

# Use of a Clonal Line of Porcine Kidney Cell Cultures for Primary Isolation and Vaccine Studies with Adenoviruses

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Received for publication 8 August 1972

The clonal line (Y15) of porcine kidney stable cells provided a recovery system for adenovirus T4 from specimens from adults with respiratory illnesses that was as sensitive as human embryo kidney cultures. Adenoviruses T7 from adults, and T1, 2, 3, and 5 from children could be readily isolated in porcine kidney cell cultures. The latter were useful for adenovirus vaccine studies in that infectivity titers of live virus vaccine and neutralization antibody responses after vaccination were equal to those obtained in human embryo kidney cultures.

A clonal line of porcine kidney stable cells for assay of Japanese encephalitis virus was described by Inoue and Yamada in 1963 (1). This was developed because of alteration in susceptibility of the porcine kidney cell line to Japanese encephalitis virus during serial cultivation. Similar problems with growth of adenoviruses in HeLa, KB, and HEp-2 cell lines were encountered for several years in this laboratory and were also noted by Rose, who recommends the use of human embryo kidney (HEK) cultures (2). Because of unavailability or high cost of HEK cultures, the use of this cloned line of porcine kidney cells was investigated for isolation, live virus vaccine-infectivity titration, and neutralization antibody determinations with several adenovirus types.

## MATERIALS AND METHODS

**Cell cultures.** The clonal line (Y-15) of porcine kidney stable cells was obtained from B. H. Sweet, Gulf South Research Institute, at the 842nd passage. For growth, 10% fetal bovine serum in Eagle medium with 200 units of penicillin, 100  $\mu$ g of neomycin, and 25 units of nystatin per ml were used. For virus studies, serum was 2% fetal bovine that had been heated at 56 C for 30 min. KB and HEp-2 cells obtained from commercial sources were handled similarly. WI-38 cells (diploid) were propagated in Eagle medium with 10% fetal bovine serum and 50  $\mu$ g of Aureomycin per ml. HEK cultures were prepared from 0.5% trypsin-dispersed cells. Growth and maintenance media were similar to the above.

**Vaccines.** Live oral adenovirus (types 1, 2, 4, 5, and 7) vaccines prepared for the Infectious Disease Branch, National Institute of Allergy and Infectious

Diseases, by Wyeth Laboratories were held in the refrigerator prior to use. Methods for types 4 and 7 vaccine studies were described in detail by W. M. Gooch and W. J. Mogabgab (*Arch. Environ. Health, in press*).

**Specimens for virus isolation.** Pharyngeal washings, swabs, and rectal swabs were collected in 0.5% lactalbumin hydrolysate and refrigerated, and the fluid was quick frozen and stored in ampoules at -65 C.

**Serum neutralization.** Sera were heated at 56 C for 30 min for neutralization titers. Virus,  $10^4$  tissue culture infective dose<sub>50</sub>, and diluted sera were held at 37 C for 3 hr prior to inoculating cultures. Antibody titers were expressed as the initial dilution of serum-inhibiting virus effect.

**Virus isolation.** Two cell cultures were inoculated with 0.2 ml and incubated in stationary racks at 35 C as long as cells were viable. This was at least 2 weeks for porcine kidney (PK-Y15) and HEK cultures, and 1 week for HEp-2, KB, and WI-38 diploid human embryo lung cultures. Culture fluids were changed at weekly intervals. Second passages were held for 1 week because initial experiments did not reveal increased yield after that time. Adenoviruses detected by cytopathic effects were identified with type-specific animal sera.

## RESULTS

**Comparison of porcine kidney cells with other cultures for isolation of adenoviruses, T4, and 7 from adults.** As many T4 adenoviruses were recovered in PK (Y15) cell cultures as in HEK for the years 1968-70 (Table 1). Most were found in the first passage in the former, and all were found on the first passage in the latter. Considerably more T4 and 7 viruses were

obtained in porcine kidney cultures than in KB, HEp-2, or WI-38 cell cultures. Porcine kidney and HEK could be incubated without significant degenerative changes for more than 2 weeks. This probably explains the advantage of these cells over KB and HEp-2 cells for isolation of adenoviruses. WI-38 cell cultures were not useful for recovery of these adenoviruses.

**Isolation of adenoviruses (types 1, 2, 3, and 5) from pharyngeal and rectal swabs from children.** Porcine kidney cloned cells

isolated more adenoviruses, types 1, 2, 3, and 5, from specimens from children than monkey kidney, HEp-2, or WI-38 cell cultures (Table 2). Moreover, all viruses were obtained by a single passage in PK (Y15), in contrast to the other cultures used. In all of the systems utilized, 4 to 5 T3 isolations were missed.

**Infectivity titrations of live vaccine virus.**

Because of the low order of infectivity of live vaccine virus in WI-38 cell cultures despite 12 previous passages in these cultures, it has been

TABLE 1. Comparison of cell cultures for isolation of adenovirus types 4 and 7 from pharyngeal washings from military personnel with adenovirus respiratory illnesses<sup>a</sup>

Adenovirus	1967-68				1968-70		1970-71
	PK(Y15) <sup>b</sup>	HEp-2	KB	WI-38	PK(Y15)	HEK <sup>c</sup>	PK(Y15)
Type 4							
Passage 1	4	0	0	0	15	17	39
Passage 2	0	1	0	0	2		10
Passage 3		0	1				
Type 7							
Passage 1	17	0	1	1	0	0	8
Passage 2	0	4	2	0	0		3
Passage 3	1	8	3				
Total type 4 and 7	22	13	7	1	17	17	60
Total tested	30	100	130	130	40	39	70

<sup>a</sup> As determined by complement-fixing antibody response.

