CHAPTER III

RESULTS

3.1 Monocyte-derived dendritic cell morphology

After 6 days of incubation, monocytes that cultured in complete medium, supplemented with (rh) GM-CSF and (rh) IL-4 developed into immature monocyte-derived dendritic cells. Then LPS was added to the culture. Immature MoDCs were activated with LPS for 2 days. At day 8, most of immature dendritic cell turned into mature MoDcs. The morphology of mature MoDCs in complete culture medium is shown in Figure 3.1.

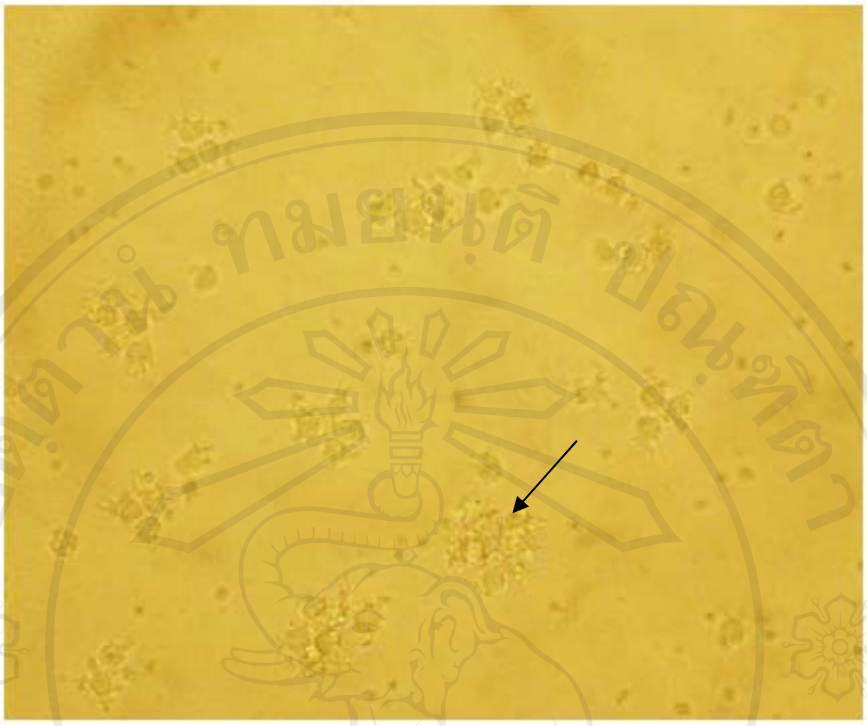

Figure 3.1 The picture of mature MoDCs in culture medium at day 8. Most of MoDCs developed into mature dendritic cells. The shape is less roundish with longer dendrites and large size as compared to immature DCs. They can attach the tissue culture flask loosly. The mature MoDCs also preferred to stay in cluster (arrow).

3.2 Monocyte-derived dendritic cell purity and viability

Before using MoDCs as an effector cells in T cell proliferation assays, MoDC purity was determined by flow cytometry using FC500 flow cytometer (Beckman Coulter). Mature MoDCs have larger size than they were in normal monocytes state. The data was confirmed in the forward scatter (FSC) and side scatter (SSC) dot plot in Figure 3.2A. The purity of MoDCs was assessed by using PC5 conjugated anti-HLA-DR and FITC conjugated anti-CD3, anti-CD14, anti-CD16, anti-CD19 and anti-CD20. MoDCs were identified as the cells that positive for HLA-DR and negative for other lineage markers used for staining as shown in Figure 3.2B. The MoDCs were also stained with propidium iodide (PI) to monitor cell viability as shown in Figure 3.2C. Cells that positive for PI are counted as dead cells. All of generated MoDCs had more than 90% purity and more than 95% of cell viability.

Three groups of blood samples were involved (see in Chapter 2) in this study. Monocytes from all of these groups were able to transform into mature MoDCs. MoDC purity and viability were ranged between 90-99% and 98-99% respectively. However, the numbers of MoDCs generated from HIV-1 infected patients were less than those of HIV-1 negative volunteers.

