CHAPTER V
DISCUSSION

Exposure to HIV does not inevitably result in productive infection and it is now established that a spectrum of variability exists in the susceptibility to HIV (Rowland-Jones et al., 1995 (a), Sheare G., 1996 (a)). Resistance to HIV infection is observed in different categories of high risk individuals including sexual partners of HIV-infected individuals (heterosexual and male homosexual) (Mazzoli et al., 1997, Clerici et al., 1992), commercial sex workers (CSWs) (Rowland-Jones et al., 1995 (a)), neonates of HIV-seropositive mothers (Clerici et al., 1993 (c), De Maria et al., 1994) and HIV-1 exposed health care workers (Clerici et al., 1994(a), Pinto et al., 1995). These individuals are referred to as highly exposed persistently seronegative (HEPS), exposed uninfected (EU), or exposed seronegative (ES).

Exposure to defective HIV strains, subinfectious dose of the virus, innocuous pathogens, and a genetic predisposition to activating immunologic defense are possible explanations for resistance to HIV infection. The genetic basis of HIV resistance has been illustrated by data on CCR5Δ32 genotyping. Nevertheless, as the majority of highly exposed seronegative individuals tested are not homozygous for this deletion, other resistance factors must exist (Lui R., 1996, Rugeles MT., 2002). In accordance with a previous report, HIV-1 resistance has been associated with various HIV-1–specific immune responses including T-helper responses. The lowest Th1 cytokine production and high levels of IL-4 expression were detected in HIV-1-seropositive patients (Clerici M., 1994), and plasma and mucosal IgA, production of some cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFN-γ) from T helper type 1 (Th1) after stimulation with HIV peptides (De Maria et al., 1994, Cheynier et al., 1992) and CTL epitope responses (Bernard et al., 1999) suggested that resistance may be immune mediated in HEPS individuals.

Therefore, anti-HIV-1/2, HIV-1 antigen (p24), and proviral-DNA were tested in 19 HIV-1 sero-discordant couples with a history of frequent unprotected sexual intercourse over the course of 1 year to confirmation 19 uninfected HEPS individuals and 17 HIV-1 seropositive sex partners from Sanpatong Hospital between 2001 and 2002. In addition, to understand the role of immune response to HIV in HEPS persons and their HIV seropositive sex partners, anti-HIV classes (IgG, IgA and IgM) and intracellular Th1 (IL-2, interferon-gamma)/Th2 (IL-4) cytokine staining were investigated and compared with those of normal controls.

It was demonstrated by third-generation EIA (MEIA or EIA) and the gelatin particle agglutination test that all 17 HIV-1 infected sex partners had a reactive HIV-1 antibody (100%), whereas all 19 HEPS persons had a non-reactive HIV-1 antibody (100%) at screening, and at 12 months follow-up. However, the limitation of EIA has

been its inability to detect infection in patients during the ''window period'', which is from initial virus acquisition to the time when antibodies become detectable. Additionally, an unusual patient who developed AIDS despite testing negative for antibodies was reported (Reimer L.,1997, Centers for Disease Control and Prevention., 1995). To confirm that the HEPS group does not include such an unusual patient or there is not a ''window period'', the p24 antigen was investigated in all subjects. In this study, the p24 antigen was undetectable in all groups at screening and during one-year of trimonthly follow-ups. This data supported that the ''window period'' of HIV-infection did not occur in the HEPS group. Additionally, undetectable p24 antigen in the HIV-infected sex partners suggested that no viremia was observed and p24 antigen did not correlate with low level CD4 cells counts, clinical symptoms or progression to AIDS. Nishanian reported that acid treatment in immune-complex dissociation (ICD) HIV-p24 Ag assay increases sensitivity by 3-8 fold as compared to a standard assay by dissociating the immune complex, destroying the Ab and freeing the p24 Ag for detection (Nishanian P., 1990). However, if the immune-complex dissociation (ICD) HIV-p24 Ag assay had been performed in the HIV-infected sex partners in this study, p24 antigen may be observed, especially in HIV-infected subjects, who have low CD4 cells counts, clinical symptoms or progression to AIDS.

