Viral Susceptibility of a Cell Line Derived from the Pig Oviduct

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ABSTRACT

Seventeen of 24 RNA viruses and eight of nine DNA viruses replicated in a cell line derived from a pig fallopian tube. The following RNA viruses grew poorly in it: the virus of transmissible gastroenteritis of pig and the swine influenza, Sendai and bovine para-influenza type 3 viruses. Among other RNA viruses an untyped swine para-myxovirus and some picornaviruses, rhabdoviruses and toga-viruses attained high titers and produced an extensive cytopathic effect. Among the DNA viruses a porcine adeno, equine rhinopneumonitis, infectious bovine rhinotracheitis, pseudorabies and porcine cytomegalo viruses replicated in pig fallopian tube cells as well as in other cells generally used to grow them.

RÉSUMÉ

Les auteurs ont réussi à cultiver 17 virus ARN, sur un total de 24, et huit virus ADN, sur un total de neuf, dans une lignée cellulaire provenant des trompes de Fallope d'une truie. Parmi les virus ARN, ceux de la gastro-entérite transmissible et de l'influenza du porc, ainsi que le virus Sendai et le para-influenza 3 des bovins, s'y multiplièrent de façon médiocre; par contre, un para-myxovirus porcin non typé, ainsi que quelques virus picorna, rhabdo et toga, s'y développèrent de façon luxuriante et produisirent un effet cytopathogène marqué. Quant aux virus ADN, un adénovirus porcin, ceux de la rhino-pneumonite équine, de la rhino-trachéite bovine et de la maladie d'Aujeszky, ainsi qu'un virus cytomégalique porcin, se multiplièrent aussi bien dans cette lignée cellulaire que dans celles qu'on utilise ordinairement pour les cultiver.

INTRODUCTION

In a previous communication (3) some biological characteristics of a newly established cell line derived from pig fallopian tube (PFT) were described. Its morphology up to the 50th (± 10) subculture was epithelial-like whereas the subsequent subcultures were fibroblast-like. Subsequently, karyotypic alterations (Bouillant, Genest, Greig, unpublished data) and C-type virus (4) have been observed in the 90th and subsequent subcultures. No other virus contaminants have yet been found in this cell line.

The purpose of this study was to determine the capacity of some viruses of importance in animal diseases to replicate in the PFT cell line. It was undertaken as a portion of a more comprehensive project designed to fully characterize this pig cell line (3, Bouillant, Genest, Greig, unpublished data).

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MATERIALS AND METHODS

Cell Cultures

Procedures for culturing the PFT cell line have been described (3). Only PFT epithelium-like cells of the 20 to 40th subculture and fibroblast-like cells of the 70th to 110th subculture were used. Primary pig kidney (PPK) and pig thyroid (PPTh) cells, primary bovine fetal kidney cells (PBFK) and primary chicken embryo fibroblasts (PCEF) were prepared as reported elsewhere (11, 12, 16, 17).

Viruses

The 25 viruses which were found to replicate in PFT cells are listed in Tables I and II, while the eight viruses which failed to replicate are reported in the text only. The classification of Andrewes (1) was followed. Most of the virus strains were obtained from the virus collection of our Institute. Swine pox virus was purchased from the American Type Culture Collection, Rockville, Maryland. The swine vesicular disease (SVD) and Visna viruses were kindly provided by Dr. J. Callis[1] and Dr. H. Thormar[2] respectively. Foot and mouth disease (FMD) virus assays were conducted by Dr. B. Larenaudie[3].

All tests were carried out in duplicate in 25 cm^2 plastic flasks[4] containing 1 x 10^5 epithelial-like and 2 x 10^6 fibroblast-like cells respectively. One ml of virus was added to each flask at multiplicity of infection listed in Tables I and II. Virus inoculum was absorbed at 37°C for one hour. Then the inoculum was discarded and replaced by 5 ml of maintenance medium. Each flask was incubated at 37°C for five days, until a maximal CPE was observed in PFT cells. The fluids of the final passages in PFT cells were then collected and titrated again on a cell system indicated in Tables I and II. Heparinized blood from a pig infected with hog cholera (HC) or African swine fever (ASF) was diluted from 10^{-1} to 10^{-5} and each dilution was directly inoculated in triplicate onto PFT cells.

