IV. RESULTS

A. Isolation and purification of human peripheral blood monocytes

Monocytes were isolated from peripheral blood of normal persons or tuberculosis patients by the method of Jones et al.(1989) with some modification. The number of white blood cells were counted and percent of monocytes in whole blood were determined by differential count. Our results showed that the number of white blood cell and the percent of the monocytes which determined by differential count in both normal persons, (Table 1) and tuberculosis patients (Table 3) were in normal range between 5-10 x 10^6 cells/ml.

The peripheral blood mononuclear cells (PBMC) were performed by the Ficoll-Hypaque gradient centrifugation method and the number of white blood cell including the viability and the non-specific esterase staining which represent the approximate number of the monocytes in the PBMC were determined.

The results showed that the average numbers of white blood cell in PBMC in normal persons (Table 2) and tuberculosis patients (Table 4) were $2.57x10^6 \pm 0.41$ (mean±SE) and $2.32x10^6 \pm 0.36$ cells/ml, respectively. The percents of the viability of the PBMC in normal persons and tuberculosis patients were 99.6±0.06 and 98.6±0.42, respectively. The percents of cells in PBMC which had positive result for non-specific esterase staining in normal persons and tuberculosis patients were 16.86±1.80% (Table 2 and Figure 1) and 12.67±2.73% (Table 4 and Figure1), respectively. The number of adherent cells,the percentage of viability and the non-specific esterase were also determined. The results showed that the number of adherent cells in normal and tuberculosis patients were $2.40x10^6 \pm 0.31$ and $1.98x10^6 \pm 0.49$, respectively. The percentages of the viability of the adherent cells in normal persons and tuberculosis patients were 98.40±0.06% and 97.80±0.42%, respectively. The purity of the adherent cells was determined by the non-specific esterase staining. The results showed that the percentages of the non-specific esterase positive cells in normal persons and tuberculosis patients were 92.83±0.39% and 89.68±1.20%, respectively (Table 2 and 4).

The percent recovery of the monocytes in adherent cells was determined by compared the number of monocytes in adherent cells with the number of the monocytes in whole blood and the number of non-specific esterase positive cells in PBMC respectively. The results showed that the percent recovery of the monocytes in adherent cells when compared with the number of the monocytes in whole blood in normal persons and tuberculosis patients were 115.53±19.21% and 36.79±20.2%, respectively . The percent recovery of the monocytes in adherent cells when compared with the number of the non-specific esterase positive cells in the PBMC in normal persons (Table 5) and tuberculosis patients (Table 6) were 77.29±4.95% and 101.36±9.98%, respectively.

These data demonstrated that the number of white blood cells in both normal persons and tuberculosis patients were normal and the average percent of the viable cells in PBMC and the adherent cells in both normal persons and tuberculosis patients which used in the experiment is more than 95%. The average percentages of purity of monocytes in adherent cells in both normal persons and tuberculosis patients which used in the experiment were more than 85%.

B. Infection of monocyte-derived macrophages with *Mycobacterium tuberculosis*

To determine the optimum multiplicity of infection (MOI) of the mycobacteria per cell and the optimum time of infection, macrophages were infected with *M. tuberculosis* H37Ra at MOI 5, 10 and 20 and at time 60, 120, and 180 min. The percentage of *M. tuberculosis*-ingested macrophages were measured by acid fast stain. The control of phagocytosis function of the cells was *C.albicans*-ingested macrophages at MOI 10, 120 min showed the average percent phagocytosis 41.60±3.83%. *Candida albicans* was used to test the phagocytosis function of macrophages at MOI 10 and incubated for 120 min with or without 10% pooled human AB serum. The results showed that the percentages of phagocytosis with or without serum were 79.20±0.53% and 41.60±3.85%, respectively. These results demonstrated that the function of phagocytosis of the macrophages was normal and the quality of the pooled human AB serum was good to support phagocytosis.

When macrophages were incubated with *M. tuberculosis*, the results showed that the average percentages of phagocytosis at 60 min at MOI 5, 10, and 20 were 4.33±0.60%, 5.50±1.26%, and 5.34±0.16%, respectively. the average percentages of phagocytosis at 120 min at MOI 5, 10, and 20 were 9.16±1.42%, 10.67±2.45%, and 10.34±2.35%, respectively. the average percentages of phagocytosis at 180 min at MOI 5, 10, and 20 were 9.16±1.20%, 14.50±0.29%, and 14.83±0.60%, respectively (Table 7 and Figure 2, 3). These results demonstrated that at MOI 5 the phagocytosis was reach a plateau at 180min. Percentage of phagocytosis was increased in MOI 10 and 20 when increase, however, at MOI 10 and 20 showed similar results in every experiment. These results indicated that the optimal MOI is 10 mycobacteria per cell but the time of infection may be longer than 180 min because the percent phagocytosis at that time was not reach a plateau.

To determine the optimum time of the infection, the macrophages were infected with *M. tuberculosis* H37Ra at MOI 10 and the time of infection was 60, 120, 180, and 240 min. Because of the low number of *M. tuberculosis*-ingested macrophages was found, the pooled human AB serum was used to increase the percent phagocytosis in this experiment. The results showed that the highest percent phagocytosis was at 240 min of the infection time (Table 8 and Figure 4, 5) and when the pooled human AB serum was added, the percent phagocytosis was significantly increased (11.34±2.13% to 31.74±1.92%) ($p<0.05$).

These results demonstrated that the optimal MOI was 10 mycobacteria per macropage and the optimum time of infection was 240min. The significant increase of percent phagocytosis was found when the pooled human AB serum was added during the uptake period.

C. Determination of the apoptotic cell in *Mycobacterium tuberculosis*-infected macrophages.

The macrophages were infected with *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv at MOI 10 for 240 min in the case of with or without 10% pooled human AB serum. The percentage of *M. tuberculosis*-ingested macrophages was determined by acid fast stain. The control of phagocytosis function of the cells was *C. albicans*-ingested macrophages at MOI 10, 120 min showed the average percent phagocytosis

was 41.60±3.83%. Quality control of pooled human AB serum, *C. albicans* was used at MOI 10, 120 min under the condition with 10% pooled human AB serum. The result showed that the average percent phagocytosis was 79.20±0.53 %. This result demonstrated that the function of phagocytosis of the macrophages was normal and the quality of the pooled human AB serum was good to support phagocytosis. The results showed that the percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv by the macrophages from normal persons were 13.87±0.24% and 12.00±.06%, respectively. When 10% pooled human AB serum was added in the test, the percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv increased to 33.74+1.62% and 32.57+1.22%, respectively (Table 9 and Figure 6). The percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv by the macrophages from the tuberculosis pateints without serum were 12.94±1.85% and 11.20±0.72%, respectively. When serum was added the percentages of phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv also increased to 32.50±0.58% and 30.06±1.18%, respectively (Table 10). Comparison of the percent phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv between normal persons and tuberculosis patients was not found significant different either without or with 10% pooled human AB serum (Figure 7).

These data indicated that there was no different in the number of both *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv-ingested cells between the normal persons and tuberculosis patients and the percent phagocytosis was enhanced by adding 10% pooled human AB serum.

To determined the optimum incubation time for detecting apoptosis of *M. tuberculosis*-infected cells, the macrophages were infected with *M. tuberculosis* H37Ra at MOI 10 for 240 min and the extracellular organisms were removed by washing with the medium for 5 times. Cells were incubated at 37°C, 5% CO_2 for 48, 72 and 120 h. The apoptotic cells were detected by staining with AnnexinV FLUOS staining kit (Roche) and were analyzed by fluorescence microscope. The positive control is Actinomycin D (50μg/ml)-treated macrophages for 19h and the average percent apoptosis from three experiments was 82.20±3.83 (Figure 8). The percentages of apoptosis of *M. tuberculosis* H37Ra-infected macrophages detected at 48 h were 8.33±0.43 and 6.89±1.45 in the case of phagocytosis without or with 10%

pooled human AB serum, respectively. At 72 h, the percentages of apoptosis were 9.58±1.05 (without 10% pooled human AB serum) and 5.82±1.01 (with 10% serum pooled human AB serum). At 120 h, the percentages of apoptosis were 4.76±0.84 (without 10% pooled human AB serum) and 1.10±0.38 (with 10% serum pooled human AB serum), respectively (Table 11 and Figure 9, 10). The percentages of apoptosis at 48 h in either without or with 10% pooled human AB serum-enhanced phagocytosis showed significantly ($p<0.05$) higher than the control, uninfected cell (Table 11). However, there were no significant different of the percentages of apoptosis at 48, 72 and 120 h of incubation. These results demonstrated that the optimum incubation time for detecting apoptosis of *M. tuberculosis*-infected cells was 48 h after the infection.

In the case of phagocytosis using normal persons cells without 10% pooled human AB serum, , the percentages of apoptosis of *M. tuberculosis* H37Ra-infected cells was 8.33±0.43 and *M. tuberculosis* H37Rv-infected cells was 4.74±0.20, respectively. However, when 10% pooled human AB serum was added, there was no significant different in the percentages of apoptosis of *M. tuberculosis* H37Ra-infected cells (6.89±1.45) and *M. tuberculosis* H37Rv-infected cells (4.46±0.15) (Table 12 and Figure 11, 12). Similar with the normal persons, there was significant different in the number of the apoptotic cells in tuberculosis patients when compared between *M. tuberculosis* H37Ra-infected cells and *M. tuberculosis* H37Rv-infected cells. In the case of phagocytosis without 10% pooled human AB serum, the percent apoptosis of *M. tuberculosis* H37Ra-infected cells was 12.94±3.12 and *M. tuberculosis* H37Rv-infected cells was 5.30±1.46 and the control uninfected cells was 1.12±0.39. Likewise, the phagocytosis with 10% pooled human AB serum was significantly different in the number of the apoptotic cells in tuberculosis patients when compared between *M. tuberculosis* H37Ra-infected cells and *M. tuberculosis* H37Rv-infected cells. The percentages of apoptosis of *M. tuberculosis* H37Ra-infected cells was 12.13±1.02 and *M. tuberculosis* H37Rv-infected cells was 5.49±0.98 and the control uninfected cells, which incubated with 10% pooled human AB serum was 1.10±0.08 (Table 13 and Figure 13, 14). There was no significant different in the percentages of apoptosis of *M. tuberculosis* H37Ra-infected cells or *M. tuberculosis* H37Rv-infected cells between normal and tuberculosis patients. The

control of both groups also was no significantly different in the percentages of apoptotic cells (Figure 15).

