Swine Interferon II. Induction in Pigs with Viral and Synthetic Inducers

V. E. Vengris and C. J. Maré*

ABSTRACT

The production of interferon by pigs in response to viral and synthetic inducers was studied. The inducers used included polyriboinosinic-polyribocytidylic acid (Poly I:C), swine influenza virus and pseudorabies virus. Following intravenous inoculation of pigs with the inducers, sera were examined for interferon by the plaque-reduction method in porcine kidney (PK_{15}) cell cultures using vesicular stomatitis virus as the challenge inoculum. It was shown that pigs can produce interferon in response to each of these inducers. The pseudorabies virus used in this investigation was found to be a better interferon inducer than the swine influenza virus.

The interferon produced in pigs was identified as an interferon because it was pH stable, non-dialyzable, sensitive to trypsin, non-sedimentable and possessed broad-spectrum antiviral activity as well as host-species specificity.

RÉSUMÉ

Cette expérience visait à étudier la production d'interféron chez des porcs, en réponse à certains agents viraux et synthétiques. Les agents utilisés à cette fin comprenaient l'acide polyriboinosinique - polyribocytidylique (Poly I:C), ainsi que les virus de l'influenza du porc et de la pseudo-rage. Après avoir injecté ces agents à des porcs, par la voie intra-veineuse, on rechercha la présence d'interféron dans leur sérum au moyen de la méthode de la réduction des plages dans des cultures de cellules rénales de porc (PK_{15}) ensemencées avec le virus de la stomatite vésiculaire. On démontra que les porcs injectés avec l'un ou l'autre des agents employés élaborèrent de l'interféron. Le virus de la pseudo-rage utilisé au cours de l'expérience s'avéra un meilleur provocateur de la production d'interféron que le virus de l'influenza du porc.

On identifia comme tel l'interféron produit chez les porcs, en se basant sur les critères suivants: stabilité de son pH, impossibilité de le dialyser ou de le faire sédimenter, sensibilité à la trypsine, action inhibitrice à l'endroit de plusieurs virus et spécificité d'espèce.

INTRODUCTION

The interferon system has become recognized as a major natural defense mechanism against viral diseases. It is believed to play an important role in the recovery of host animals from viral infections and serves to limit virus spread through the blood stream (1, 6).

Interferon production has been induced in many animal species but there is little information on the ability of the pig to produce interferon in response to various stimuli. Torlone *et al* (20) reported the successful induction of interferon in pigs infected with hog cholera virus but did

*Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa 50010.

This study was supported by training grant T1-AI-250-07 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.

Submitted December 29, 1971.

not determine whether the pig could produce interferon in response to other inducers.

The purpose of this experiment was to investigate the interferon-producing capacity of swine in response to various inducers. The inducers used included polyriboinosinic-polyribocytidylic acid (Poly I:C), a synthetic double-stranded ribonucleic acid, swine influenza virus (SIV), a single-stranded enveloped RNA virus, and pseudorabies virus (PRV), a double-stranded enveloped DNA virus.

MATERIALS AND METHODS

The cell cultures, media, viruses, and procedures for interferon assay and characterization have been described (21).

Polyriboinosinic-polyribocytidylic Acid

Poly I:C[1] was stored at 4°C in vials until used and was used as an interferon inducer in pigs.

Experimental Pigs and Interferon Induction

The pigs used for interferon production in response to Poly I:C and pseudorabies virus (strain PRV-Pa) were of mixed breed and were obtained from a private pig producer. They were shown to be free of PRV antibodies prior to inoculation. The pigs used for interferon production in response to SIV were of the Hampshire breed and were obtained from the respiratory-disease-free swine herd maintained at the Veterinary Medical Research Institute, Iowa State University.

The pigs were of both sexes and ranged in weight from 10.4 kg to 19.4 kg at the start of the experiment. They were housed individually in 2.5 x 2.5 x 3 foot isolation cages, with air tight doors and virus retaining filters on the intake and exhaust ports.

Pigs were inoculated with the appropriate virus or Poly I:C into the ear veins. The dosage levels are indicated in Tables I and II. Before inoculation, and at specified times after inoculation, blood samples were collected for interferon assay using the orbital sinus bleeding technique (9). The rectal temperatures were recorded twice daily.

Serum Preparation for Interferon Assay

After firm clots were formed the sera were harvested and clarified by centrifugation for 15 minutes at 2,000 rpm. The serum samples were then dialyzed, centrifuged, filtered, stored and assayed as described previously (21).

