32, CCR5m303, CCR2-64I, SDF1-3'A). For the CCR5-Δ32 and CCR5-m303 mutation, the mechanism of resistance is quite clear; they result in lack of expression of a functional CCR5 protein and abrogation of its HIV co-receptor function for M-tropic HIV-1 strains.

The situation is more complex for the CCR2-64I mutation. Indeed, although some HIV co-receptor activity has been attributed to CCR-2, its significance in this respect is minor when compared to CCR5 and CXCR4. In addition, the CCR2-64I substitution is a conservative substitution located in a transmembrane domain of CCR-2 protein, which is not a part of the HIV-binding site. Thus most probably, the mechanism of protective action of the CCR2-64I mutation is indirect. In this respect, an interesting hypothesis has been suggested by Kostrikis *et al.*[102] These authors demonstrated the presence of a polymorphism in a regulatory region of the CCR5 gene (C to T substitution at position number 59653, GeneBank Accession number U95626), which was in complete linkage disequilibrium with CCR2-64I. If it is suggested that this polymorphism can suppress the expression of the CCR-5 protein, then the actual determinant of the CCR2-64I associated resistance could be the CCR-5/C to T 59353 substitution, while the closely-linked CCR2-64I (both CCR-2 and CCR-5 gene are located in close proximity on chromosome 3) is only a "tracking" marker.

The protective effect of the SDF1-3'A mutation is recessive, i.e., it is observed only in homozygotes and no difference is apparent in this respect between wild type and heterozygote individuals.[16] The extent of protection in SDF1-3'A homozygotes is approximately twice as strong as that conferred by either CCR5-Δ32 or CCR2-64I[16]. The mechanism of the protective effect remains unknown. At the same time, a reasonably plausible hypothesis has been suggested. It implies that the 3' untranslated region of SDF-1 gene serves as a target for *cis*-acting factors upregulating expression of SDF-1 protein. As a result, more of the protein is available to bind CXCR4, and the receptor becomes less available for T-tropic HIV-1 strains, which are associated with disease progression

One of the most obvious questions in the framework of genetic resistance to HIV/AIDS is how frequent the relevant mutations are in different racial and ethnic groups. Knowledge of mutation frequencies is essential for gaining insights about their origin, as well as the nature of selective factors responsible for the accumulation of these genetic polymorphisms in human populations. In addition, such information might

be helpful in modeling the dynamics of HIV/AIDS epidemics as well as in estimating the cost-effectiveness of HIV/AIDS resistance genotyping in various ethnic groups.

Previous studies of the allele frequency of CCR2-64I and SDF1-3'A have not contained Thai people as subjects. In addition, Thais are a distinct ethically and geographically defined population in South East Asia and the modern population of Thailand is composed of a majority of peoples of Tai (Dai) lineage, as well as an admixture of a number of minority Mongoloid populations from the northern mainland (especially southern Chinese) and the southern island groups of South East Asia. [103,104,105] The population of Bangkok is a reflection of this ethnic mixture, having a predominance of Central Thai, with a large southern Chinese minority. This population is experiencing an HIV epidemic, spreading largely through heterosexual contact.. The current study was undertaken to determine the frequency of these two alleles in the general Thai population, to ascertain how relevant they are to the ongoing HIV epidemic in Thailand. Subjects were drawn randomly from blood donors at the Thai Red Cross National Blood Center as these represented a good cross-section of the healthy Thai population.

The CCR5-Δ32 allele has been reported very rarely outside Caucasian groups. In the Thai population, we found no evidence for the CCR5-Δ32 in 200 subjects (Nookhai, S., *et al.*, manuscript submitted for review) indicating that the phenomena of resistance to HIV infection and long term non-progression to HIV disease (that have been observed amongst Thai people) are not significantly contributed by this allele.

The CCR2-64I allele is broadly distributed across world populations (Shown in table IX). The frequency of 15.7 % detected in the Thai population is in concordance with previous incident in other Asian populations. The genotype frequencies of this allele fall within the range consistent with Hardy-Weinberg equilibrium (10.7%-20.8%), indicating an absence of strong selective pressure. From the data in this study, we shown that more than 70 % of the Thai people do not have a mutation in the CCR2 gene (Figure VI).