These data demonstrated that the percentages of apoptosis of *M. tuberculosis* H37Ra-infected cells in both normal persons and tuberculosis patients was significant higher than the percent apoptosis in *M. tuberculosis* H37Rv-infected cells. However, there was no significant different in the percentages of apoptosis of *M. tuberculosis* H37Ra-infected cells and *M. tuberculosis* H37Rv-infected cells when compared between normal persons and tuberculosis patients.

Table 1. Number and differential count of white blood cell in peripheral blood from normal persons.

Subject number	WBC (cells/ml)	Differential count (%)				
		Neu	Lym	Mo	Eo	Ba
N1	8.91×10^6	62.34	29.34	2.66	5.66	0
N2	7.00×10^6	57.67	40.33	1.33	0	0.67
N3	5.03×10^6	56.34	37.33	4.66	1.67	0
Mean ± SE	6.9×10^6 ± 1.12	58.78 ± 1.82	35.67 ± 3.28	2.88 ± 0.97	2.44 ± 1.68	0.22 ± 0.22

WBC = White blood cell count
Neu = Neutrophils
Lym = Lymphocytes
Mo = Monocytes
Eo = Eosinophils
Ba = Basophils

Table 2. Number of white blood cells, viable count and non-specific esterase positive cell in peripheral blood mononuclear cells (PBMC) and adherent cells in normal persons.

Subject number	PBMC			Adherent cell		
	WBC (cells/ml)	Viability (%)	NSE+ cell (%)	WBC (cells/ml)	Viability (%)	NSE+ cell (%)
N1	3.37×10^6	99.60	20.30	2.96×10^6	97.80	93.6
N2	2.0×10^6	99.60	14.00	1.86×10^6	99.60	92.60
N3	2.36×10^6	99.80	16.30	2.39×10^6	97.80	92.30
Mean $\pm$ SE	2.57×10^6 $\pm$ 0.41	99.66 $\pm$ 0.06	16.86 $\pm$ 1.80	2.40×10^6 $\pm$ 0.31	98.40 $\pm$ 0.60	92.83 $\pm$ 0.39

NSE + cell = Cells give positive result for non-specific esterase staining

WBC = White blood cell count

Table 3. Number and differential count of white blood cell in peripheral blood from tuberculosis patients.

Subject number	WBC (cells/ml)	Differential count (%)				
		Neu	Lym	Mo	Eo	Ba
P1	12.2 x10^6	60.67	32.00	5.67	1.66	0
P2	9.37 x10^6	53.66	39.34	5.33	1.00	0.67
P3	7.72 x10^6	48.33	43.34	4.00	3.56	0.67
Mean ± SE	9.76 x 10^6 ± 1.31	54.22 ± 3.57	38.23 ± 3.32	5.00 ± 0.51	2.07 ± 0.77	0.45 ± 0.22

WBC = White blood cell count

Neu = Neutrophils

Lym = Lymphocytes

Mo = Monocytes

Eo = Eosinophils

Ba = Basophils

Table 4. Number of white blood cells, viable count and non-specific esterase positive cell in peripheral blood mononuclear cells (PBMC) and adherent cells in tuberculosis patients.

Subject number	PBMC			Adherent cell		
	WBC count (cells/ml)	Viability (%)	NSE+ cell (%)	WBC count (cells/ml)	Viability (%)	NSE+ cell (%)
P1	2.56×10^6	99.40	18.00	2.76×10^6	96.00	90.67
P2	2.80×10^6	98.40	9.00	2.10×10^6	98.00	87.34
P3	1.61×10^6	98.80	11.00	1.07×10^6	99.40	91.34
Mean ± SE	2.32×10^6 ± 0.36	98.60 ± 0.42	12.67 ± 2.73	1.98×10^6 ± 0.49	97.80 ± 0.99	89.68 ± 1.20

NSE + cell = Cells give positive result for non-specific esterase staining

WBC = White blood cell count

Table 5. Percent recovery of adherent cells compared with number of monocytes in whole blood and number of non-specific esterase positive cells in peripheral blood mononuclear cell of normal person.

Subject numbers	Total Monocytes in whole blood (cells/50ml)	Total NSE+ cell in PBMC (cells/30ml)	Total NSE+cell in adherent cell (cells/5ml)	% Recovery of adherent cell compared with the number of monocytes in	
				Whole blood	PBMC
N1	11.85×10^6	20.52×10^6	13.85×10^6	116.80	67.40
N2	4.65×10^6	8.40×10^6	8.61×10^6	148.17	81.63
N3	11.72×10^6	11.54×10^6	11.03×10^6	81.64	82.84
Mean ± SE	9.40×10^6 ± 2.38	13.48×10^6 ± 3.63	11.16×10^6 ± 1.51	115.53 ± 19.21	77.29 ± 4.95

PBMC = Peripheral blood mononuclear cell

NSE + cell = Cells give positive result for non-specific esterase staining

SE = Standard error of the mean

Table 6. Percent recovery of adherent cells with the number of monocytes in whole blood and the number of non-specific esterase positive cells in peripheral blood mononuclear cell of tuberculosis patients.

Subject numbers	Total Monocytes in whole blood (cells/50ml)	Total NSE+ cell in PBMC (cells/30ml)	Total NSE+cell in adherent cell (cells/5ml)	% Recovery of adherent cell compared with the number of monocytes in	
				Whole blood	PBMC
P1	34.58×10^6	13.82×10^6	12.51×10^6	36.17	90.52
P2	24.97×10^6	7.56×10^6	9.17×10^6	40.81	121.30
P3	15.44×10^6	5.31×10^6	4.88×10^6	33.93	92.28
Mean ± SE	24.99×10^6 ± 5.52	8.89×10^6 ± 2.54	8.85×10^6 ± 2.20	36.97 ± 2.02	101.36 ± 9.98

PBMC = Peripheral blood mononuclear cell

NSE + cell = Cells give positive result for non-specific esterase staining

SE = Standard error of the mean

Table 7. Percent phagocytosis of macrophages from normal persons to *M. tuberculosis* H37Ra at various multiplicity of infections (MOI) and incubation times.

Subject number	Phagocytosis (%)								
	60 min			120 min			180 min		
	MOI			MOI			MOI		
	5	10	20	5	10	20	5	10	20
N1	3.5	4.0	5.5	7.5	9.0	7.5	7.5	14.0	14.5
N2	2.5	4.5	5.0	8.0	7.5	8.5	8.5	14.5	14.0
N3	3.0	8.0	5.5	12.0	15.5	15.0	11.5	15.0	16.0
Mean ± SE	4.33 ± 0.60	5.50 ± 1.26	5.34 ± 0.16	9.16 ± 1.42	10.67 ± 2.45	10.34 ± 2.35	9.16 ± 1.20	14.50 ± 0.29	14.83 ± 0.60

Table 8. Percent phagocytosis of macrophages from normal persons to *M. tuberculosis* H37Ra at the multiplicity of infection 10 in 10% pooled human AB serum with various incubation times.

Subject number	Phagocytosis (%)							
	Without 10% pooled human AB serum				With10% pooled human AB serum			
	60 min	120 min	180 min	240 min	60 min	120 min	180 min	240 min
N1	6.4	9.8	13.6	15.4	12.6	23.0	26.2	28.2
N2	2.6	5.4	10.2	8.2	5.2	24.6	28.6	34.8
N3	2.8	5.6	7.2	10.4	10.8	21.4	20.8	32.2
Mean ± SE	3.93 ± 1.23	6.94 ± 1.43	10.34 ± 1.85	11.34 ± 2.13	9.54 ± 2.23	23.00 ± 0.92	25.20 ± 2.30	31.74 ± 1.92

Multiplicity of infection (MOI) is 10 mycobacteria per macrophage

Table 9. Percent phagocytosis of macrophages from normal persons to *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv in 10% pooled human AB serum.

Subject number	Phagocytosis (%)					
	Without 10% pooled human AB serum			With10% pooled human AB serum		
	H37Ra*	H37Rv**	*C. albicans*	H37Ra*	H37Rv**	*C. albicans*
N1	14.2	10.4	34.2	30.6	34.8	78.4
N2	13.4	14.0	43.6	34.6	32.3	90.2
N3	14.0	11.6	59.6	36.0	30.6	93.0
Mean ± SE	13.87 ± 0.24	12.00 ± 1.06	45.80 ± 7.41	33.74 ± 1.62	32.57 ± 1.22	87.20 ± 4.47

Multiplicity of infection (MOI) is 10 mycobacteria per macrophage

Time of infection is 240 min

* = $p < 0.05$

** = $p < 0.05$

Table 10. Percent phagocytosis of macrophages from tuberculosis patients to *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv in 10% pooled human AB serum.

Subject number	Phagocytosis (%)			
	Without 10% pooled human AB serum		With10% pooled human AB serum	
	H37Ra*	H37Rv**	H37Ra*	H37Rv**
P1	16.6	11.0	32.3	28.3
P2	10.6	12.0	31.6	29.6
P3	11.6	10.6	33.6	32.3
Mean ± SE	12.94 ± 1.85	11.20 ± 0.72	32.50 ± 0.58	30.06 ± 1.18

Multiplicity of infection (MOI) is 10 mycobacteria per macrophage

Time of infection is 240 min

* = $p < 0.05$

** = $p < 0.05$

Table 11. Percent apoptotic cells of *M. tuberculosis* H37Ra-infeced macrophages from normal persons.

