RESULTS

A. Determination of optimum condition for IL-2 production

In our pilot study, culture supernatants at the final 1:2 dilution gave the highest counts per minute (CPM). Thus, this dilution was assay for IL-2 activity.

1. Determination of optimum PHA-P concentration

Stimulation IL-2 production by various concentration of PHA-P were evaluated. The mean of IL-2 production by PBMC in different concentration of PHA-P stimulated culture were (mean ± SEM) 4,666 ± 1,292 at 0.25 µg/ml, 7,805 ± 618 at 0.5 µg/ml, 11,128 ± 793 at 1.0 µg/ml, and decreased to, 10,246 ± 981 at 2.0 µg/ml 6,059 ± 187 at 4.0 µg/ml, and 3,554 ± 309 at 8.0 µg/ml (Table 3 and Figure 1). It was found that PHA-P at 1.0 µg/ml gave the maximum stimulation for IL-2 production. Therefore, this concentration was chosen to be used in this study.

2. Determination of optimum cells concentration

Stimulation IL-2 production by various concentration of PBMC were evaluated. The mean of IL-2 production by various concentration of PBMC were (mean ± SEM) 5,881 ± 951 at 0.25 x 10^6 cells/ml, 8,883 ± 866 at 0.5 x 10^6 cells/ml, 11,972 ± 1,213 at 1.0 x 10^6 cells/ml, and decreased when the concentration of cells is higher, 9,986 ± 961 at 1.5 x 10^6 cells/ml, 7,816 ± 708 at 2.0 x 10^6 cells/ml, and 4,726 ± 316 at 4.0 x 10^6 cells/ml (Table 4 and

Table 3 IL-2 production by various concentration of PHA-P

PHA-P concentration (μg/ml)	IL-2 at 1:2 dilution (CPM)			
	Exp I	Exp II	Exp III	Mean ± SEM
0.25	6,607[a]	5,174	2,217	4,666 ± 1,292[b]
0.5	8,939	6,812	7,676	7,809 ± 618
1.0	10,797	9,952	12,637	11,128 ± 793
2.0	10,401	8,474	11,862	10,246 ± 981
4.0	6,417	5,786	5,974	6,059 ± 187
8.0	4,105	3,036	3,522	3,554 ± 309

a = Mean of CPM in triplicate cultures.

b = Mean ± SEM of three experiments.

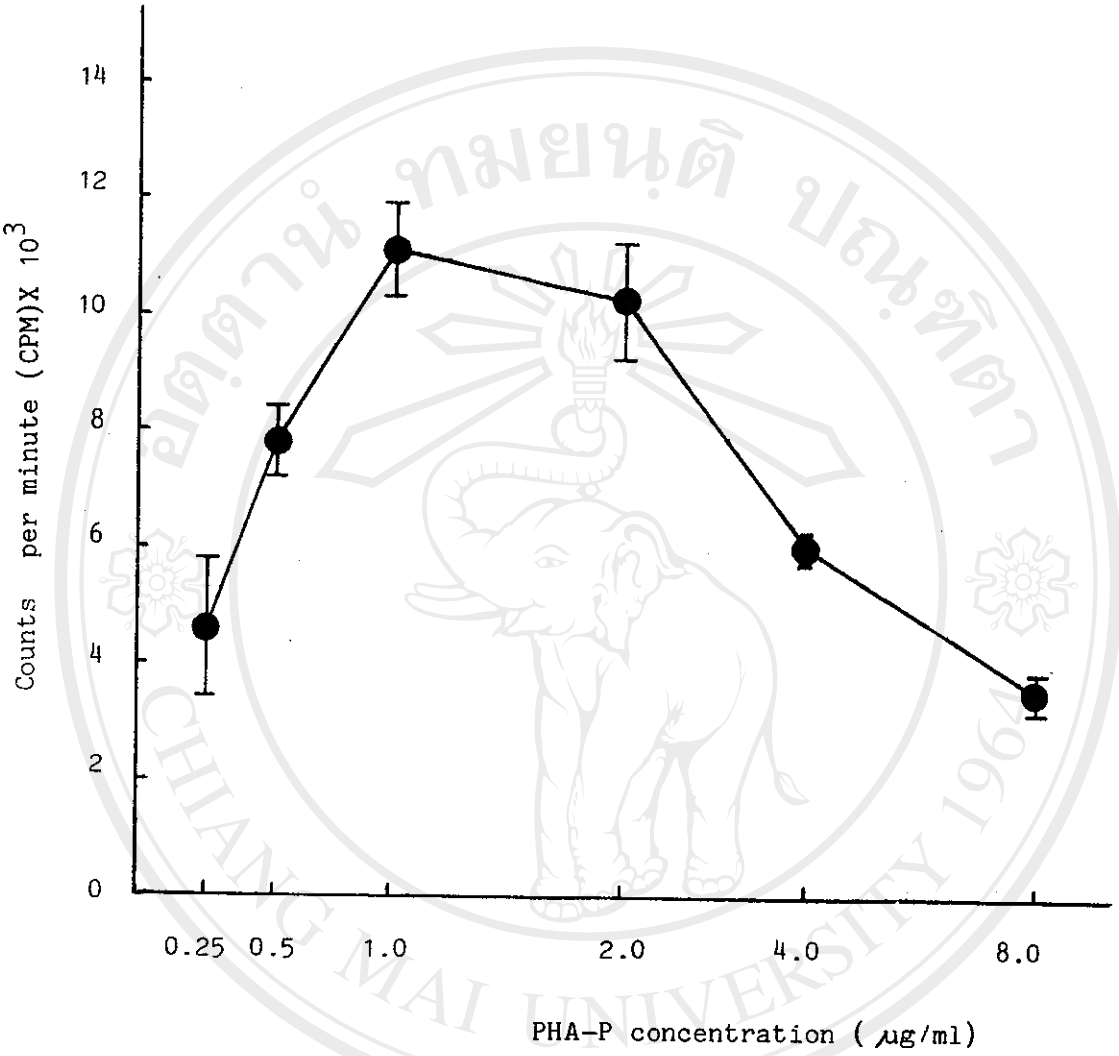

Figure 1 IL-2 production by various concentration of PHA-P. The line represent mean ± SEM (●) of three normal subjects.

Table 4 IL-2 production by various concentration of PBMC.

PBMC concentration (cells/ml)	IL-2 at 1:2 dilution (CPM)			
	Exp I	Exp II	Exp III	Mean ± SEM
0.25×10^6	7,697[a]	5,464	4,481	5,881 ± 951[b]
0.5×10^6	9,986	9,489	7,175	8,883 ± 866
1.0×10^6	13,937	12,220	9,758	11,927 ± 1,213
1.5×10^6	10,364	11,429	8,166	9,986 ± 961
2.0×10^6	8,863	8,117	6,468	7,816 ± 708
4.0×10^6	5,227	4,142	4,810	4,726 ± 316

a = Mean of CPM in triplicate cultures.

b = Mean ± SEM of three experiments.

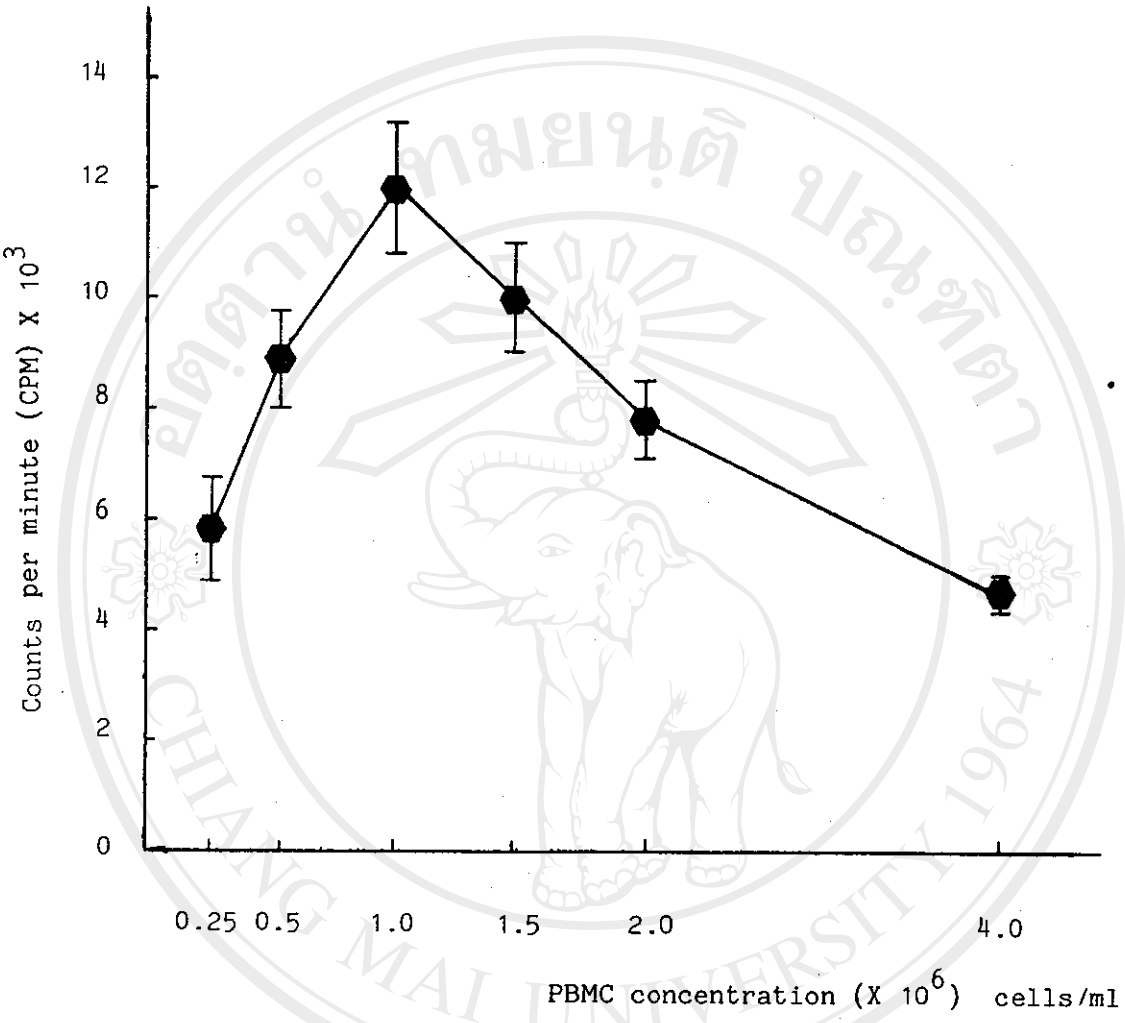

Figure 2 IL-2 production by various concentration of PBMC. The line represent mean ± SEM (⬢) of three normal subjects.

Figure 2). It was found that the final concentration of PBMC 1.0 x 10^6 cells/ml gave the highest IL-2 production. Therefore, this concentration was chosen to be used in this study.

