Swine Interferon I. Induction in Porcine Cell Cultures with Viral and Synthetic Inducers

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ABSTRACT

The production of interferon by porcine kidney (PK_{15}) cell culture in response to viral and synthetic inducers was studied. The inducers used included a synthetic double-stranded polyribonucleotide, polyriboinosinic-polyribocytidylic acid (Poly I:C), swine influenza virus and three strains of pseudorabies virus. Following exposure to these inducers cell culture fluids were examined for interferon by the plaque-reduction method.

The Poly I:C and the swine influenza virus induced production of interferon by PK_{15} cell cultures, whereas, all three strains of pseudorabies virus at the two concentrations tested failed to induce production of interferon in vitro.

The antiviral substance produced in PK_{15} cells was identified as an interferon because it was pH stable, non-dialyzable, sensitive to trypsin, non-sedimentable, relatively heat stable, host-species specific and it possessed broad-spectrum antiviral activity. The latter was demonstrated by inhibition of vesicular stomatitis, vaccinia and pseudorabies viruses. Differences in interferon activity against the different viruses were observed.

RÉSUMÉ

Cette expérience visait à étudier l'élaboration d'interféron par des cultures de cellules rénales de porc (PK_{15}) en réponse à certains agents viraux et synthétiques. Les agents utilisés à cette fin comprenaient un polyribonucléotide synthétique à double chaîne: l'acide polyriboinosinique - polyribocytidylique (Poly I:C), le virus de l'influenza du porc et trois souches du virus de la pseudo-rage. Après avoir fait subir l'action de ces agents aux cultures cellulaires, on vérifia la présence d'interféron dans leur liquide en utilisant la méthode de la réduction des plages.

L'acide Poly I:C et le virus de l'influenza du porc provoquèrent l'élaboration d'interféron par les cultures cellulaires PK_{15}, tandis que les trois souches du virus de la pseudo-rage, aux deux concentrations utilisées, ne provoquèrent pas la production d'interféron in vitro.

On identifia la substance antivirale élaborée par la lignée cellulaire PK_{15} comme étant de l'interféron, en se basant sur les critères suivants: stabilité de son pH, impossibilité de la dialyser ou de la faire sédimenter, sensibilité à la trypsine, thermostabilité relative, spécificité d'espèce et action inhibitrice à l'endroit de plusieurs virus. La démonstration de cette dernière propriété s'effectua par l'inhibition des virus de la stomatite vésiculaire, de la vaccine et de la pseudo-rage. On observa des variations dans l'activité de l'interféron à l'endroit des différents virus.

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INTRODUCTION

Interferon production has been induced in many different animal species and cell cultures by viral and non-viral stimuli (7, 9) but there is little information on the response of swine systems to interferon inducers. There are two reports of interferon production by suspension cultures of swine leukocytes in response to phytohemagglutinin (11, 12).

In this study the ability of porcine kidney cell cultures (PK_{15}) to produce interferon in response to viral and synthetic inducers was investigated. The inducers used included polyriboinosinic - polyribocytidylic acid (Poly I:C) a synthetic double-standed ribonucleic acid, swine influenza virus (SIV) a single-stranded enveloped RNA virus, and pseudorabies virus (PRV) a double-stranded enveloped DNA virus. Poly I:C has been reported to be a potent interferon inducer in rabbit (1), mouse (2), rat (5), dog (4), human (4), hamster (4), and chicken embryo (3) cell cultures. No report of the response of swine cell cultures to Poly I:C has appeared in the literature. Interferon production in response to PRV *in vivo* or *in vitro* in swine has not been reported. The interferon-inducing ability of SIV has also not been studied in swine systems.

Since the interferon system has become recognized as a major natural defence mechanism against viral disease it is important that the interferon-producing ability of swine cell cultures in response to different inducers be determined. This knowledge will serve as background information in the study of interferon production in the intact swine host.

MATERIALS AND METHODS

CELL CULTURES

A porcine kidney cell line (PK_{15}) was used for interferon production, assay and characterization. The cells were grown in Eagle's basal medium (BME) with Earle's salts, supplemented with 10% newborn calf serum[1] and 0.11% sodium bicarbonate. The maintenance medium was the same, except that the concentration of serum was reduced to 2%.

The Madin-Darby bovine kidney (MDBK) cells which were used for interferon characterization were grown and maintained in the same medium described for PK_{15} cells.

The baby hamster kidney (BHK) cell line was also used for the interferon characterization. The cells were grown in BHK-21 medium[2] supplemented with 10% tryptose-phosphate broth, 10% newborn calf serum and 0.11% sodium bicarbonate.