PCR is a powerful technique for in vitro amplification of specific DNA fragments and has already been widely used for the detection of HIV-1 in blood from HIV-1 seropositive persons or individuals at risk for HIV-1 infection (Albert J., 1990, Psallidopolous MC, 1989). The one problem of false negatives in PCR based diagnosis is high genetic variation in the HIV-1 genome. In 1996, Sutthent R. et al. developed a nested PCR reagent kit by using several primer sets to detect HIV-1 subtype B and E, which were commonly found in Thailand (Sutthent R. 1996). In this study, multiplex nested HIV-1 PCR, the special kit for detecting HIV-1 subtype E, was investigated in HEPS persons and their sex partners to confirm HIV-1 infection. The specific primer for the gag gene in nested PCR gave 100% sensitivity in HIV-1 seropositive sex partners compare to 56.86 % from that for the *pol* gene. This finding was similar to Sutthent's studies and several other reports (Sutthent R., 1996, Albert J. 1990, McCutchan FE. 1992). However, no HIV-1 proviral DNA was found in HEPS individuals, despite the 12 months follow up. This data supported that HEPS subjects were uninfected with HIV-infection while their sex partners were infected.

In another study describing uninfected HIV-exposed individuals, Clerici and co-workers (2002) reported that the serum IgA protecting against infection recognized HIV epitopes located at the transmembrane protein, while the HIV-positive individuals recognized mainly gp120 and gp41 (Clerici M., 2002). In the study by Sligh et al., FIFA was a highly sensitive test (Sligh JM., 1989) and suitable for detecting classes of HIV-1 antibodies. Hu and coworkers reported that IgM was the first marker detected followed subsequently by p24 antigen and IgA (Hu YW., 1996). Sera from HEPS persons, HIV-infected individuals and healthy normal controls were analysed for the presence of HIV-specific IgG, IgA and IgM in this study. IgG HIV-antibody was detected in all HIV-infected individuals; IgA and IgM antibodies were

randomized in each subject on each visit. The fluorescence intensity of the serum HIV-1-specific antibody may vary at each visit. The random detection of IgA and IgM classes in HIV-seropositive sex partners was surprising because IgA has the famouse role in responsing to infection at mucosal system and IgM response was shown that continuous viral activation of the immune system. However, random IgA detection may be caused from short half life of it, since some previous reports demonstrated more predominant IgG than IgA antibody (Williams SB., 2002).

No HIV-antibody class was detectable in HEPS persons, despite the 12 months follow up. The results of this study demonstrated none humoral immune response in HEPS individuals. The data presented in this report suggested that the HIV antibody class IgA do not affect HIV-1 resistance in the HEPS group and there was no detection of an IgM, surrogate marker for HIV infection in the window period. Although HIV-1-specific neutralizing activity mediated by IgA antibodies has been demonstrated in the serum of HEPS individuals (Mazzoli S., 1999, Devito C., 2000, Burnett PR., 1994), purification of IgA before detection may be necessary to increase sensitivity. However, HIV-1 infection occurs predominantly through sexual contact at mucosal surfaces. Therefore, HIV-1–specific humoral and cellular immune responses localized at the genital tract may play an important role in HIV-1 protection. Thus, many previous reports detected a greater role of mucosal IgA in HEPS persons than that of serum IgA (Williams SB., 2002, Mazzoli S., 1997, Mazzoli S., 1999, Devito C., 2000, Beyrer C., 1999). Moreover, IgG antibody response can mask IgA antibody response, presumably by competing with IgA for binding sites on HIV-1 antigen (Kual R., 1999), and the frequency of serum IgA may therefore be underestimated.