3. Determination of optimum incubation time

Stimulation IL-2 production by various incubation time were evaluated. The mean of IL-2 production by PBMC at different incubation time were (mean ± SEM) 1,985 ± 521 at 4 hours, 2,846 ± 855 at 6 hours, 4,504 ± 421 at 8 hours, 8,554 ± 355 at 12 hours, 11,985 ± 1,365 at 18 hours, and started to decline, 9,072 ± 857 at 24 hours, 2530 ± 336 at 48 hours, and 139 ± 109 at 72 hours (Table 5 and Figure 3). It was found that the production of IL-2 induced by PHA-P was detectable at short period of incubation time (4 hours) after the cultures, reached a peak at 18 hours, and followed by a progressive decline in activity to nealigible values at 72 hours. Therefore, 18 hours incubation time was chosen to be used in this study.

Table 5 IL-2 production by various incubation time.

PBMC concentration (cells/ml)	IL-2 at 1:2 dilution (CPM)			
	Exp I	Exp II	Exp III	Mean ± SEM
4	2,875[a]	1,069	2,010	1,985 ± 521[b]
6	2,975	1,305	4,258	2,846 ± 855
8	3,706	5,137	4,670	4,504 ± 421
12	9,213	7,996	8,454	8,554 ± 355
18	14,265	9,545	12,144	11,985 ± 1,365
24	10,412	7,475	9,329	9,072 ± 857
48	3,035	1,892	2,662	2,530 ± 336
72	20	41	356	139 ± 109

a = Mean of CPM in triplicate cultures.

b = Mean ± SEM of three experiments.

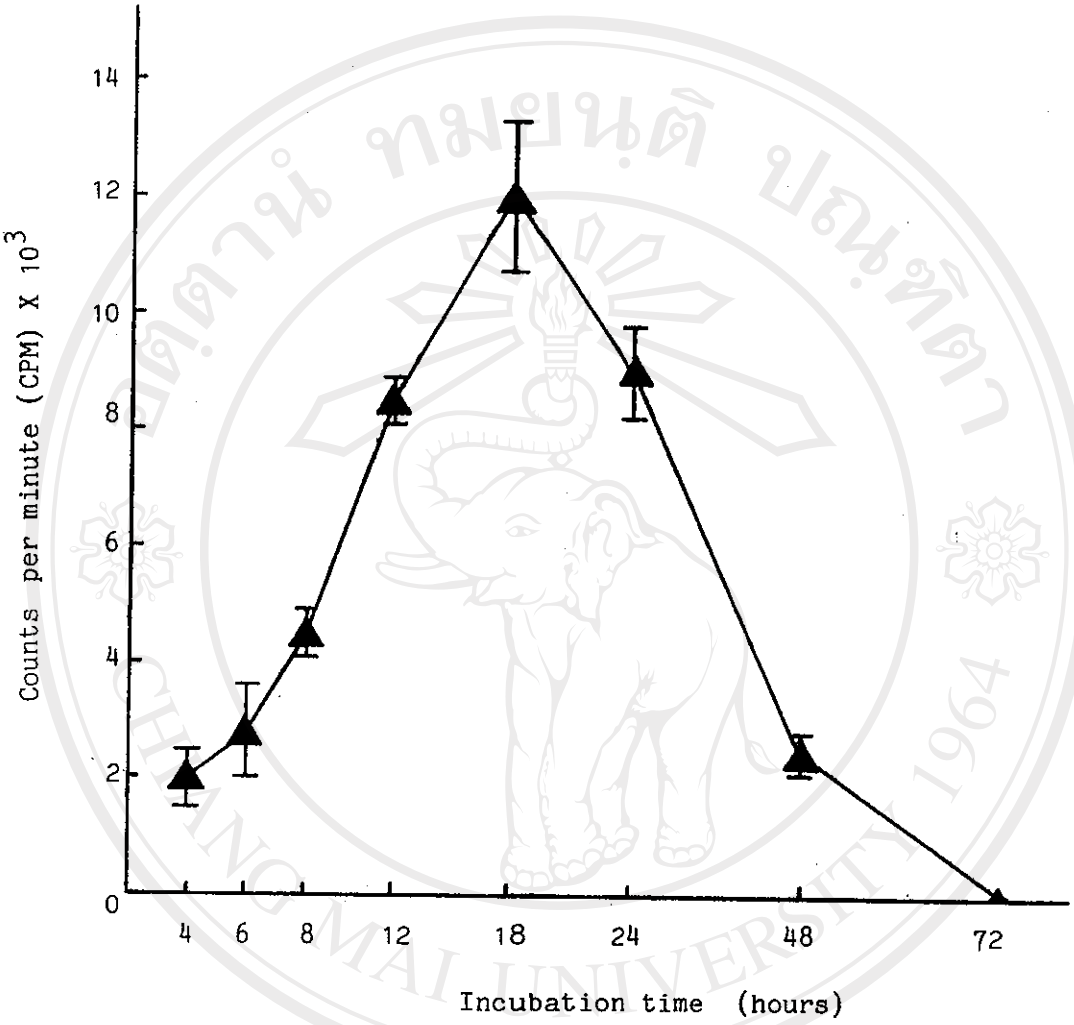

Figure 3 IL-2 production by various incubation time. The line represent mean ± SEM (▲) of three normal subjects.

B. Titration of Con A concentration for AR^+ cells preparation

In order to differentiate the hyperfunction of suppressor T cells (AR^+ cells) of SLE patients from normal subjects, it is important to select the Con A concentration, to prepare AR^+ cells, which will not gave maximum suppression. To obtain the minimum concentration of Con A for AR^+ cells preparation, various concentration of Con A were used to stimulate T cells. The activation of Con A induced suppressor T cells which were separated by autologous erythrocyte rosette formation is depend on the dose of Con A (Table 6). The mean percentage of IL-2 suppression by AR^+ cells (0.125×10^6 cells/ml) co-culture with rested PBMC (0.5×10^6 cells/ml) at different concentration of Con A were (mean ± SD) 6 ± 4 at 5 µg/ml, 11 ± 6 at 10 µg/ml, 21 ± 2 at 20 µg/ml, and 24 ± 3 at 40 µg/ml (Table 7 and Figure 4). By statistical analysis, the mean percentage of IL-2 suppression by AR^+ cells preparation from 5 µg/ml and 10 µg/ml of Con A were significantly different from thos of Con A 20 µg/ml and 40 µg/ml ($p < 0.05$). However, the mean percentage of IL-2 suppression by AR^+ cells preparation by concentration of Con A between 5 µg/ml and 10 µg/ml or 20 µg/ml and 40 µg/ml were not significantly different ($p > 0.05$). The mean percentage of IL-2 suppression by AR^+ cells (0.25×10^6 cells/ml) co-culture with rested PBMC (0.5×10^6 cells/ml) at differenct concentration of Con A were (mean ± SD) 13 ± 5 at 5 µg/ml, 29 ± 4 at 10 µg/ml, 31 ± 8 at 20 µg/ml, and 35 ± 6 at 40 µg/ml (Table 7

and Figure 4). By statistical analysis, the mean percentage of IL-2 suppression by AR^+ cells preparation from 5 μg/ml of Con A were significantly different from those of Con A at 10 μg/ml, 20 μg/ml, and 40 μg/ml ($p < 0.05$). However, the mean percentage of IL-2 suppression by AR^+ cells preparation by different concentration of Con A at 10 μg/ml, 20 μg/ml, and 40 μg/ml were not significantly different ($p > 0.05$). The mean percentage of IL-2 suppression by AR^+ cells (0.5×10^6 cells/ml) co-culture with rested PBMC (0.5×10^6 cells/ml) at different concentration of Con A were (mean ± SD) 25 ± 6 at 5 μg/ml, 41 ± 11 at 10 ug/ml, 59 ± 13 at 20 μg/ml, and 72 ± 14 at 40 μg/ml (Table 7 and Figure 4). By statistical analysis, the mean percentage of IL-2 suppression by AR^+ cells preparation from 5 μg/ml of Con A were significantly different from those of Con A at 10 μg/ml, 20 μg/ml, and 40 μg/ml ($p < 0.05$). However, the mean percentage of IL-2 suppression by AR^+ cells preparation by different concentration of Con A at 10 μg/ml, 20 μg/ml, and 40 μg/ml were not significantly different ($p > 0.05$). The overall results indicated that minimum concentration of Con A to show suppressive activity was 5 μg/ml. This concentration was chosen for AR^+ cells preparation in this study.

The inhibitory action of AR^+ cells was confirmed by the addition of an increasing number of AR^+ cells to a constant rested PBMC. A dose dependent of IL-2 secretion by rested PBMC was observed. The more AR^+ cells were added, the more pronounced was the inhibition (Table 7 and Figure 4).

Table 6 Optimum Con A concentration for preparation of AR^+ cells.

	Added AR^+ cells (cells/ml)	Rested PBMC (cells/ml)	IL-2 production (1:2;CPM) by adding AR^+ cells from different Con A concentration			
			5 μg/ml	10 μg/ml	20 μg/ml	40 μg/ml
Exp I	0.125×10^6	0.5×10^6	10,845[a] (2)	10,677(4)	8,566(23)	8,088(27)
	0.25×10^6	0.5×10^6	10,277 (7)	7,410(33)	6,780(39)	6,411(42)
	0.5×10^6	0.5×10^6	8,868(20)	5,154(53)	2,897(74)	1,399(87)
	-	0.5×10^6	11,103	11,103	11,103	11,103
Exp II	0.125×10^6	0.5×10^6	8,444(10)	8,027(15)	7,480(21)	7,417(21)
	0.25×10^6	0.5×10^6	7,839(17)	6,732(29)	6,692(29)	6,571(30)
	0.5×10^6	0.5×10^6	6,434(32)	5,753(39)	4,667(50)	3,786(60)
	-	0.5×10^6	9,443	9,443	9,443	9,443
ExpIII	0.125×10^6	0.5×10^6	8,727(7)	8,090(14)	7,686(18)	7,022(25)
	0.25×10^6	0.5×10^6	7,967(15)	7,048(25)	7,184(24)	6,306(33)
	0.5×10^6	0.5×10^6	7,133(24)	6,493(31)	4,365(54)	2,868(69)
	-	0.5×10^6	9,420	9,420	9,420	9,420
Mean	0.125×10^6	0.5×10^6	9,339[b]	8,931	7,911	7,509
	0.25×10^6	0.5×10^6	8,694	7,063	6,885	6,429
	0.5×10^6	0.5×10^6	7,478	5,800	3,976	2,684
	-	0.5×10^6	9,989	9,989	9,989	9,989

a = Mean of CPM in triplicate cultures.

b = Mean of three experiments.

( ) = % Suppression

Table 7 Optimum Con A concentration for preparation of AR^+ cells.

Added AR^+ cells (cells/ml)	Rested PBMC (cells/ml)	Mean % suppression by adding AR^+ cells from different Con A concentration			
		5 µg/ml	10 µg/ml	20 µg/ml	40 µg/ml
0.125×10^6	0.5×10^6	6 ± 4[a]	11 ± 6	21 ± 2	24 ± 3
0.25×10^6	0.5×10^6	13 ± 5	29 ± 4	31 ± 8	35 ± 6
0.5×10^6	0.5×10^6	25 ± 6	41 ± 11	59 ± 13	72 ± 14

a = Mean ± SD of three expeniments from three normal subjects.