Subject number	Apoptosis of *M. tuberculosis* H37Ra-infected cells (%)							
	Without 10% pooled human AB serum				With10% pooled human AB serum			
	48h*	72h	120h	UF*	48h**	72h	120h	UF**
N1	9.05	7.97	3.83	1.73	9.50	4.63	7.74	1.93
N2	8.36	9.22	4.00	0.33	4.47	5.00	2.68	0.97
N3	7.57	11.55	6.44	0.75	6.70	7.83	2.56	0.68
Mean ± SE	8.33 ± 0.43	9.58 ± 1.05	4.76 ± 0.84	0.94 ± 0.41	6.89 ± 1.45	5.82 ± 1.01	4.30 ± 1.70	1.10 ± 0.38

UF = Uninfected cell as control

* = $p < 0.05$

** = $p < 0.05$

Table 12. Percent apoptotic cells of *M. tuberculosis* H37Ra-infeced macrophages or *M. tuberculosis* H37Rv-infeced macrophages from normal persons after 48 hours of incubation.

Subject number	Apoptosis of *M. tuberculosis* -infected cells (%)					
	Without 10% pooled human AB serum			With10% pooled human AB serum		
	H37Ra *	H37Rv*	*UF*	H37Ra	H37Rv	*UF*
N1	9.05	5.05	1.73	9.50	4.28	1.93
N2	8.36	4.36	0.33	4.47	4.35	0.97
N3	7.57	4.80	0.75	6.70	4.76	0.68
Mean ± SE	8.33 ± 0.43	4.74 ± 0.20	0.94 ± 0.41	6.89 ± 1.45	4.46 ± 0.15	1.10 ± 0.38

UF = Uninfected cell as control

* = $p < 0.05$

Table 13. Percent apoptosis of *M. tuberculosis* H37Ra-infected macrophages or *M. tuberculosis* H37Rv-infeced macrophages from tuberculosis patients.

Subject number	Apoptosis of *M. tuberculosis* -infected cells (%)					
	Without 10% pooled human AB serum			With 10% pooled human AB serum		
	H37Ra *	H37Rv*	*UF*	H37Ra **	H37Rv**	*UF*
P1	16.34	5.67	0.50	13.46	6.67	0.97
P2	15.77	7.64	1.85	12.80	6.25	1.26
P3	6.72	2.60	1.02	10.12	3.54	1.07
Mean ± SE	12.94 ± 3.12	5.30 ± 1.46	1.12 ± 0.39	12.13 ± 1.02	5.49 ± 0.98	1.10 ± 0.08

UF = Uninfected cell as control

* = $p < 0.05$

** = $p < 0.05$

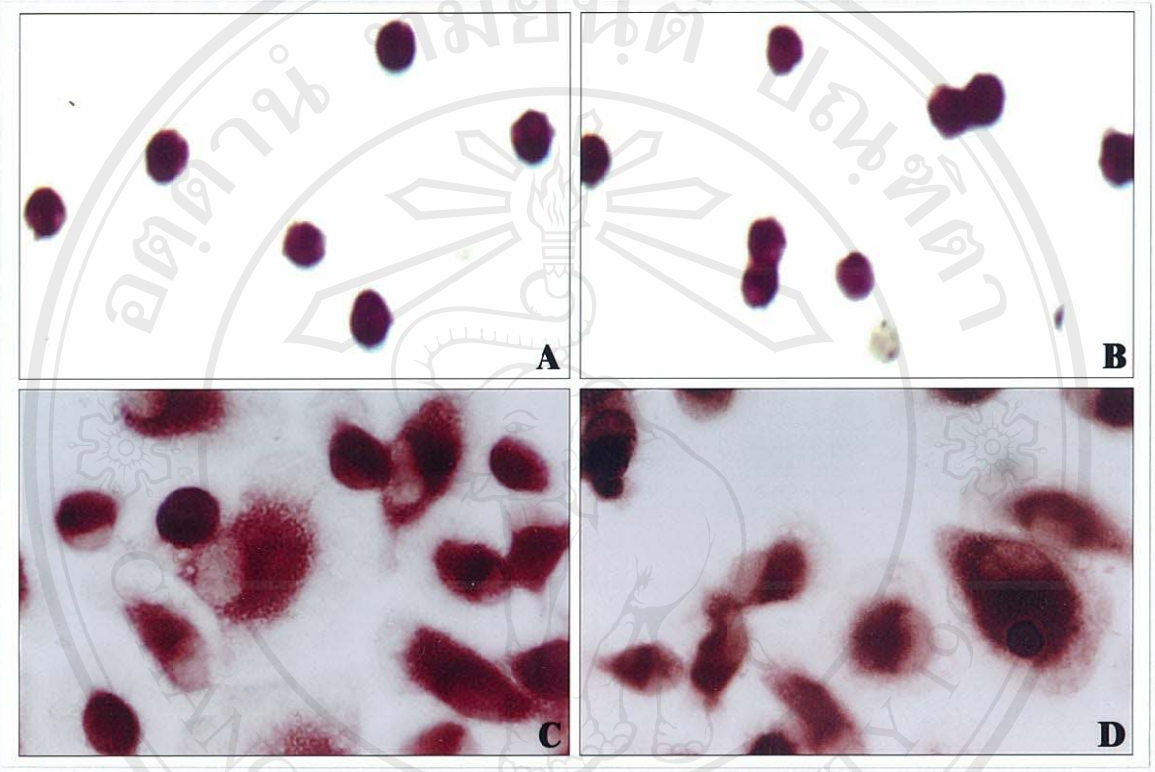

Figure 1. Non-specific esterase positive cells in adherent cells of normal persons (A: day 0, C: day 5) and tuberculosis patients (B: day 0, D: day 5). (Magnification 1000x)

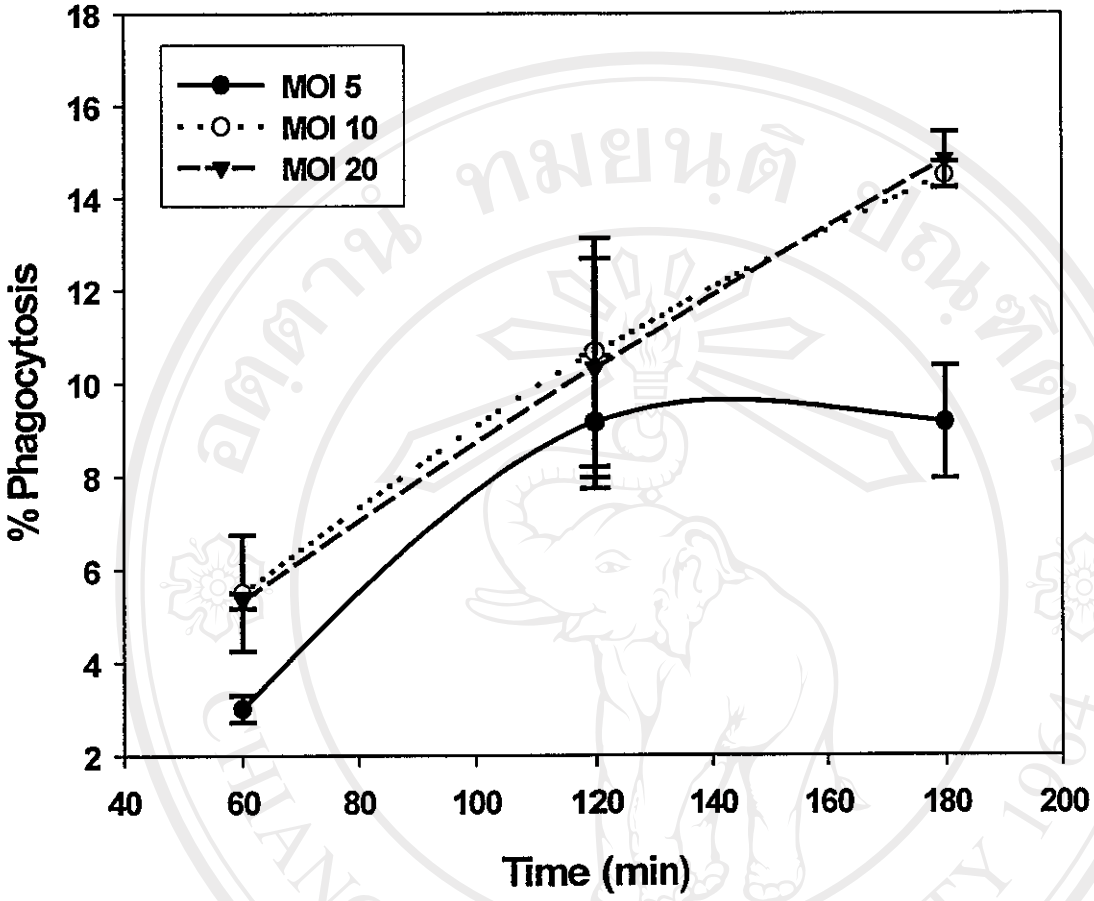

Figure 2. Percent phagocytosis of normal macrophages to *M. tuberculosis* H37Ra in various MOIs and incubation times.

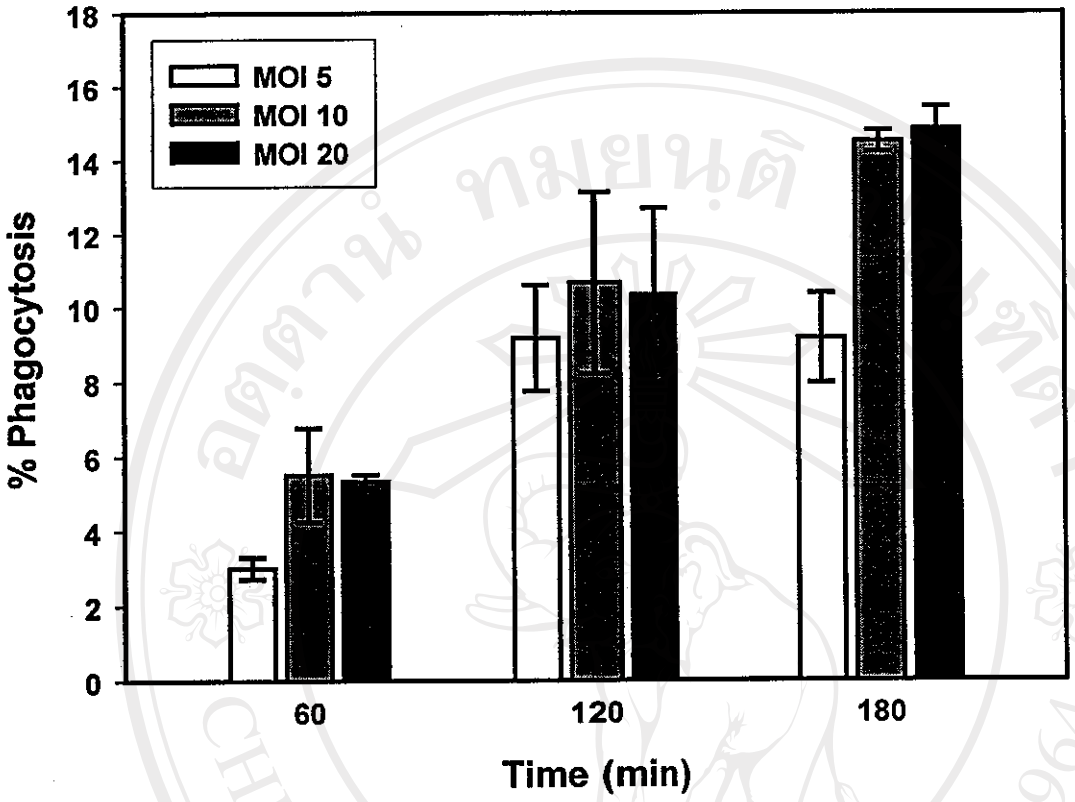

Figure 3. Comparison of percent phagocytosis of macrophages from normal persons to *M. tuberculosis* H37Ra at various MOI and incubation time.

