

The Sensitivity of Aedes albopictus Cells to the Growth of Arboviruses of
and in the Recovery of Arboviruses from Human and Mosquito Origin

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LLC-MK2 cells are routinely used in this laboratory as a host in the recovery of arboviruses from natural specimens. One-day old mice are used in conjunction with cell cultures for the recovery of the viruses that grow poorly in those cell lines. The purpose of this study was to compare the growth of arboviruses recovered in Thailand in Aedes albopictus cell culture and in LLC-MK2 cell culture. A second purpose is to compare the efficiency of isolation of arboviruses from natural sources in A. albopictus and LLC-MK2 cell lines.

Material and Method: The Singh line of A. albopictus cells was cultured in Mitsuhashi and Maramorsch (M-M) medium. LLC-MK2 cells grown and maintained by standard techniques served as reference host for virus growth. Fourteen specimens from natural sources or low passaged laboratory strains were included in the studies which are listed in Table 1. In each experiment described, volumes of similar dilutions of specimens tested were inoculated into monolayer cell cultures of A. albopictus and LLC-MK2 cells grown in one-ounce prescription bottles. The temperature for cell outgrowth and virus growth of LLC-MK2 cells is 37°C. Aedes albopictus cells were cultured and maintained at 27°C and after virus inoculation the cells were maintained at 32°C. In subsequent experiments, at various intervals half volumes of maintenance medium was aspirated from infected cultures for virus titration and replaced with the same volume of maintenance medium. In the first experiment only (the growth of laboratory strain (40783) of JE virus, Table 2) both the cells and fluid phase were pooled as a source of viruses for infectivity determinations. Viral titrations were performed in LLC-MK2 cells by plaque technique.

The growth of laboratory strain (40783) of JE virus in LLC-MK2 and A. albopictus cells.

JE (40783) strain of JE virus recovered from the brain of a fatal human case of encephalitis, was used at the sixth mouse passage level to infect Culex tritaeniorhynchus by feeding them on chicks infected at one day of age. Infected mosquito pools were diluted serially and inoculated onto LLC-MK2 and A. albopictus cells. Simultaneous plaque titration of the dilutions of mosquito pools on LLC-MK2 cells was performed. The inoculated doses varied from 115,000 to 1.2 PFU. The results in Table 2 show that the peak virus titers were obtained at all dilutions in both cell cultures before 7 days with the exception of the lowest dilution in A. albopictus cells. Virus titers obtained in A. albopictus cells between 3-7 days after inoculation generally were 1-2 logs higher than those obtained in LLC-MK2 cells.

The recovery of JE viruses from known infected mosquito pools.

Three wild culicine mosquito pools (BKM1022, BKM 1096 and BKM977) obtained from the Chiangmai Valley, from which JEV had previously been isolated, were used undiluted to infect LLC-MK2 and A. albopictus cells. As shown in Table 3, peak virus titers obtained in A. albopictus cells exceeded by one to two log peak titers obtained in LLC-MK2 cells for two virus strains, and the titer was similar in both cell lines for the third virus strain.

The recovery of Wesselsbron and BKM 705 (Simbu Group) virus from known infected mosquito pools.

BKM705, isolated from a wild mosquito pool of Culex vishnui, was identified as a Simbu group virus. A strain of Wesselsbron virus (BKM660) was isolated from Aedes lineatopennis. The original infected mosquito pools were used to infect cell cultures. As shown in Table 4, although growth of the Simbu group strain was slower in A. albopictus cells, peak virus titers exceeded those in LLC-MK2 cells by more than one log₁₀. Growth of the Wesselsbron strain was faster in A. albopictus cells and peak titers attained in this insect cell line exceeded that in LLC-MK2 cells by one or two log₁₀.

The preceding experiments indicated that certain low passage arbovirus strains indigenous to Thailand grew to higher titers in A. albopictus cell monolayers than in LLC-MK2 cell monolayers. The following experiments were undertaken to compare the sensitivity of isolation of Thai arboviruses in the two cell lines. In these experiments, specimens containing virus were serially diluted ten-fold and each dilution was inoculated into LLC-MK2 and A. albopictus cells. The cells were washed at 1 1/2 hours to remove unadsorbed virus in the inoculum and fed with maintenance medium. Aliquots of maintenance medium were aspirated from cultures of each dilution daily for 9 days post inoculation and inoculated undiluted into LLC-MK2 cells for plaque assay. Dilutions showing ≥ 100 PFU in plaque assay were considered positive.

The sensitivity of LLC-MK2 cells and Aedes albopictus cells in the recovery of JE virus from human brain and mosquito pools.

In one experiment a 20% suspension of human brain specimen containing JEV virus 40783 was used. JEV virus was isolated from undiluted through the 10⁻³ dilution of the brain suspension in LLC-MK2 cells, while virus was isolated from undiluted through the 10⁻⁸ dilution of the same suspension in A. albopictus cells (Table 5). In a second experiment, a C. tritaeniorhynchus pool containing JEV virus was used. The highest dilution of this pool yielding ≥ 100 PFU was the 10⁻² dilution in both cell lines (Table 5).

