Growth Activity of Porcine Adenoviruses in Primary Porcine Testicular Cell Cultures

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Haig *et al.* [4] first isolated porcine adenovirus (PAV) from rectal swabs of a piglet with diarrhea in 1964. Since that time several authors have reported the isolation of PAV from various tissues and feces [2, 7, 8, 9, 10, 12]. At present, PAV is divided into 4 serotypes by the cross neutralization test [3]. The authors [6] also isolated 2 strains of PAV from nasal swabs of pigs with respiratory illness on a farm in the Kinki District of Japan in 1987.

Bovine adenoviruses (BAV) have been divided into 2 biological types; one showing growth activity in primary bovine kidney (BK) and the other indicating growth activity in bovine testicular (BT) cell cultures [1, 11].

This report describes the growth activity of several strains of PAV in primary porcine testicular (PT) cell cultures.

The following viruses were used in this examination; the newly isolated strains of HNF61 and HNF70 and the strains of 25R in serotype 1, A47 in serotype 2, 6618 in serotype 3 and München in serotype 4 of PAV. These virus strains were passaged 3rd to 5th generations on primary porcine kidney (PK) cell cultures in our laboratories. These viruses were propagated well in PK cell cultures. The infective titers of HNF61, HNF70, 25R, A47, 6618 and München strains were 7.5, 8.0, 7.0, 7.25, 7.5 and 6.25 $\log_{10}$ TCID₅₀/ml, respectively. Primary PK and PT cell cultures were prepared in glass tubes ($\phi 13 \times 110$ mm) as described previously [5]. Serial ten-fold dilutions of virus were made in maintenance medium, and 0.1 ml of each dilution was inoculated into 4 PT cell culture tubes. The cell cultures were added to 0.75 ml of maintenance medium, which consisted of Eagle's minimum essential medium (MEM) containing 0.11% bovine serum albumin, 200 units/ml penicillin G, 200 μ g/ml streptomycin, 100 μ g/ml kanamycin and 5 μ g/ml fungizone. The cell cultures were maintained in a roller drum at 37°C for 7 days after inoculation and examined for cytopathic

effect (CPE). The cell culture fluid was harvested until showing CPE-positive at grade +++. No cell culture fluid with CPE was harvested 7 days after inoculation. This fluid was passaged 5 generations by the same method. The harvested fluid of each generation was determined for infective titer by using PK and PT cell cultures. The A47 and 6618 strains grew in PT cell cultures with CPE. These PAV virus strains clearly showed CPE with rounding cells in the first generation of PT cell cultures. This finding continued to the 5th generation. Infective titers were 6.25–6.5 $\log_{10}$ TCID₅₀/ml for the A47 strain and 6.5–7.5 $\log_{10}$ TCID₅₀/ml for the 6618 strain in PT cell cultures (Table 1). The 5th passaged viruses of these strains in PT cell cultures were completely neutralized against guinea pig anti-serum of the A47 and 6618 strains by the cross-neutralization test as described previously [6]. Microscopic examination of the infected PT cells stained with hematoxylin and eosin revealed intranuclear inclusion bodies at the 5th passage level. Consequently, we thought that the CPE resulted from infection of the A47 and 6618 strains. However, the HNF61, HNF70, 25R and München strains did not show any CPE in the PT cell cultures during the 5th passage level. The infective titers of these strains in PK cell cultures decreased gradually from 3.5–5.5 $\log_{10}$ TCID₅₀/ml in the 1st generation to 2.0–2.5 $\log_{10}$ TCID₅₀/ml in the 2nd generation, and no virus infectivity could be detected in the culture fluids from the 3rd through the 5th generations (Table 1).

From the above results, it was proven that some strains of PAV have growth activity with CPE in PT cell cultures, like BAV which are divided into 2 distinct groups according to its propagation in BK or BT cell cultures. The A47 strain of serotype 2 and the 6618 strain of serotype 3 grew with CPE in PT cell cultures in the present study. However, we could not clarify whether the growth activity of PAV strains in PT cell cultures depended on common property of strain in serotypes 2 and 3 of PAV. Accordingly, the growth activity of PAV in PT cell cultures

Table 1. Growth ability of PAV in PT cell cultures

Strain	serotype	Number of generation				
		1	2	3	4	5
HNF61	isolate	— ^{a)}	—	—	—	—
		2.5 ^{b)}	1.0	0.0	0.0	0.0
HNF70	isolate	—	—	—	—	—
		4.5	1.5	0.0	0.0	0.0
25R	1	—	—	—	—	—
		2.5	1.0	0.0	0.0	0.0
A47	2	+	+	+	+	+
		5.25	5.25	5.5	5.25	5.5
6618	3	+	+	+	+	+
		5.5	6.0	6.5	5.5	5.75
München	4	—	—	—	—	—
		3.0	1.5	0.0	0.0	0.0

a) +: CPE positive, —: CPE negative.

b) Virus infective titer in PK cell cultures (log₁₀ TCID₅₀/ml).

Table 2. Cross neutralization test with PT passaged viruses

strain	(Serotype)	Antiserum				
		25R (1)	A47 (2)	6618 (3)	München (4)	HNF70 (Isolate)
A47	(2)	<1.5 ^{a)}	4.5	<1.5	<1.5	<1.5
6618	(3)	<1.5	<1.5	5.0	<1.5	<1.5

a) Neutralization antibody (log-index) by virus dilution method.

must be determined for many strains of all serotypes of PAV.

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要 約

豚アデノウイルスの豚精巣培養細胞における増殖性(短報): 平原 正・安原寿雄・松井 修・福山新一・山中盛正・出水田昭弘・吉木研一・児玉和夫・中井正久・佐々木文存((株)微生物化学研究所)——豚アデノウイルスの豚精巣培養細胞での増殖性について調べた結果, 2型のA47株と3型の6618株は明瞭な細胞変性効果を伴って増殖した。しかし, 1型の25R株, 4型のMünchen株および新分離株のHNF 61とHNF 70株は増殖しなかった。従って, 豚アデノウイルスには牛アデノウイルスと同様, 腎細胞増殖型と精巣細胞増殖型の存在が明らかにされた。しかし, その増殖性は血清型に共通した性質か否かは, 更に検討する必要がある。