Virus growth was evaluated by one or more of the following criteria: cytopathic effect (CPE) including staining for inclusion bodies, hemagglutination and immunofluorescence. Fluids from infected PFT cells were titrated in PFT, PPK, PPTh, PBFK, PCEF or other reference cells. For instance, titrations of Visna virus were conducted in primary ovine choroid plexus cells (36). Equine coital exanthema (ECE) virus was titrated in equine dermis (NHL-6) cell line obtained from the American Type Culture Collection (CCL 57) and primary pig testicle cells (PPT) were used to assay the pig cytomegalovirus (25). All togaviruses were assayed in PCEF cells with the exception of Powassan virus which was inoculated into mice. The growth of HC and ASF viruses was evaluated by immunofluorescence. Titers were calculated by the method of Reed and Muench (32) and expressed as log 10 per ml.

RESULTS

Seven RNA viruses belonging to six separate groups of viruses did not replicate to detectable levels in PFT cells as evaluated by one or more of the above tests as well as reinoculation of reference cells. Of these, three were assayed on both epithelial-like and fibroblast-like cells, namely hemagglutinating encephalitis virus of pig (strain HEV 2), para-influenza 2 virus (strain Greer) and Newcastle disease virus (strains B 1 and G.B., Texas). The remaining four viruses were assayed only on fibroblast-like cells, i.e. blue tongue virus (strain California 10), bovine virus diarrhea (strain NADL), Sindbis virus (strain A-339) and Visna virus (strain 796).

Seventeen RNA viruses replicated in PFT cells (Table I). However the titers of virus in the fluids were not always similar to those of the fluids of cells routinely used for each virus. Low titers were found in PFT cells infected with the virus of transmissible gastroenteritis of pig (TGE). It appeared that TGE virus replicated in these cells as indicated by immunofluores-

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[4]Tissue culture flask, cat. no. 3012, Falcon Plastics, Oxnard, California.

cence, minimum and transient CPE and low titers which, nevertheless, precluded their usage in the routine diagnosis of TGE. With the exception of swine para-influenza virus which produced a titer of 7.0 all the myxoviruses tested replicated poorly in PFT cells. A similar observation was made with bovine para-influenza 3 virus (SF4) where a titer of 2.5 was detected in PFT cells. A titer of 5.0 or greater was obtained in the PFT cells infected with most of the picornaviruses and rhabdoviruses. The equine encephalitides viruses were also cytopathogenic and replicated to high titers in PFT cells. Although no CPE was observed in PFT cells inoculated with HC infected blood evidence of virus growth was detected by immunofluorescence but with less intensity than in PPK cells.

As shown in Table II eight DNA viruses replicated in PFT cells while ECE virus did not. The porcine adenovirus grew and produced a characteristic CPE (22) but titers were relatively low. The herpes viruses, except for ECE virus, replicated with the production of CPE in PFT cells. ASF virus did not induce a CPE in PFT cells but evidence of virus growth was detected by immunofluorescence. Parvovirus replication was shown by the presence of nuclear inclusion bodies and immunofluorescence in cells and by hemagglutination