Growth of dengue viruses in LLC-MK2 and A. albopictus cells.

Dengue virus of low (1 or 2) LLC-MK2 passage was used. As shown in Table 6, three of five dengue viruses of human and mosquito origin tested were detected with greater sensitivity in A. albopictus, while one human strain of dengue 3 virus did not grow in A. albopictus cells.

Growth of Tembusu virus (BKM3999) in LLC-MK2 cells, A. albopictus cells and one-day old mice.

The purpose of this experiment was to compare the growth of a Tembusu virus strain in A. albopictus cells and LLC-MK2 cells. One-day old mice were used as reference host for the growth experiment of Tembusu virus because high mouse passage of Tembusu virus has been found to grow poorly in MK2 cells. The results of this experiment are as follows:

1. Undiluted fluid cultures tested for the presence of virus by plaque titration in LLC-MK2 cells seems to interfere with the growth of Tembusu virus grown in the same cells. Virus was demonstrated on the first day after inoculation with dilutions containing 10^{4.0} to 10^{1.0} mouse LD₅₀, but no virus in these dilutions was detected on the following days;

2. When undiluted fluid of infected A. albopictus cells was titrated there was no evidence of interference;

3. The highest log dilution of inoculating virus which infected A. albopictus cells was 8.0 and for MK2 cells log 5.0.

Further studies are in progress in the assay of virus collected from infected LLC-MK2 cells to see whether the interfering effect will disappear on dilution. The interference in infected LLC-MK2 cells may be the effect of an interferon-like substance, since it acts only on the homologous cell system in plaque assay. A. albopictus cells may or may not produce a similar interfering substance in the stage of virus growth which could not be detected since A. albopictus cells were not used as the homologous system for plaque assay in these experiments.

Table 1.

Histories of Specimens Used in Comparative Growth Studies in LLC—MK2 Cells and A. albopictus Cells

Specimen	Origin of Viruses	Host Passage	Level of Passage	Virus Identification
40783	Human brain	suckling mouse brain	6	Japanese encephalitis
		one-day old chick	1	
		Culex tritaeniorhynchus	1	
40783	Human brain	—	—	Japanese encephalitis
BKM 977-70	Culex tritaeniorhynchus	—	—	Japanese encephalitis
BKM 1022-70	C. fuscocephala	—	—	Japanese encephalitis
BKM 1096-70	C. tritaeniorhynchus	—	—	Japanese encephalitis
BKM 1410-70	C. tritaeniorhynchus	—	—	Japanese encephalitis
BKM 705-70	C. vishnui (subgroup)	—	—	Simbu group
BKM 3999-68	C. annulus	Suckling mouse brain	2	Tembusu
BKM 660-66	Aedes linneatopennis	—	—	Wesselsbron
CH 69-42101	Human serum	MK2 cells	1	D1
KS 69 E 718	A. aegypti	MK2 cells	1	D2
CH 69-40411	Human serum	MK2 cells	1	D3
CH 69-39153	Human serum	MK2 cells	1	D3
KS 68-1M474	A. aegypti	MK2 cells	2	D4

Table 2. Growth of 40783 (JE) Virus in LLC-MK2 and A. albopictus Cells

Day after inoculation	LLC-MK2 cells										A. albopictus cells						
	virus titer (log 10) detected from different inoculated PFU					virus titer (log 10) detected from different inoculated PFU					virus titer (log 10) detected from different inoculated PFU			virus titer (log 10) detected from different inoculated PFU			
	115000 PFU inoculated	11500 PFU inoculated	1150 PFU inoculated	115 PFU inoculated	11.5 PFU inoculated	1.2 PFU inoculated	115000 PFU inoculated	11500 PFU inoculated	1150 PFU inoculated	115 PFU inoculated	11.5 PFU inoculated	1.2 PFU inoculated	115000 PFU inoculated	11500 PFU inoculated	1150 PFU inoculated	115 PFU inoculated	11.5 PFU inoculated
1	4.3	4.7	4.3	3.3	<1.0	<1.0	5.3	4.0	4.4	<1.5	<1.0	5.3	4.0	4.4	<1.5	<1.0	<1.0
3	11.0	10.8	9.3	11.3	7.0	3.0	12.7	12.5	11.9	3.3	7.5	12.7	12.5	11.9	3.3	7.5	<1.0
5	9.0	7.0	6.0	6.0	6.0	5.7	9.0	11.0	3.0	10.5	11.2	9.0	11.0	3.0	10.5	11.2	<1.0
7	6.0	5.3	5.0	4.3	4.8	5.3	6.0	7.5	7.3	9.5	6.5	6.0	7.5	7.3	9.5	6.5	<1.0
9	3.3	3.7	3.3	3.3	3.3	3.0	3.3	6.8	6.5	7.5	5.3	3.3	6.8	6.5	7.5	5.3	<1.0
11	4.0	4.3	5.0	6.8	5.0	6.3	2.0	4.3	5.7	4.3	4.3	2.0	4.3	5.7	4.3	4.3	6.3
13	5.7	7.3	7.5	6.8	5.3	8.3	5.7	8.0	7.4	6.3	6.0	5.7	8.0	7.4	6.3	6.0	6.0
15	4.3	4.7	4.7	4.7	4.3	4.7	4.3	4.7	4.7	5.0	4.7	4.3	4.7	4.7	5.0	5.0	4.7