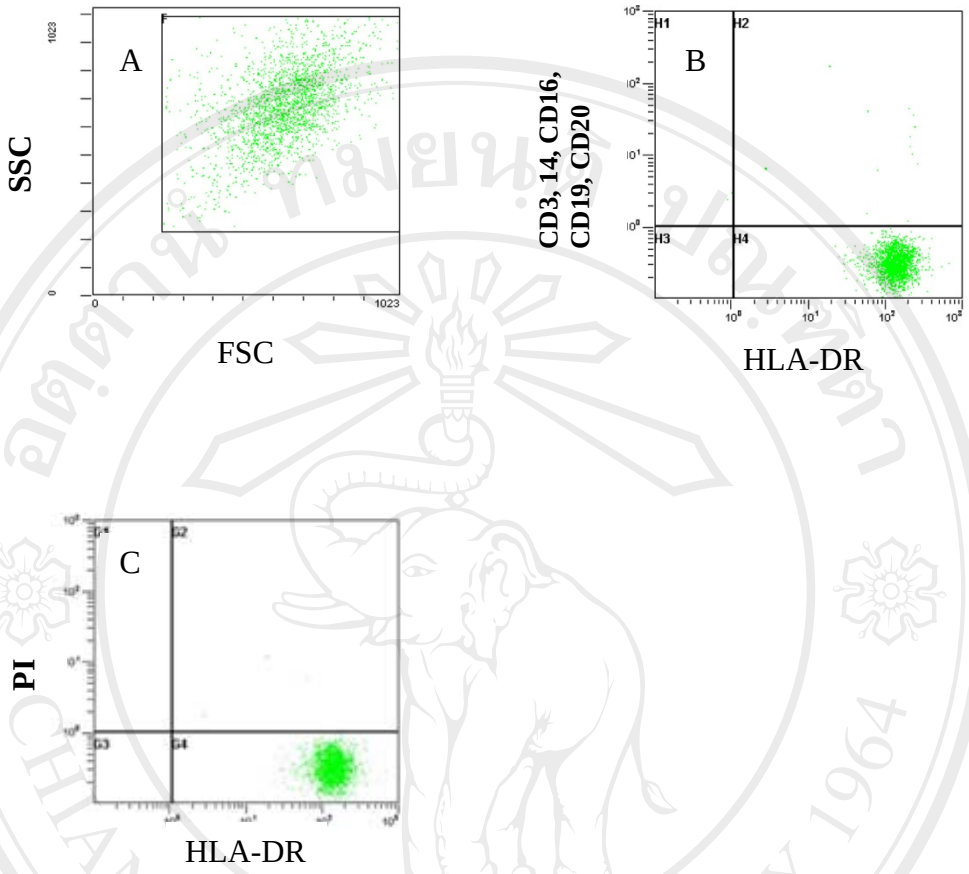

Figure 3.2 The dot plot results of MoDC purity and viability. FSC versus SSC dot plot has shown the larger size of MoDCs (A). More than 90% of cells are positive for HLA-DR and negative for other lineage markers (B). Less than 5% of cells that positive for PI indicated dead cells (C). The results are representatives of all experiments showing similar results from all samples.

3.3 Purified T cell purity

The frozen PBMCs were isolated from healthy normal individual and then processed to T cell purification by using Human Pan T cell Isolation Kit II (Miltenyi Biotec). The purity of isolated cells were assessed for phenotype by using the PC5 conjugated anti-CD3 and FITC conjugated anti-CD4 as shown in Figure 3.3.

The results showed that the purity of isolated cells (CD3 positive cells) were ranged between 85-95%. Viability of isolated cells was higher than 99%. These data suggested that purified T cells have a good purity and viability to use in the MoDC-T cell co-culture.