In this experiment, intracellular cytokines could be measured by intracellular cytokine staining and flow cytometric analysis. Flow cytometry allows a clear picture of cytokine production on the cellular level. With this technique, the difference between cellular subsets was observed and the amount of cytokines produced rapidly determined by large populations of cells and multiple colour detection, which facilitated more comprehensive analysis. Flow cytometry shows the percentage of cells from given populations that are positive for a cytokine and the relative amount of production. The procedure requires an antibody to cell-surface antigens to isolate a specific population of cells. Human lymphocyte express the CD-3 surface antigen at high levels, and they are used commonly in flow cytometry for identifying and gating lymphocyte populations. In this study, a whole blood incubation procedure was used to closely simulate the natural physiological state of the cells under investigation. In accordance with a previous report (Pala P., 2000), it was found that the use of PMA and ionomycin resulted in a much larger down-regulation of CD4, but minimal decrease in the percentage of positive cells.

Since the two subtypes of Th cells were first found by Mosmann et al. in 1986 (Mosmann TR., 1997), many diseases have been characterized as having a Th1/Th2 imbalance. In HIV infection, Clerici and Shearer, suggested initially that imbalance in the Th1-type and Th2-type responses contributed to immune dysregulation, and resistance to HIV infection and/or progression to AIDS was dependent on a Th1→Th2 dominance (Clerici and Shearer). IL-2, IFN-γ (type 1

cytokines) and IL-4 (type 2 cytokines) were chosen to measure the Th1/Th-2 profile induced by mitogenic stimulation. Although other cytokines could be used to represent the Th1/Th2 profile, many previous reports supported that IL-2, IFN-γ and IL-4 have been the clearest and most representative markers of the two phenotypes. Moreover, the mutually exclusive production of cytokines by a single cell has been most clearly shown for these markers (Jung T., 1995).

In this study, cytokine (IL-2, IFN-γ and IL-4) measurement was compared between HEPS persons, HIV-infected sex partners and healthy controls at visit 3 (6 months), 4 (9months) and 5 (12months) of follow up. The amount of each cytokine production differed between HEPS persons, HIV-infected sex partners and healthy conrols, with intersubject variations greater for HIV-infected sex partners than HEPS persons and healthy controls. None of the subjects showed intrasubject variation in the amount of each cytokine production (IL-2, IFN-γ and IL-4) in CD4+ T cells at each visit, despite 6 months follow up. However, the typical course of HIV infection in about 80 % to 90 % of HIV-infected persons and its median survival time is approximately 10 years (Cao Y., 1995). Therefore, the follow up time in this experiment may be too short, thus, the change in production of cytokines in each subject was not found, and a longitudinal evaluation of cytokine production may be necessary in HEPS individuals and HIV-infected sex partners (Canaris AD., 1998).

HIV infection produces a progressive reduction in the CD4 pool and a very precocious functional impairment of this subset. The most striking abnormalities seen are the inability to produce in vitro cytokines such as IL-2, IFN-γ and others (Clerici, M., 1994(b), Clerici, M., 1996). Intersubject variation in cytokine production was observed in both HEPS individuals and HIV–infected sex partners. According to previous reports, a much clearer decrease in production of IL-2 and IFN-γ in CD4+ T cells in HIV-infected sex partners was detected, while no change in production of IL-2 and IFN-γ in CD4+ T cells in HEPS persons was reported when compared with healthy controls (Sousa AE.. 1998, Fan J., 1993, Kein SA., 1997). Although the data were normalized, an impaired ability of CD4+ T cells to synthesize IL-2 and IFN-γ in HIV-infected sex partner individuals still remained in comparison with HEPS individuals and normal controls. The normalized data (% cytokine-producing CD4+ T cells/total CD4+ T cells) were useful for comparison of HIV-infected sex partners with other groups but not for comparison of HEPS and normal control group. This may be caused from the difference of the level of CD4+ T cells in each group, especially in HIV-infected sex partners with very low level of CD4+ T cells that show clear normalized data. From the data of CD4+ T cells, an impaired ability of CD4+ T cells to synthesize IL-4 in HIV-infected sex partners remained in comparison with HEPS individuals and normal controls. Conversely, when the data of IL-4-producing CD4+ T cells with the total CD4+ population were normalized, no decrease in IL-4 expression was found. Similar to reports on cytokine expression in HIV-infected persons (Meyaard L., 1996, Sousa AE, 2001), no decrease in IL-4 expression were found, and only a modest reduction in IL-2 and IFN-γ expression were seen. The normal range of IL-4 expression was too low when compared with IL-2 and IFN-γ expression, since demonstration of the significant difference was not clear. These