Table 3. Recovery of JE Viruses from Known Infected Mosquito Pools

Day after Inoculation	BKM 1022		BKM 1096		BKM 977	
	Virus titer (log 10) detected in:		Virus titer (log 10) detected in:		Virus titer (log 10) detected in:	
	LLC-MK2	A. albo	LLC-MK2	A. albo	LLC-MK2	A. albo
1	not done	4.0	<1.0	<1.0	<1.0	<1.0
3	6.0	9.5	3.7	9.3	<1.0	<1.0
5	6.6	7.0	7.5	8.2	<1.0	<1.0
7	7.0	7.0	9.5	8.3	5.0	6.0
9	6.0	4.0	5.7	7.0	5.6	6.6
11	3.5	3.5	3.0	7.0	5.0	6.3
13	<1.0	3.0	3.7	5.0	3.5	6.4
15	4.0	5.0	3.5	5.0	4.5	6.0

Table 4.
Recovery of Wesselsbron and Simbu Group Virus from Known Infected Mosquito pools

Day after Inoculation	BKM 705 (Simbu group)		BKM 660 (Wesselsbron)	
	Virus titer (log 10) detected in:		Virus titer (log 10) detected in:	
	LLC-MK2	A. albo.	LLC-MK2	A. albo.
1	<1.0	<1.0	<1.0	<1.0
3	3.5	<1.0	0.7	2.4
5	5.7	<1.0	1.5	4.6
7	4.5	5.7	2.2	5.0
9	4.5	7.0	3.0	5.5
11	1.7	7.0	3.0	4.5
13	<1.0	5.7	2.5	4.0
15	<1.0	5.5	<1.0	<1.0

Table 5.
The Sensitivity of LLC-MK2 Cells and A. albopictus Cells in the Recovery of JE
Viruses from Human Brain and Mosquito Pool

Log 10 dilution of material	Human brain 40783		Mosquito pool 1410	
	Day when ≥ 100 PFU first detected in undiluted fluid of cells after inoculation		Day when ≥ 100 PFU first detected in undiluted fluid of cells after inoculation	
	LLC-MK2	<u>A. albopictus</u>	LLC-MK2	<u>A. albopictus</u>
0	1	1	9	1
1	1	3	3	3
2	1	3	3	5
3	3	3	0	0
4	3	3	0	0
5	0	5	0	0
6	0	5	0	0
7	0	9	0	0
8	0	9	0	0
9	0	0	0	0

0 = No detectable plaque in undiluted fluid of infected cell culture

Table 6. Growth of Dengue Viruses in LLC-MK2 and A. albopictus Cells

Virus	LLC-MK2		<u>A. albopictus</u>	
	Highest log ₁₀ dilution which infect cells	Day when ≥ 100 PFU detected after inoculation	Highest log ₁₀ dilution which infect cells	Day when ≥ 100 PFU detected after inoculation
42101 (Dengue 1)	3.0	3	4.0	5
KS-69E718 (Dengue 2)	3.0	1	4.0	3
CH69 39153 (Dengue 3)	3.0	5	4.0	11
40411 (Dengue 3)	3.0	3	0	7
KS68 18M-474 (Dengue 4)	4.0	7	4.0	5

Table 7. Growth of Tembusu Virus (8KM 3999-68) in LLC-MK2 Cells, A. albopictus Cells and One-day Old Mice

Dilution of Virus log 10	LLC-MK2 cells								A. albopictus cells							
	PFU detected in undiluted fluid infected with diluted (log 10) virus								PFU detected in undiluted fluid infected with diluted (log 10) virus							
	-1	-2	-3	-4	-5	-6	-7	-8	-1	-2	-3	-4	-5	-6	-7	-8
Day after inoculation																
1	> 100	> 100	> 100	> 100	20	0	0	0	> 100	> 100	> 100	> 100	> 100	12	2	0
3	0	0	0	4	63	20	4	0	> 100	> 100	> 100	> 100	> 100	> 100	32	8
5	0	0	0	2	> 100	70	32	0	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
7	0	0	0	0	10	16	32	0	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
9	0	0	0	0	0	8	1	0	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
11	0	0	0	0	0	0	0	0	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
13	0	0	0	0	0	0	0	0	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Mouse LD50 in dilution	10,000	1,000	100	10	1	0	0	0	10,000	1,000	> 100	10	1	0	0	0