

## Mink Lung Epithelial Cells: Unique Cell Line That Supports Influenza A and B Virus Replication

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**We have demonstrated for the first time that a mink lung epithelial cell line (Mv1Lu) supports the replication of influenza A and B viruses, including the recently isolated H5N1 avian and human Hong Kong strains, to titers comparable to those in MDCK cells. These results suggest that Mv1Lu cells might serve as an alternative system for the isolation and cultivation of influenza A and B viruses and may be useful for vaccine development.**

Isolation of influenza viruses in embryonated eggs or cell culture is critical for epidemiologic investigation of outbreaks and for vaccine production. The ability to culture influenza was critical in the recognition of the H5N1 avian influenza outbreak in humans in 1997. This recent emergence of lethal avian influenza viruses in humans justifies our need for reliable methods of isolating and identifying influenza A and B viruses from clinical samples. There is overwhelming evidence that the growth of influenza A and B viruses in eggs can lead to the selection of variants containing antigenic and structural changes in the hemagglutinin (HA) molecule (7, 12, 14). In addition, passaging of mammalian influenza viruses in eggs can result in a change in the receptor specificity from the mammalian  $\alpha$ -2,6-galactose oligosaccharide to the avian  $\alpha$ -2,3-sialic acid linkage (6). Finally, the lack of reliable high-quality eggs is a serious limitation in their use.

Many attempts have been made to find suitable alternatives to the use of eggs for isolating influenza virus from clinical samples and for virus propagation. Influenza viruses can infect a variety of primary and continuous cell lines; however, most cells do not support productive viral replication (1a, 2, 4, 8, 11). Currently, Madin-Darby canine kidney (MDCK) epithelial cells are widely used for viral studies since they support the growth and isolation of virus (2, 11, 13). During the course of our studies assessing the role of cytokines in influenza A viral pathogenesis (15), we found that a mink lung epithelial cell line (Mv1Lu [ATCC CCL-64]) supports the replication of influenza A and B viruses to titers comparable to those in MDCK cells. In these studies, we examined the usefulness of the Mv1Lu cell line for the isolation and replication of numerous influenza A and B viruses, including the recently isolated human and chicken Hong Kong H5N1 isolates.

To examine the use of Mv1Lu cells for propagation of influenza viruses, we first compared the replication of reference strains of mammalian and avian influenza A and B viruses in Mv1Lu cells and MDCK cells. MDCK (passage 3 to 30) or Mv1Lu (passage 41 to 60) cells were seeded at  $5 \times 10^5$  cells per well in six-well tissue culture plates and allowed to grow to confluency. The cells were washed twice with phosphate-buff-

ered saline and then infected at a multiplicity of infection of 0.01 and allowed to incubate for 1 h at 37°C with 5% CO<sub>2</sub>. After 1 h, the cells were washed with minimum essential medium (MEM) and further incubated in MEM containing 5% bovine serum albumin and 0.3 (Mv1Lu) or 1 (MDCK)  $\mu$ g of TPCK (L-1-tosylamide-2-phenylethyl chloromethyl ketone)-treated trypsin per ml. Mv1Lu cells are more sensitive to trypsin; therefore, the optimal concentration of trypsin was determined by dose response assay (data not shown). Aliquots of supernatants were removed at 0, 8, 24, 48, and 72 h postinfection (p.i.), and the viral yield was determined by hemagglutination and 50% tissue culture infectious dose (TCID<sub>50</sub>) assays on MDCK cells. The following viruses were obtained from the influenza virus repository at the University of Wisconsin—Madison: avian strains A/Mallard/NY/6874/78 (H3N2), A/Mallard/Wisconsin/994/82 (H5N2), and A/Turkey/Ontario/7732/66 (H5N9) (Ty/Ont); mammalian strains A/PR/8/34 (H1N1), A/Swine/Indiana/1726/88 (H1N1) (Sw/Ind), A/Udorn/307/72 (H3N2) (Udorn), A/Aichi (H3N2), and A/Seal/Massachusetts/92; and influenza B virus strains B/David Breeze/44, B/Lee/40, and B/Hong Kong. Viruses were propagated in the allantoic cavities of 11-day-old embryonated chicken eggs for 48 to 72 h at 35°C; the allantoic fluid was harvested, centrifuged for clarification, and stored at –70°C.