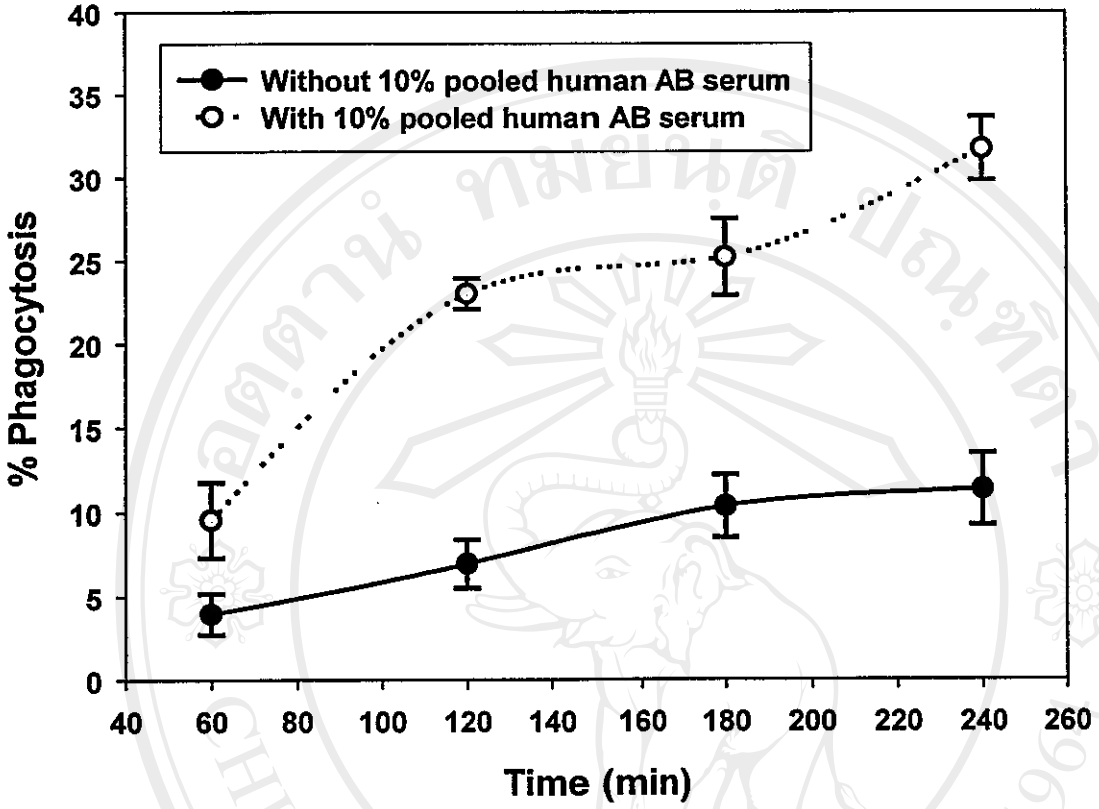

Figure 4. Percent phagocytosis of macrophages from normal persons to *M. tuberculosis* H37Ra in 10 percent pooled human AB serum.

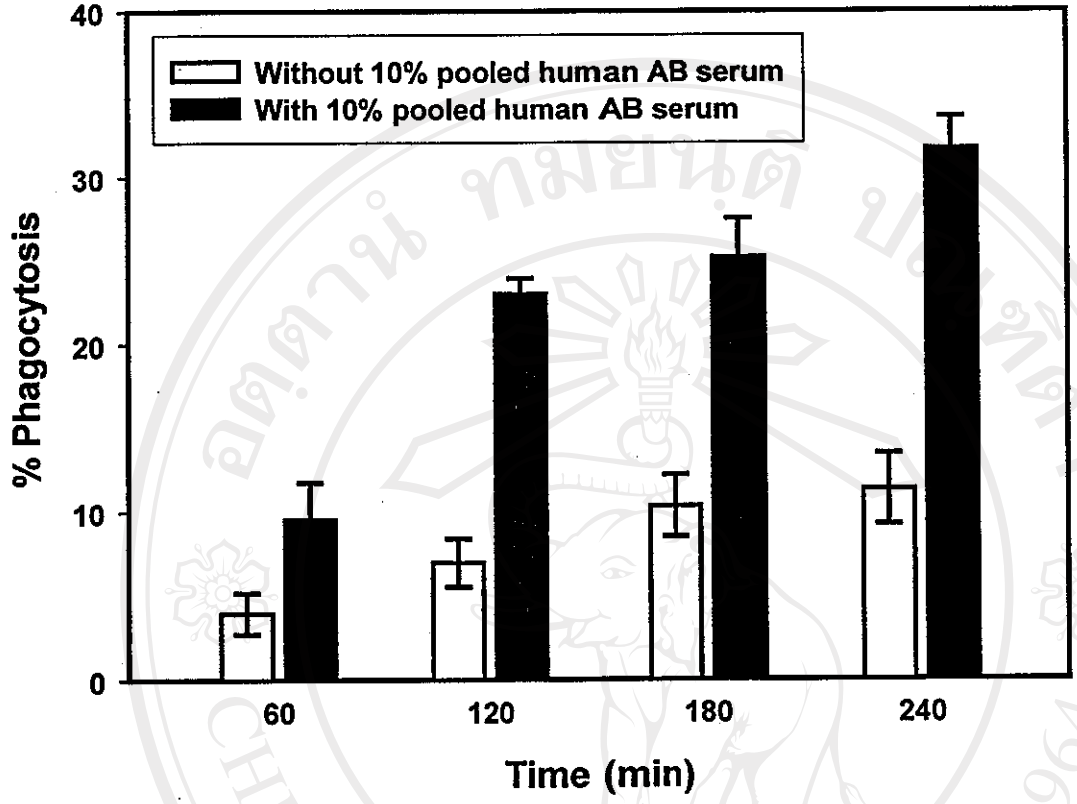

Figure 5. Percent phagocytosis of macrophages from normal persons to *M. tuberculosis* H37Ra in 10 percent pooled human AB serum.

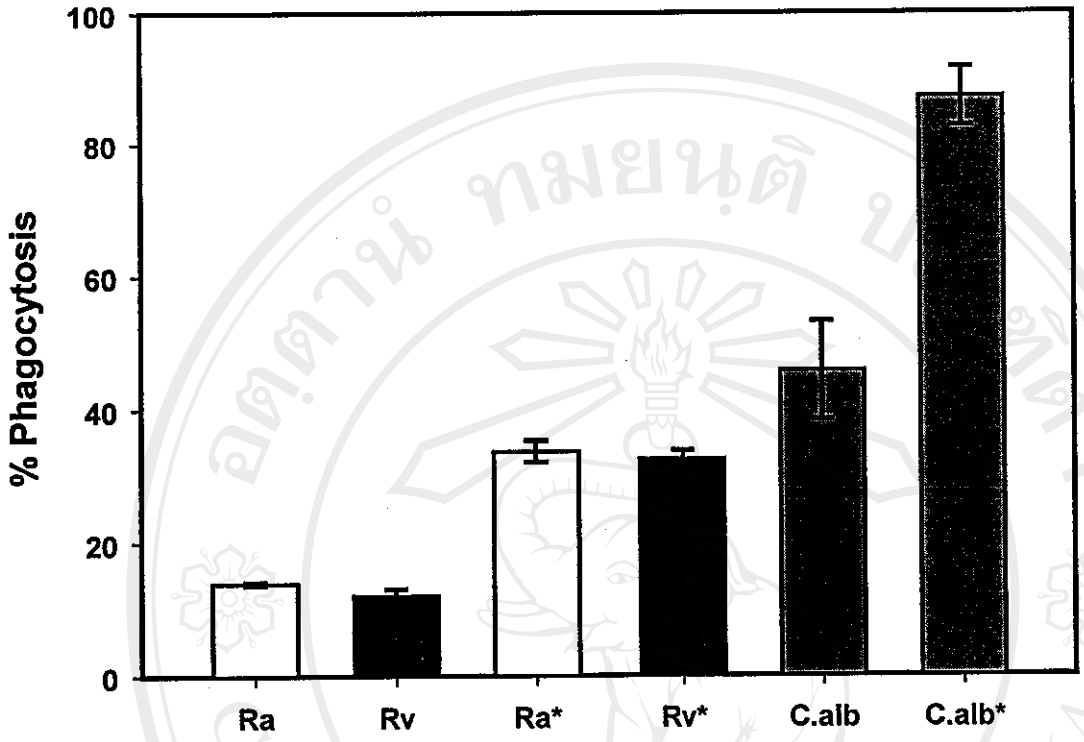

Figure 6. Comparison of percent phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv with or without 10% pooled human AB serum in macrophages of normal persons.

