CHAPTER V

RESULTS

Study of cell growth

Growth of Vero, HEp-2 and Jurkat cells were studied as followed. The seeding density for both Vero and HEp-2 cells was $5x10^4$ cells and $1x10^5$ cells for Jurkat cells. These cells were cultured in 24 well-plate with GM. Cells were counted under the light microscope and cell viability was determined by using tryphan blue staining every 24 hours for six days.

The results suggested that the approximately doubling time were 24 hours for Jurkat and HEp-2 cells and 48-72 hours for Vero cells. (Table 2 and Figure 4) The data represented the results from two independent experiments. Each experiment was done in duplicate.

The confluence monolayer of Vero cells was observed on day 3 after seeding while HEp-2 cells was on day 2. After that, the cells, shape and size, were changed due to the crowded cells. On day 3, HEp-2 cells started to detach form surface because of high cell density. Jurkat cells, which grow as suspension cell culture, the number of cells can incréase up to 10^6 cells/ml. The number of dead cells increased after long term culture. According to Figure 4, the log phase of each cell growth should be within three days after seeding and then the growth of cells were slow down. Therefore, the cells age two to three days old will be used throughout this study.

Table 2. Growth rate of Vero, HEp-2 and Jurkat cells

Day	Number of cells (mean±SD x 10^4)*								
	Vero cell			HEp-2 cell			Jurkat cell		
	Viable	Dead	Total	Viable	Dead	Total	Viable	Dead	Total
0	5	-	5	5	-	5	10	-	10
1	6.67±0.62	0.67±0.47	7.5±0.41	9.67±0.1	0.5±0.4	10.3±0.94	23±2.1	0.75±0.25	23±1.5
2	11.3±0.47	1±0	12.1±0	15±1.4	1.83±0.24	17±1.41	44±2.5	2.3±0.75	46±2.1
3	24±0	2.3±0.23	26±0.47	24.7±0.47	1.83±0.24	26.3±0.47	69±5.2	4.2±0.21	73±5.8
4	30.7±0.94	4.17±0.24	36±0.82	36.3±1.7	4±1.1	40.7±2.62	92±1	5.6±0.17	97±0.58
5	33.3±0.94	7.17±0.24	40.3±0.93	51±2.16	7.5±1.1	58.3±3.1	88±1.0	8.7±1.4	97±1.1
6	47.7±0.47	9.83±0.85	58.7±0.94	45.7±3.3	14.3±2.5	60±2.55	120±5.8	29±0.26	150±5.8

* Data was from two independent experiments (duplication each).

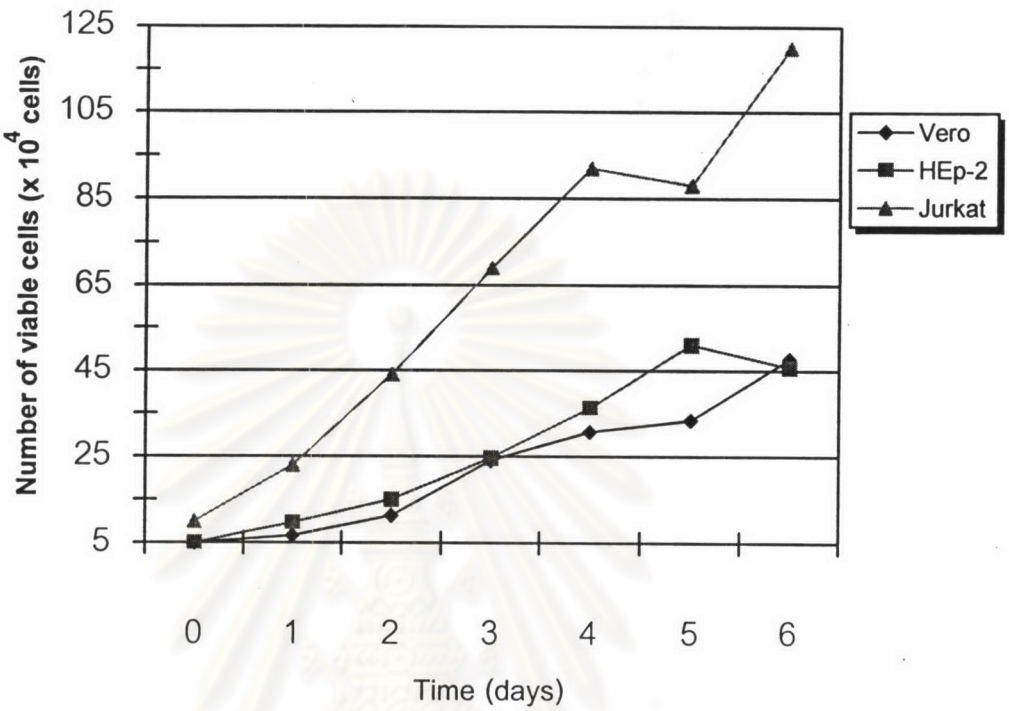


Figure 4. Growth curve of Vero, HEp-2 and Jurkat cells.

HSV replication in Vero, HEp-2 and Jurkat cells

To determine the ability of different cell types in supporting the replication of HSV-1 and HSV-2, Vero, HEp-2 and Jurkat cells were infected with either HSV-1 or HSV-2 at MOI of 0.1, 1 and 5. The viral production in cells and supernatant collected at 12, 24 and 48 h.p.i. were determined by plaque titration assay.

The yield of HSV-1 and HSV-2 production among these three cell types were shown in Table 3 and 4. Vero cells showed highest susceptibility whereas the viruses replicated poorly in Jurkat cells. Virus titers of HSV-1 infected Vero cells in all three MOI reached 10^6 PFU/ml by 24 h.p.i., while one to four $\log_{10}$ step declined in the titers obtained from HEp-2 and Jurkat cells. In this study, the yield productions of both HSV-1 and HSV-2 in Vero cells were shown higher than those in HEp-2 and Jurkat cells. The more inoculum size was added the more viral progenies were produced (Table 3-4 and Figure 5-6). The new virions could be detected at 12 h.p.i. Comparing the efficiency of replication between HSV-1 and HSV-2, HSV-2 consistently was one $\log_{10}$ lower than those obtained from HSV-1 KOS in Vero and HEp-2 cells. Interestingly, the difference was observed in Jurkat cells. The viral yield productions of both HSV-1 and HSV-2 were hardly detected especially at MOI 0.1 and 1 (Figure 5B and 6B) but at MOI 5, HSV-2 could grow better than HSV-1. Moreover, number of viruses held inside Jurkat cells was low compared to those inside Vero and HEp-2 cells (Figure 5-6), except at 48 h.p.i., viruses were found in supernatant more than inside the cells in all cell types. (Table 3 and 4)

Table 3. Efficiency of HSV-1 growth in different cell types at various MOI

Cell type	MOI (PFU/ml)	Hours post infection	Amount of viruses ($x10^4$ PFU/ml)*		
			Inside cell	Supernatant	Total
Vero	0.1	12	1.36	0.10	1.46
		24	67.5	41.8	109.3
		48	570	613	1183
	1	12	63	9	72
		24	149	60.3	209.3
		48	647	697	1344
	5	12	71.2	11.7	82.9
		24	228	210	438
		48	1150	1750	2900
HEp-2	0.1	12	1.56	0.06	1.62
		24	1.24	1	2.24
		48	3.93	146	150
	1	12	9.5	0.33	9.83
		24	52.3	1.83	54.13
		48	61.9	445	506.9
	5	12	21	2.35	23.35
		24	74.3	14	88.3
		48	105	231	336
Jurkat	0.1	12	0.0005	0.0005	0.0010
		24	0.0095	0.0314	0.0409
		48	0.0311	0.0475	0.0786
	1	12	0.0010	0.0151	0.0161
		24	0.0312	0.1150	0.1462
		48	0.1410	0.1840	0.3250
	5	12	0.0022	0.0289	0.0311
		24	0.0412	0.5230	0.5642
		48	0.3610	0.6610	1.0220