Overlay medium for interferon assays consisted of 2x BME containing 10% newborn calf serum and 0.22% sodium bicarbonate, mixed in equal parts with 2.2% Noble agar[3]. A neutral-red agar overlay containing 1:10,000 neutral red and 1% agar was used as the vital strain to differentiate the virus plaques.

VIRUSES

Vesicular stomatitis virus, New Jersey strain (VSV-NJ) of unknown passage level, was used at the second and third passage levels on the PK_{15} cells for interferon assays.

Swine influenza virus, Shope strain (SIV) of undetermined passage level, was used for interferon induction. The virus was propagated by allantoic-cavity inoculation of 12-day-old chicken embryos and the allantoic fluid was harvested at 96 hours post inoculation from the dead or live eggs. SIV-containing allantoic fluid with a titer of 320 chicken-erythrocyte hemagglutinating units per ml was used as an interferon inducer.

Three strains of pseudorabies virus (PRV) each at two different concentrations were used in attempts to induce interferon production in PK_{15} cells. The strain designations and passage levels of the PRV

[1]Grand Island Biological Company, Grand Island, New York.

[2]Grand Island Biological Company, Grand Island, New York.

[3]Difco Laboratories, Inc., Detroit, Michigan.

strains used are as follows: PRV-Pa[4], 15 passages on MDBK, 3 on PK_{15} cells; PRV-Dr[5], 11 passages on MDBK, 2 on PK_{15} cells; PRV-Be[6], 8 passages on MDBK, 2 on PK_{15} cells. All three strains of PRV were purified and concentrated by centrifugation (14).

Vaccinia virus[7] of undetermined passage level on chicken embryos followed by three passages on PK_{15} cells was used for interferon characterization experiments.

All viral preparations were stored at -90°C before use.

TABLE I. Induction of Interferon in PK_{15} Cell Culture by Poly I:C and SIV

Inducer	Interferon Titer[a] per ml Hours of Incubation						
	2	4	6	8	10	12	24
Poly I:C (50 μg/ml)	2	8	16	16	16	16	8
SIV	4	16	32	32	32	32	8

[a]Interferon titers are expressed as the reciprocal of the dilution which produces 50% reduction in the number of viral plaques

TABLE II. Effect of Poly I:C Concentration on Interferon Titer in PK_{15} Cells

Concentration of Poly I:C μg/ml	Interferon titer[a] per ml after six Hours
0	0
0.625	0
1.25	0
2.5	0
5	2
10	8
20	8
40	8
80	32
160	128
320	256

[a]Interferon titers are expressed as the reciprocal of the dilution which produces 50% reduction in the number of viral plaques

[4]Obtained from Dr. L. N. Brown, Iowa Veterinary Diagnostic Laboratory, Iowa State University.

[5]Isolated by Dr. C. J. Maré, College of Veterinary Medicine, Iowa State University.

[6]Isolated by Dr. C. J. Maré, College of Veterinary Medicine, Iowa State University.

[7]Obtained from Dr. C. H. Cunningham, Michigan State University, East Lansing, Michigan.

Polyriboinosinic-polyribocytidylic Acid

Poly I:C[8] was stored at 4°C in vials until used. The drug was diluted in maintenance medium to the appropriate concentrations (Tables I, II, and III) and was used as the interferon inducer in PK_{15} cell cultures.

Interferon Induction

One-day-old PK_{15} cell-culture monolayers grown in 30 ml plastic tissue-culture flasks were used. The cell monolayers were covered with 1 ml of the viral inducers or 0.8 ml Poly I:C at the appropriate concentration and incubated for one hour at 37°C. They were then thoroughly washed with the maintenance medium and replenished with 5.0 ml of the maintenance medium. A sufficient number of cell culture flasks were used to allow collection of the fluids at different times after treatment.

The culture fluids were collected, dialyzed in cellulose tubing at 4°C against 0.1M KCl-HCl buffer (pH 2) for 24 hours and then against 0.01M phosphate buffered saline (PBS) at pH 7.5 for 24 hours. They were then centrifuged for 15 minutes at 2,000 rpm, filtered through 300 nm Millipore filters[9] and stored at 4°C until assayed for interferon activity.