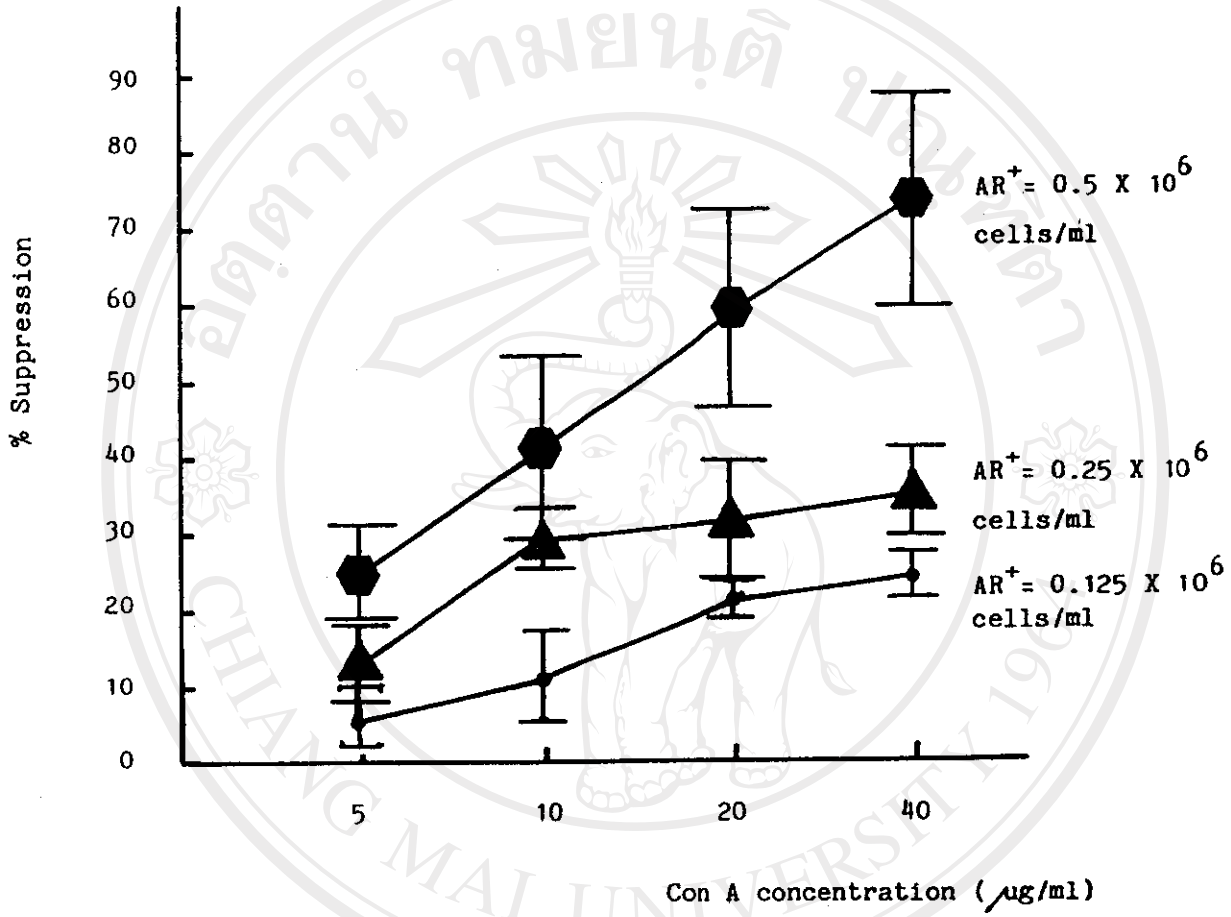

Figure 4 Optimum Con A concentration for preparation of AR^+ cells (● = mean ± SD).

C. To determine the inability of AR^+ cells to absorb IL-2 containing in culture supernatants

In this study, it is necessary to find out whether the AR^+ cells will absorb the IL-2 production in the co-culture. If the AR^+ cells absorbed the IL-2 production, the decreased IL-2 activity would see in the study. This could be contribute to the suppressive activity of AR^+ cells. Therefore, the ability of AR^+ cells absorb IL-2 containing in culture supernatants were determined. The mean of IL-2 activity remained in culture supernatants at different concentration of AR^+ cells adding were 20,496 CPM at 0.125×10^6 cells/ml, 19,394 CPM at 0.25×10^6 cells/ml, 19,390 CPM at 0.5×10^6 cells/ml, and 20, 661 CPM no cells adding (TAble 8). It was found that there was no significant difference of each culture supernatants to stimulate 3 days Con A blast in the presence of different concentration of AR^+ cells and in absence of AR^+ cells ($p > 0.05$). Therefore, AR^+ cells could not absorb IL-2 containing in culture supernatants. However, IL-2 containing in culture supernatants at the high cells concentration had little decrease. These may be due to IL-2 binding to IL-2 receptor on activated T cells but could not internalized to the cells (Fujii et al., 1986). These results clearly indicated that AR^+ cells did not absorb IL-2 containing in culture supernatants.

Table 8 Effect of AR^+ cells absorb IL-2 in culture supernatants.

Added AR^+ cells (cells/ml)	Standard IL-2 (0.5 ml)	IL-2 at 1:4 dilution (CPM)				% Absorption
		Exp I	Exp II	Exp III	Mean	
0.125×10^6	+	21,499[a]	18,255	21,734	20,496[b]	2 ± 2[c]
0.25×10^6	+	18,984	17,991	21,208	19,394	6 ± 5
0.5×10^6	+	19,946	17,960	20,265	19,390	6 ± 6
-	+	21,200	19,258	21,525	20,661	-

a = Mean of CPM in triplicate culture.

b = Mean of three experiments.

c = Mean ± SD of three experiments.

The percent absorption was calculated as follows :

$$\% \text{ Absorption} = \left\{1 - \frac{\text{CPM of standard IL-2 stimulate Con A blast in presence } AR^+ \text{ cells}}{\text{CPM of standard IL-2 stimulate Con A blast in absence } AR^+ \text{ cells}}\right\} \times 100$$

D. To determine the inability of AR^+ cells to decrease PHA-P in culture supernatants

In this study, it is necessary to find out whether the AR^+ cells will decrease PHA-P in culture medium. If the AR^+ cells did not decrease PHA-P in culture medium, the simularity IL-2 production could see in the study. Therefore, the ability of AR^+ cells decrease PHA-P in culture medium were determined. The mean of IL-2 production by remaining PHA-P, after adding different concentration of AR^+ cells, stimulated rested PBMC were 7,946 CPM at 0.125×10^6 cells/ml, 7,981 CPM at 0.25×10^6 cells/ml, 7,724 CPM at 0.5×10^6 cells/ml, and 8,381 CPM no cells adding (Table 9). It was found that there was no significant difference of stimulating activity of each culture supernatants to stimulate 3 days Con A blast ($p > 0.05$). Therefore, this results indicated that PHA-P remaining in culture supernatants were sufficient to stimulate rested PBMC to produce IL-2 and decreased IL-2 secretion was not due to decrease PHA-P to stimulated rested PBMC.

Table 9 Effect of AR^+ cells decrease PHA-P in culture suspernatants.

Supernatant[a] (0.8 ml)	Rested PBMC 0.5×10^6 cells/ml	IL-2 at 1:2 dilution (CPM)			
		Exp I	Exp II	Exp III	Mean
0.125×10^6	+	7,529[b]	6,195	10,113	7,946[c]
0.25×10^6	+	7,124	6,331	10,488	7,981
0.5×10^6	+	7,166	6,260	9,747	7,724
-	+	7,594	6,349	11,200	8,381

$$AR^+ \text{ cells} + \text{PHA-P} \xrightarrow[\text{cfg}]{18 \text{ hr}} \text{Supernatant}^{(a)}$$

b = Mean of CPM in triplicate cultures.

c = Mean of three experiments

E. Comparison between AR^+ cells and AR^- cells to suppress IL-2 secretion

In this study, it is necessary to find out whether decreased IL-2 secretion by adding AR^+ cells were due to crowding effect. Thus, AR^- cells were used instead of AR^+ cells in the co-culture. If the decreased IL-2 secretion were due to crowding effect, adding AR^- cells could suppress IL-2 secretion. Therefore, the ability of AR^+ cells and AR^- cells to suppress IL-2 secretion were determined. The mean percentage of IL-2 suppression by adding AR^+ cells and AR^- cells were (mean ± SD) 31 ± 5 and -3.6 ± 0.6 respectively (Table 10). Thus, this result indicated that when adding of AR^+ cells to autologous rested PBMC resulted in marked diminution of IL-2 secretion but adding of AR^- cell to autologous rested PBMC had no suppression of IL-2 secretion (Table 10 and Figure 5). This clearly indicated that AR^+ cells suppress IL-2 secretion not due to crowding effect. Moreover, AR^+ cells and AR^- cells could not secrete IL-2 in culture supernatants during culture period.

F. Comparison between IL-2 production by freshly isolated PBMC from normal subjects and SLE patients

IL-2 production by PHA-P stimulated freshly isolated PBMC from thirteen normal subjects and thirteen SLE patients (Table 11) were studied by using the 3 days Con A blast as IL-2 dependent target cells. The mean ± SEM IL-2 activity in the culture

Table 10 Comparison between AR^+ cells and AR^- cells to suppress IL-2 secretion.

MMC and CY treated cells ($0.5x10^6$/ml)	Rested PBMC ($0.5x10^6$/ml)	IL-2 at 1:2 dilution (CPM)				% Suppression
		Exp I	Exp II	Exp III	Mean	
AR^+	+	4,782[a]	4,607	8,693	6,027[b]	31 ± 5[c]
AR^-	+	7,483	7,387	12,236	9,035	-3.6 ± 0.6
-	+	7,194	7,084	11,858	8,712	-
AR^+	-	723	1,014	1,012	916	-
AR^-	-	776	1,030	1,120	975	-
-	-	871	1,122	1,098	1,030	-

AR^+ = MMC and CY treated autorosetting T cells.

AR^- = MMC and CY treated non-rosetting cells.

a = Mean of CPM in triplicate cultures.

b = Mean of three experiments.

c = Mean ± SD of three experiments.

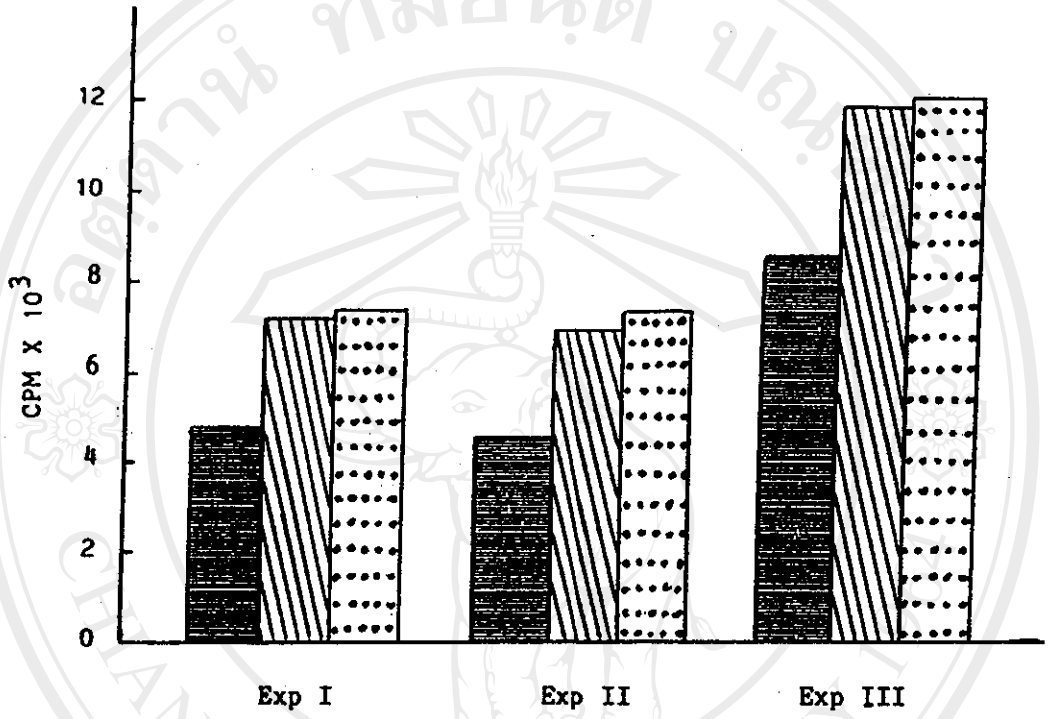

Figure 5 Comparison between AR^+ cells and AR^- cells to suppress IL-2 secretion. adding AR^+ cells, ▬; adding AR^- cells, ⁙; no cells adding, ▨.