Ra = *M. tuberculosis* H37Ra
Rv = *M. tuberculosis* H37Rv
Ra* = *M. tuberculosis* H37Ra with 10% pooled human AB serum
Rv* = *M. tuberculosis* H37Rv with 10% pooled human AB serum
C.alb = *C. albicans*
C.alb* = *C. albicans* with 10% pooled human AB serum

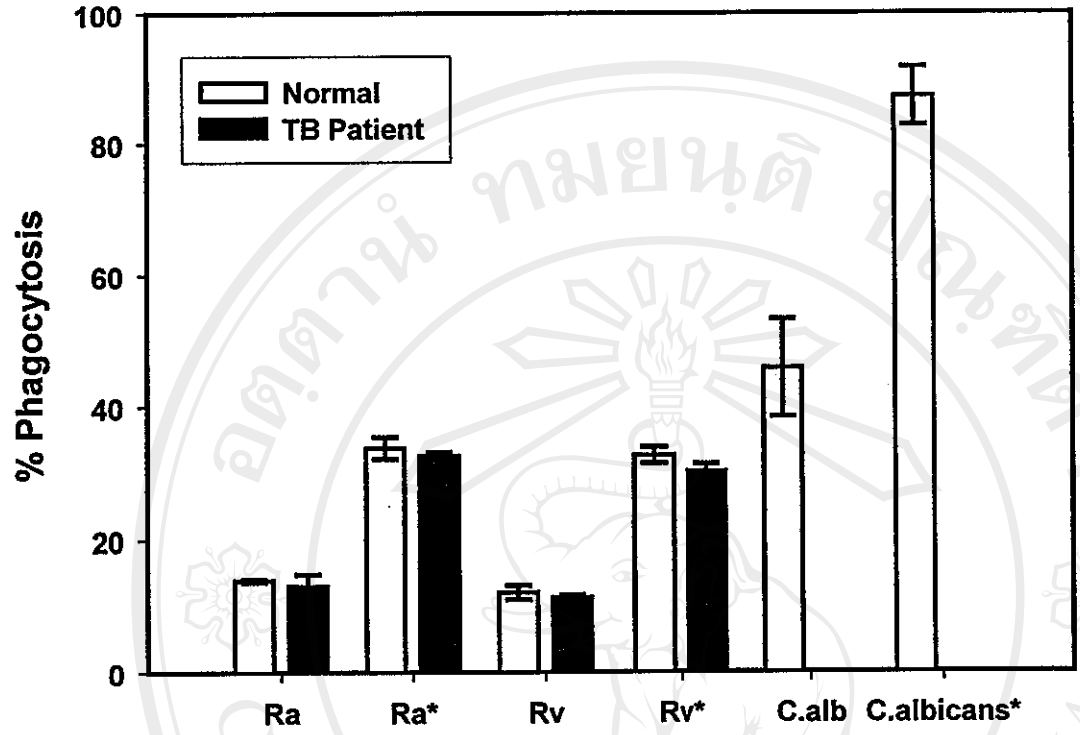

Figure 7. Comparison of percent phagocytosis of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv with or without 10% pooled human AB serum in macrophages of normal persons and tuberculosis patients.

Ra = *M. tuberculosis* H37Ra

Rv = *M. tuberculosis* H37Rv

Ra* = *M. tuberculosis* H37Ra with 10% pooled human AB serum

Rv* = *M. tuberculosis* H37Rv with 10% pooled human AB serum

C.alb = *C .albicans*

C.alb* = *C. albicans* with 10% pooled human AB serum

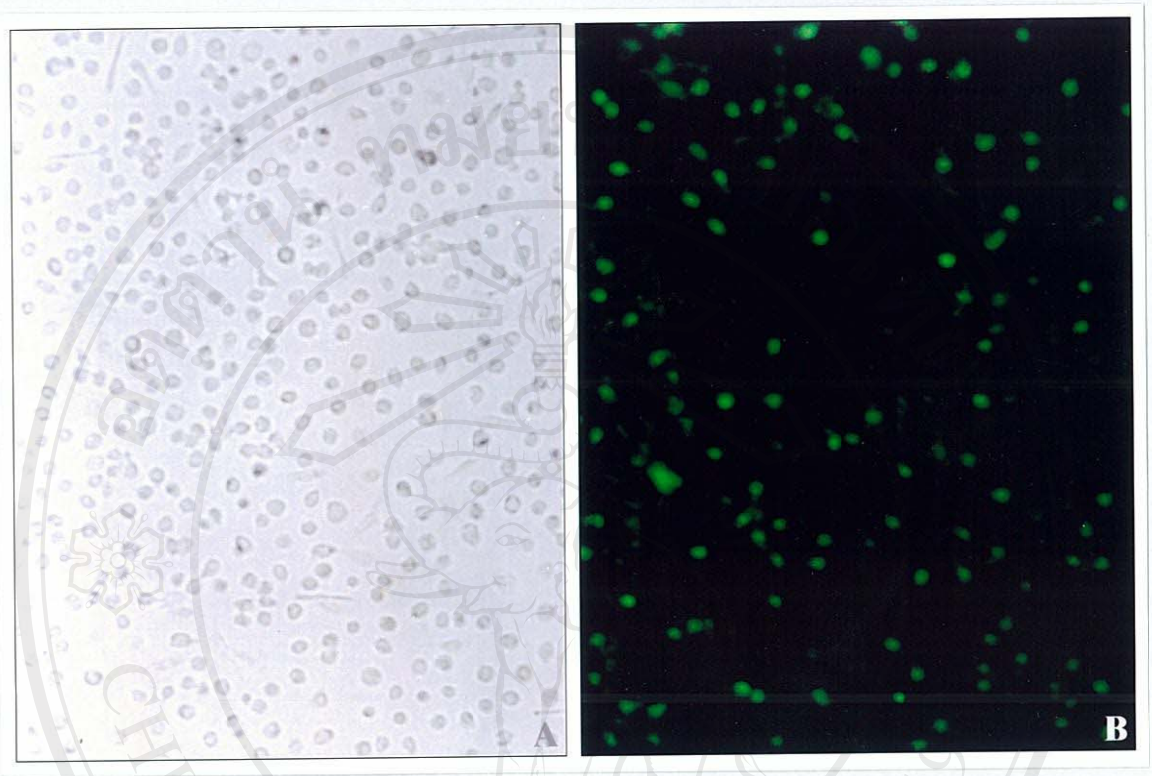

Figure 8. Apoptosis of Actinomycin D (50 μg/ml)-treated macrophages for 19 h. (Magnification 100x)

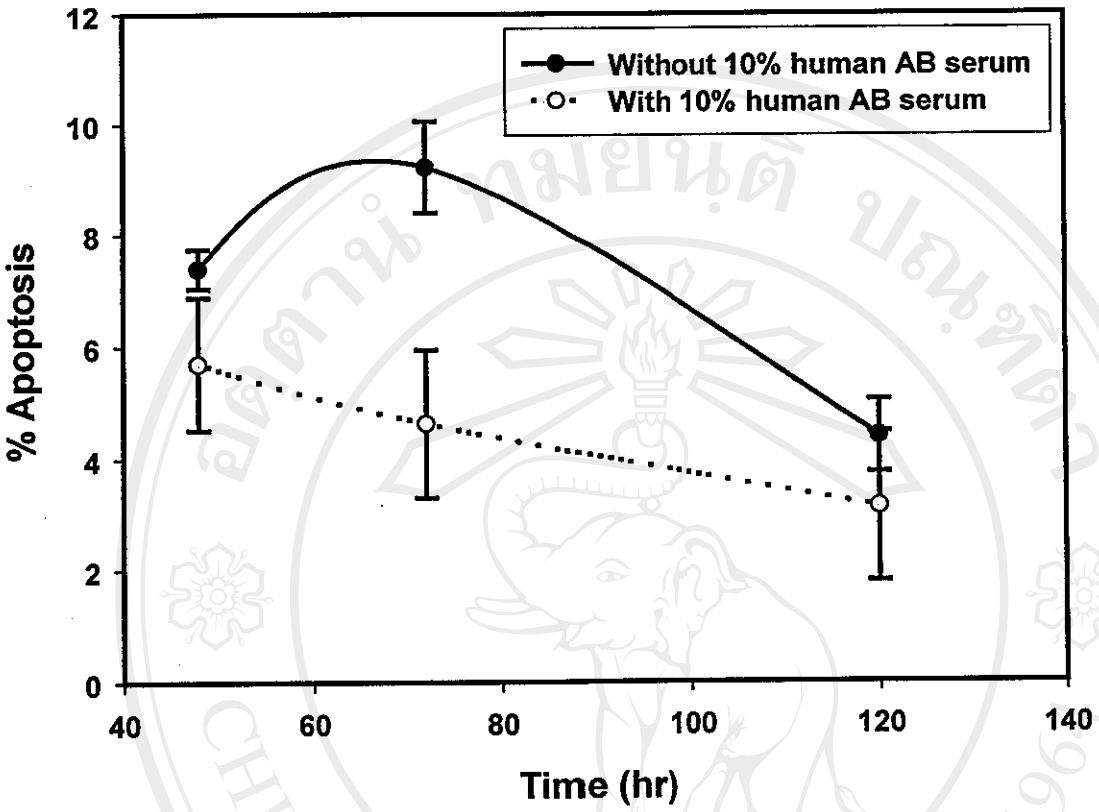

Figure 9. Percent apoptosis of *M. tuberculosis* H37Ra-infected macrophages from normal persons at 48, 72 and 120 h.