* Data was mean average from two independent experiments

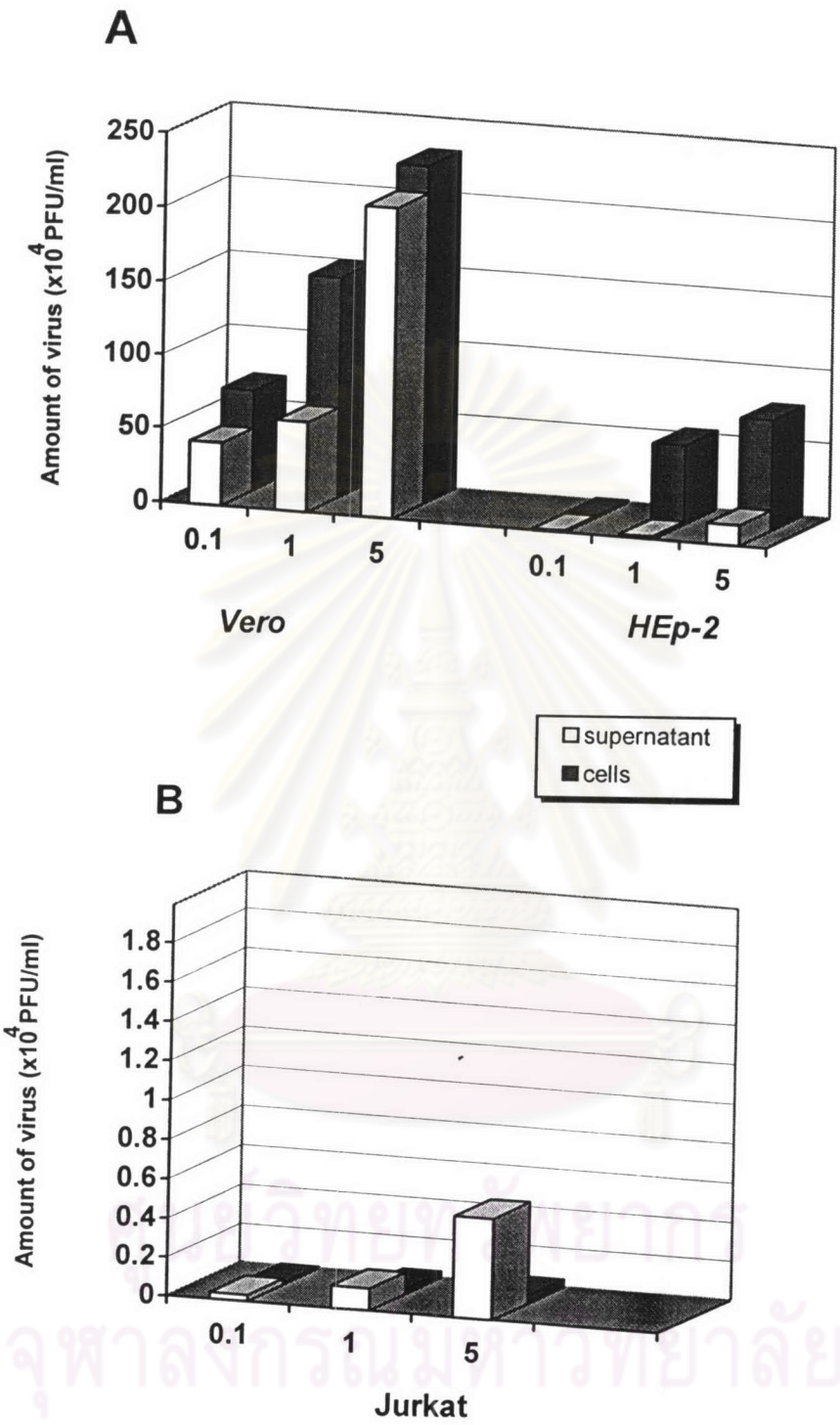


Figure 5. The amount of HSV-1 produced in Vero, HEp-2 cells (A) and Jurkat cells (B) from cells and supernatant at 24 h.p.i.

Table 4. Efficiency of HSV-2 growth in different cell types at various MOI

Cell type	MOI (PFU/ml)	Hours post infection	Amount of viruses ($x10^4$PFU/ml)*		
			Inside cell	Supernatant	Total
Vero	0.1	12	2.09	0.0550	2.14
		24	16.4	1.65	18.05
		48	36.3	61.3	97.6
	1	12	3.5	0.075	3.575
		24	33.2	3.46	36.66
		48	75.6	93	169
	5	12	5.14	2.06	7.2
		24	125	4.95	130
		48	139	149.5	288
HEp-2	0.1	12	0.0650	0.0075	0.0725
		24	0.345	0.0099	0.355
		48	10	60	70
	1	12	0.222	0.0175	0.239
		24	1.13	0.0230	1.15
		48	28.1	50	78.1
	5	12	0.59	0.0725	0.621
		24	1.99	0.27	2.26
		48	26.9	65.8	92.7
Jurkat	0.1	12	0.0037	0.0068	0.0105
		24	0.0015	0.0920	0.0935
		48	0.0032	0.0900	0.0932
	1	12	0.0050	0.0070	0.0120
		24	0.0294	0.1150	0.1440
		48	0.0170	0.2150	0.2320
	5	12	0.0055	0.0327	0.0382
		24	0.1150	1.43	1.545
		48	0.1390	1.53	1.669

