CHAPTER I
INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) is a clinically multifaceted disease induced by human immunodeficiency virus (HIV) infection. HIV belongs to the retrovirus family, a family of RNA viruses, Genus Lentivirus. HIV is subdivided into two distantly related types, HIV-1 and HIV-2. HIV-1 is the predominant worldwide isolate from individuals with AIDS or at high risks for the development of AIDS. HIV-2 is endemic among people in West Africa.

The known routes of transfer of the AIDS virus are blood, blood products, intimate sexual activity, and transmission from mother to child. However, transmission through sexual contact has accounted for 75 to 85 percent of with the human immunodeficiency virus (HIV) infections so far.

After transmission of HIV, about 80% to 90% of HIV-infected persons are "typical progressors" (TPs) and a course of HIV disease has a median survival time of approximately 10 years. However, approximately 6% of HIV-infected individuals experience a rapid decline in CD4+ T-cell levels within 2 to 3 years and develop full-blown AIDS within 3 years after primary HIV infection. These individuals are "rapid progressors" (RPs). In addition, a small percentage of infected persons (5%) do not experience clinical progression of HIV infection and have stable CD4+ T-cell counts for many years (7 or more years) despite lack of therapy. These individuals are "Long-Term Nonprogressors" (LTNPs).

Although progression to AIDS occurs in most HIV-infected individuals, it is now evident that a small percentage of individuals is HIV-1 highly exposed persistently seronegative (HEPS) even though they have been highly exposed to the virus. HEPS persons, multiple exposed to HIV but HIV seronegative, have been observed in the sex partners of HIV-infected persons, female commercial sex workers, intravenous drug users, blood transfusions, infants born to HIV-seropositive mothers and healthy care workers occupationally exposed to HIV-contaminated body fluids.

The mechanisms of resistance to HIV in HIV-exposed individuals is still unclear. Exposure to HIV not resulting in infection may depend on a complex array of features contributed by the pathogen and host such as: HIV-1-specific cellular immunity; a particularly fortunate genetic constitution; low inoculum level or defective virions, viral exposure insufficient to initiate infection, interference with infection by a more virulent strain or an immune response that confers protection or restricts high levels of replication; or a combination of all these factors. Either inherited or acquired factors may be the underlying mechanism of resistance to HIV. Studies focusing on HEPS populations have helped to identify both genetic and cellular factors associated with resistance to infection.

HIV infection occurs not only through interaction of the virion envelope glycoproteins (gp120/41) with the CD4 molecule on the target cells, but also with its

co-receptors, namely, chemokine receptors. Macrophage-tropic (M-tropic) strains of HIV-1 use the CC chemokine receptor, CCR5, for entry and replicate mainly in macrophages and also in CD4+ T-cells. The CCR5 co-receptor is used by almost all primary HIV-1 isolates regardless of viral genetic sub-type. Heterozygosity of the mutated allele does not protect from infection, but is associated with a slower course of disease progression. Homozygosity for a 32-bp deletion mutation of a CCR-5 locus (Δ32CCR-5) is a genetically acquired HIV resistance factor, but not found in all HEPS persons. As the resistance conferring homozygous condition is present in only 1% of caucasians, it is clear that this mutation represents just part of the picture. HIV-1 infection can occur in persons who have an absence of inherited CCR5 coreceptor defects, which implies that other mechanisms of protection must also be involved.

Many previous reports demonstrated increasing evidence that a substantial proportion of people with documented HIV exposure, who remain uninfected, generate a range of immune responses to the virus. These responses include HIV-1-specific cellular immunity, T helper or cytolytic responses, and resistance to HIV infection that correlates with detection of HIV-specific and type l cytokine-secreting T helper lymphocytes (Th) in the peripheral blood. Acquired factors may be other mechanisms that play a role in resistance to HIV infection in HEPS persons, including production of interleukin-2 (IL-2) and interferon-gamma (IFN-γ) from T helper type 1 (Th1) after stimulation with HIV peptides, HIV-specific IgA antibody at the mucosa surface, HIV-specific cytotoxic T cell (CTL) response and other role of immunity against HIV in HEPS persons.