TABLE I. Susceptibility of the Pig Cell Line to RNA Viruses

Virus	PFT Subculture[a]	MOI[b]	CPE[c]	IB	HA	FA	Ref. Cells and Virus Titer/ml from PFT Fluids[d]	Remarks
A. Corona								
1. Transmissible Gastroenteritis of pig								
— Ames strain	32	ND[e]	±			+	ND	
	85	0.1	±			+	PTh:2.0	
— Purdue strain	85	0.3	±			+	PTh:2.5	
	86	0.3	±			+	PPK:1.8	
B. Orthomyxo								
1. Swine influenza								
— Shope strain	23	0.05	+		+		PFT:2.5	
	104	0.05	+		+		ND	
C. Para-myxo								
1. Swine para								
— untyped strain	23	0.1	+		+		PFT:7.0	
	80	0.1	+		+		PFT:7.0	
2. Para 1								
— Sendai strain	22	0.1	+		+		PFT:3.5	
	104	0.1	+		+		ND	
3. Bovine para 3								
— SF4 strain	27	0.1	+				PFT:2.5	
	104	0.1	+				ND	
D. Picorna								
1. Foot and Mouth Disease								
— type A	90	ND	+				PPK: > 5.0	
— type C	90	ND	+				PPK: > 5.0	
— type O	90	ND	+				PPK: > 5.0	
2. Pig enterovirus PE-1								
— ADRI strain	29	0.1	+				PFT: 6.7	
	104	0.1	+				ND	
3. Swine Vesicular Disease	75	0.04	+				PPK:8.0	
4. Teschen								
— Konratice strain	33	0.1	+				ND	
	84	0.01	+				PPK:5.6	
— French strain	86	0.01	+				PPK:2.5	
5. Vesicular Exanthema of Swine								
— Nebraska strain	33	0.04	+				ND	
	89	0.04	+				PPK:6.5	

TABLE I. (Continued)

Virus	PFT Sub-culture[a]	MOI[b]	CPE[c]	IB	HA	FA	Ref. Cells and Virus Titer/ml from PFT Fluids[d]	Remarks
E. Rhabdo								
1. Rabies								
— ERA strain	20	0.01	+	+	+	+	ND	
	70	0.01	+	+	+	+	PFT:5.5	see ref. 5
2. Vesicular Stomatitis								
— NJ type	23	0.1	+				PFT:7.0	
	89	0.1	+				PPK:6.5	
F. Toga								
1. Eastern Equine Encephalitis								
— Quebec 72 strain	76	5.0	+				CEF:9.5	
2. Powassan								
— McLean isolate	20	ND	+				ND	Undiluted fluids from PFT cells killed mice I.C.
3. Venezuelan Equine Encephalitis								
— TC-83 strain	74	0.2	+				CEF:9.0	
4. Western Equine Encephalitis								
— California strain	60	0.2	+				CEF:6.5	
G. Unclassified								
1. Hog Cholera								
— ADRI strain	100	ND	−			+	ND	Infected blood at a dilution of 10^{-5}

[a]Epithelial-like cells: 20 to 40th subcultures, fibroblast-like cells: 70 to 110th subcultures
[b]MOI: multiplicity of infection
[c]CPE: cytopathic effect (+: 50% or more of cell destruction of the monolayer, ±: transient and less than 5% of cell destruction, −: no cell destruction), IB: inclusion body, HA: hemagglutinating activity, FA: fluorescent antibody
[d]Reference cells: cells employed for the titration of virus in harvested PFT cell fluids, titer expressed as $\log_{10}$ per ml
[e]ND: not done

of guinea pig red blood cells in the supernatant fluid. Swine pox virus induced CPE with characteristic inclusion bodies (14, 23) and attained titer of 5.0 or greater.

DISCUSSION

Widespread application of cell culture in biological research and virus disciplines requires the development of well characterized cell lines for diagnosis and research (37). Fedoroff's recommendations (13) pertaining to characterization of new cell lines were followed in reporting some biological characteristics of the PFT cell line (3) and studies concerning the growth of this cell line under different conditions (Bouillant, Genest, Greig, unpublished data).

Although pig cell lines have been initiated and used in some laboratories (10, 19, 20, 28, 30, 31, 33, 34, 35, 38) no comprehensive evaluation of the replication of viruses in any of these cell lines has yet been reported. Therefore, a survey of various viruses in the PFT cell line was conducted not only to further characterize this line but also to explore its potential for diagnosis and research on animal diseases. No attempt was made to compare titers obtained in PFT cells with those of reference cells.