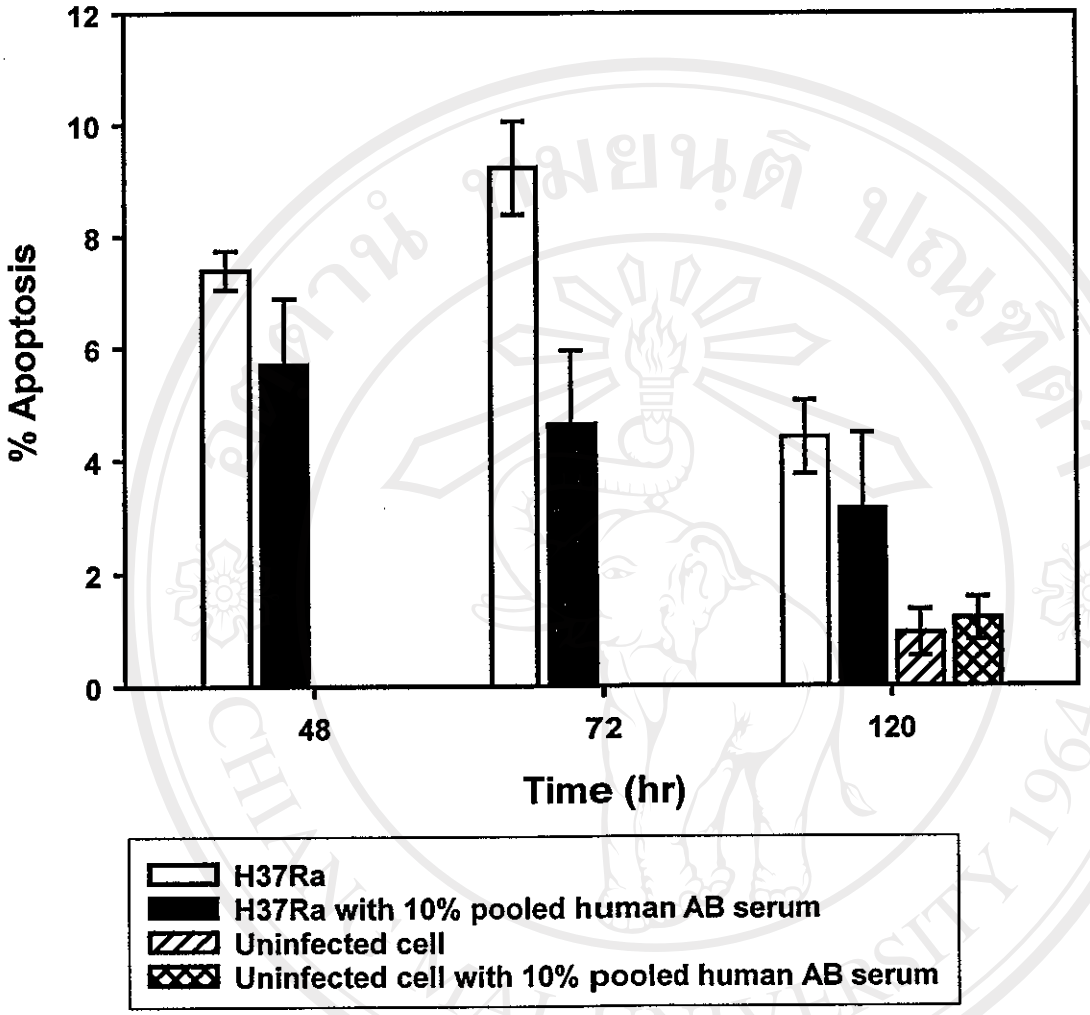

Figure 10. Comparison of percent apoptosis of *M. tuberculosis* H37Ra-infected macrophage from normal persons in 0% and 10% pooled human AB serum.

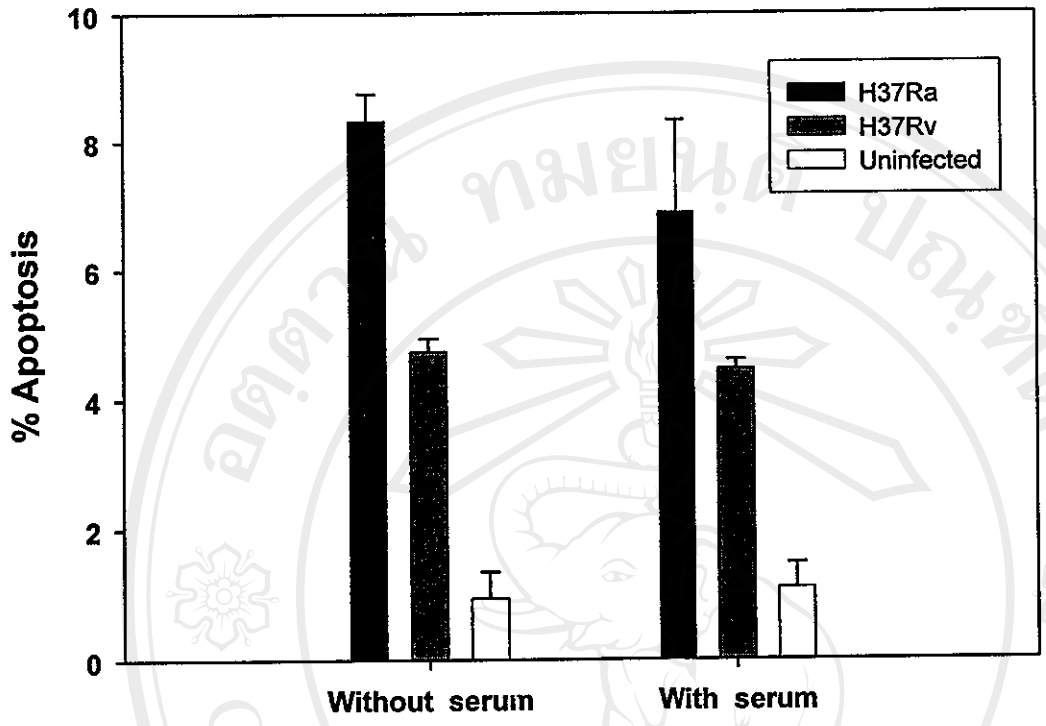

Figure 11. Comparison of percent apoptosis of *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv-infected macrophage in normal persons.

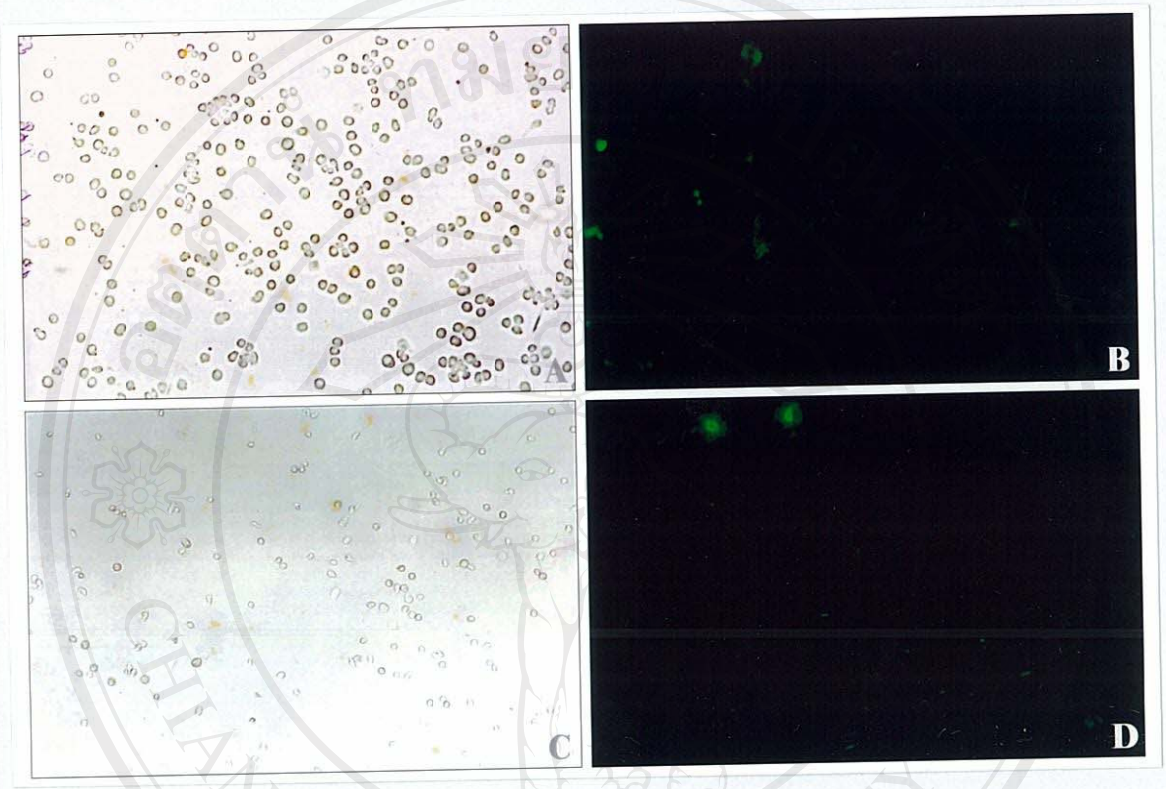

Figure 12. Apoptosis of uninfected macrophages in normal persons without pooled human AB serum (A, B) or with 10% pooled human AB serum (C, D). (Magnification 100x)