Interferon Assay

Serial two-fold dilutions of the dialyzed and centrifuged interferon were made in maintenance medium. One ml of each dilution was inoculated into each of two well-drained (35 x 10 mm) wells of a cell culture plate[10] containing one-day-old PK_{15} cell monolayers and allowed to remain in contact with the cells for 18 to 22 hours in a 5% CO_2 atmosphere at 37°C. Control cultures were treated overnight with 1 ml of the maintenance medium. Following the incubation period, fluids were aspirated, and 0.5 ml of VSV-NJ calculated to contain 50 to 100 pfu/ml was added to each well. After adsorption at room temperature for one hour, excess viral fluids were aspirated and the cultures were overlaid with

[8]Microbiological Associates, Bethesda, Maryland.

[9]Millipore Filter Corporation, Bedford, Massachusetts.

[10]Linbro Chemical Company, New Haven, Connecticut.

TABLE III. Properties of PK_{15} Cell Culture Interferon

Tretment	Results: Induced by SIV	Results: Induced by Poly I:C 320 μg/ml
1. Dialysis for 48 hours at 40°C		Non-dialyzable
2. Acidification to pH2		Activity retained
3. Activity against different viruses		Broadspectrum activity
Virus		Titer[a]
VSV-NJ	32	256
PRV-DR	2	32
PRV-Pa	—	16
PRV-Be	—	32
Vaccinia	8	128
4. Ultracentrifugation		Non-sedimentable
Titer before ultracentrifugation	16	256
Titer after ultracentrifugation	16	128
5. Trypsin sensitivity		Activity destroyed
Control titer	16	256
Trypsin (2 μg/ml) for 2 hrs at 37°C	0	0
6. Activity against VSV-NJ in heterologous cells		Not active
Titer on PK_{15}	32	128
Titer on BHK	0	2
Titer on MDBK	0	0
7. Heat stability		Relative stable
Control titer	16	256
Titer after heating 37°C for 6 hrs	16	256
Titer after heating 37° C for 12 hrs	4	128
Titer after heating 56°C for 30 min	4	64

[a]Interferon titers are expressed as the reciprocal of the dilution which produced 50% reduction in the number of viral plaques

overlay medium. When plaques became visible after incubation at 37°C for several days, a neutral red agar overlay was added. The interferon titers were expressed as the reciprocal of the dilution which produced 50% reduction in the number of plaques as compared with the number in control cultures.

Characterization of Interferon

All samples were dialyzed for 24 hours against 50 volumes of 0.1M KC-HC1 buffer (pH 2) at 4°C in dialysis tubing. This was followed by a similar dialysis against PBS (pH 7.5) for 24 additional hours.

Representative samples were then tested for species specificity by assaying the antiviral substance produced in PK_{15} cells on PK_{15}, MDBK and BHK cells. The antiviral substance was tested for lack of viral specificity by evaluation of antiviral effect against VSV-NJ, PRV-Pa, PRV-DR, PRV-Be and vaccinia viruses on PK_{15} cells by the plaque-reduction method. Heat stability was checked by heating at 37°C and 56°C as indicated in Table III. The samples were ultracentrifuged at 100,000 g for 60 minutes and the supernatant fluid was assayed for antiviral activity using VSV-NJ.

Sensitivity to proteolytic enzymes was tested by incubating a mixture of the interferon sample and trypsin[11] (2 μg/ml in physiological saline) for two hours at 37°C. After the incubation interferon was assayed for antiviral activity against VSV-NJ on PK_{15} cells. Suitable controls were included with each test.

RESULTS

Interferon Induction by Poly I:C

A solution of Poly I:C containing 50 ug/ml was incubated for one hour in PK_{15} cells. A sufficient number of cell culture flasks were used so that the fluids could be collected at two, four, six, eight, ten, 12

[11]Trypsin 1:250, Difco Laboratories, Detroit, Michigan.

and 24 hours after the treatment and assayed using VSV-NJ virus. Low levels of interferon were detected in cell culture fluids collected at two hours and the maximum titer of 16 was detected at six hours post exposure. The results are summarized in Table I.

Poly I:C concentrations ranging from 0.625 to 320 μg/ml were used to induce interferon production. The culture fluids were collected six hours after treatment and assayed using VSV-NJ virus. Poly I:C concentrations of 0.625 to 2.5 μg/ml did not induce any detectable interferon formation in PK_{15} cell culture. Poly I:C at a concentration of 5 μg/ml induced a low level of interferon.

A positive correlation was observed between Poly I:C concentration and the titer of interferon induced in PK_{15} cell culture. The results are presented in Table II.