Table 11 Clinical data of SLE patients.

Patient No.	Age (yr)	Disease activity	Prednisone mg/day	Disease duration (yr)
1	15	1	0	4
2	22	1	0	1
3	22	1	0	3
4	25	3	0	5
5	25	3	0	9/12
6	25	3	0	5/12
7	26	3	0	5
8	26	3	0	10/12
9	27	3	0	2/12
10	30	2	50	2
11	37	3	0	1
12	43	3	0	1
13	47	1	0	4/12

supernatants from SLE patients and normal subjects were 15.32 ± 1.24, 27.19 ± 2.80 U/ml respectively (Table 12 and Figure 6). It was found that freshly isolated PBMC from SLE patients produced significantly less IL-2 activity in comparison with those from normal subjects ($p < 0.01$). By linear regression analysis the decreased IL-2 production was not correlated with the disease activity ($r = -0.10$), and the duration of disease ($r = -0.50$) ($p > 0.05$; Figure 7). Therefore, the decreased IL-2 production in SLE patients is unlikely to be the result of the disease activity or disease duration.

G. Comparison between freshly isolated and rested PBMC to produce IL-2

Thirteen normal subjects and thirteen SLE patients were determined ability of freshly isolated and rested PBMC to produce IL-2 in vitro. The IL-2 activity produced by freshly isolated PBMC of normal subjects in PHA-P stimulated cultures were 27.19 ± 2.80 U/ml. In contrast, the IL-2 activity produced by 2 days rested PBMC of normal subjects in PHA-P stimulated culture were 26.46 ± 2.59 U/ml (mean ± SEM ; Table 13 and Figure 8). The mean of IL-2 production by freshly isolated and rested PBMC of normal subjects were not significantly different ($p > 0.05$).

Table 12 Comparison between IL-2 production by freshly isolated PBMC from normal subjects and SLE patients.

Subject or Patient No.	Normal subjects		SLE patients	
	Age (yr)	IL-2 (U/ml)	Age (yr)	IL-2 (U/ml)
1	18	40.61	15	14.36
2	23	46.65	22	17.08
3	24	13.39	22	15.39
4	24	15.38	25	9.81
5	24	35.35	25	13.39
6	24	28.72	25	12.50
7	26	17.68	26	7.18
8	27	25.00	26	18.30
9	28	32.98	27	17.67
10	32	19.61	30	24.15
11	37	21.02	37	20.31
12	38	24.15	43	16.49
13	44	32.99	47	12.50
Mean ± SEM		27.19 ± 2.80		15.32 ± 1.24

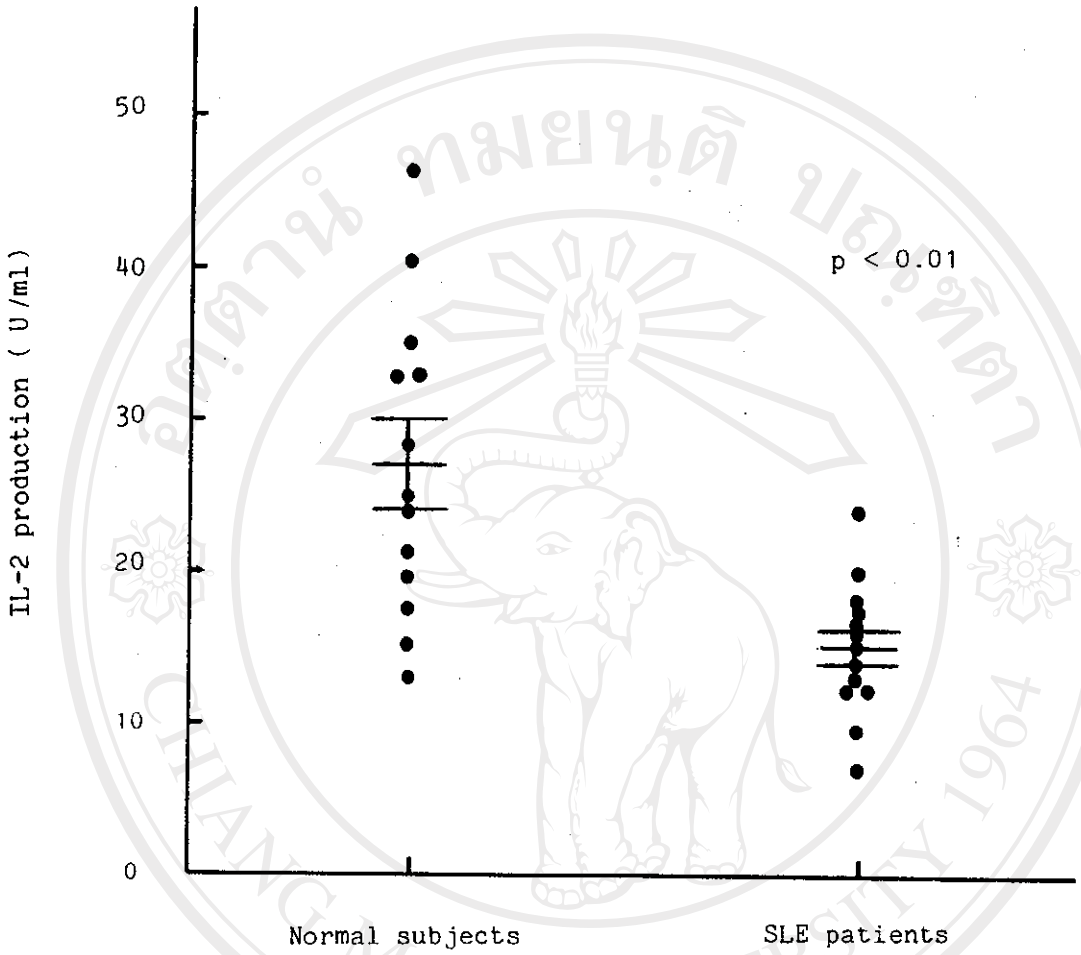

Figure 6 Comparison between IL-2 production by freshly isolated PBMC from normal subjects and SLE patients ($\overline{\overline{\text{T}}}$ = Mean ± SEM).

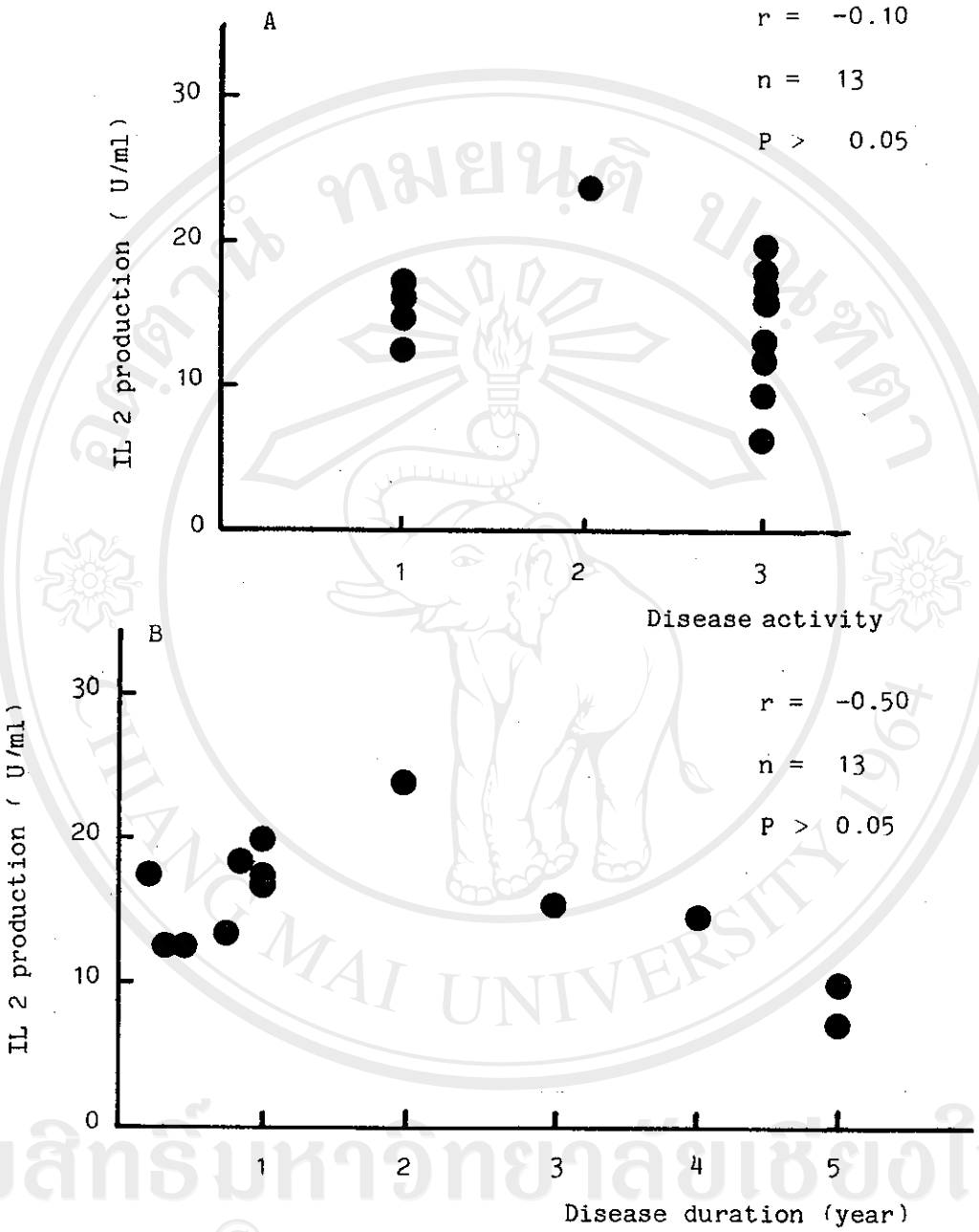

Figure 7 A) Correlation between IL 2 production by freshly isolated PBMC and disease activity of SLE patients.

B) Correlation between IL 2 production by freshly isolated PBMC and disease duration of SLE patients.

Table 13 Comparison between freshly isolated and rested PBMC from normal subjects to produce IL-2.