A, C = Light microscopy

B, D = Fluorescent microscopy

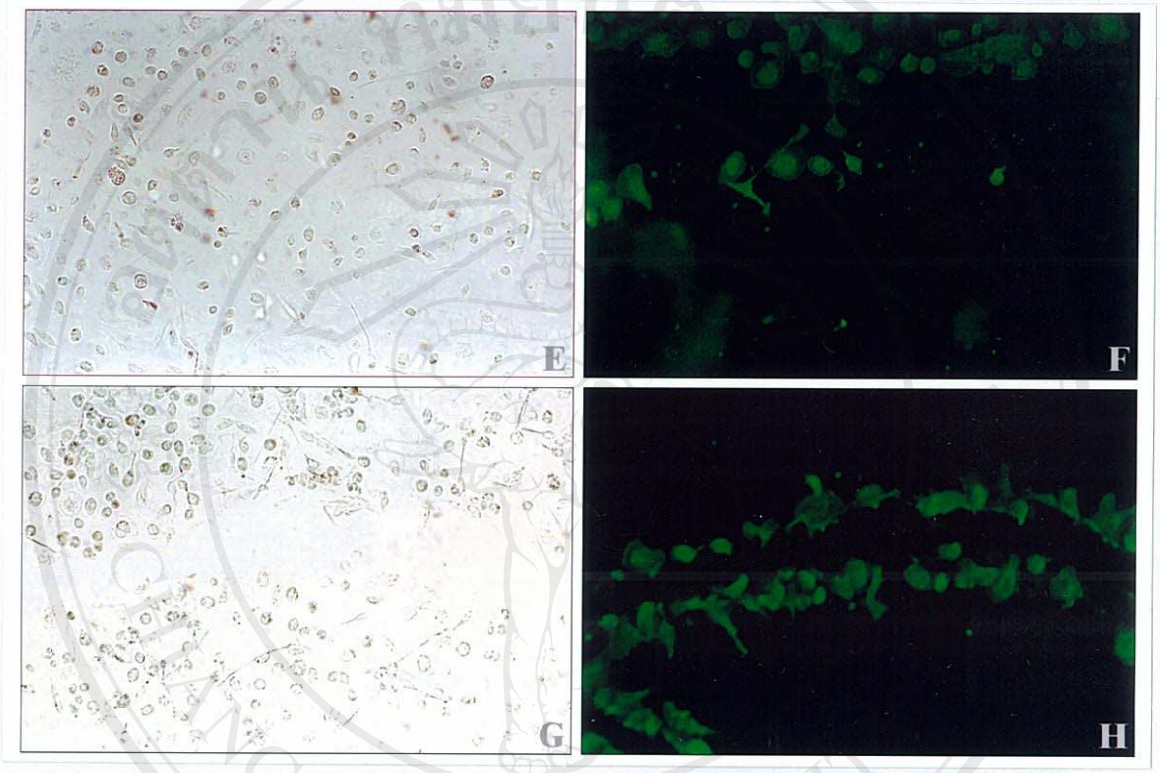

Figure 12 a. Apoptosis of *M. tuberculosis* H37Ra-infected macrophages in normal persons without pooled human AB serum (E, F) or with 10% pooled human AB serum (G, H). (Magnification 100x)

E, G = Light microscopy

F, H = Fluorescent microscopy

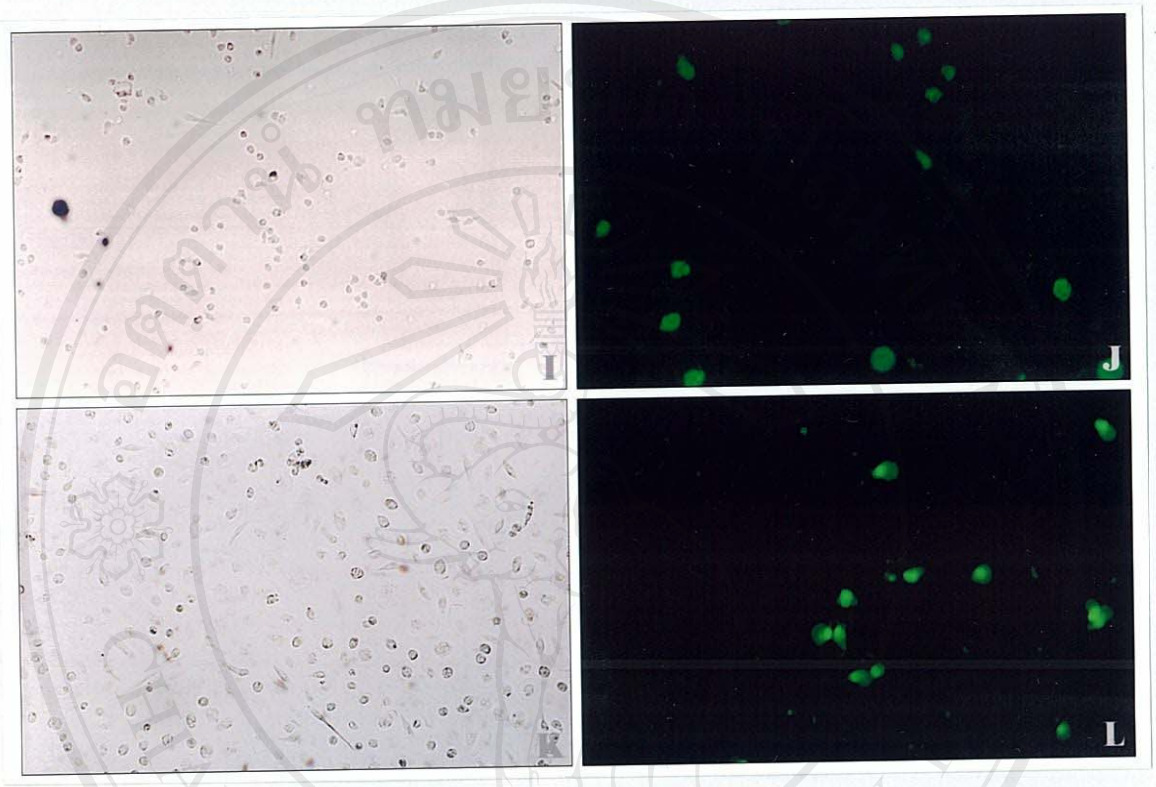

Figure 12 b. Apoptosis of *M. tuberculosis* H37Rv-infected macrophages in normal persons without pooled human AB seru (I, J) or with 10% pooled human AB serum (K, L). (Magnification 100x)

I, K = Light microscopy

J, L = Fluorescent microscopy

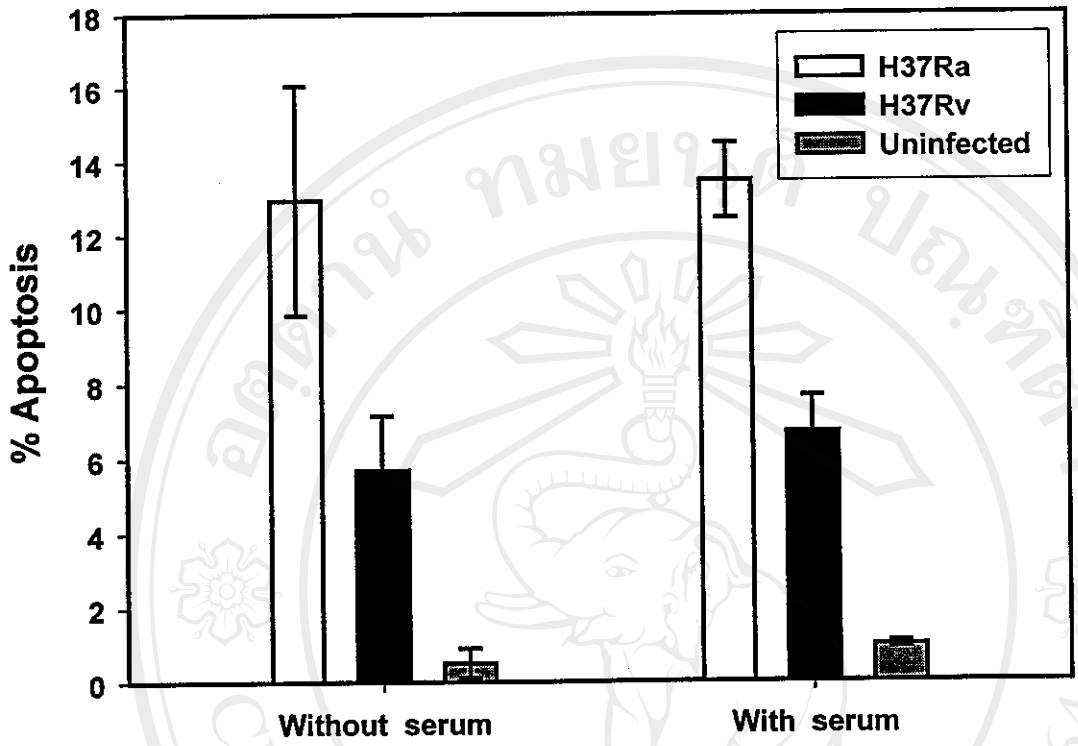

Figure 13. Comparison of percent apoptosis of *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv-infected macrophages in tuberculosis patients.

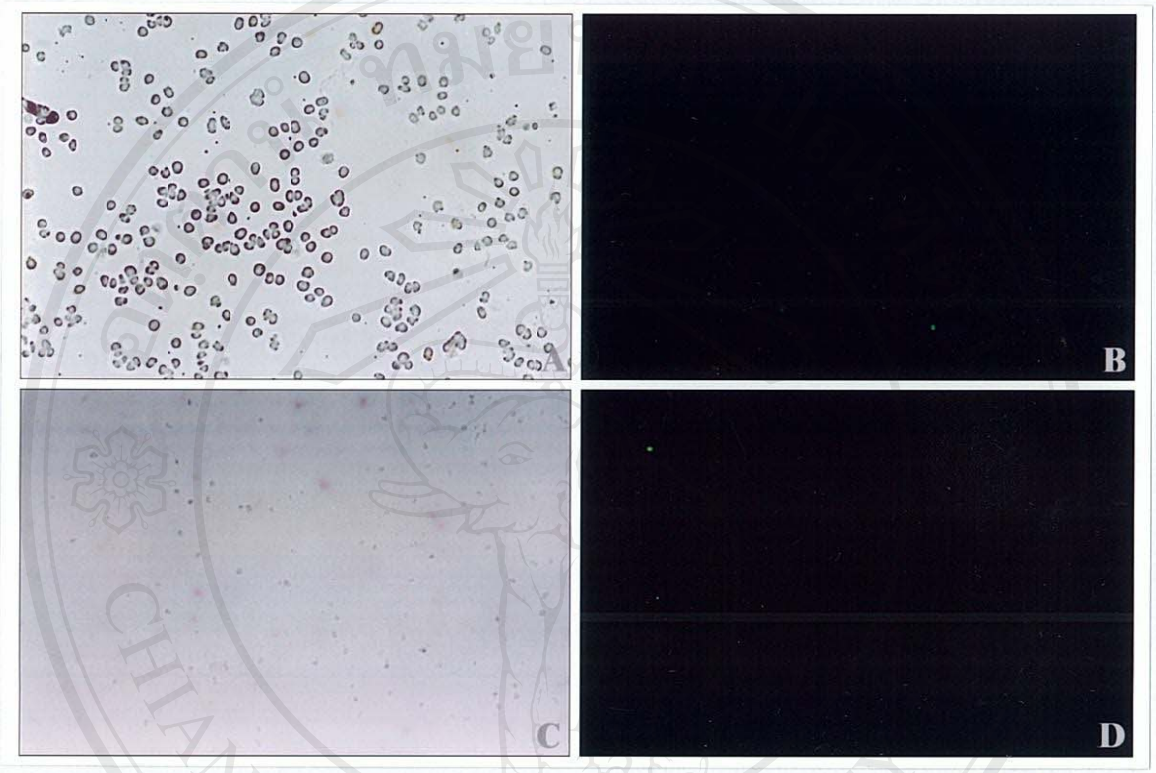

Figure 14. Apoptosis of uninfected macrophages in tuberculosis patients without pooled human AB serum (A, B) or with 10% pooled human AB serum (C, D). (Magnification 100x)