Interferon Induction by Pseudorabies virus

Three strains of pseudorabies virus, namely PRV-Pa, PRV-DR and PRV-Be at the following high and low titers were used: PRV-Pa 9.1 x 10^7 pfu/ml and 9.1 x10^2 pfu/ml; PRV-DR 1.6 x 10^8 pfu/ml and 1.6 x 10^2 pfu/ml; PRV-Be 2.0 x 10^8 pfu/ml and 2.0 x 10^2 pfu/ml. Cell culture fluids were collected at two, six, 12, 24, 36, and 48 hours after virus adsorption and assayed for interferon activity on PK_{15} cell cultures using VSV-NJ.

All three strains of pseudorabies virus at the two concentrations tested failed to induce the production of detectable interferon on PK_{15} cells. An observable cytopathic effect (CPE) started after 16 hours following PRV inoculation at high (10^7-10^8 pfu/ml) concentrations and by 36 hours all the cells showed CPE. Observable CPE started later when low (10^2 pfu/ml) concentrations of PRV were used but by 48 hours complete CPE was observed.

Interferon induction by swine influenza virus

One ml of SIV virus Shope strain with a titer of 320 hemagglutinating units per ml was inoculated per flask. The cell culture fluids were collected at two, four, six, eight, ten, 12 and 24 hours after exposure. A low level of interferon was detected in cell culture fluids, collected at two hours post exposure. A maximal titer of 32 was detected at six hours. The results are summarized in Table I.

Characterization of interferon

The viral inhibitor produced in PK_{15} cells in response to Poly I:C and SIV was characterized as being acid-resistant, nondialyzable, non-sedimentable by centrifugation at 100,000 g for 60 minutes, trypsin sensitive, relatively heat stable, active against different viruses and possessed species specificity. The results of these tests are summarized in Table III.

DISCUSSION

In this study Poly I:C has been successfully used to induce interferon in porcine kidney (PK_{15}) cell cultures. A positive correlation was observed between the concentration of Poly I:C and the titer of interferon produced in PK_{15} cell cultures. No plateau was reached even at a 320 μg/ml concentration of Poly I:C. A minimum Poly I:C concentration of 5 μg/ml was required to induce a detectable interferon level in PK_{15} cell culture. This concentration of Poly I:C is much higher than that required for interferon induction in primary rabbit kidney, dog kidney and human embryonic kidney cell cultures (4). It is known that cell cultures vary in their sensitivity to an inducer with respect to their species of origin but one variable which should not be overlooked is the fact that the source of Poly I:C used by different investigators may have been different and that variations in sensitivity of cell systems may be due to this fact.

All three strains of pseudorabies virus at the two concentrations tested failed to induce production of interferon *in vitro*. Results of this study confirm earlier reports (6, 8, 10) that herpesviruses are poor interferon inducers *in vitro*. Apparently the failure of PRV to induce interferon in PK_{15} cell cultures is due to the rapid damage of the host cells by PRV replication prior to interferon synthesis. Swine influenza virus induced interferon production in PK_{15} cells. A low level of interferon was detected at two hours post exposure and the maximal titer was reached at six hours post exposure.

With both Poly I:C and SIV detectable interferon was produced on PK_{15} cells at two hours post treatment and the maximal titer was reached six hours post exposure. Interferon production in response to Poly I:C is much greater than to SIV at the virus dose used.

The antiviral substance produced in PK_{15} cells was found to fulfill the biological criteria to be classified as an interferon (9). It was a macromolecule of relatively small size as indicated by its lack of sedimentation at 100,000 g for one hour and the fact that it was non-dialyzable. It possessed stability over a broad range of pH and sensitivity to trypsin the latter indicating that the active antiviral substance was protein in nature. The antiviral property was partially inactivated by heating at 56°C for 30 minutes. Broad-spectrum antiviral activity was demonstrated by inhibition of VSV-NJ, PRV-DR, PRV-Pa, PRV-Be and vaccinia viruses. Differences in interferon activity against the different viruses were observed. All three strains of pseudorabies virus were less sensitive to interferon action than VSV-NJ or vaccinia.

A narrow host-species specificity was shown by treating heterologous BHK and MDBK cell cultures with the antiviral substance produced on PK_{15} cells. In contrast to the findings of Torlone *et al* (13) who found that swine interferon is active in primary calf kidney cell cultures, the MDBK cell line used in these experiments as well as the BHK cells did not respond to treatment with the antiviral substance produced on PK_{15} cells.

These characteristics justify the conclusion that the antiviral substance produced by the cells in response to Poly I:C and SIV is in fact an interferon (9).

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