Subject No.	IL-2 production (U/ml)	
	freshly isolated PBMC	rested PBMC
1	40.61	37.89
2	46.65	40.61
3	13.39	18.95
4	15.38	18.95
5	35.35	26.79
6	28.72	32.99
7	17.68	11.66
8	25.00	23.32
9	32.98	34.15
10	19.61	17.07
11	21.02	23.32
12	24.15	20.30
13	32.99	37.99
Mean ± SEM	27.19 ± 2.80	26.46 ± 2.59

Table 14 Comparison between freshly isolated and rested PBMC from SLE patients to produce IL-2.

patient No.	IL-2 production (U/ml)	
	freshly isolated PBMC	rested PBMC
1	14.36	13.40
2	17.08	15.93
3	15.39	12.50
4	9.81	8.84
5	13.39	20.30
6	12.50	10.88
7	7.18	7.43
8	18.30	15.39
9	17.67	18.95
10	24.15	25.00
11	20.31	23.32
12	16.49	14.36
13	12.50	13.40
Mean ± SEM	15.32 ± 1.24	15.36 ± 1.47

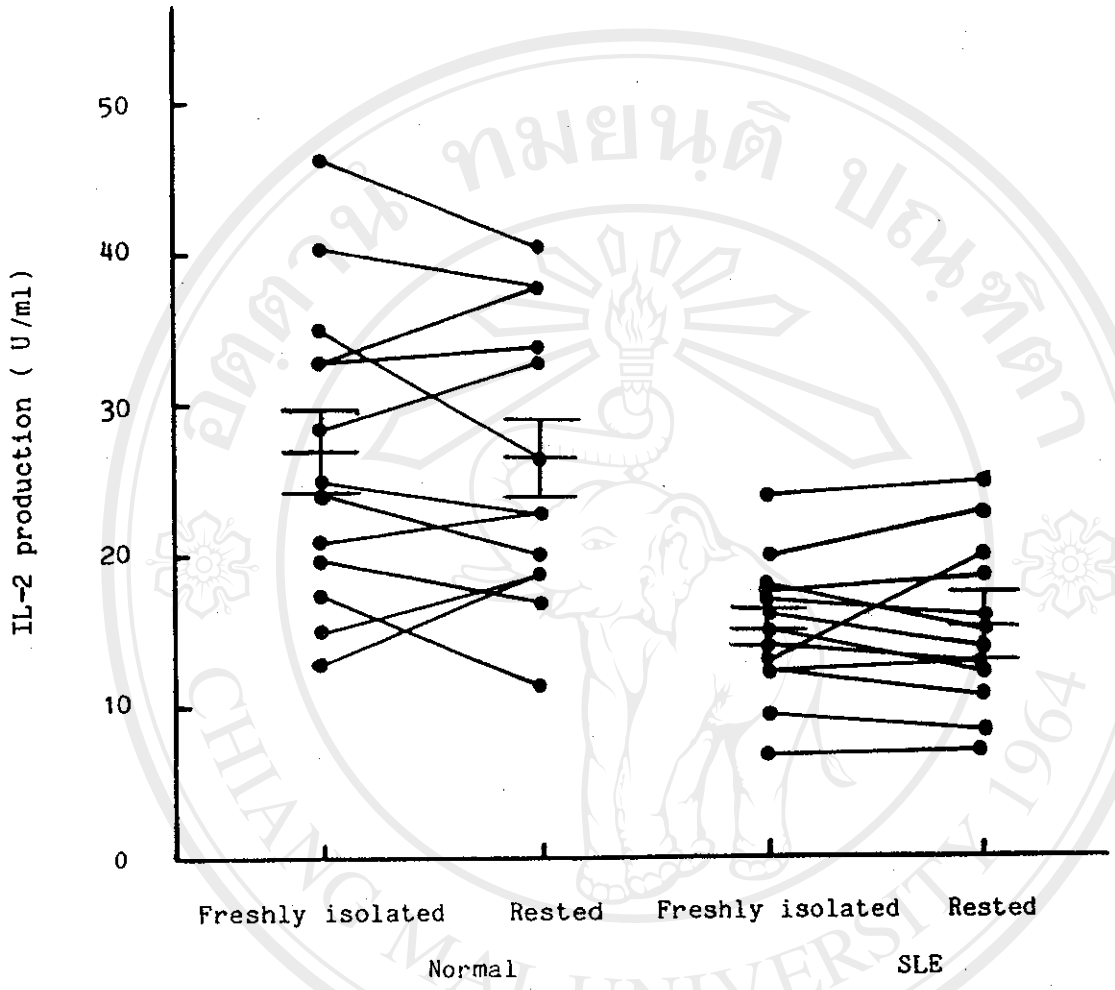

Figure 8 Comparison between freshly isolated and rested PBMC from normal subjects and SLE patients to produce IL-2 (∓ = Mean ± SEM).

The IL-2 production by freshly isolated and rested PBMC from thirteen SLE patients were also investigated. The IL-2 activity produced by freshly isolated PBMC of SLE patients were 15.32 ± 1.24 U/ml. In contrast, The IL-2 activity produced by 2 days rested PBMC of SLE patients were 15.36 ± 1.47 U/ml (mean ± SEM ; Table 14 and Figure 8). The mean of IL-2 production by freshly isolated and rested PBMC of SLE patients were not significantly different ($p > 0.05$). Therefore, the decreased IL-2 production in our SLE cases were not due to exhausted T cells.

H. <u>The percentage of autorosetting T cells of normal subjects and SLE patients</u>

After Con A stimulation, PBMC of normal subjects and SLE patients were further rosetted with autologous erythrocytes. T cells binding three or more autologous RBC were regarded as autorosetting T cells and 200 PBMC were counted. The percentage of autorosetting T cells of thirteen normal subjects and twelve SLE patients were enumerated. It was found that the percentage of autorosetting T cells were (mean ± SD) 37.77 ± 1.83 in normal subjects and 25.08 ± 3.34 in SLE patients (Table 15 and Figure 9). The mean percentage of autorosetting T cells of SLE patients were significantly less than those of normal subjects ($p < 0.001$).

Table 15 Comparison between percentage of autorosetting T cells of normal subjects and SLE patients.

Subject or Patient No.	% Autorosetting T cells	
	Normal	SLE
1	41	26
2	38	30
3	37	29
4	36	20
5	40	21
6	40	23
7	37	24
8	36	30
9	39	24
10	35	27
11	36	-
12	38	24
13	38	23
Mean ± SD	37.77 ± 1.83	25.08 ± 3.34

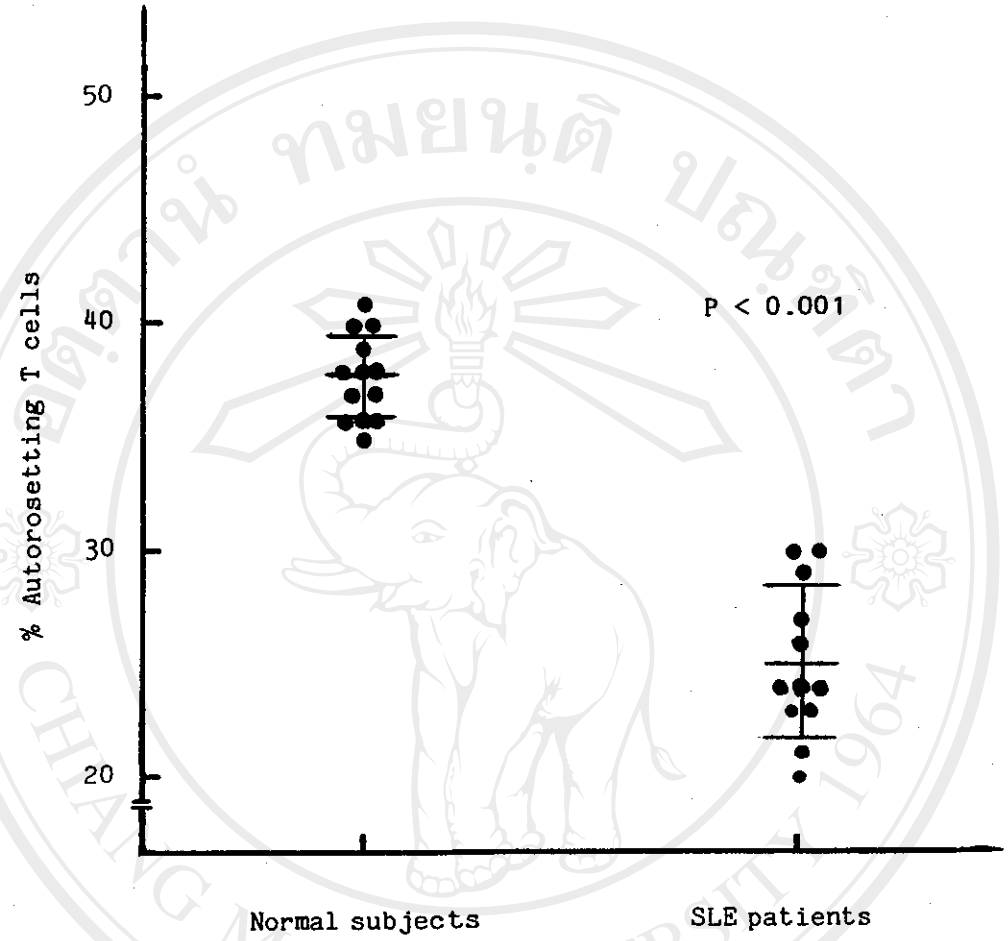

Figure 9 Comparison between % autorosetting T cells of normal subjects and SLE patients (⊣⊢ = Mean±SD).

By linear regression analysis the percentage of autorosetting T cells of SLE patients was not correlated with disease activity ($r = -0.24$), disease duration ($r = -0.13$), and IL-2 production by freshly isolated PBMC of SLE patients ($r = 0.57$) ($P > 0.05$; Figure 10, 11). Therefore, circulating blood of SLE patients had significantly decreased number of suppressor T cells. This defect was not due to the disease activity or disease duration.