The demonstration of HIV-1-specific IgA directed against envelope (Env) glycoproteins suggests a potential role for IgA in viral neutralization, as functional IgA serves in a defensive capacity against other human viral pathogens. Kozlowski and co-workers showed an increased production of HIV-1-specific polymeric serum IgA (sIgA), due to the rise of both classes, IgA1 and IgA2 during early HIV-1 infection. In patients with AIDS and symptomatic HIV-1 infection, a declining titer of sIgA antibodies against these antigens could be seen. IgA antibodies seem to be more effective in neutralizing HIV-1 as opposed to IgG.

Clerici and Shearer suggested initially that an imbalance in the Th1-type and Th2-type responses contributes to the immune dysregulation associated with HIV infection, and that resistance to HIV infection and/or progression to AIDS is dependent on a Th1-->Th2 dominance. Thl-like cytokines play a protective role early in the response and are gradually replaced by 'nonprotective' Th2-like cytokines. Several studies have shown that peripheral-blood mononuclear cells appear in presumably HIV-1-exposed persons, but apparently uninfected individuals proliferate and secrete interleukin-2 on exposure to T-helper-cell-epitopes. The role of Thl cells is less clear than that of CTLs, but interferon-gamma (IFN-γ) (produced by Thl cells and CTLs) has direct antiviral effects and enhances the effectiveness of the MHC class I pathway of antigen presentation. In addition, Thl cells are probably necessary for the induction and maintenance of CTL responses and memory. Moreover, many previous reports have indicated that cellular immunity rather than inheritance of the Δ32CCR5 mutation accounts more often for persistentl HIV-1-resistant cases.

Therefore, to understand the immune response to HIV-1 infection in HEPS persons, HEPS individuals and their couples were identified from 19 HIV-1 sero-discordant couples with a history of frequent unprotected sexual intercourse over the

course of 1 year. Also, detection for the anti-HIV-1/2, HIV-1 antigen (p24) and proviral-DNA was carried out. EDTA whole blood of HEPS individuals, their couples and normal control individuals, who were all negative for the anti-HIV-1/2, HIV-1 antigen (p24) and proviral-DNA, was collected. DNA extraction was performed from whole blood. Then, the *gag, pol* and beta-globin genes were amplified directly by multiplex nested-PCR from extracted DNA.

In addition, intracellular cytokine staining (ICCS) was determined to investigate the Th1 (IL-2, IFN-γ)/Th2 (IL-4) ratio. Whole blood was collected in a blood collection tube containing sodium heparin. Activation of lymphocyte cells by Phorbal 12-Myristate 13-Acitate (PMA) and ionomycin was performed in the presence of Brefeldin-A (BFA), which inhibits intracellular transport so antigens and cytokines produced during the activation will be retained inside the cell. Cell surface staining was performed by the direct immunofluorescent technique using the monoclonal antibody (mAb) to the CD4 molecule. Then, the red cells and cytokine staining were lysed by using fluorochrome-conjugated intracellular mAbs to detect the Th1 (IL-2, IFN-γ) and Th2 (IL-4).

In this data, the researcher sought to demonstrate the immune response of cytokines and antibody response in HEPS individuals, and confirm that HEPS individuals are uninfected with HIV. This study may lead to an explaination on the role of cytokines and antibody response in resistance to HIV infection in HEPS, and an understanding of the mechanism of resistance, guide to prevention and development of new therapeutic and vaccine strategies HIV in the future.

Aims of the study

1. To evaluate intracellular cytokine levels in HEPS persons, their HIV-1 seropositive sex partners compare to HIV-1 seronegative persons.
2. To confirm the infection of HIV in HEPS persons, their HIV-1 seropositive sex partners and HIV-1 seronegative persons.
3. To investigate HIV antibody classes (IgG, IgA and IgM) in HEPS persons, their HIV-1 seropositive sex partners and HIV-1 seronegative persons.