CHAPTER IV

DISCUSSION AND CONCLUSION

Although the natural immune responses to human immunodeficiency virus type 1 (HIV-1) is not effective for eradicating the virus, vigorous HIV-1-specific $CD4^+$ Th1 cell responses have been shown to be associated with the control of viremia and long-term nonprogression in infected individuals [160-162]. Early intervention with highly active antiretroviral therapy (HAART) during or shortly after acute infection was also associated with enhanced HIV-1-specific $CD4^+$ Th1 responses [163, 164]. In contrast, at a later stage, HAART leads to the decline of HIV-1-specific $CD4^+$ Th1-cells and $CD8^+$ cytotoxic T lymphocyte (CTL) responses. Thus the functional capacities of HIV-1-capturing antigen presenting cells (APCs), which are required for the induction of the immune responses, are progressively lost along the course of infection [165-167]. The role of DCs in the transmission of HIV-1 to T cells has been demonstrated in vitro. However, the extent to which DC remains phenotypically and functionally unaltered during HIV-1 infection is not clear. Given the efficiency of DCs for stimulating T cell-dependent immune responses, alterations in DC number and function could be important factors in the development of T cell functional impairment in HIV-1 disease. However, monocyte-derived DCs may still be in good function [11].

In vitro techniques are now available to permit the differentiation and expansion of DCs from monocytes using GM-CSF and IL-4. These MoDCs have been suggested to be good candidates for clinical uses in enhancing immune responses in human [168]. However, there is a controversy whether these DCs arise from proliferating precursors or simply from differentiation of monocytes. Cavanagh *et al* had reported that the yield of DCs derived from monocytes in the presence of GM-CSF and IL-4 cannot be expanded beyond the number of starting monocytes. Their studies showed that proliferation in the culture resulted from a small number of contaminating progenitor cells, not from proliferation of DCs or their monocyte precursors [169].

In our study, we also found that monocytes which were maintained in complete medium supplemented with GM-CSF and IL-4 can develop into MoDCs. Our study included the blood samples from HIV-1 negative volunteers, HIV-1 infected patients who had $CD4^+$ T cells more than 200 cells/µl and HIV-1 infected patients who had $CD4^+$ T cells less than 200 cells/µl. All of monocytes from those samples can be manipulated into MoDCs. All generated MoDCs had more than 90% and 95% in purity and viability respectively. One thing we have noticed about these generated MoDCs was that the difference in the numbers of MoDCs from HIV-1 infected patients. The numbers of MoDCs from HIV-1 negative blood samples were greater than from those of HIV-1 infected patients with $CD4^+$ T cell counts either more or less than 200 cells/µl. We also found that HIV-1 infected patients who had $CD4^+$ T cells less than 200 cells/µl had the lowest number of MoDCs. Since our method used the whole PBMCs and left monocytes to adhere to the surface of tissue culture flask, the number of monocytes in each sample may provide an explanation. Monocytes also express CD4 molecules on their surfaces that make them become the targets of HIV-1

as well. We found that HIV-1 infected patients who had $CD4^+$ T cells less than 200 cells/µl had the numbers of starting monocytes less than those of HIV-1 negative volunteers. In this study we also found that the yield of DCs derived in the presence of GM-CSF and IL-4 cannot be expanded beyond the number of starting monocytes.

In order to use MoDCs in the application of therapeutic vaccine, there is a guideline for a good generated MoDC as described in Chapter 1. In our study we did not use CD83, CD80, CD86 and CCR7 as the markers of mature MoDCs. However, more than 90% of our MoDCs were strongly positive for MHC class II, HLA-DR. We also determined the viability of generated MoDC by flow cytometry and found that less than 5% of generated MoDCs were counted for dead cells by staining with PI.

In order to control HIV-1 virus we need the vaccines that can elicit HIV-1 specific $CD4^+$ Th1 cell and $CD8^+$ cytotoxic T lymphocyte (CTL) responses. Ex vivo generated MoDCs may be the hope to this achievement. Rissoan *et al* had reported that T cells originally cultured with MoDCs secreted large amounts of IFN-γ but little amounts of IL-4, IL-5 and IL-10, suggesting that MoDCs can induce Th1 cells [105].

We further studied the function of generated MoDCs in inducing allogenic T cell proliferation and cytokine production. In this study, we compared the function of MoDCs between those of HIV-1 negative blood samples and HIV-1 infected patients by flow cytometry. For cytokine production, we found that T cells isolated from normal blood donors co-cultured with generated MoDCs from both HIV-1 negative volunteers and HIV-1 infected patients secreted higher amounts of IFN-γ compare to T cells which were not cultured with MoDCs ($p<0.05$). There was no difference in the percentages of IFN-γ producing T cells when T cells were co-cultured with MoDCs

from either HIV-1 negative volunteers or HIV-1 infected patients (p=0.240). We found a very few IL-4 producing T cells which were stimulated with MoDCs from both groups of blood samples. These results supported the data that ex vivo generated human MoDCs can induce Th1 cells and these MoDCs are very promising for using in therapeutic applications. Tanaka *et al* had reported that dendritic cells can induce naïve T cell differentiation into Th1 or Th2 depending on the ratio between stimulators and responders. They found that low MoDC/T cell ratio (1:300) may trigger T cells to Th2 effectors whereas high MoDC/T cell ratio (1:4) can trigger T cells to undergo Th1 effectors [170].

We used high ratio between MoDCs and T cells (1:10) and MoDCs in our experiments also triggered T cells differentiate into Th1 effectors.

For T cell proliferation assay, we used CFSE dye to track the proliferation of T cells. The results showed that ex vivo generated MoDCs can induce allogeneic T cell proliferation by determining proliferation indexes and mean fluorescence intensities. T cells which were stimulated with MoDCs from HIV-1 negative samples and HIV-1 infected patients, showed no difference in inducing T cell proliferation (p=0.058 and p-0.366 respectively).

In conclusion, we successed the ex vivo generation of MoDC in our labolatory. The generated MoDCs have good phenotypic and viability in both groups of HIV-1 negative and HIV-1 infected blood samples. Our data also supported that human MoDCs can induce T cells into Th1 effectors and secrete proinflamatory cytokine, IFN-γ. The results also supported that there is no difference between the function of MoDC which were generated from HIV-1 negative volunteers and HIV-1 infected patients. Finally, our study provides some informations about the normal function of MoDC generated from HIV-1 infected patients in Thailand.