data suggested that reduction in IL-2 and IFN-γ-producing cells was not equivalent to a low CD4+ T cell count or CD4+ T cell reduction in synthesizing IL-2 and IFN-γ. The change in cytokine production patterns was not observed in HEPS and healthy cytokines. Also, in two HIV-infected sex partners, IL-2 and IFN-γ expression was within the normal range (01P and 15P), as seen in HEPS individuals and normal controls, according to the normal range of CD4+ T cell counts.

A significantly increased proportion of CD8+ T cells was observed with the ability to express large amounts of IFN-γ in HIV-infected sex partners, compared with HEPS persons and healthy controls, and the levels of these cytokines remained stable through out the study period. However, the normalized data indicated that the large number of IFN-γ producing CD8+ T cells was caused by the increase number of CD8+ T cells, but not from increase in IFN-γ synthesis. Perhaps more surprising is the close relationship between HIV-infected persons and production of 'high' levels of IFN-γ by CD8+ T cells. This indicates that low CD4+ T cell numbers are associated with activated CD8+ T cells, which are in turn associated with HIV-infection, to increase the amounts of CD8+ T cells. The "Th1→Th2 Switch" hypothesis in HIV infection is not clear. Romagnani et al. (Romagnani S., 2000) suggested preferential HIV replication in T cells producing Th-2 type cytokines rather than a "Th1→Th2 Switch" during HIV replication, as suggested by Clerici and Shearer (Clerici M., 1993(b)) and according to other reports (Granziosi C., 1994). Romagnani concluded that there was no switch or shift from a dominant-Th1 (IFN-γ) to a dominant-Th2 (IL-4) cytokine profile, whereas the data from other laboratories favor a Th1-to-Th2 shift (Romagnani S.,2000). The data in this study partially supported the hypothesized Th1-to-Th2 shift with advancing HIV-associated disease. (Clerici, M., 1994(a), Clerici, M., 1996, Douglas SD., 2003). It is better if data of each subset group were compared at each visit, instead of one visit of control group.

Like other studies of HEPS persons, this study is limited by the small number of subjects. Other study limitations include those inherent in the methodology. For example, the use of specimens from vagina fluid allowed us to detect HIV-specific IgA, although supporting findings of the HIV-1–IgA role in HIV resistance, virus-suppressive activity and other host factors may also contribute to protection against HIV infection. The ploblem of this study were 1) the exact time of infection of HIV-infected sex partners of HEPS persons 2) the frequency of sexual contact of HEPS persons with their HIV-infected sex partners 3) they are still have continous sexual contact or not?. This question will be solved when the role of cellular-cytotoxic T cell (CTL) in HEPS individuals are studied. But unfortunately, HIV-specific CTL is not done in this study. Because HIV-specific CTL play a major role in contolling virus level through the asymtomatic period. The CTL activity towards conserved virus epitopes that response to repeated exposure of HIV, should be investigated in HEPS groups. Moreover, genetic host factors—namely, inheritance of mutant chemokine receptors or ligands, such as CCR5-Δ32, and CCR5 promoter polymorphisms, as well as the HLA type—affect resistance and susceptibility to infection and subsequent clinical course. The role of humoral immunity, mucosal

immunity, and other local factors in determining the course of HIV infection and resistance in HEPS need to be studied in future.