At 48 h p.i., all of the influenza A virus reference strains had replicated to comparable levels in MDCK and Mv1Lu cells (Table 1). Similar results were seen with influenza B viruses at 72 h p.i. (Table 1). The strains were positive by hemagglutination assay, infectivity assays, and indirect immunofluorescent-antibody labeling (data not shown). Growth curves show the similar kinetics of replication of a highly pathogenic avian strain (Ty/Ont), two mammalian strains (Sw/Ind and Udorn), and an influenza B virus strain (B/David Breeze/44) in MDCK and Mv1Lu cells (Fig. 1). These studies suggest that Mv1Lu cells support the replication of mammalian and avian reference strains of influenza A and B viruses tested at levels similar to MDCK cells (Table 1). It is worth noting that, of all the strains tested, only the equine viruses (Equine/Kentucky/1/81 [H3N8] and Equine/Prague [H7N7]) failed to replicate in Mv1Lu cells (data not shown). The reason for this is unclear.

The viruses tested above were first propagated in embryonated eggs, a method shown to alter the receptor specificity and structure of the HA molecule (6, 7, 10). Therefore, we tested the replication of clinical isolates in Mv1Lu cells. Viruses

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TABLE 1. Efficiencies of influenza A and B virus replication in Mv1Lu and MDCK cells<sup>a</sup>

Virus strain	Mv1Lu cells		MDCK cells	
	CPE	TCID <sub>50</sub> titer	CPE	TCID <sub>50</sub> titer
A/PR/8/34 (H1N1)	None	1.75	+++	3.62
Sw/Ind	None	5.75	+++	5.25
A/Mallard/NY/6874/78 (H3N2)	++	5.125	+++	3.25
Udorn	+++	4.75	+++	5.75
A/Aichi (H3N2)	None	0.75	++	1.375
A/Seal/Massachusetts/92 (H3N3)	++	5.5	+++	4.25
A/Mallard/Wisconsin/994/82 (H5N2)	None	1.75	+++	2.25
Ty/Ont	None	3.32	+++	3.18
B/David Breeze/44	++	2.37	+++	2.37
B/Lee/40	+	5.5	+++	6.25
B/Hong Kong	++	4.659	+++	6.25

<sup>a</sup> HA positivity and TCID<sub>50</sub> titers were determined at 48 h p.i. (influenza A) and at 72 h p.i. (influenza B). TCID<sub>50</sub> titers were determined on MDCK cells. CPE key: +, very little CPE; ++, holes in the monolayer; +++, monolayer destruction.

from nasopharyngeal swabs of infected individuals were isolated, and confluent monolayers of MDCK or Mv1Lu cells were infected with H1N1, H3N2, and B strains (12 total) obtained from the Wisconsin State Laboratory of Hygiene (Madison, Wis.). Supernatants were harvested at 24, 48, and 72 h p.i., and infectivities were assessed by hemagglutination assay and TCID<sub>50</sub> analysis on MDCK cells (Table 2). All of the clinical isolates replicated in Mv1Lu cells (Table 2 and data not shown). Interestingly, we found that two of the H1N1 strains failed to replicate in MDCK cells while replicating in Mv1Lu cells (Table 2). Finally, we found that the human and chicken H5N1 strains isolated during the recent Hong Kong outbreak replicated to comparable titers in the Mv1Lu and MDCK cells

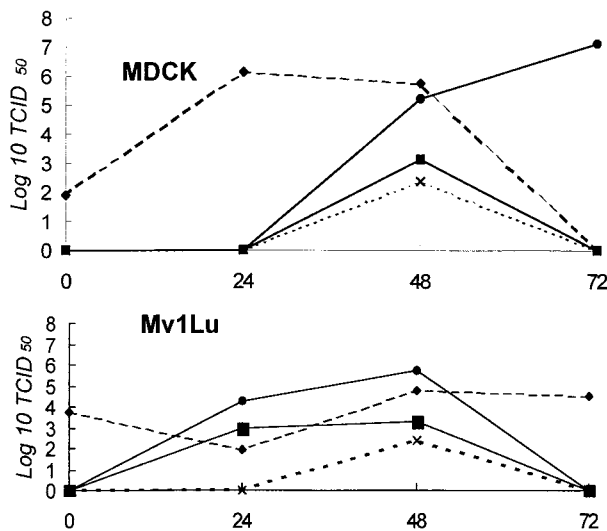


FIG. 1. Growth curves in Mv1Lu and MDCK cells. Confluent monolayers of MDCK or Mv1Lu cells were infected with Ty/Ont (■), Sw/Ind (●), Udorn (◆), or B/David Breeze/44 (×) with a multiplicity of infection of 0.01. Aliquots were removed at 0, 24, 48, and 72 h p.i., and infectivity was determined by TCID<sub>50</sub> assays on MDCK cells. Results are representative of at least five separate experiments.