* Data was mean average from two independent experiments

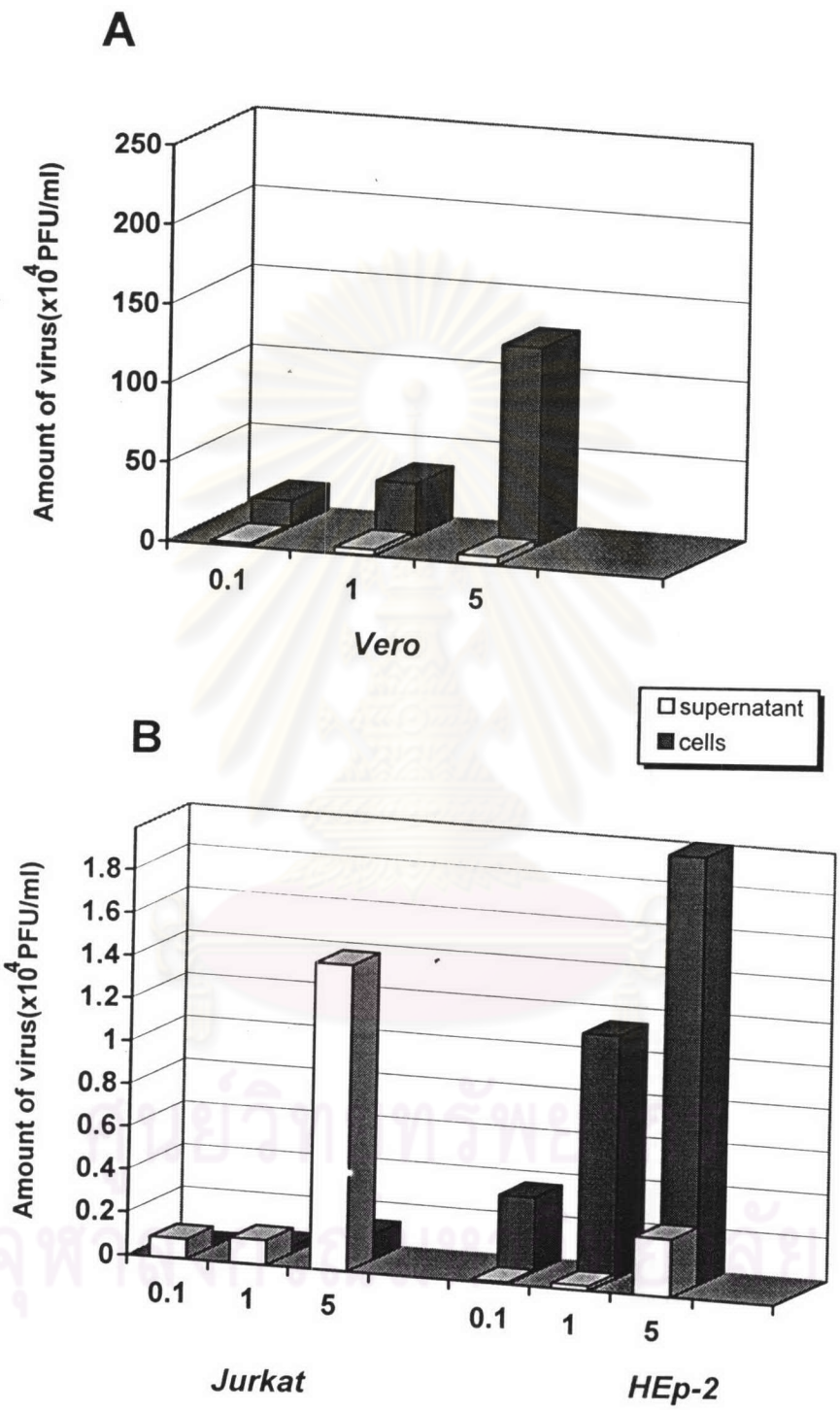


Figure 6. The amount of HSV-2 produced in Vero (A), HEp-2 cells and Jurkat cells (B) from cells and supernatant at 24 h.p.i.

Viral replication in PHA-activated Jurkat cells

It has been shown that PHA activation enhances viral growth in peripheral blood leukocytes (22), therefore to determine whether Jurkat cells activated by PHA can efficiently support viral growth. The Jurkat cells were activated with PHA for one, two and three days. The activated cells were then infected with either HSV-1 or HSV-2 at inoculum size of MOI = 5. The number of cells began at $1x10^6$ cells. After adsorption, the virus was allowed to replicate in culture fed with MM. Viral production in cells and supernatant were determined by plaque titration assay at 24 and 48 h.p.i. The viral production could be demonstrated within 24 h.p.i in one day stimulated Jurkat cells (Table 5). Pre-activated Jurkat of one, two or three days did not change the yield of HSV-1 production but the longer pre-activation of Jurkat cells the higher amount of HSV-2 production was shown (Table 5).

To compare the viral production in PHA-activated Jurkat cells with Vero, HEp-2 and non-activated Jurkat cells, $2x10^5$ starting cells and MOI 5 of inoculum size were used. The results in Table 6 showed that after PHA activation, the ability of Jurkat cells to support HSV-1 replication was 12 and 37 times greater than non-activated cells at 24 and 48 h.p.i. Furthermore, the new HSV-1 viruses remained inside the cells similar to Vero and HEp-2 cell. However, the yield of HSV-2 production was lower than HSV-1 and no difference in viral growth efficiency between PHA-activated cells and Jurkat cells was observed (Figure 7).

Table 5. Efficiency of HSV-1 and 2 growth in PHA activated Jurkat cells ($1x10^6$cells) at MOI=5

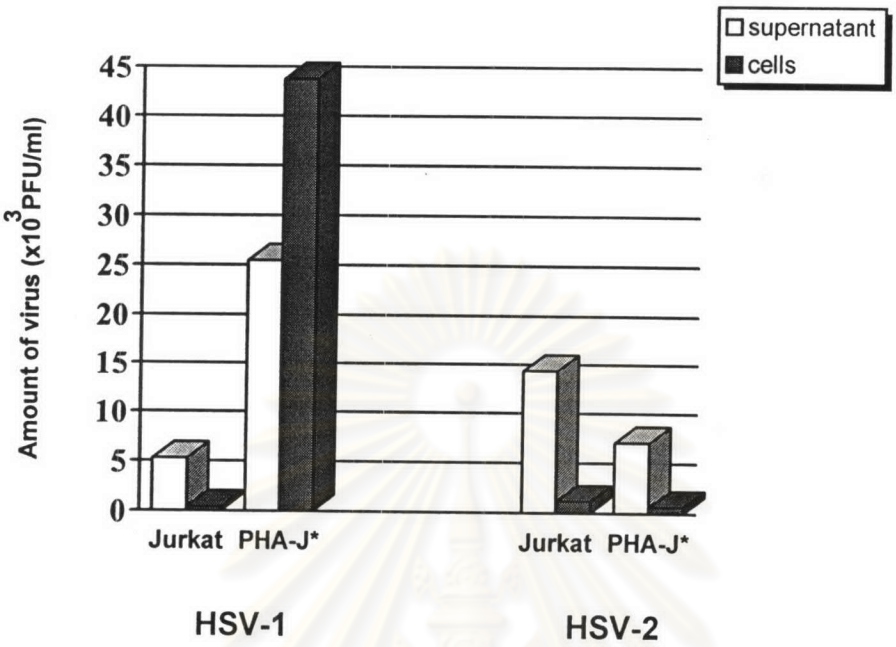
HSV type	Day after activation	Hours post infection	Amount of viruses ($x10^4$PFU/ml)*		
			Inside cell	Supernatant	Total
HSV-1	1	24	75.75	406.5	482.25
		48	200	387.5	587.5
	2	24	152	255	407
		48	89.7	335	424.7
	3	24	123	190	313
		48	125	328	453
HSV-2	1	24	0.5	13.5	14
		48	0.5	24.25	24.75
	2	24	1.5	30.25	31.75
		48	1	67.5	68.5
	3	24	0.5	80.0	80.5
		48	9.65	650	660

*Data was mean average from two independent experiments.

Table 6. Efficiency of HSV-1 and HSV-2 growth in normal Jurkat and PHA-activated Jurkat cells at MOI = 5 and $2x10^5$ starting cells

Cell type	Type of virus	Hours post infection	Amount of viruses ($x10^3$PFU/ml)*		
			Inside cell	Supernatant	Total
Jurkat	HSV-1	24	0.412	5.23	5.642
		48	3.61	6.61	10.22
	HSV-2	24	1.15	14.3	15.45
		48	1.39	15.3	16.69
PHA-activated Jurkat	HSV-1	24	43.75	25.5	69.25
		48	207.25	175	382.25
	HSV-2	24	0.625	5	5.625
		48	1.875	7	8.875

* Data was mean average from two independent experiments



* PHA-J = PHA-activated Jurkat cells

Figure 7. The amount of HSV-1 and HSV-2 produced in Jurkat and PHA activated Jurkat cells in cells and supernatant at 24 h.p.i.

The kinetic expression of HSV proteins

HSV protein expression

The above observation indicated that ability of each cell type to support HSV replication was different. This fact might be due to, different cell types have different factors in promoting viral growth. Thus, the kinetics study of HSV-1 and HSV-2 protein synthesis in HEp-2 and Jurkat infected cells were performed by mean of the indirect immunofluorescence assay. Here, the polyclonal antibodies to HSV-1, HSV-2 and HSV immediately early protein, ICP0, ICP22 and ICP47 were used. The amount of HSV inoculum throughout this study was at MOI 5. After inoculation, cells were collected at two, four, six, nine and 24 h.p.i. The infected cells were observed, scored and recorded for fluorescent pattern, localization and intensity of the fluorescent-conjugated antiserum. The staining patterns were defined as peripheral, granular, filamentous, perinuclear, cytoplasmic, diffuse, periphery membrane and whole cell staining shown in Figure 8.

Using rabbit anti HSV-1 and HSV-2, fluorescent staining in HEp-2 cells could be seen at two hours with granular pattern on cell membrane [Figure 9(a)]. After four hours viral protein located mostly in nucleus (rarely in cytoplasm), and at six hours, they appeared in both nucleus and cytoplasm. At nine hours, the staining was found in nucleus, cytoplasm and membrane [Figure 9(a-h)]. The pattern of staining did not significantly differ between HSV-1 and HSV-2 infected cells except at six hours, proteins located mostly in cytoplasm and the staining intensity in HSV-1 infected cells was brighter than that in HSV-2 infected cells. In addition, the number of positive cells observed in HSV-1 infected HEp-2 cells was more than those in HSV-2 infected cells (75% vs 50% at nine hours). At 24 h.p.i., the infected cells were detached from the cover slip and lysed therefore, no data was available, assuming 100 percentage of cells were infected.

