CHAPTER V

DISCUSSION

Progressive decline in CD4+ T lymphocytes is the hallmark of HIV infection.[4] This leads to progressive immunodeficiency and clinical manifestations of AIDS.[54,55] Several *in vitro* studies have shown the impairment of antigen-induced IL-2 production in HIV-1 infected patients.[87, 131]

IL-2 is a cytokine with a MW of 15.5 kDa secreted by activated T cells. *In vitro* studies have shown that IL-2 augments HIV-1 specific T-cell proliferation,[4] decreases apoptosis of PBMC obtained from HIV-infected patients[132], improves viral suppression by CD8+ T lymphocytes[133], and induces B-cell activation and antibody synthesis. IL-2 decreases chemokine receptor expression including CCR-5 on macrophages and lead to a marked decrease in viral replication[134]. In contrast, IL-2 has been shown to increase chemokine receptor expression on CD4+ cells, thus possibly improving the survival of memory CD4+ T lymphocytes.[135, 136]

Although highly active antiretroviral therapy (HAART) is recommended as current milestone treatment of HIV-1 infection, the immune restoration after HAART remains limited.[137,138] Therefore, the rationale of adding IL-2 to antiretroviral therapy is warranted for clinical investigation. Several randomized controlled clinical trials have demonstrated the ability of IL-2 to produce a marked and sustained increase in CD4+ T-cell counts in HIV-infected patients on antiretroviral therapy (ART).[129,140,141,142]

The HIV-NAT study protocol 004 (HIV-NAT 004) is a phase II, randomized controlled trial for evaluation of safety and efficacy of three different doses of subcutaneous (s.c.) IL-2 in Thai patients infected with HIV-1. The study has been conducted by the AIDS Research Center, Thai Red Cross Society and Division of Allergy and Clinical Immunology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, and sponsored by the U.S. NIH.

The stored PBMC samples of this cohort have provided us with an opportunity to investigate the *in vivo* effects of IL-2 on IL-2 and other cytokine and chemokines expressions among patients treated with ART alone or ART plus sc. IL-2.

There have been several approaches to investigate the roles of cytokines or chemokines in any diseases including HIV infection. The common approaches are measuring serum levels or studying *in vitro* production by PBMC (with or without stimulation). The others include the analysis of gene expression, immunohistochemistry and the study of intracytoplasmic cytokine production using flowcytometric analysis. Each approach varies as to its advantages and disadvantages. Serum cytokine measurement is the easiest method to study cytokines in peripheral blood and any body fluid samples, as there are commercial ELISA kits available for most of the currently known cytokines. However, in general, most serum cytokine levels are very low because they are synthesized at the physiological locations required, have a short half-life and may bind with soluble cytokine receptors or antagonists. Therefore, serum cytokine levels usually have poor correlation with disease activity and pathogenesis, unless the disease is an active systemic disease. For diseases locally restricted pathology, local fluid analysis of cytokines provides more reliable results. *In vitro* production studies of cytokines serve to investigate whether that particular cytokine is autonomously produced or its production is either increased or decreased under certain stimulants (mitogens, cytokines or antigens) compared with the normal control. This approach is usually more sensitive than the direct measurement of cytokines from serum or body fluid, nevertheless, it requires tissue culture facilities and skill. The interpretation of the correlation of these *in vitro* findings and the *in vivo* phenomena should be made carefully. Intracytoplasmic cytokine analysis using flowcytometer has become more popular in this field.[90,91] The greatest advantage of this technique lies in its capacity to analyze on a single cell basis, also it can phenotype the cells by staining cellular markers and it can perform quantitative analysis.

Cytokine gene expression in cells or tissues can be analyzed either by RT-PCR or Northern blot or *in situ* hybridization. Among those RT-PCR is the most sensitive

approach. Ideally, RT-PCR analysis of cells or tissues obtained from the active site of the disease is much more reliable than that of peripheral blood mononuclear cells (PBMC), unless the disease or the immune activation is systemic as seen in HIV infection. Apart from the techniques used to investigate the role of chemokines, there might be another reason for these conflicting data. An alternative explanation to the observed differences in the level of chemokines in HIV infection may be related to the redundancy of chemokine production by various cells.

A few studies have investigated the effects of IL-2 therapy on changes of cytokines and chemokines in HIV-infected patients. Results showed that IL-2 therapy can induce the reconstitution of the CD4/CD45RA (naive) lymphocyte subsets and can recover the ability of these cells to produce IL-2, IL-4 and IFN-γ *in vitro*.[45]

In this study, RT-PCR method was used to assess mRNA expression of cytokines and chemokines in PBMC samples. We studied the effects of IL-2 given subcutaneously in combination with ART on two cytokines: IL-2, IL-18, and two chemokines: RANTES and MIP-1α

At the baseline, mRNA expression of IL-18, RANTES and MIP-1α was detected in all PBMC samples obtained from both groups of patients. In the ART control group, at week 8 of the study, there were no significant differences in the proportion of gene expression of these cytokines and chemokines as compared to baseline. The study performed during the third cycle of IL-2 administration in the ART plus IL-2 group also showed no significant differences in the proportion of gene expression of these cytokines and chemokines.

Serum RANTES levels have been shown elevated in HIV-infected patients treated with HAART [30, 142]. The discrepancy of our findings may be on the account of the use of different assays. However, it may be more likely due to the difference in antiretroviral regimens, as the majority of patients in our study received dual nucleoside RT inhibitors. It should be noted that RT-PCR used in the present study was a qualitative method, then the quantitative difference if existing could not be observed. Generally, various considerations should also be taken into account in

clinical research on cytokines, for instance, the characteristics of each cytokine which include: cell source, peak, half-life, activity and regulation; timing of specimen collection, sample preparation and storage, type of assay and its limitations. And it must be emphasised that results obtained with one technical approach may not correlate well with others.

Of noted, at week 24 of the study in this cohort (HIV-NAT 004), the majority of patients in both the IL-2+ART and ART groups had a viral load (plasma HIV-RNA) below the limit of detection at 500 copies/ml (by Quantiplex™, Chiron). And there was no significant difference in mean plasma HIV-1 RNA levels among the groups. Our chemokine gene expression findings showed no significant increase after IL-2 therapy, therefore, did not support the role of RANTES and MIP-1α in controlling viral bursts in this setting. The possible mechanisms contributing to the blunt of viral burst in the IL-2 + ART group, although the ART regimen was not as potent as HAART, may include enhanced expression of CD8+ cell associated suppressive factors, CD8+ CTLs, and decreased CCR-5 expression on monocytes/macrophages.[134]

The results showed that at week 0, there was no IL-2 mRNA expression in PBMC samples obtained from either the ART or the ART plus IL-2 group. However, at week 16 of the study (pre-cycle 3 of IL-2 administration), the proportion of patients with IL-2 mRNA expression in PBMC was significantly higher in the ART plus IL-2 group than that in the ART group (50% versus 0%), P=0.03. It is note worthy that when samples were analyzed at cycle I, the IL-2 mRNA expression was significantly increased in a dose-response fashion as indicated by 12.5% in the 1.5 MIU bid group, 71 % in the 4.5 MIU bid group (p=0.04) and 77 % in the 7.5 MIU bid group, respectively (p= 0.01). These results show a nice dose-response relation and also suggest that IL-2 at a dose of 4.5 MIU bid or above should be considered an effective dosage for IL-2 therapy in HIV infection.

For future study, in particular to quantitatively compare the IL-18, RANTES and MIP-1α gene expression, an quantitative RT-PCR should be used.