As shown in Tables I and II response of PFT cells to virus infection was similar in epithelial-like and fibroblast-like cells. This parallelism of virus susceptibility is consistent with the concept that a cell line can retain some of its biological character-

TABLE II. Susceptibility of the Pig Cell Line to DNA Viruses

Virus	PFT Sub-culture[a]	MOI[b]	CPE[c]	IB	HA	FA	Ref. Cells and Virus Titer/ml from PFT Fluids[d]	Remarks
A. Adeno								
1. Swine								
— Kasza strain	25	0.01	+	+	+		ND[e]	
	89	0.01	+	+	+		PPK:3.5	
B. Herpes								
1. Equine Rhino-pneumonitis								
— Kentucky strain	80	0.1	+	ND		+	BFK:6.0 PFT:5.5	
2. Infectious Bovine Rhinotracheitis								
— Colorado strain	21	0.01	+	+			PPK:6.7 PFT:5.5	
	89	0.01	+	+			PPK:4.5 PFT:4.7	
3. Pseudo-Rabies								
— Aujeszky strain	26	0.01	+	+			PPK and PFT:8.5	
	87	0.01	+	+			PPK:8.5	
4. Pig Cytomegalo								
— ADRI strain	25	0.01	+	+			ND	
	89	0.01	+	+			PPT:4.5	
C. Irido								
1. African Swine Fever								
— Spencer strain	30	ND	−			+	ND	Undiluted infected blood
	70	ND	−			+	ND	Infected blood at a dilution of 10^{-5}
D. Parvo								
1. Swine	77	ND	−	+	+	+	ND	
E. Pox								
1. Swine								
— Kasza strain	25	0.2	+	+		+	PPK:5.7 PFT:5.2	
	77	0.2	+	+		+	ND	

[a]Epithelial-like cells: 20 to 40th subcultures, fibroblast-like cells: 70 to 110th subcultures
[b]MOI: multiplicity of infection
[c]CPE: cytopathic effect (+ : 50% or more of cell destruction of the monolayer, − : no cell destruction), IB: inclusion body, HA: hemagglutinating activity, FA: fluorescent antibody
[d]Reference cells: cells employed for the titration of virus in harvested PFT cells fluids, titer expressed as $\log_{10}$ per ml, ND: not done
[e]ND: not done

istics through a large number of subcultures (Bouillant, Genest, Greig, unpublished data). The potential of the PFT cell line for virus investigation has been shown already for the titration of rabies virus and for its neutralization (5). Results showed that 25 out of 33 viruses were capable of replication in this cell line. Pig viruses replicated in PFT cells to the same order of magnitude as in PPK cells with the exception of HEV and TGE. SPI, picorna, rhabdo, herpes and swine pox viruses grew as well in PFT cells as in other reference cells. The fact that ECE virus did not grow in PFT cells is in agreement with previous work indicating that it will replicate only in equine cells (15, 24 and A. Girard, unpublished data). The isolation of ASF and HC viruses from infected blood, confirmed by immunofluorescence but not by CPE, merits further investigation. However it should be pointed out that in the case of HC and ASF the intensity of fluorescence observed was not as high in PFT cells as in PPK cells. Among togaviruses, equine encephalitides viruses grew as well in PFT cells as in reference cells. All four viruses causing similar vesicular diseases in pig (FMD, SVD, vesicular exanthema of swine and vesicular stoma-

titis viruses), attained high titers in PFT cells. Among the eight viruses which did not replicate to a detectable level in PFT cells only one, HEV, is a pig virus. It is of interest to note that TGE, another pig coronavirus, did grow in PFT cells although titers were lower than in two other cell systems (11).

In these studies it was found by immunofluorescence that the PFT cell line was not contaminated by HC and porcine parvoviruses as has been shown with some other cell lines (7, 9, 20, 35). Bovine viruses which may have been introduced into cultures by fetal bovine serum (27) were not detected in this cell line. Karyotypic alterations and C-type viruses have been shown in spontaneously and in virus transformed cell lines (31) and especially in other pig cell lines (2, 6, 8, 26, 33, 38). Some karyotypic modifications were also observed in the PFT cell line (Bouillant, Genest, Greig, unpublished data) and C-type viruses were induced by 5-bromodeoxyuridine as early as the 90th subculture and spontaneously released in the 217th subculture as shown by electron microscopy and reverse transcriptase assay (4). Because some C-type viruses are known to be etiological agents of leukemias, lymphomas and sarcomas in several species (18) the PFT cell line should not be used in vaccine production (29) until it is proved to be free of oncogenic viruses and safe from adverse effects in animals. However, as demonstrated, the PFT cell line has potential value in diagnostic virology and research.

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