The protein expression observed in HSV-infected Jurkat cells was slightly distinct from those described in HEp-2 cells (Figure 10). At four h.p.i., the staining still located mainly in nucleus with high intensity and some staining appeared on cell membrane, none in cytoplasm part. The fluorescent staining in cytoplasm was shown at six h.p.i. It seemed that HSV multiplication step in Jurkat cell delayed at least two hours comparing to that in HEp-2 cells. The pattern of HSV staining between

HSV-1 and HSV-2 infected Jurkat cells were not different unless the low intensity was observed in HSV-2 infected cells. At 24 h.p.i., the number of HSV-infected Jurkat cells was lower than in HEp-2 (10-20% vs 100%).

Previous experiment indicated that PHA-activated Jurkat cells supported growth of HSV better than non-activated cells. Here, the kinetic HSV protein expression in PHA-activated Jurkat cells revealed the same finding as in HEp-2 infected cells (Figure 10). Increasing of the number of HSV-infected PHA-activated Jurkat cells was demonstrated approximately 50% although it was still lower than that of HEp-2 cells (100%).

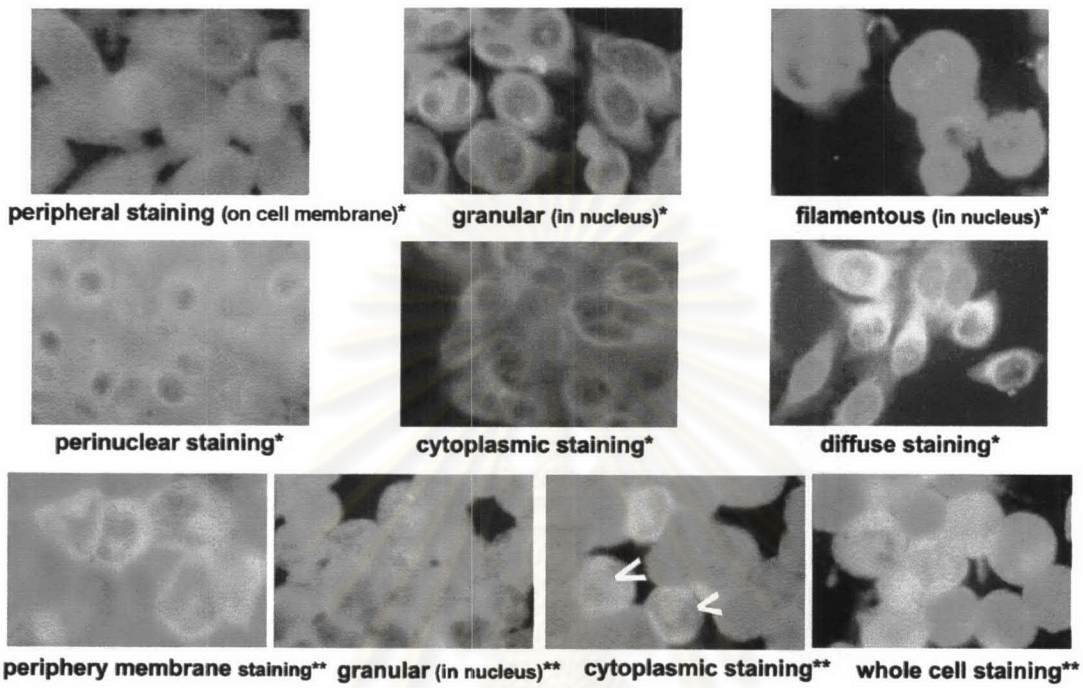


Figure 8. The staining patterns of HSV-1 and HSV-2 infected cells.

* HEp-2 infected cells, magnify 400X

** Jurkat infected cells, magnify 1000X

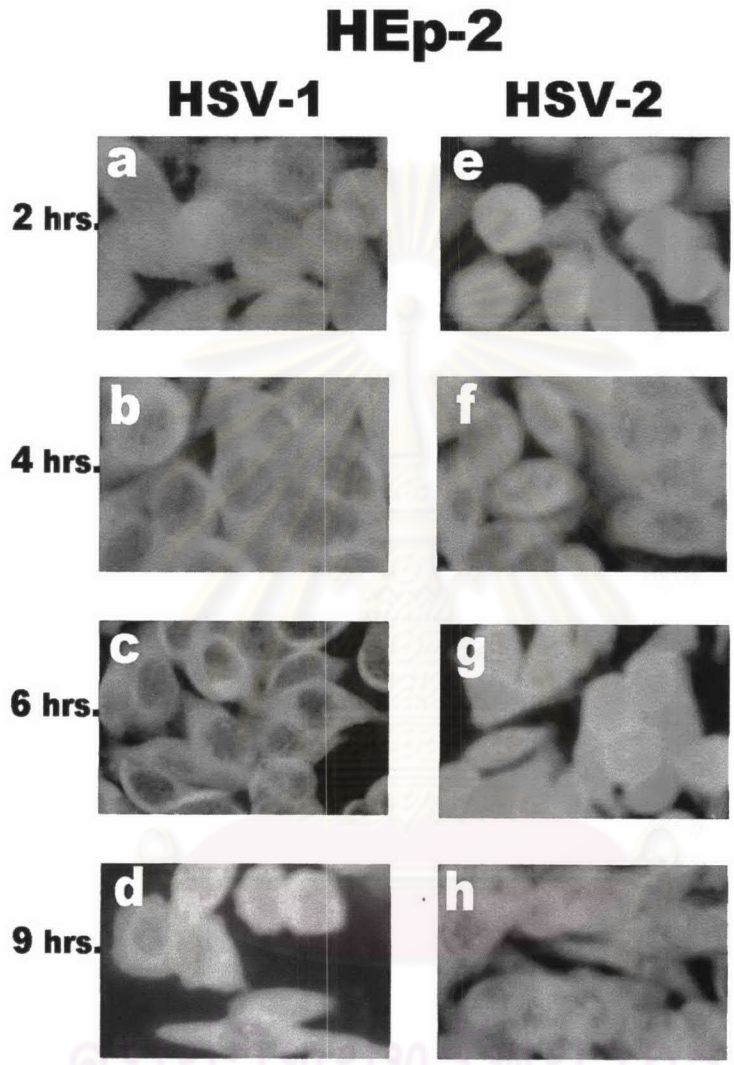


Figure 9. Fluorescent staining of HSV-1 infected HEp-2 cells at 2 (a), 4 (b), 6 (c), 9 (d) and HSV-2 infected HEp-2 cells at 2 (e), 4 (f), 6 (g), and 9 h.p.i. (h) at MOI of 5, using rabbit anti HSV-1 and HSV-2 antibodies, respectively at magnification of 400X.