I. Suppressive activity of AR^+ cells from normal and SLE to suppress IL-2 secretion of autologous rested PBMC.

Four normal subjects and four SLE patients were studied for suppressive activity of AR^+ cells to suppress autologous rested PBMC to secrete IL-2. It was found that the IL-2 activity of both normal subjects and SLE patients was decreased when the number of added AR^+ cells were increased (Table 16, 17 and Figure 12, 13). The percentage of suppression of IL-2 secretion of each added AR^+ cells was determined. The mean percentage of IL-2 suppression by AR^+ cells in four normal subjects were (mean ± SD) 8 ± 2 at 0.125×10^6 cells/ml, 20 ± 7 at 0.25×10^6 cells/ml, and 26 ± 8 at 0.5×10^6 cells/ml (Table 18 and Figure 14). The mean percentage of IL-2 suppression by AR^+ cells in four SLE patients were (mean ± SD) 8 ± 3 at 0.125×10^6 cells/ml, 17 ± 5 at 0.25×10^6 cells/ml, and 25 ± 2 at 0.5×10^6 cells/ml (Table 18 and Figure 14). By statistic, there were no significant difference in the mean percentage of IL-2

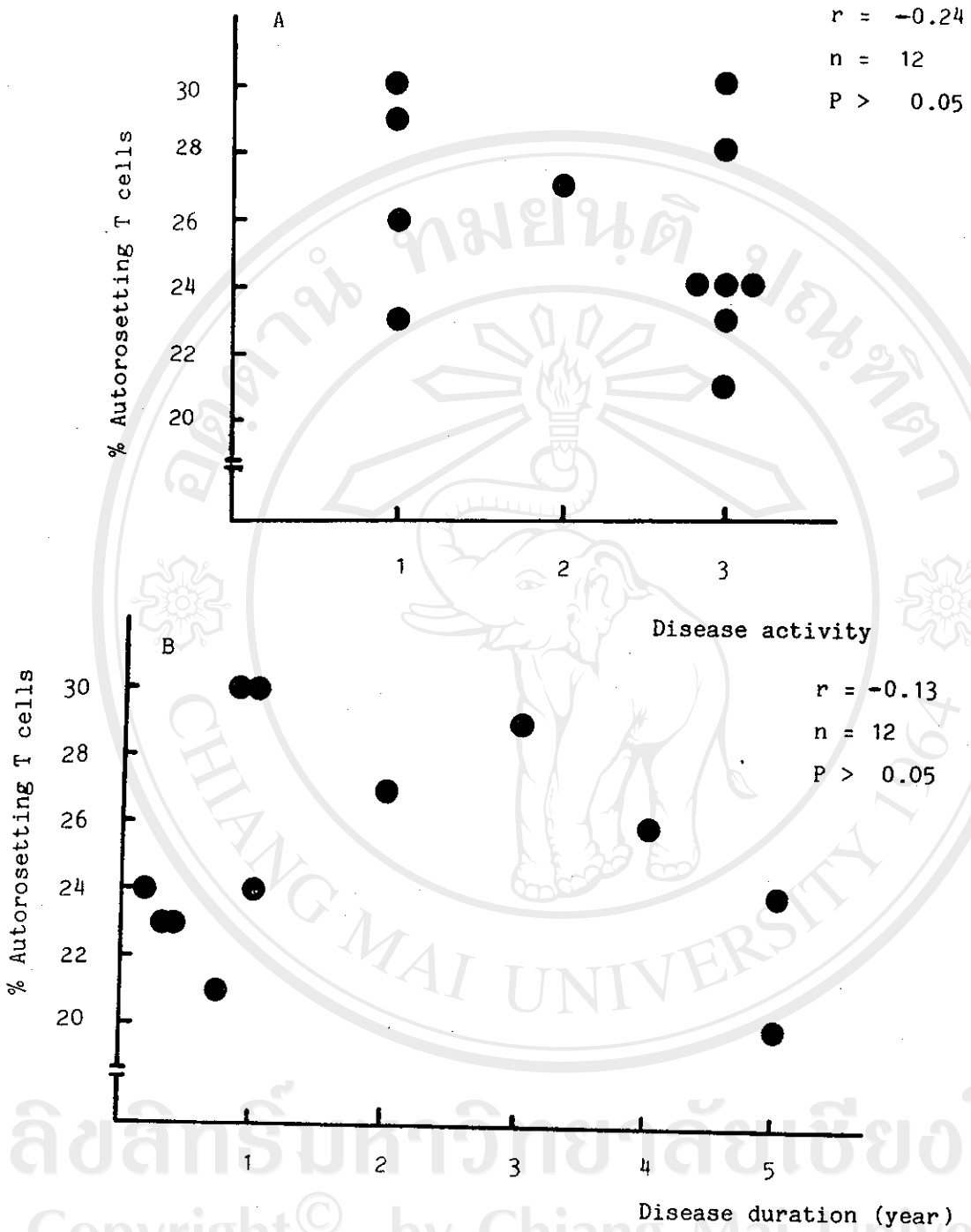

Figure 10 A) Correlation between % autorosetting T cells and disease activity of SLE patients.

B) Correlation between % autorosetting T cells and disease duration of SLE patients.

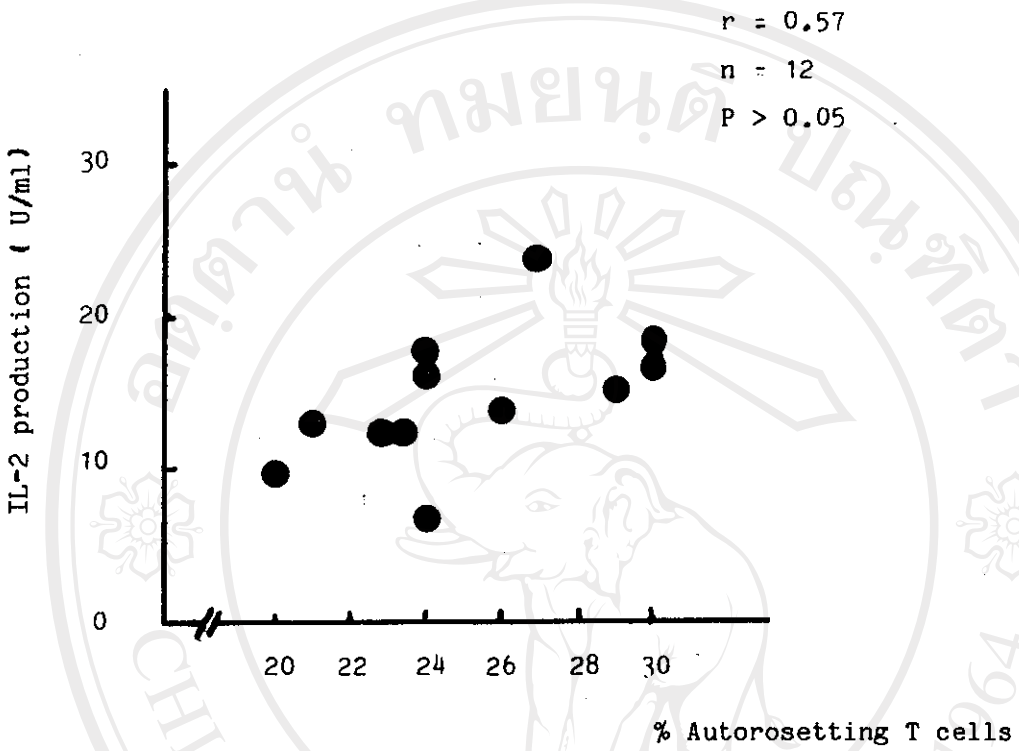

Figure 11 Correlation between IL-2 production by freshly isolated PBMC and % autorosetting T cells of SLE patients.

Table 16 Suppressive activity of AR^+ cells from normal subjects to suppress autologous rested PBMC to secrete IL-2.

Subject No.	Autologous rested PBMC (cells/ml)	Added AR^+ cells (cells/ml)	IL-2 secretion (U/ml)	% Sup.
2	0.5×10^6	-	25.00	-
	0.5×10^6	0.125×10^6	23.32	7
	0.5×10^6	0.25×10^6	20.31	19
	0.5×10^6	0.5×10^6	18.95	24
8	0.5×10^6	-	12.50	-
	0.5×10^6	0.125×10^6	11.66	7
	0.5×10^6	0.25×10^6	10.15	18
	0.5×10^6	0.5×10^6	9.47	24
11	0.5×10^6	-	10.88	-
	0.5×10^6	0.125×10^6	10.15	7
	0.5×10^6	0.25×10^6	9.47	13
	0.5×10^6	0.5×10^6	8.84	19
12	0.5×10^6	-	9.15	-
	0.5×10^6	0.125×10^6	7.97	12
	0.5×10^6	0.25×10^6	6.47	29
	0.5×10^6	0.5×10^6	5.63	38

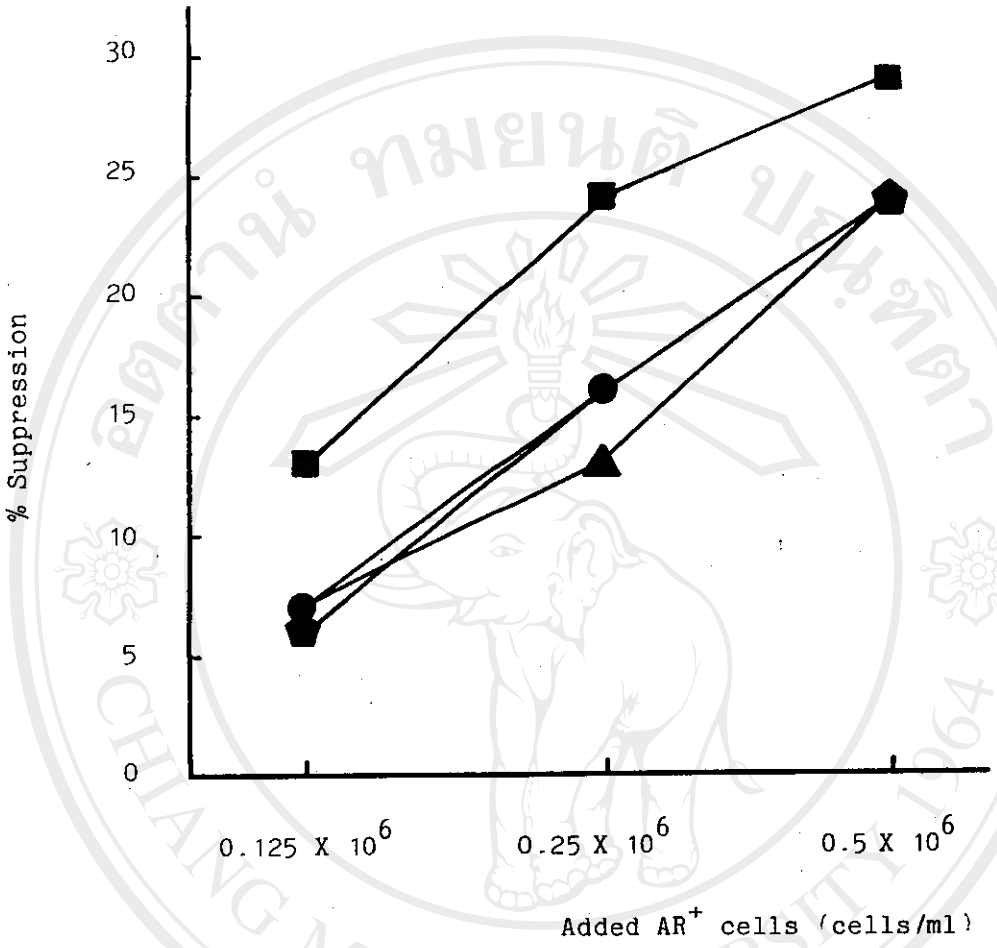

Figure 12 Suppressive activity of AR^+ cells from normal subjects to suppress autologous rested PBMC to secrete IL-2.

Table 17 Suppressive activity of AR^+ cells from SLE patients to suppress autologous rested PBMC to secrete IL-2.