RESULTS

Interferon Induction by Polyriboinosinic-polyribocytidylic Acid (Poly I:C)

The experimental pigs were inoculated intravenously with Poly I:C using the ear veins. The dosage levels per kilogram of body weight are indicated in Table I. Pigs number 2 and 3 were restimulated with Poly I:C administered intravenously on the seventh day after the first stimulation. Pig number 4 was used as a control (Table II).

Circulating interferon was detected and found at maximal titer by postinoculation hour two (Table I). Reduced or not titers of serum interferon were observed by the sixth hour after inoculation.

Pigs number 2 and 3 restimulated by the intravenous administration of Poly I:C on the seventh day after the first stimulation produced lower interferon titers than after first induction (Table II). Neither pyrogenicity nor toxicity was observed in pigs inoculated intravenously with Poly I:C.

Interferon Induction by PRV-Pa

The experimental pigs were inoculated intravenously with one ml of PRV-Pa containing 3.7 x 10^7 pfu/ml. The one control pig was inoculated with 1.0 ml of saline G (16). Results are summarized in Table I.

A detectable low level of interferon was produced by six hours post inoculation in one pig, and all the pigs, except the control, produced interferon by 24 hours after inoculation. Due to electrical failure, resulting in an inadequate air circulation in the isolation units, two pigs (9 and 10) died of suffocation. Maximal interferon titers were reached in two pigs (8 and 11) at 72 hours post inoculation. Pig number 7 reached peak titer at 24 hours. The serum interferon levels diminished thereafter but

[1]Microbiological Associates, Bethesda, Maryland.

TABLE I. Serum Interferon Titers of Pigs Given Intravenous Injections of Synthetic and Viral Inducers.

Inducers	Pig No.	Sex	Body Weight kg	Dose of Inducer	Control Inoculum[a] ml	Interferon Titers[b] per ml Hours Postinoculation 0	2	6	12	24	48	72	96	120	144	168
Synthetic				mg/kg												
Poly I;C	1	M	13.6	.3	0	0	4	0	0	0	0	0	—	—	—	—
	2	M	17.2	.1	0	0	128	2	0	0	0	0	—	—	—	—
	3	F	14.5	.3	0	0	64	4	0	0	0	0	—	—	—	—
	4	F	16.8	.1	0	0	4	0	0	0	0	0	—	—	—	—
	5	M	19.4	.1	0	0	16	2	0	0	0	0	—	—	—	—
	6	M	15.0	0	2	0	0	0	0	0	0	0	—	—	—	—
Viral agents				ml												
PRV-Pa[c]	7	F	11.3	1.0	0	0	0	2	—	64	32	32	32	Died		
	8	F	10.4	1.0	0	0	0	0	—	64	64	128	32	Died		
	9	F	12.3	0	1.0	0	0	0	—	0	Died[d]					
	10	F	13.6	1.0	0	0	0	0	—	16	Died[d]					
	11	F	11.8	1.0	0	0	0	0	—	4	4	64	16	2	2	0
SIV[e]	12	F	14.5	0	1.5	0	0	0	0	0	0	0	0	—	—	0
	13	M	14.8	1.0	0	0	0	2	4	2	2	0	0	—	—	0
	14	M	14.5	2.0	0	0	0	0	0	0	0	0	0	—	—	0
	15	M	13.7	2.0	0	0	2	2	4	8	4	4	0	—	—	0
	16	M	13.2	1.0	0	0	0	2	0	0	0	0	0	—	—	0

[a]Pig No. 6 Physiological saline
Pig No. 9 Saline G
Pig No. 12 Normal allantoic fluid
[b]Interferon titers are expressed as the reciprocal of the dilution which produced 50% reduction in the number of viral plaques
[c]Titer of 3.7 x10^1 pfu/ml
[d]Died of suffocation
[e]Titer of 320 HA units/ml

TABLE II. Serum Interferon Titers of Pigs Restimulated with Poly I:C Intravenously

Pig No.	Sex	Body Weight kg	Poly I:C Dose mg/kg	Physiological Saline Dose ml	Interferon Titer Hours Postinoculation 0	2	6	12	24	48	72
2	M	19.8	.1	0	0	16	2	2	0	0	0
3	F	18.6	.1	0	0	32	0	0	0	0	0
4	F	19.0	0	2	0	0	0	0	0	0	0

in pig number 11 the titer remained at detectable levels through 144 hours after inoculation. Pigs number 7 and 8 had detectable interferon in their sera until 96 hours post inoculation. Both died displaying typical symptoms of pseudorabies infection on the fifth day after the inoculation of virus. Pig number 11 showed symptoms characteristic of PRV infection and recovered without treatment.