TABLE 2. Replication of human (H1N1) influenza A and B virus clinical samples in Mv1Lu and MDCK cells

Cell type	Sample	Titer at time of harvest <sup>a</sup>					
		24 h		48 h		72 h	
		HA	TCID <sub>50</sub>	HA	TCID <sub>50</sub>	HA	TCID <sub>50</sub>
MDCK	96 559	0	0	0	0	0	0
	96 1547	0	>5.0	+/-	>5.0	+	>5.0
	96 3806	0	0	0	0	0	0
Mv1Lu	96 559	0	0	0	1.75	0	5.125
	96 1547	0	2.75	0	4.625	0	4.0
	96 3806	0	0	0	2.25	0	4.5

<sup>a</sup> TCID<sub>50</sub> assays were performed on MDCK cells.

(Table 3). These studies suggest that a wide range of subtypes are capable of infecting and replicating in Mv1Lu cells. In addition, Mv1Lu cells support the replication of clinical isolates of influenza A and B virus strains, including those that do not replicate in MDCK cells. Unlike Vero cells, viruses do not have to be adapted for growth in Mv1Lu cells (3, 4). This may be important for the isolation of clinical strains.

In these studies, we describe the use of a mink lung epithelial cell line for the replication and isolation of influenza viruses. Mv1Lu cells were originally isolated from trypsinized lungs of unsexed fetal Aleutian mink and are reverse transcriptase negative (1). The cells are useful for focus-forming assays for murine and feline sarcoma viruses (5) and are susceptible to herpes simplex virus, reovirus type 3, and vaccinia virus (1). They are resistant to adenovirus type 5, coxsackieviruses A-9 and B-5, and poliovirus type 2 infections (1). We propose that Mv1Lu cells may serve as a valuable cell line for influenza virus isolation and identification for the following reasons: (i) they support the replication of laboratory and clinical isolates of viruses to titers similar to the standard MDCK system and are commercially available, making them accessible to any laboratory or institution; (ii) they support the replication of clinical isolates that fail to replicate in MDCK cells; (iii) flow cytometry analysis shows that Mv1Lu cells contain the  $\alpha$ -2,6 and  $\alpha$ -2,3 receptors necessary for influenza virus entry at levels similar to those of MDCK cells (79 versus 64%, respectively, for  $\alpha$ -2,3 and 80 versus 83% for  $\alpha$ -2,6); and (iv) mink can be experimentally infected with influenza virus and serve as a model system for human influenza virus pathogenesis (9), suggesting that Mv1Lu cells may be useful in studying the cell biology of influenza virus pathogenesis including the identification of cellular receptors and cell death pathways.

It is worth noting that differences were found in the cytopathic effects (CPE) produced in Mv1Lu cells with certain

TABLE 3. Replication of human and chicken influenza A virus Hong Kong strains in Mv1Lu and MDCK cells

Cell type	Sample	Titer at time of harvest <sup>a</sup>							
		18 h		24 h		48 h		72 h	
		HA	TCID <sub>50</sub>	HA	TCID <sub>50</sub>	HA	TCID <sub>50</sub>	HA	TCID <sub>50</sub>
MDCK	CK 220	0	3.6	16	3.6	64	5.4	64	4.8
	HK 156	0	2.4	4	5.4	32	5.4	64	4.8
Mv1Lu	CK 220	0	3.6	0	7.2	128	6.0	64	4.8
	HK 156	0	2.4	0	5.4	64	4.8	256	4.2

<sup>a</sup> TCID<sub>50</sub> assays were performed on MDCK cells.

influenza A virus strains, and the appearance of CPE was also delayed compared to MDCK cells (data not shown). Because of variability in CPE, we were unable to use Mv1Lu cells for plaque assays or TCID<sub>50</sub> analysis. All of the reported TCID<sub>50</sub> data was generated by testing Mv1Lu cell supernatants on MDCK cells. The ability of Mv1Lu cells to support viral replication to high titers with delayed onset of cell death suggests that they may provide adequate quantities of virus for vaccine development. However, further studies examining the usefulness of Mv1Lu cells for vaccine production will have to be undertaken.

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