Patient No.	Autologous rested PBMC (cells/ml)	Added AR^+ cells (cells/ml)	IL-2 secretion (U/ml)	% Sup.
3	0.5×10^6	-	5.44	-
	0.5×10^6	0.125×10^6	4.74	13
	0.5×10^6	0.25×10^6	4.12	24
	0.5×10^6	0.5×10^6	3.85	29
7	0.5×10^6	-	2.21	-
	0.5×10^6	0.125×10^6	2.06	7
	0.5×10^6	0.25×10^6	1.86	16
	0.5×10^6	0.5×10^6	1.67	24
8	0.5×10^6	-	4.12	-
	0.5×10^6	0.125×10^6	3.85	6
	0.5×10^6	0.25×10^6	3.45	16
	0.5×10^6	0.5×10^6	3.12	24
13	0.5×10^6	-	9.47	-
	0.5×10^6	0.125×10^6	8.84	7
	0.5×10^6	0.25×10^6	8.25	13
	0.5×10^6	0.5×10^6	7.18	24

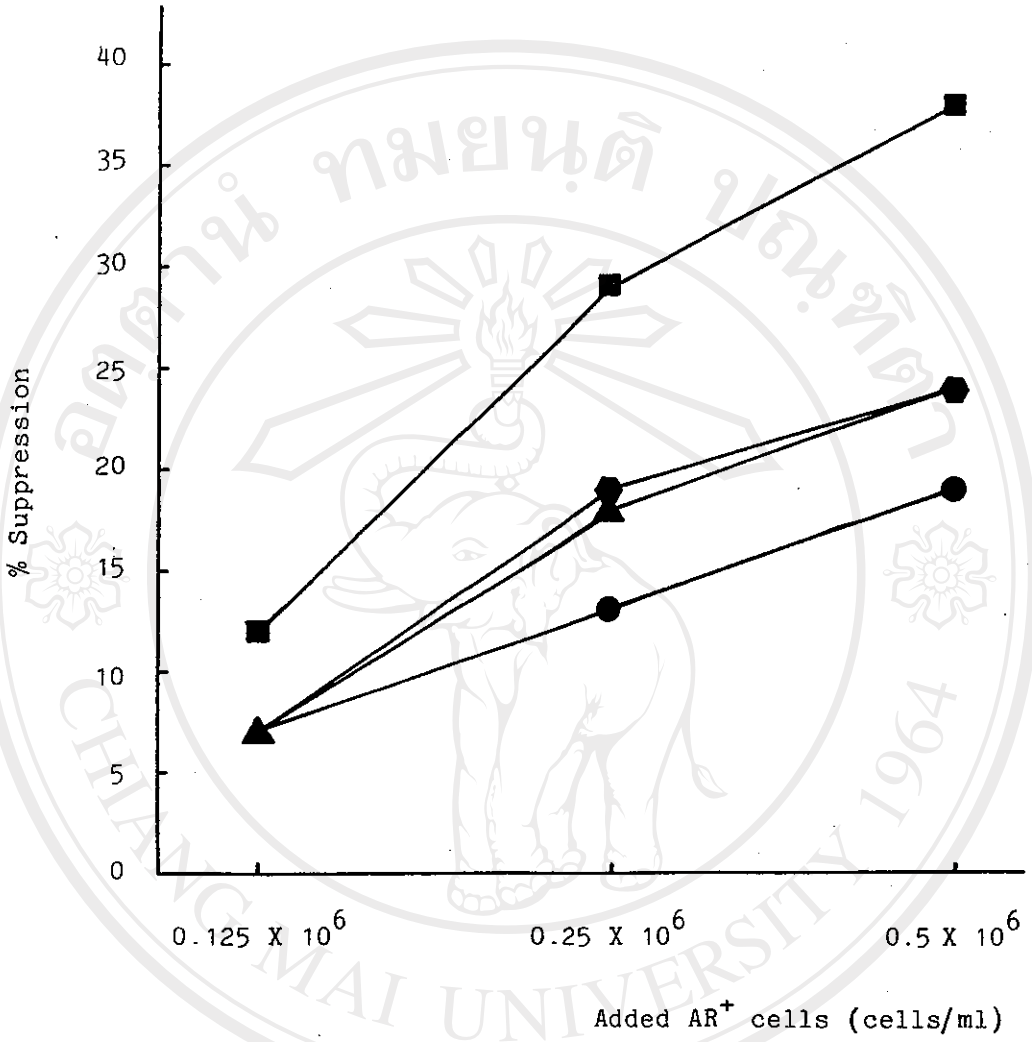

Figure 13 Suppressive activity of AR^+ cells from SLE patients to suppress autogous rested PBMC to secrete IL-2.

Table 18 Mean percentage of autologous suppression of IL-2 secretion by adding AR^+ cells of normal subjects and SLE patients.

Autologous rested PBMC (cells/ml)	Added AR^+ cells (cells/ml)	Mean % suppression by adding AR^+ cells	
		Normal	SLE
0.5×10^6	0.125×10^6	8 ± 2^a	8 ± 3
0.5×10^6	0.25×10^6	20 ± 7	17 ± 5
0.5×10^6	0.5×10^6	26 ± 8	25 ± 2

a = Mean ± SD of four subjects.

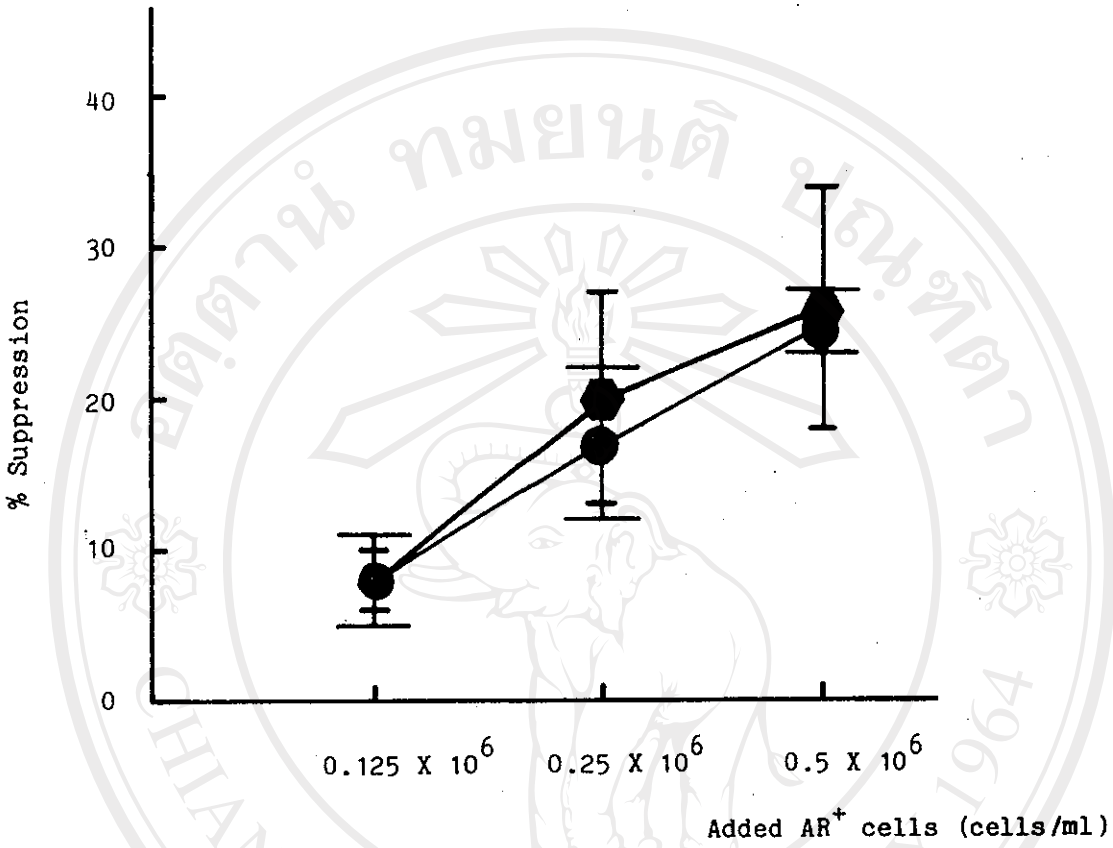

Figure 14 Mean percentage of autologous suppression of IL-2 secretion by adding AR^+ cells of normal subjects and SLE patients.

●, mean ± SD of four normal subjects.

●, mean ± SD of four SLE patients.

suppression by AR^+ cells between normal subjects and SLE patients ($p > 0.05$; Table 18 and Figure 14). Therefore, AR^+ cells of SLE patients have suppressor potential to suppress autologous rested PBMC to secrete IL-2, indifferent from AR^+ cells of normal subjects. In conclusion, the AR^+ cells of SLE patients were not hyperfunction.

J. Suppressive activity of AR^+ cells from normal and SLE to suppress IL-2 secretion of heterologous rested normal PBMC

Five normal subjects and five SLE patients were studied for suppressive activity of AR^+ cells to suppress heterologous rested normal PBMC to secrete IL-2. It was found that the IL-2 activity of both normal subjects and SLE patients was decreased when the number of added AR^+ cells were increased (Table 19, 20 and Figure 15, 16). The percentage of suppression of IL-2 secretion of each added AR^+ cells were determined. The mean percentage of IL-2 suppression by AR^+ cells in five normal subjects were (mean $\pm$ SD) 9 ± 4 at 0.125×10^6 cells/ml, 16 ± 5 at 0.25×10^6 cells/ml, and 26 ± 5 at 0.5×10^6 cells/ml (Table 21 and Figure 17). The mean percentage of IL-2 suppression in five active SLE patient were (mean $\pm$ SD) 9 ± 4 at 0.125×10^6 cell/ml, 18 ± 4 at 0.25×10^6 cells/ml, and 26 ± 7 at 0.5×10^6 cells/ml (Table 21 and Figure 17). By statistic, there were no significant difference in the mean percentage of IL-2 suppression by AR^+ cells between normal subjects and SLE patients ($p > 0.05$; Table 21 and Figure 17). Therefore, AR^+ cells of SLE patients have normal suppressor potential to suppress heterologous rested normal PBMC to secrete IL-2.

Table 19 Suppressive activity of AR^+ cells from normal subjects to suppress heterologous rested normal PBMC to secrete IL-2.