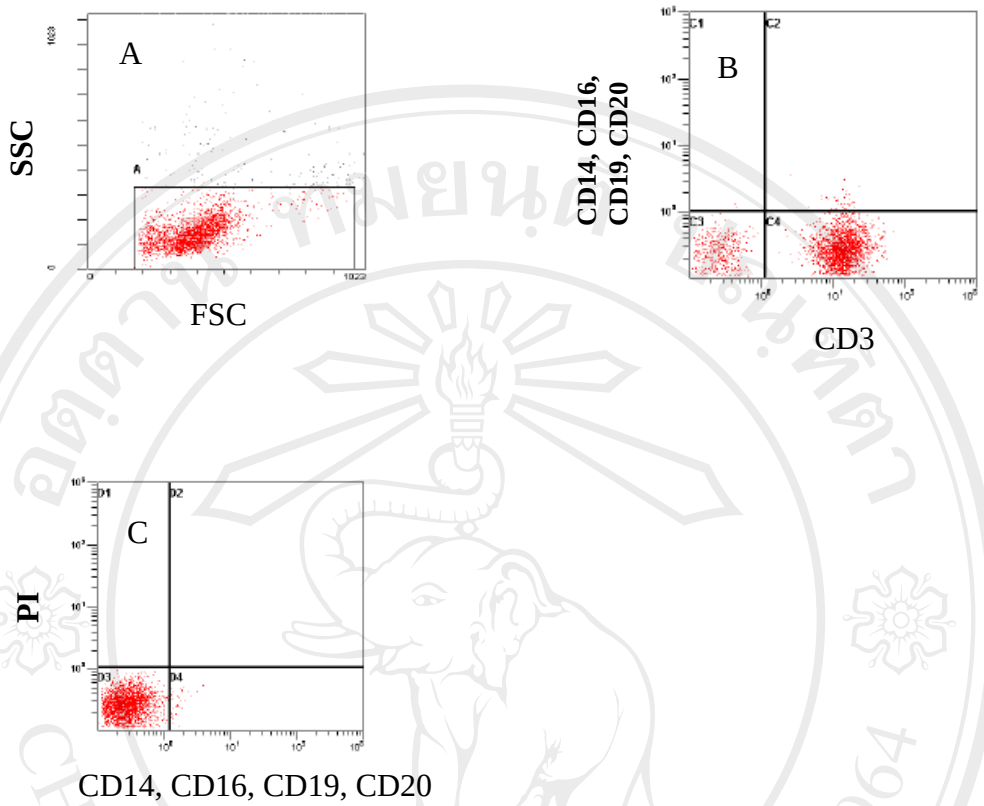

Figure 3.3 T cell purity and viability Most of isolated cells had shown to be lymphocyte population from FSC and SSC dot plot (A). More than 85% of gating cells were positive for CD3 (B). Less than 1% of cells were positive for PI (C). The results are representatives of all experiments showing similar results from all samples.

3.4 Intracellular cytokine assay

After 7 days of co-culture between purified T cells from normal volunteers and MoDCs from either HIV-1 negative volunteers or HIV-1 infected patients, T cells were assessed for their intracellular cytokine productions as described in 2.2.4 and 2.2.5 and the results have shown in Figure 3.4 and 3.5. Intracellular interferon-γ (IFN-γ) and interleukin-4 (IL-4) were used as markers of Th1 and Th2 respectively in order to study the function of MoDC in inducing cytokine production of T cells. The percentages of IFN-γ producing cells after co-culture with MoDCs generated from HIV-1 negative and HIV-1 positive groups were 11.7±6.9 and 17.4±13.9 respectively. The percentages of IL-4 producing cells of HIV-1 negative and HIV-1 positive groups were 0.8±1.2 and 1±1.2 respectively.

The percentages of interferon γ and IL-4 productions from $CD4^+$ T cells which were stimulated with MoDCs from HIV-1 negative volunteers (n=10) were compared with percentages of cytkine production from those of HIV-1 positive patients (n=10). The results were analyzed by using program SPSS version 15.0 using Mann-Whitney U Test. The results suggested that there was no significant difference between the productions of interferon γ from these two groups (p=0.240).

The percentages of IL-4 production from $CD4^+$ T cells which were stimulated with MoDCs from HIV-1 negative volunteers were also not significantly different from those of HIV-1 infected patients (p=0.366).

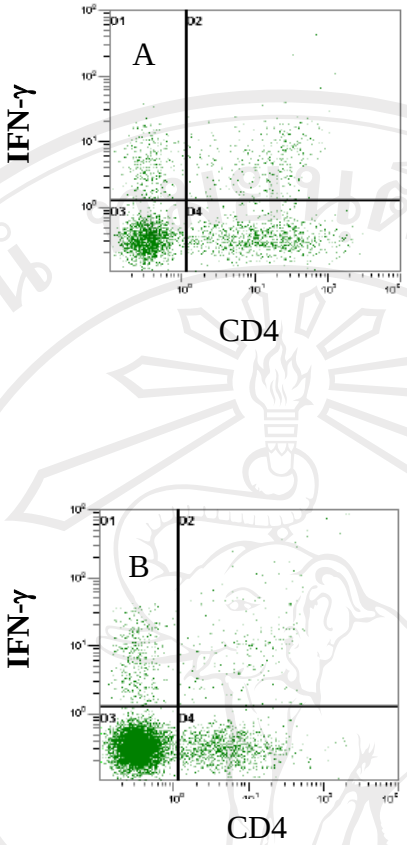

Figure 3.4 Dot plot results of IFN-γ secretion from T cells. T cells were co-cultured with MoDCs from either HIV-1 negative or HIV-1 infected groups. The percentage of IFN-γ producing cells from T cells co-cultured with MoDCs (A) was higher than that from T cells cultured without MoDCs (B). The results are representatives of all experiments showing similar results from both HIV-1 negative and HIV-1 infected groups.