<sup>b</sup> Clonal line of porcine kidney cells.

<sup>c</sup> Primary human embryo kidney cultures.

TABLE 2. Comparison of cell cultures for isolation of adenovirus types 1, 2, 3, and 5 from pharyngeal (PS) and rectal swabs (RS) from children with adenovirus respiratory illnesses, 1966-69

Adenovirus	PK (Y15)		MK		WI-38		HEp-2		Total isolates	
	PS	RS	PS	RS	PS	RS	PS	RS	PS	RS
Type 1										
Passage 1	4 <sup>a</sup>	2	3	2	0	0	1	0	4	2
Passage 2	0	0	0	0	0	0	1	0		
Passage 3	0	0	0	0	0	0	0	1		
Type 2										
Passage 1	5	4	0	0	0	0	0	0	5	4
Passage 2	0	0	5	0	0	0	2	1		
Passage 3	0	0	0	0	0	0	0	1		
Type 3										
Passage 1	6	1	6	1	0	0	2	0	8	4
Passage 2	0	0	1	0	0	0	4	2		
Passage 3	0	0	0	0	0	0	0	0		
Type 5										
Passage 1	4	1	2	0	0	0	3	0	4	1
Passage 2	0	0	0	1	0	0	1	0		
Passage 3	0	0	0	0	0	0	0	0		

<sup>a</sup> No. positive on each passage.

necessary to titrate infectivity in HEK cultures. In Table 3, comparisons are shown with cloned porcine kidney cell line cultures. Similar infectivity titers were found in both systems. An index of stability of these vaccine viruses can also be obtained by the serial determinations that were done in HEK cultures.

**Effect of cell cultures employed on neutralization antibody titers.** Cell line cultures such as HEp-2 frequently did not allow demonstration of neutralization antibody responses (Table 4). In contrast, both HEK and PK (Y15) cultures were suitable for determination of antibody responses after vaccine types 4 and 7.

### DISCUSSION

The utility of a cell line for isolation of adenoviruses, especially when large numbers of specimens are involved, is quite obvious. HEK is scarce, and cultures are expensive when purchased. Whether cloning of HeLa, HEp-2, or KB cells would succeed in providing suitable cultures for this purpose is not known. However, PK (Y15) cells appear to be more stable, and the length of incubation time can be correlated with the percentage of adenovirus isolates obtained.

That neutralization antibody responses frequently could not be demonstrated in HEp-2 cell cultures was probably the result of the

TABLE 3. *Infectivity titrations of live adenovirus vaccine in primary human embryo kidney and cloned porcine kidney cell line cultures*

Vaccine lot no., adenovirus type	Tissue culture infective dose <sub>50</sub> , log <sub>10</sub>				
	HEK determination dates				PK (Y15) deter- mination dates
	1/70	6/70	6/71 <sup>a</sup>	9/71 <sup>a</sup>	7/71 <sup>b</sup>
16 CX 01 401, 1				7.1	7.8
16 CVII 01 301, 2				5.8	5.9
16 CI 00 801, 4	5.5	4.0	4.1		3.8
16 CVIII 01 501, 5				7.0	7.9
16 CV 01 104, 7	6.5	7.0	7.1		5.7

<sup>a</sup> By manufacturer.

<sup>b</sup> Mean of five determinations.

TABLE 4. *Effect of cell culture on neutralization titers after live oral adenovirus vaccine*

Subject no.	Cell Cultures		
	HEp-2	HEK	PK(Y15)
Adenovirus T7			
AV1	<2/<2 <sup>a</sup>	<2/>4	2.8/11.3
AV9	<2/<2	<2/>4	<2/8
AV18	<2/<2	<2/>4	<2/5.7
AV19	<2/<2	<2/>4	<2/16
AV26	<2/<2	<2/>4	<2/16
AV36	<2/<2	<2/>4	<2/11.3
A14	<2/<2	<2/4	<2/4
A17	<2/<2	<2/>4	<2/8
A21	<2/<2	<2/>4	<2/>16
Adenovirus T4			
A36	<2/<2	<2/>4	<2/2.8
A80	<2/<2	<2/>4	<2/5.7
A82	<2/<2	<2/>4	<2/11.3
A85	<2/<2	<2/>4	<2/5.7
A88	<2/<2	<2/>4	<2/>16
A89	<2/<2	<2/2.8	2.8/>16
A91	<2/<2	<2/2	4/11.3
A118	<2/<2	<2/>4	<2/16
A122	<2/<2	<2/>4	<2/>16
A123	<2/<2	<2/>4	<2/16

<sup>a</sup> Reciprocal neutralization titers, pre- and post-vaccination.

quantity of virus required in this system. At least 10 times as much virus was needed for the challenge dose as was employed in cloned porcine kidney or HEK cultures. It is possible that excess antigen blocked antibody and precluded demonstration of neutralization.

### ACKNOWLEDGMENTS

This investigation was supported by contract PH 43 63 75 from the Infectious Disease Branch, National Institute of Allergy and Infectious Diseases, and by the Infectious Disease Research Fund, Department of Medicine, Tulane University School of Medicine.

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