CHAPTER I

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) belongs to the *Lentivirus* subfamily of retroviruses that causes the acquired immune deficiency syndrome (AIDS). AIDS was first reported in 1981 and has become a major epidemic worldwide. By killing or impairing cells of the immune system, HIV-1 progressively destroys the body's ability to fight against infections and certain cancers. Individuals infected with HIV-1 are also susceptible to opportunistic infections caused by different microbes that normally do not cause illness in healthy people.

The rate of HIV-1 disease progression exhibits a remarkable variation among different individuals. After begin infected, most HIV-1 infected persons, accounting for 80% to 90% developed the disease and died within approximately 10 years. They are known as a "typical progressors" (TPs). However, about 5% of those who have stable CD4 count and low viral load show no symptoms of disease for a certain number of years (8 to 10 years at least) referred to as a "long-term nonprogressors" (LTNPs). Another, 5% to 10% of those HIV-1 infected persons who have rapid increase of the viral loading and decrease in CD4 count develop disease in 2 to 3 years; they are known as a "rapid progressors" (RPs).

Some individuals with high risk against infection like commercial sex workers (CSWs), sex partners of HIV-1 infected persons (heterosexual and male homosexual), infants born to HIV-1 infected mothers as well as intravenous drug users who shared needles with HIV-1 infected persons remain HIV-1 seronegative despite multiple repeated exposures to the virus. These subjects are known as highly exposed persistently seronegative (HEPS), exposed uninfected (EU) or exposed seronegative (ES).

Multi-factors including host and viral factors might influence resistibility to infection and rate of disease progression. A number of host genetic variants have now been identified and shown to have an association with HIV-1 disease progression and resistance to infection. Genetic polymorphisms in host chromosomes including HLA class I, cytokines (e.g., IL4-589T and IL10-

5'A), chemokine receptors (e.g., CCR5Δ32, CCR5-m303, CCR5-893(-) and CCR2-V64I) and chemokine ligands (e.g. SDF-1-3'A and RANTES-403A/-28G) have been studied in different ethnic groups. In addition, exposing to a low inoculum or virus with altered infectivity may also lead to disease transmission limiting. In such cases, exposing to virus may not be sufficient enough to initiate infection or may prime an immune response that confers protection or restricts the high levels of replication. HIV-1 specific cellular immunity, both T-helper and cytotoxic T lymphocytes (CTLs), as well as HIV-1 specific IgA responses have been demonstrated in several HEPS cohorts that confer protection against HIV-1 infection in those individuals.

Several genetic polymorphisms in the major co-receptor for the macrophage-tropic (M-tropic) strains of HIV-1, CC-chemokine receptor 5 (CCR5), are some of the important host genetic factors that involve in the protection against HIV-1 infection. A 32 nucleotides deletion (CCR5Δ32) in the CCR5 coding region encoding a truncated CCR5 protein is not expressed on the cell surface. The complete absence of CCR5 expression in CCR5Δ32 homozygotes is strongly protective against infection by CCR5-using (R5) HIV-1 but not the CXCR4-using (X4) HIV-1 variants. The point mutation at nucleotide position 303 (m303) in the CCR5 coding region results in encoding of a truncated receptor has also been associated with a resistance to HIV-1 infection. However, the number of the CCR5 molecules on the CD4+ lymphocyte varies among individuals with wild-type CCR5 coding alleles, this leads to the suggestions that polymorphisms in the CCR5 promoter region might influence resistibility to infection or disease progression. To date, more than 10 polymorphic nucleotides in the CCR5 promoter region have been identified, some of which affect the rate of disease progression; e.g., CCR5-59029G homozygote with lacking the CCR5Δ32 causes a slower progression to AIDS than that of CCR5-59029A homozygote while the CCR5P1 haplotype is associated with the acceleration of disease progression.

In order to understand the influence of genetic polymorphisms in the CCR5 gene with resistance to HIV-1 infection in the HEPS individuals, the nucleotide polymorphisms of CCR5 promoter and coding regions including CCR5Δ32, and CCR5-m303 and its' protein density expressed on the surface of CD4+ lymphocytes and monocytes from HEPS persons were determined. In this study, PBMCs were isolated from EDTA blood of HEPS, their HIV-1 seropositive spouses and healthy normal individuals. Then, genomic DNA was extracted. For

the CCR5 promoter polymorphism analysis, the CCR5 promoter DNA was amplified by using PCR. The amplified product was cloned into the pGEM®-T Easy vector (Promega, USA) and sequenced by the Thermo Sequenase™ Cy™5 Dye Terminator Kit (Amersham Bioscience, England). The nucleotide sequence was resolved by the Long-Read Tower Sequencer (Visible Genetic, USA). This sequence was compared to the reported sequence obtain from the GenBank database (the NIH genetic sequence database at NCBI) by using the BioEdit Sequence Alignment Editor version 5.0.9 software.

CCR5Δ32 was determined by using PCR technique with specific primers flanking the Δ32 deletion region and analyzed by size differences of the amplified products on the agarose electrophoresis. The CCR5-m303 was detected by using Nested-PCR with *Hinc*II restriction enzyme analysis.

Finally, the density of CCR5 protein on the surface of CD4+ lymphocytes and monocytes were measured by using direct immunofluorescent technique and flow cytometry. The CCR5 protein density was determined against the standard microbeads coated with known amount of fluorochrome (QuantiBRITE-PE, Becton Dickinson, USA).

Aims of the study

1. To determine the nucleotide polymorphisms of the promoter region of CCR5 gene from HEPS persons and their HIV-1 seropositive spouses.
2. To determine the CCR5Δ32 and CCR5-m303 from HEPS persons, their HIV-1 seropositive spouses and healthy normal individuals.
3. To measure the density of CCR5 protein on the surface of CD4+ lymphocytes and monocytes from HEPS persons, their HIV-1 seropositive spouses and healthy normal individuals.