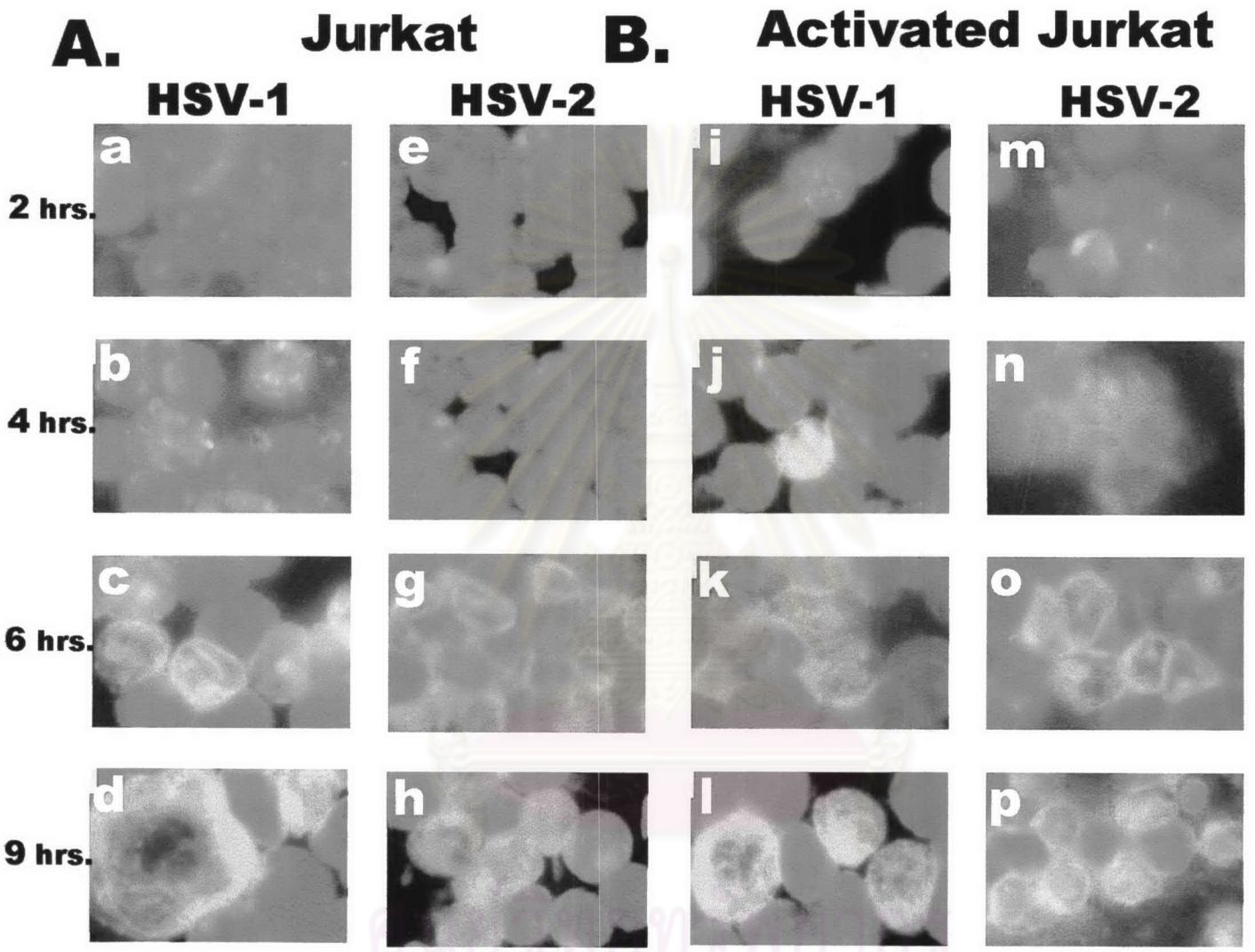


Figure 10. Fluorescent staining of HSV-1 or HSV-2 infected Jurkat (A) and PHA-acitvated Jurkat (B) cells at MOI 5 using rabbit anti HSV-1 and HSV-2 antibodies at 2, 4, 6 and 9 h.p.i. at magnification of 1000X.

Immediately HSV protein expression

The data obtained from the study of HSV protein expression suggested that the HSV replication cycle in Jurkat cells may be delayed compared to that in HEp-2 cells. It is known that HSV IE proteins are the first group of proteins can be expressed and play role in controlling HSV replication cycle. Thus, kinetic expression of three IE proteins, ICP0, ICP22 and ICP47 were done to see whether the delayed replication in Jurkat cells was caused by the delayed expression of IE proteins or not.

The results revealed that the appearance of those three IE proteins are different among HEp-2, Jurkat and PHA-activated Jurkat cells (Table 7, Figure 11,12 and 13). All three IE proteins were detected within two to four hours in HSV-1 infected cells whereas the delayed expression of those proteins (six hours) were observed in HSV-2 infected cells, ICP0 in PHA activated Jurkat cells; ICP22 in Jurkat cells; ICP47 in HEp-2 cells. As all these three proteins mainly function in nucleus part, the time of locating in nucleus was recorded (Table 8). The time of appearance was mostly the same as the time observed in nucleus except some of them was two hours delayed, ICP22 and ICP47 in HSV-1 infected Jurkat and PHA-activated Jurkat cells and ICP22 in HSV-2 infected HEp-2 cells. It was very surprised to see that ICP47 could not be detected after four hours only in HSV-2 infected PHA-activated Jurkat cells [Figure 13 (3a-d)] while the rest of it in the other cells was still remained. The intensity of IE protein staining reduced after nine hours in both HSV-1 and HSV-2 infected HEp-2 cells while in Jurkat and PHA-activated Jurkat cells, it was still high intensity (except ICP47 of HSV-2 infected Jurkat and PHA-activated Jurkat cells).

Table 7. Timing of the detected HSV-proteins (ICP0, ICP22, ICP47) in three different cell types

	HSV-1 (hours)			HSV-2 (hours)		
	HEp-2	**Jurkat**	**PHA-J**	**HEp-2**	**Jurkat**	**PHA-J**
ICP0	4	2	2	2	2	6
ICP22	2	4	4	4	6	6
ICP47	2	2	2	6	6	4

Table 8. Timing of detecting HSV-proteins located in nucleus

	HSV-1 (hours)			HSV-2 (hours)		
	HEp-2	**Jurkat**	**PHA-J**	**HEp-2**	**Jurkat**	**PHA-J**
ICP0	4	2	2	2	2	6
ICP22	2	6	6	6	6	6
ICP47	2	4	4	6	6	4