A, C = Light microscopy

B, D = Fluorescent microscopy

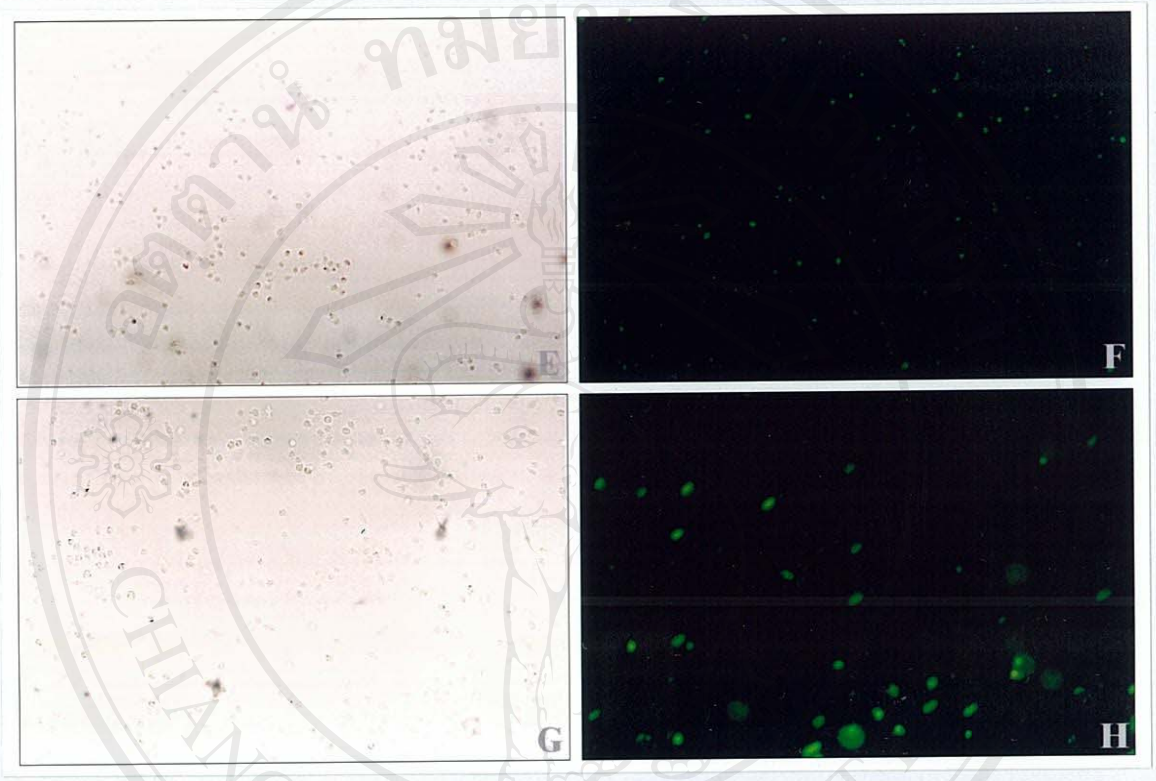

Figure 14 a. Apoptosis of *M. tuberculosis* H37Ra-infected macrophages in tuberculosis patients without pooled human AB serum (E, F) or with 10% pooled human AB serum (G, H). (Magnification 100x)

E, G = Light microscopy

F, H = Fluorescent microscopy

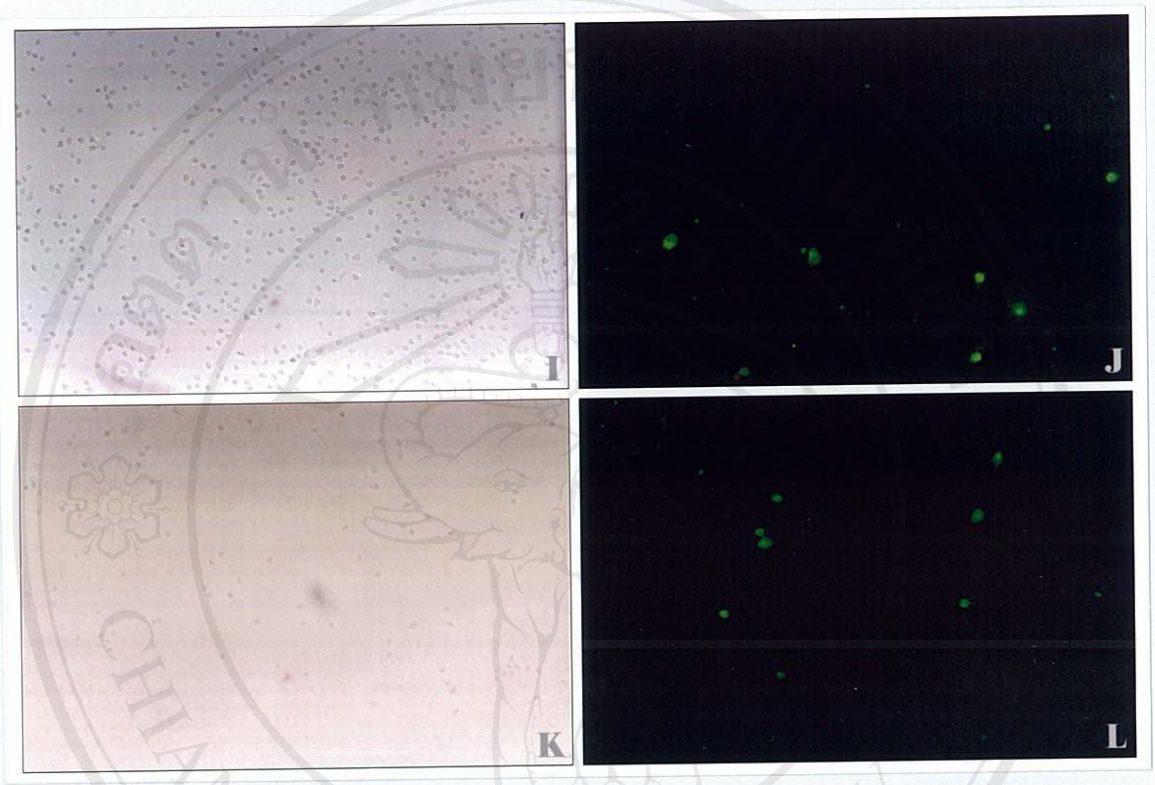

Figure 14 b. Apoptosis of *M. tuberculosis* H37Rv-infected macrophages in tuberculosis patients without pooled human AB serum (I, J) or with 10% pooled human AB serum (K, L). (Magnification 100x)

I, K = Light microscopy

J, L = Fluorescent microscopy

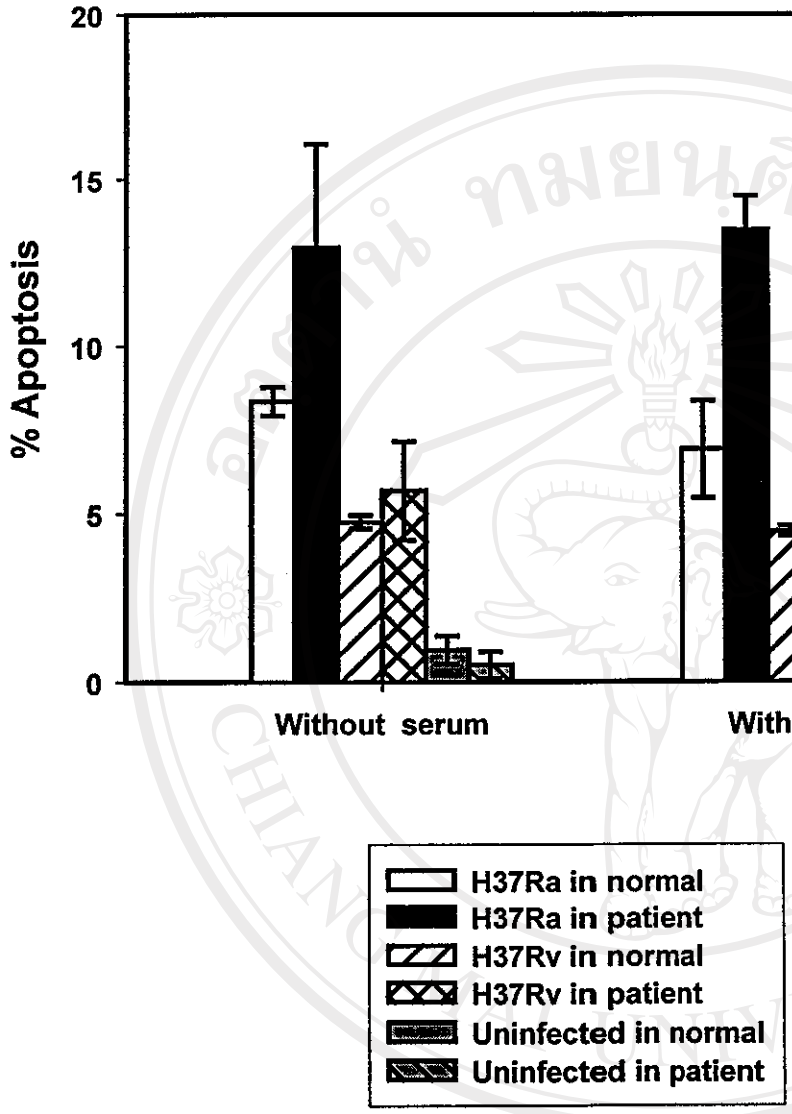

Figure 15. Comparison of percent apoptosis of *M. tuberculosis* H37Ra or *M. tuberculosis* H37Rv-infected macrophages in normal persons and tuberculosis patients.