The SDF1-3'A allele has an uneven distribution between racial groups (Shown in table X) ranging from 3% in some African populations to greater than 70% in native New Guineans. This study demonstrates a high frequency of SDF1-3'A in the Thai people (33.2%), similar to that which has been reported in other south Asian and Japanese groups. This genotype frequency is consistent with Hardy-Weinberg equilibrium (26.8%-39.7%). In our study, we found more than 50% of the Thai subjects carried this mutant allele (Shown in Figure VI). The SDF1-3'A allelic frequency in Thai population concurs well with data from the Cambodian population(32.1-33.2%).[21] A trend toward higher levels of this allele in Asian (particularly Australasian) populations may reflect a chance event during past human migrations or a response to local evolutionary pressure, such as an endemic infectious disease.

These data show that the frequency of the CCR2-64I and SDF1-3'A mutations in Thailand are not significantly different from what was predicted using data from other populations - especially Asian. Additionally, there was no evidence of linkage disequilibrium between these alleles. The CCR2 gene is located in chromosome 3q21 and SDF1 gene is located at the chromosome 10q11. Using the combination data between CCR2-64I and SDF1-3'A, we found 65% of the subject have either the CCR2-64I and/or SDF1-3'A mutation (data shown in Table VIII). Interestingly, approximately 13.5 % of the subjects have genotypes that known to be significantly associated with delayed progression to AIDS, which includes 5 % of SDF1-3'A homozygous with CCR2-64I heterozygous mutation and 8.5 % of SDF1-3'A homozygous mutation with CCR2-64I wild types (shown in table XI).

This is the largest samples in genetic survey to date undertaken to examine the frequency of CCR2-64I and SDF1-3'A alleles in a specific Asian population. It is the first study to examine these alleles in Thailand. The results confirm previous observations of the distribution of these alleles in Asian populations and indicate that CCR2-64I and SDF1-3'A are contributing to the nature of the HIV-1 epidemic amongst the Thai people.

As further genetic factors important in HIV-1 transmission and pathogenesis become known, it is likely that some of these will also vary in their frequencies in

different human populations, for reasons as yet unknown. As the Thai population is ethnically distinct, it will therefore be important to assess these new discoveries as they arise. The data in this study, and future such studies, will have implications, not only for the epidemic in Thailand; they will also contribute to the global picture of the distribution of these alleles. This information will be a great importance in developing a better understanding of the biology and pattern of epidemic spread of HIV-1.

Table IX. CCR2-64I allele frequencies in world population

Ethnicity	*n*	Allele frequency (%)	Reference
Caucasian (Unspecified)	1847	9.8	Smith *et al.* (1996)
Hispanic	207	17.2	Smith *et al.* (1996)
Arabic (Kuwait)	113	12.0	Voevodin *et al.* (1999)
African (Kenya)	235	23.0	Anzala *et al.* (1998)
African American	899	15.1	Smith *et al.* (1996)
Asian (unspecified)	40	25.0	Smith *et al.* (1996)
Japanese	122	26.2	Hizawa *et al.* (1999)
Chinese (Taiwan)	71	15.5	Shieh *et al.* (1999)
Thai	**200**	**15.7**	**Nookhai *et al.* (2000)**

Table X. SDF1-3'A allele frequencies in world population.

Ethnicity	*n*	Allele frequency (%)	Reference
Caucasian (Unspecified)	1835	21.1	Winkler *et al.* (1998)
Caucasian (Northern)	23	21.7	Su *et al.* (1998)
Caucasian (Italian)	37	14.9	Su *et al.* (1998)
Hispanic	131	16.0	Winkler *et al.* (1998)
Arabic (Kuwait)	108	26.0	Voevodin *et al.* (1999)
African	11,20,35	2.9-9.1	Su *et al.* (1998)
African American	859	5.7	Winkler *et al.* (1998)
Asian (Unspecified)	37	25.7	Winkler *et al.* (1998)
Chinese (Northern)	45	24.4	Su *et al.* (1998)
Native American	20,36,40	5.6-22.5	Su *et al.* (1998)
Japanese	15	36.6	Su *et al.* (1998)
Chinese (Southern)	19	34.2	Su *et al.* (1998)
Chinese (Taiwan)	63	28.6	Shieh *et al.* (1999)
Khmer (Cambodia)	28,170	32.1-33.2	Su *et al.* (1998),Rousset *et al.* (1999)
Thai	**200**	**33.2**	**Nookhai *et al.* (2000)**
Melanesian	12	66.7	Su *et al.* (1998)
New Guinean	21,48	66.7-71.4	Su *et al.* (1998)
Australian Aboriginal	14	53.6	Su *et al.* (1998)

Table XI The percentage of genotype that influence in delay progression to AIDS

SDF-3'A	CCR2-64I	CCR5-Δ32	Percentage
Homozygous	Homozygous	Wild type	0
Homozygous	Heterozygous	Wild type	5
Homozygous	Wild type	Wild type	8.5
	Total		13.5