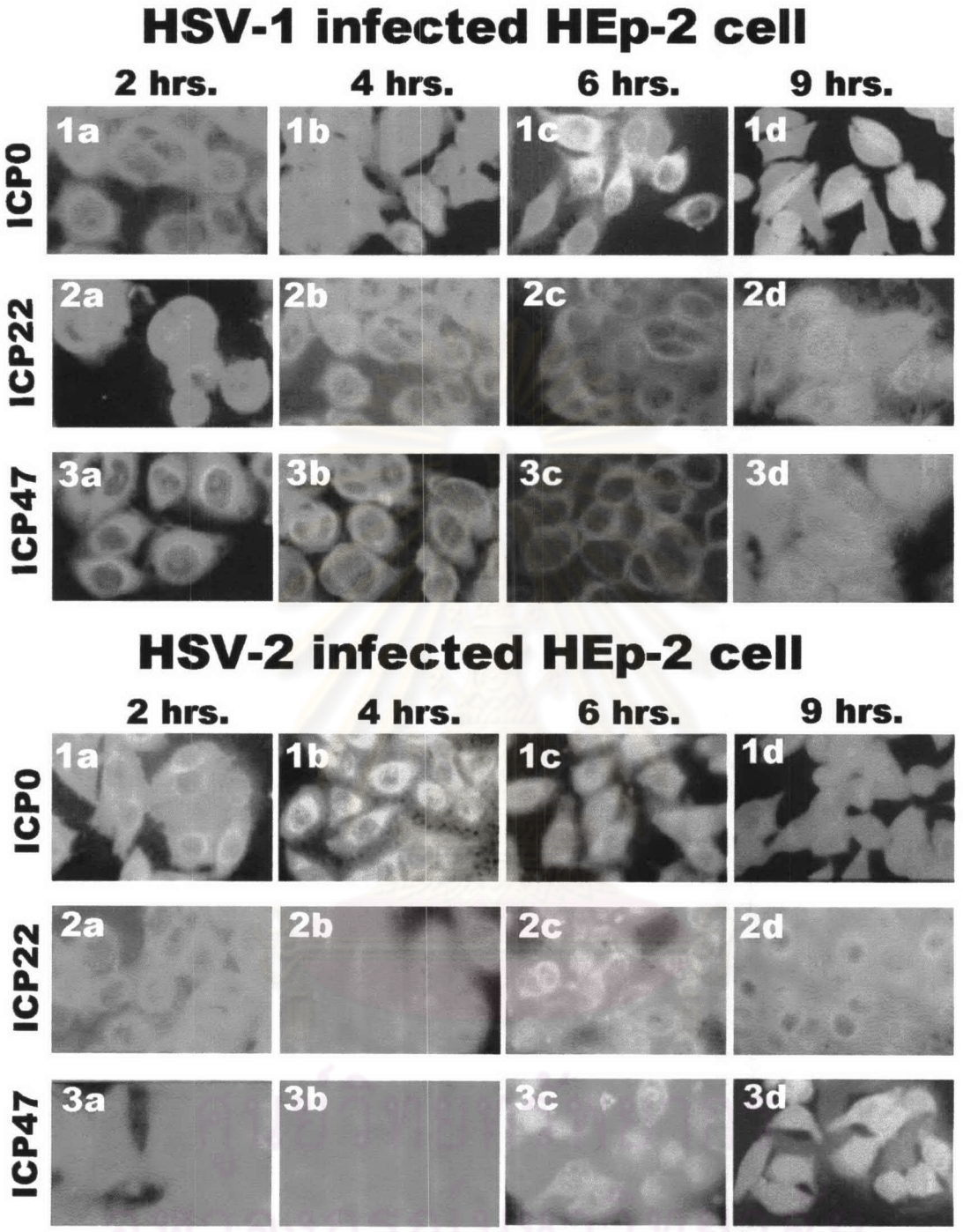


Figure 11. Fluorescent staining of HSV-1 or HSV-2 infected HEp-2 cells at 2, 4, 6, and 9 h.p.i. at MOI 5, using rabbit anti HSV ICP0, ICP22 and ICP47 antibodies, respectively (magnify of 400X).

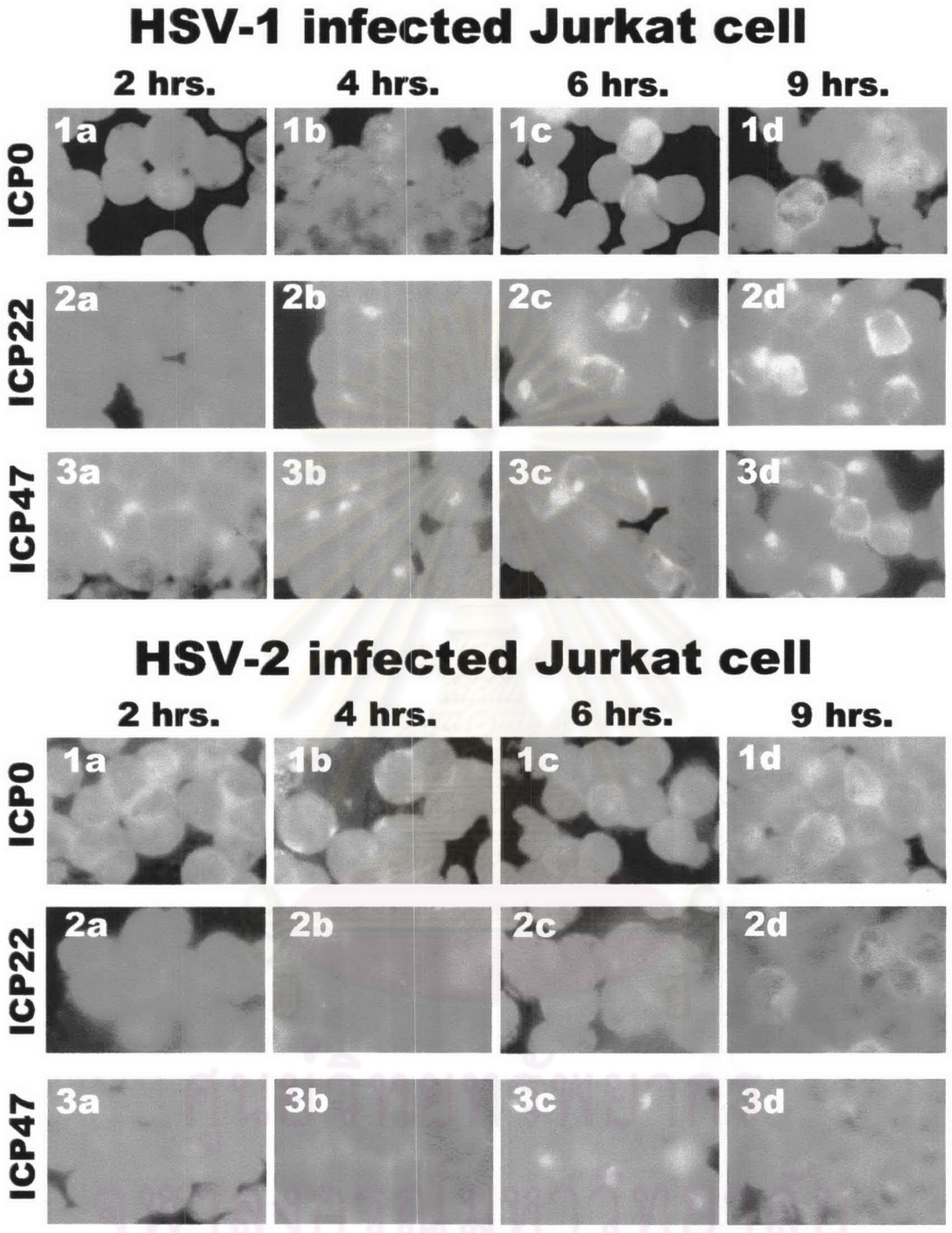


Figure 12. Fluorescent staining of HSV-1 or HSV-2 infected Jurkat cells at 2, 4, 6, and 9 h.p.i. at MOI 5, using rabbit anti HSV ICP0, ICP22 and ICP47 antibodies, respectively (magnify of 1000X).