Subject No.	Heterologous rested PBMC (cells/ml)	Added AR^+ cells (cells/ml)	IL-2 secretion (U/ml)	% Sup.
4	0.5×10^6	-	9.47	-
	0.5×10^6	0.125×10^6	8.25	13
	0.5×10^6	0.25×10^6	7.18	24
	0.5×10^6	0.5×10^6	6.70	29
5	0.5×10^6	-	23.32	-
	0.5×10^6	0.125×10^6	21.76	7
	0.5×10^6	0.25×10^6	19.61	16
	0.5×10^6	0.5×10^6	18.95	19
9	0.5×10^6	-	25.00	-
	0.5×10^6	0.125×10^6	24.15	3
	0.5×10^6	0.25×10^6	22.53	10
	0.5×10^6	0.5×10^6	17.08	32
10	0.5×10^6	-	14.86	-
	0.5×10^6	0.125×10^6	13.87	7
	0.5×10^6	0.25×10^6	12.94	13
	0.5×10^6	0.5×10^6	11.26	24
13	0.5×10^6	-	12.50	-
	0.5×10^6	0.125×10^6	10.88	13
	0.5×10^6	0.25×10^6	10.15	19
	0.5×10^6	0.5×10^6	9.47	24

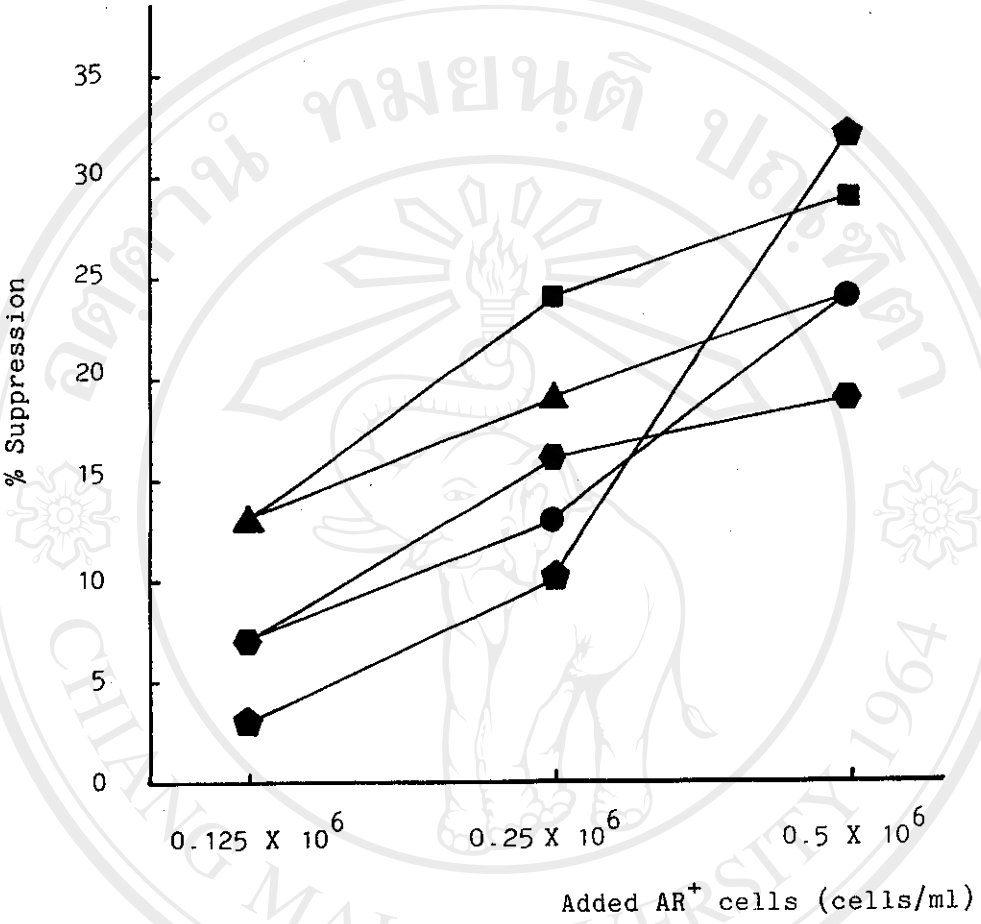

Figure 15 Suppressive activity of AR^+ cells from normal subjects to suppress heterologous rested normal PBMC to secrete IL-2.

Table 20 Suppressive activity of AR^+ cells from SLE patients to suppress heterologous rested normal PBMC to secrete IL-2.

Patient No.	Heterologous rested PBMC (cells/ml)	Added AR^+ cells (cells/ml)	IL-2 secretion (U/ml)	% Sup.
2	0.5×10^6	-	6.93	-
	0.5×10^6	0.125×10^6	6.04	13
	0.5×10^6	0.25×10^6	5.26	24
	0.5×10^6	0.5×10^6	4.27	38
4	0.5×10^6	-	12.50	-
	0.5×10^6	0.125×10^6	11.66	7
	0.5×10^6	0.25×10^6	10.88	13
	0.5×10^6	0.5×10^6	10.15	19
5	0.5×10^6	-	18.95	-
	0.5×10^6	0.125×10^6	18.30	3
	0.5×10^6	0.25×10^6	15.39	19
	0.5×10^6	0.5×10^6	13.87	27
10	0.5×10^6	-	13.40	-
	0.5×10^6	0.125×10^6	12.07	10
	0.5×10^6	0.25×10^6	11.26	16
	0.5×10^6	0.5×10^6	10.15	24
12	0.5×10^6	-	7.69	-
	0.5×10^6	0.125×10^6	6.70	13
	0.5×10^6	0.25×10^6	6.25	18
	0.5×10^6	0.5×10^6	5.83	24

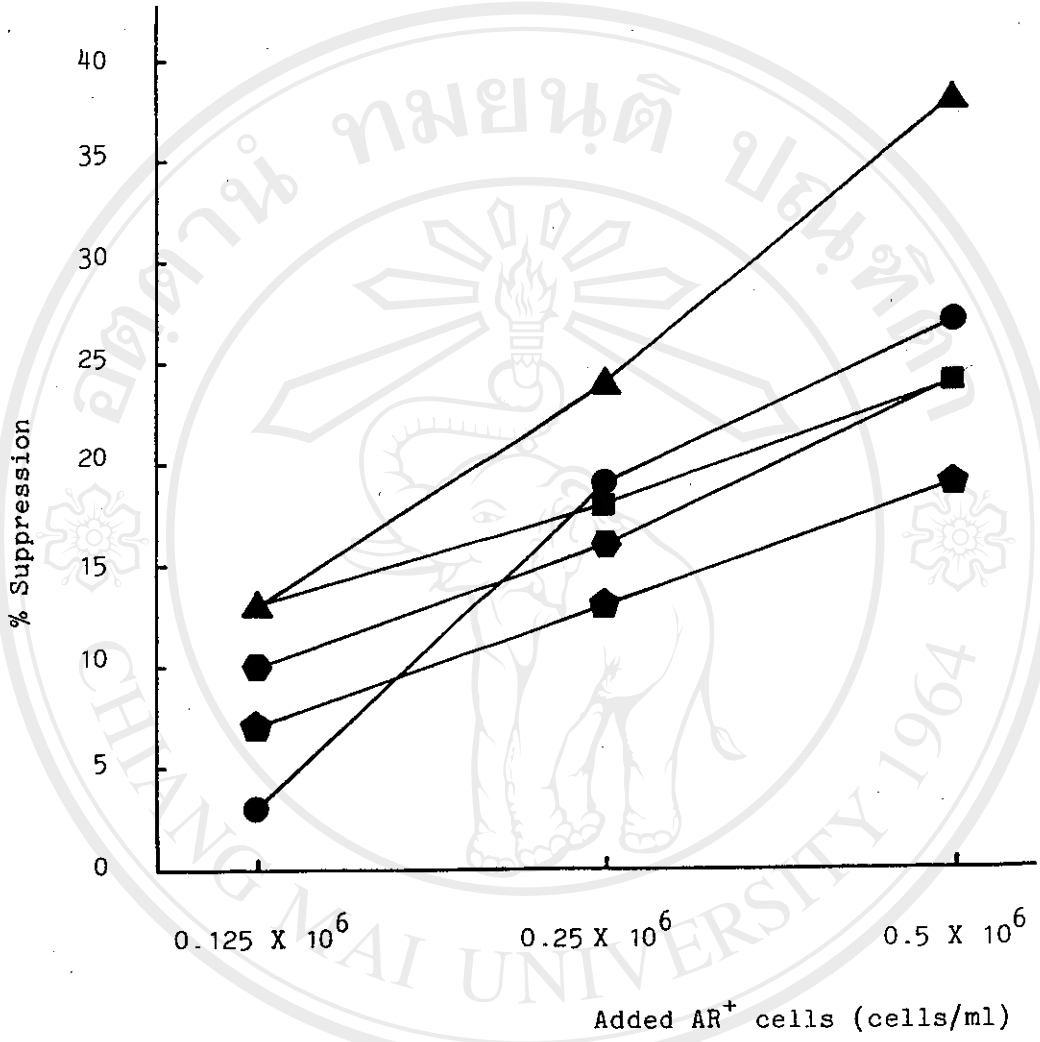

Figure 16 Suppressive activity of AR^+ cells from SLE patients to suppress heterologous rested normal PBMC to secrete IL-2.

Table 21 Mean percentage of heterologous suppression of IL-2 secretion by adding AR^+ cells of normal subjects and SLE patients.

Heterologous rested PBMC (cells/ml)	Added AR^+ cells (cells/ml)	Mean % suppression by adding AR^+ cells	
		Normal	SLE
0.5×10^6	0.125×10^6	9 ± 4^a	9 ± 4
0.5×10^6	0.25×10^6	16 ± 5	18 ± 4
0.5×10^6	0.5×10^6	26 ± 5	26 ± 7

a = Mean ± SD of five subjects.

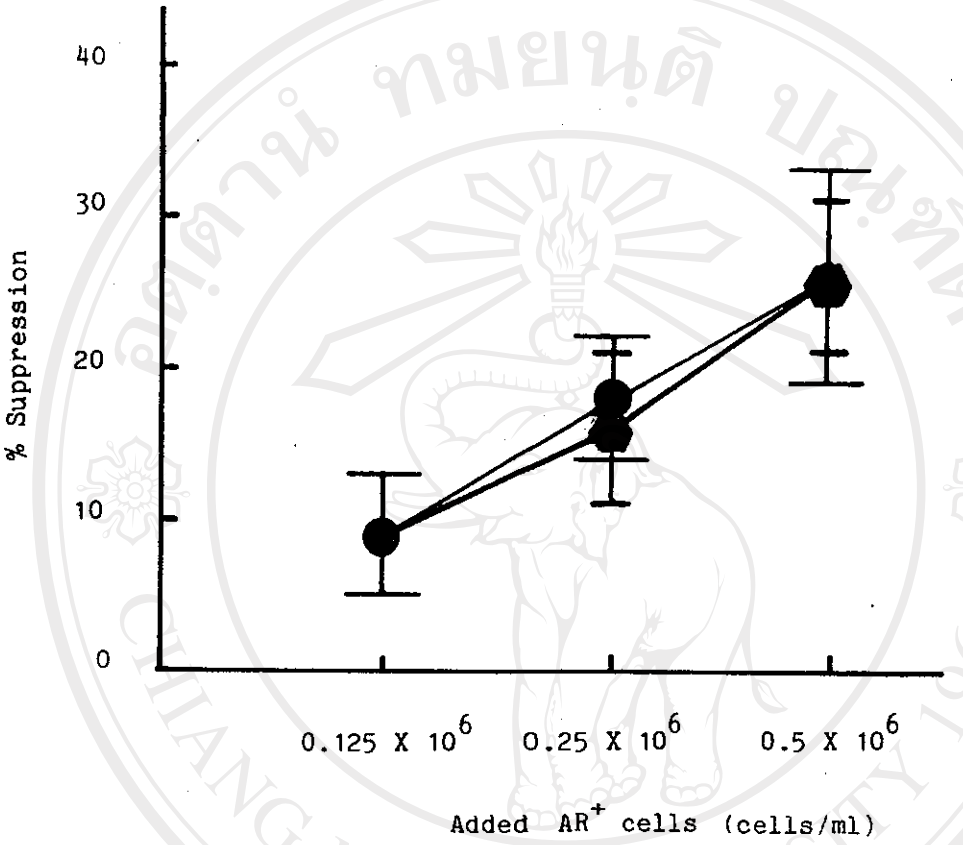

Figure 17 Mean percentage of heterologous suppression of IL-2 secretion by adding AR^+ cells of normal subjects and SLE patients.

●, mean ± SD of five normal subjects.

●, mean ± SD of five SLE patients.