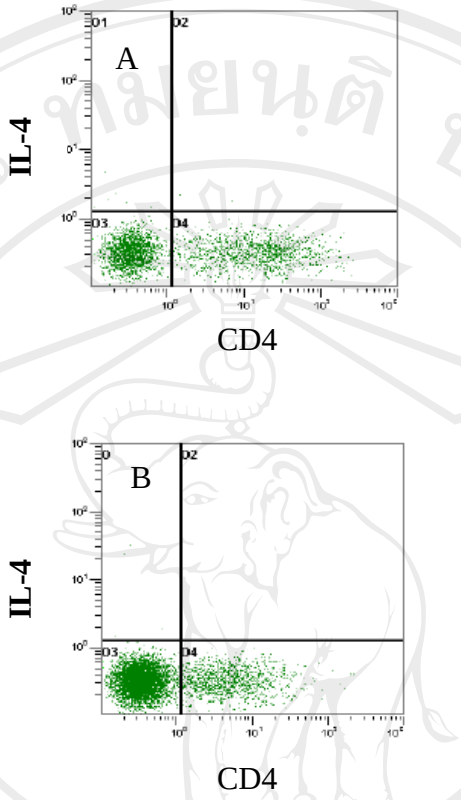

Figure 3.5 Dot plot results of IL-4 secretion from T cells. T cells were co-cultured with MoDCs from either HIV-1 negative or HIV-1 infected groups. The percentage of IL-4 producing cells from T cells co-cultured with MoDCs (A) was higher than that from T cells cultured without MoDCs (B). The results are representatives of all experiments showing similar results from both HIV-1 negative and HIV-1 infected groups.

3.5 Proliferation assay

In order to study the function of MoDCs in inducing T cell proliferation, T cells were stained with CFSE before using in the co-culture with MoDCs for tracking the proliferation of stimulated T cells as described in section 2.2.4. The proliferations of stimulated T cells were analyzed by using program FlowJo version 7.2.2 (Tree Star, Inc.) as shown in Figure 3.6. The proliferation indexes and mean fluorescence intensities (MFI) of CFSE were assessed as the results of proliferation assay. The results were compared between proliferation indexes and mean fluorescence intensities of T cells co-cultured with MoDCs from HIV-1 negative samples and T cells co-cultured with MoDCs from HIV-1 positive samples. The proliferation indexes of T cells co-cultured with MoDCs from HIV-1 negative samples and HIV-1 positive samples were 1.66±0.6 and 2.18±0.6 respectively as shown in Table 3.1 and Table3.2. The mean fluorescence intensities of CFSE from T cells co-cultured with MoDCs from HIV-1 negative samples and HIV-1 positive samples were 82±39.0 and 60±39.9 respectively as shown in Table 3.3 and Table 3.4.

The statistical analysis has shown that there was no significant difference between proliferation indexes and mean fluorescence intensities of HIV-1 negative group and those of HIV-1 infected group ($p=0.058$ and $p=0.366$ respectively).