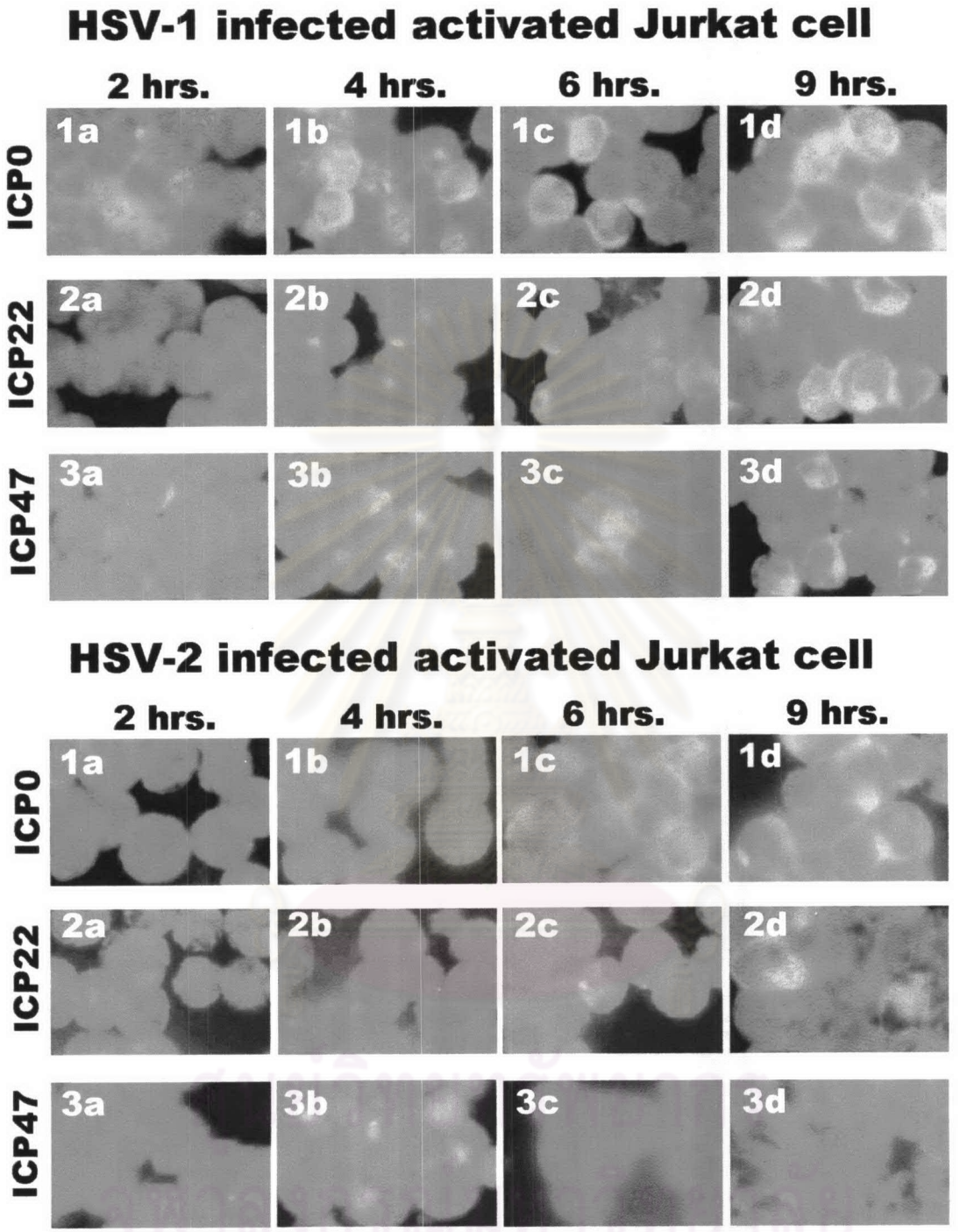


Figure 13. Fluorescent staining of HSV-1 or HSV-2 infected PHA-activated Jurkat cells at 2, 4, 6, and 9 h.p.i. at MOI 5, using rabbit anti HSV ICP0, ICP22 and ICP47 antibodies, respectively (magnify of 1000X).

Detection of HSV infected cells by Flow cytometry

The binding of FITC-conjugated antibodies to cells infected with either HSV-1 or HSV-2 was analyzed by using Flow cytometry. In this study, the amount of virus inoculum was used at MOI 5. After 24 h.p.i., HEp-2 and Jurkat cells were collected and the reaction of cells was stop by adding 0.02% EDTA solution. To allow the entry of HSV specific antibodies, cells were then fixed by using 4% w/v paraformaldehyde and permeabilised by FACS permeabilizing solution with containing 0.1% saponin. Then cells were incubated with a specific antiserum followed by incubation with the swine-anti rabbit antibody conjugated with FITC. In order to increase the specificity of detection, Jurkat cells were stained with CD3/PerCP before analysis. Uninfected control cells were included in each experiment to allow determination of the specificity of staining reaction and the overlap between positive and negative cells. A mean representative results from three independent experiments, were shown in Table 9 and Figure 14. The number of cells in each group was calculated with the Cell-Quest software and expressed as the percentage of total population (Table 9). The number of HEp-2, Jurkat and PHA-activated Jurkat cells infected with HSV-1 or HSV-2 were compared in Table 9. The percentage of cells stained nonspecifically is less than 0.1% in all cases, so that in each sample of 20,000 cells less than 20 cells were nonspecifically stained. The results in Table 9 and Figure 14 demonstrated that the percentage of positive cells of HSV-1 infected HEp-2 and Jurkat cells were different (71.58% vs 22.71%). The increasing in infected cells number was observed in PHA-activated Jurkat cells (40.07%). In contrast to HSV-1 infected cells, the number of positive HSV-2 infected HEp-2 and Jurkat cells were quite similar 57.01% and 47.15%, respectively. But, the number of positive cells was decreased in HSV-2 infected Jurkat cells after activated with PHA (29.13%). In addition, the percentage of HSV-1 infected both HEp-2 and PHA-activated Jurkat cells was higher than HSV-2 infected these cells (71.58% vs 57.01% and 40.07% vs 29.13%). Interestingly, only the percentage of HSV-1 infected Jurkat cells was lower than that of HSV-2 (22.71% vs 47.15%). However, it was found that the intensity of HSV-1 infected cells was brighter than HSV-2 infected cells in all cell types.

Table 9. Percentage of HSV-1 or HSV-2 antigen expressing cells in HEp-2, Jurkat and PHA-activated Jurkat cells at 24 h.p.i. detected by Flow cytometry.

Cell type	% of positive cells (mean±SD)*	
	HSV-1	HSV-2
HEp-2	71.58±7.45 **	57.01±5.04
Jurkat	22.71±0.39	47.15±0.76
PHA-activated Jurkat	40.07±1.62	29.13±1.59

* Data was mean average from three independent experiments

** The % of positive cells shown in the table come from the % of positive cells which already cut off the % of nonspecific cells (control)

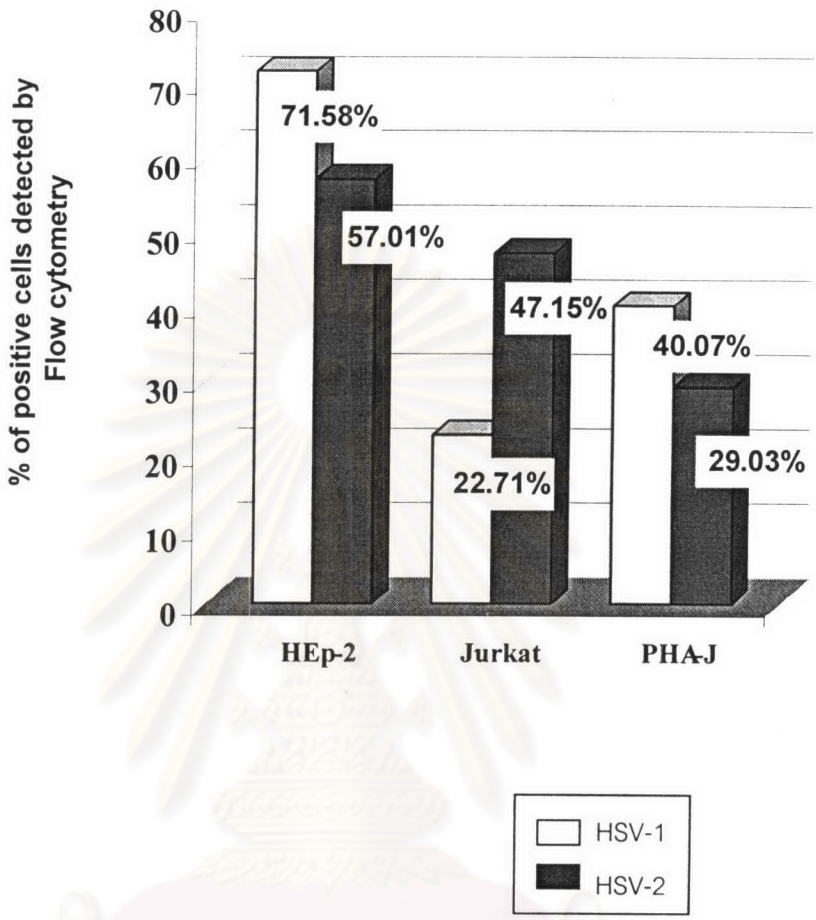


Figure 14. Graph showing the percentage of HSV-1 and HSV-2 infected HEp-2, Jurkat and PHA-activated Jurkat cells (PHA-J) at 24 h.p.i. detected by Flow cytomety.

Adsorption ability of HSV among different cell types

The fact that HSV replication in each cell type is different, not only involving with the factors of cells to promote viral growth are different, ability of virus adsorption and entry into cell might be involved. The adsorption of HSV virions to HEp-2, Jurkat and PHA-activated Jurkat cells were determined by assay the reduction of viruses titer in inoculum after adsorption procedure. The results showed in Table 10. The ability of cells in adsorption of viruses represented as the percentage of adsorbed virus per total amount of inoculum. The high percentage indicated the high ability of adsorption. It appeared that HEp-2 and PHA-activated Jurkat cells could adsorb HSV-1 better than HSV-2 (93% vs 64% and 97% vs 47%) whereas the ability of Jurkat cells to adsorb both HSV-1 and HSV-2 was the same (89%). Moreover, the data showed that HSV-2 did adsorb to 89% of Jurkat cells, but only 47% of PHA-activated Jurkat cells.

Table 10. The HSV adsorption ability of HEp-2, Jurkat and PHA-activated Jurkat cells

Cell type	Type of virus	Amount of viruses (mean ± SD x10^6PFU/ml)*			% Adsorbed virus (%) (c/a x100)
		Inoculum (a)	After adsorption (b)	Adsorbed virus (a-b=c)	
HEp-2	HSV-1	1.13±0.11	0.07±0.01	1.06±0.11	93±0.01
	HSV-2	1.75±0.25	0.63±0.15	1.12±0.09	64±0.03
Jurkat	HSV-1	1±0	0.11±0.01	0.89±0.01	89±0.01
	HSV-2	1±0	0.11±0.02	0.89±0.02	89±0.02
PHA-activated ·Jurkat	HSV-1	1±0	0.03±0.01	0.97±0.01	97±0.01
	HSV-2	1±0	0.53±0.03	0.47±0.03	47±0.02

* Data was mean average from two independent experiments (duplicate each)

HveA-mRNA expression in Jurkat cells and PHA-activated Jurkat cells

Since HveA is well known that it is only one HSV receptor presents on T lymphocytes (15) and the previous results indicated that the ability of virus adsorption between Jurkat and PHA-activated Jurkat cells were different. This might be probably caused by changing in receptors due to PHA activation. Thus an experiment was designed to observe this possibility by comparing HveA-mRNA expression in those cells. The cells were extracted according to the method described previously in Material and Methods. The concentration of the extracted RNA from both Jurkat and PHA-activated Jurkat cells in our preparation ranged from 319.64 ng/µl to 795.93 ng/µl and the average was 500.7 ng/µl.

RT-PCR assay

The detection of HveA mRNA expression was studied by RT-PCR. As showed in Figure 18, the increasing of the band intensity was demonstrated by serial two fold dilutions started from 25 ng to 200 ng of RNA extraction from Jurkat cells. Human β-actin mRNA was also done parallelly as a control of the system and used to compare the amount of amplified products. HveA mRNA expression in Jurkat cells was compared with those PHA-activated Jurkat cells. The results revealed that HveA mRNA could be detected in 10 ng of RNA extracted from PHA-activated Jurkat cells while no band of HveA gene product was observed in that from Jurkat cells. Although the HveA band was seen at 25 and 50 ng in both Jurkat and PHA-activated Jurkat cells RNA, the intensity of band were different. Higher intensity of HveA band observed in PHA-acdtivated Jurkat cells implied that there was an increase in HveA mRNA expression. The increasing of HveA mRNA expression was demonstrated while there was no increasing of β-actin mRNA (Figure 16).

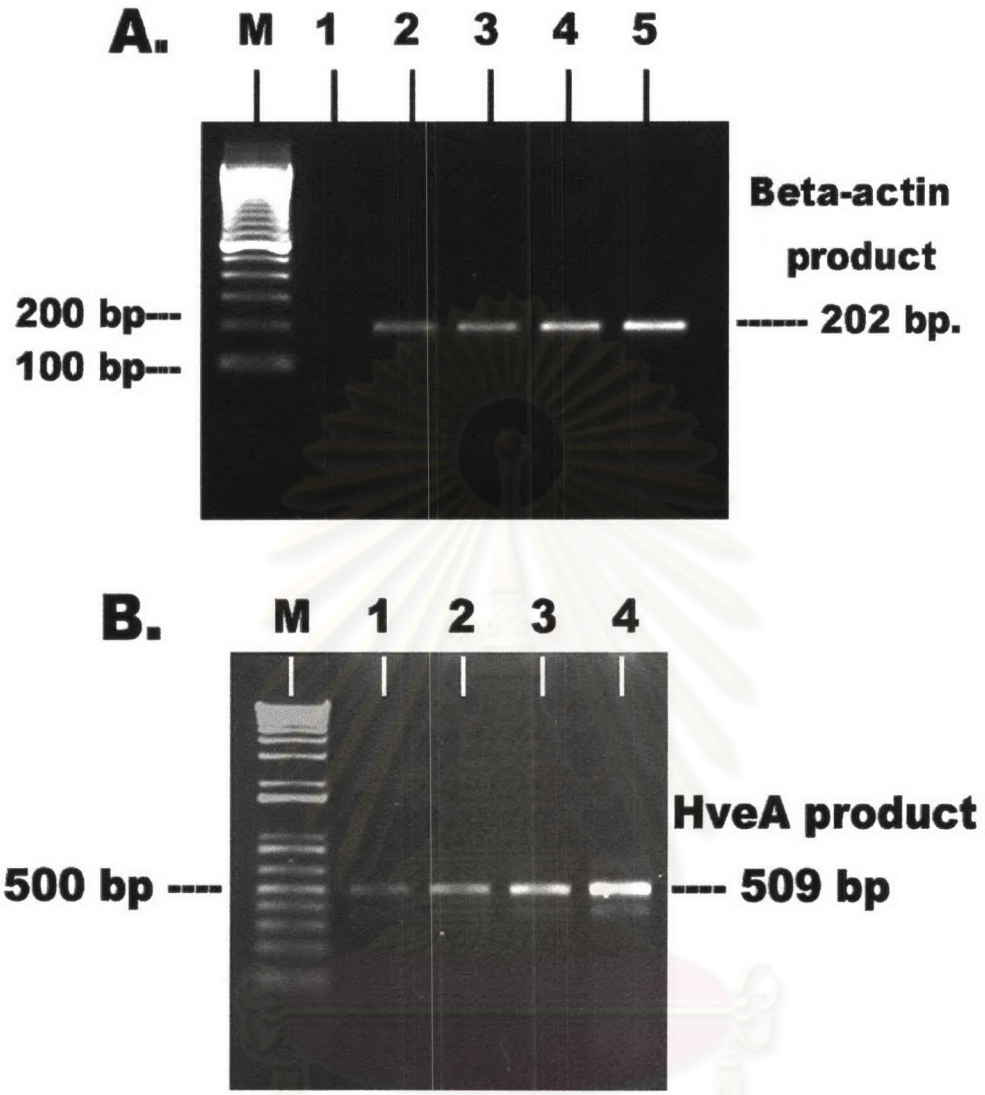


Figure 15. RT-PCR of β-actin (A) and HveA (B) from various amounts of RNA extracted from Jurkat cells

A. Lane M marker 1 kb plus 100 bp ladder
Lane 1 DW
Lane 2 Jurkat RNA 25 ng
Lane 3 Jurkat RNA 50 ng
Lane 4 Jurkat RNA 100 ng
Lane 5 Jurkat RNA 200 ng

B. Lane M marker 1 kb plus 100 bp ladder
Lane 1 Jurkat RNA 25 ng
Lane 2 Jurkat RNA 50 ng
Lane 3 Jurkat RNA 100 ng
Lane 4 Jurkat RNA 200 ng

Figure 16. Comparison of HveA product between Jurkat and PHA-activated Jurkat cells by RT-PCR

Lane M	marker 1 kb plus 100 bp ladder	
Lane 1,3	Jurkat RNA	10 ng
Lane 2,4	PHA-activated Jurkat RNA	10 ng
Lane 5,7	Jurkat RNA	25 ng
Lane 6,8	PHA-activated Jurkat RNA	25 ng
Lane 9,11	Jurkat RNA	50 ng
Lane 10,12	PHA-activated Jurkat RNA	50 ng