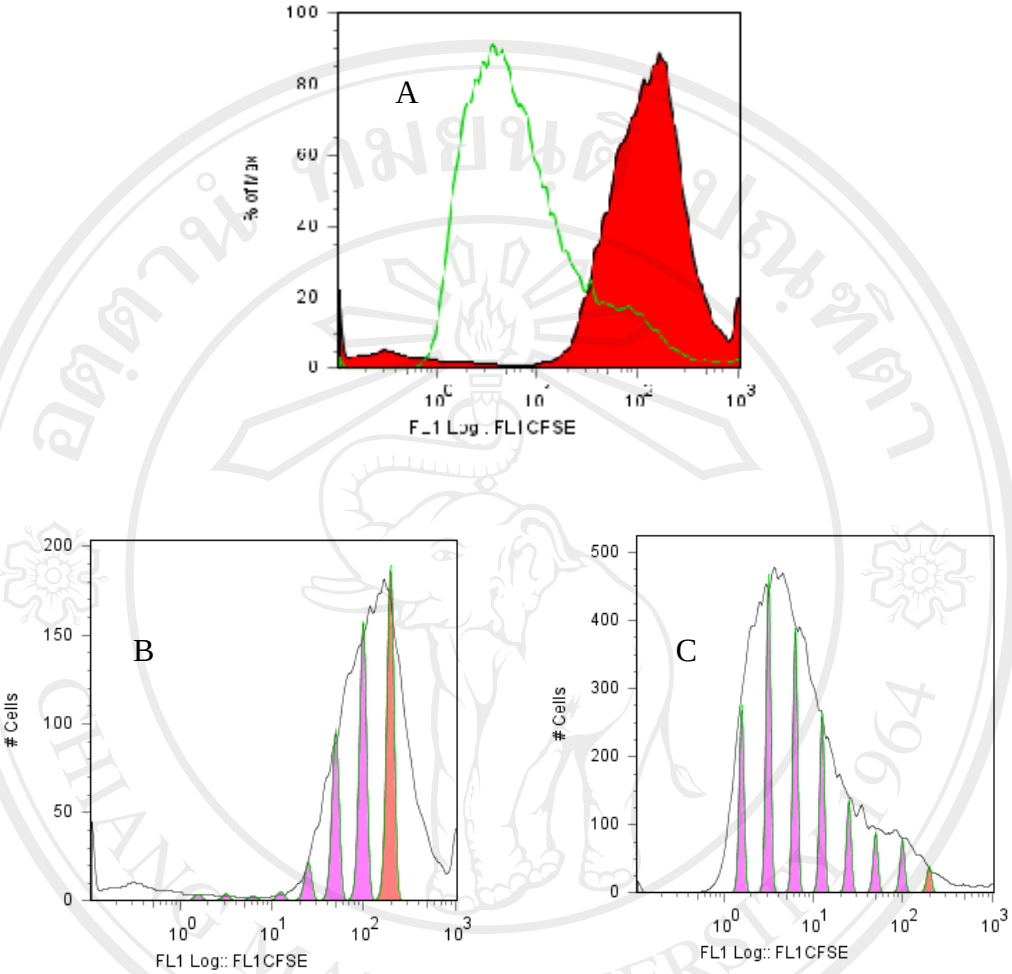

Figure 3.6 Proliferation results from T cells which were cultured with MoDCs. To compare the proliferation results the overlay CFSE histogram between T cell cultured with MoDC (green line) and without (solid red) MoDCs was assessed (A). The CFSE histogram of T cells alone was analyzed for proliferation indexes and MFI (B) and also the histogram of T cells cultured with MoDC was analyzed (C). The results are representatives of all experiments showing similar results from both HIV-1 negative and HIV-1 infected individuals.

Table 3.1 Proliferation indexes of T cells co-cultured with MoDC from HIV-1 negative volunteers

Samples	T cells alone	T cells co-cultured with MoDCs
N1	1.20	1.23
N2	1.41	2.74
N3	1.20	1.25
N4	1.57	2.70
N5	1.20	1.26
N6	1.20	1.29
N7	1.20	1.22
N8	1.20	1.28
N9	1.24	1.82
N10	1.27	1.83
Mean	**1.27**	**1.66**
SD	**0.12**	**0.60**

Table 3.2 Proliferation indexes of T cells co-cultured with MoDC from HIV-1 positive patients

Samples	T cells alone	T cells co-cultured with MoDCs
P1	1.84	2.35
P2	1.57	2.32
P3	1.57	2.75
P4	1.57	2.76
P5	1.57	2.5
P6	1.20	1.25
P7	1.20	1.28
P8	1.29	2.76
P9	1.29	2.16
P10	1.57	1.71
Mean	**1.47**	**2.18**
SD	**0.21**	**0.60**

Table 3.3 Mean fluorescence intensities of T cells co-cultured with MoDC from HIV-1 negative volunteers

Samples	T cells alone	T cells co-cultured with MoDCs
N1	132	117
N2	168	31
N3	41	33
N4	142	28
N5	132	114
N6	132	108
N7	132	110
N8	132	111
N9	140	112
N10	166	60
Mean	**131**	**82**
SD	**34.7**	**39.0**

Table 3.4 Mean fluorescence intensities of T cells co-cultured with MoDC from HIV-1 positive patients

Samples	T cells alone	T cells co-cultured with MoDCs
P1	191	43
P2	142	37
P3	142	26
P4	142	32
P5	142	24
P6	132	113
P7	132	114
P8	162	24.6
P9	163	70.9
P10	270	117
Mean	**161**	**60**
SD	**42.0**	**39.9**