All infected pigs showed a febrile response at different times post inoculation. No positive correlation between febrile response and serum interferon titers was observed.

Interferon Induction by Swine Influenza Virus

Experimental pigs were inoculated intravenously with SIV-containing allantoic fluid. Dosage levels and results are summarized in Table I.

One pig did not produce any detectable interferon in the serum. Another pig produced a low level of interferon only at six hours post inoculation. Low levels of the interferon were detectable in sera collected at six to 48 hours after inoculation of pig number 13. Pig number 15 produced higher titers of interferon, reaching a maximum titer of eight at 24 hours post inoculation.

All intravenously injected pigs showed a slight febrile response. A positive correlation between febrile response and serum interferon titers was not observed.

Characterization of Interferon

The viral inhibitor produced in pigs in response to Poly I:C, PRV-Pa, and SIV was characterized as being acid resistant, nondialyzable, non-sedimentable at 100,000 g for 60 minutes, trypsin sensitive, relatively heat stable, active against different viruses and species specific. The results are given in Table III.

DISCUSSION

A synthetic double-stranded polyribonucleotide complex, Poly I:C, has previously been shown to be an effective inducer of interferon in mice (2, 14), rabbits (3, 4), calves (17), chickens (15), hamsters (19) and man (5, 7). No report of interferon induction in swine systems in response to Poly I:C has been found in the literature.

In this study Poly I:C has been successfully used to induce interferon in pigs. The response of the pigs to Poly I:C by the production of circulating interferon, resembles that of the chicken, hamster, rabbit, mouse, man and calf and is unlike the response of the monkey, dog and guinea pig which failed to develop detectable circulating interferon following intravenous injection of Poly I:C (5).

The peak of interferon production in all five pigs occurred two hours after intravenous injection of Poly I:C. The circulating interferon decreased rapidly. This rapid appearance and early disappearance of Poly I:C-induced inhibitor is similar to the response seen in rabbits (14) and calves (17).

The magnitude and the duration of the response were not dose-related within the dose range of the drug tested and no attempt was made to demonstrate a dose-related response similar to that observed in cell-culture studies (21).

The pigs given a second injection of Poly I:C on the seventh day after initial stimulation were capable of interferon reinduction. However, the interferon titers were lower than after the first stimulation. Unfortunately, insufficient data was obtained to define the amount of Poly I:C required to bring about hyporesponsiveness or to de-

TABLE III. Properties of Swine Serum Interferon

Treatment	Results		
	Induced by Poly I:C	Induced by PRV-Pa	Induced by SIV
1. Dialysis for 48 hours at 4°C ...		Non-dialyzable	
2. Acidification at pH 2		Activity retained	
3. Activity against different viruses		Broad-spectrum activity	
Virus		Titer[a]	
VSV-NJ	32	32	—
Vaccina	16	32	—
4. Trypsin sensitivity		Activity destroyed	
Control titer	16	16	4
Trypsin (2 ug/ml) for two hours at 37°C	0	0	0
5. Heat stability		Heat stable	
Control titer	16	32	—
Titer after heating 56°C for 30 minutes	16	16	—
6. Ultracentrifugation		Non-sedimentable	
Titer before ultracentrifugation	—	—	4
Titer after ultracentrifugation ..	—	—	4
7. Activity against VSV-NJ in heterologous cells		Not active	
Titer on PK_{15}	—	16	—
Titer on MDBK	—	0	—

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

termine the duration of the refractory period. From these results it appears that if refractoriness exists in pigs it lasts less than seven days.

Neither pyrogenicity nor toxicity was observed in pigs in response to intravenous inoculation of Poly I:C at the drug dose used. This is in contrast to reports about the effect of intravenous inoculation of Poly I:C in calves (17), man (5), dogs (7), and rabbits (12). Apparently pigs are less sensitive to intravenous inoculation of Poly I:C.

Interferon production in response to PRV *in vivo* or *in vitro* in swine or other species has not been reported. In contrast to findings in porcine cell cultures (21) the intravenous injection of PRV-Pa into pigs resulted in the appearance of an antiviral substance in the serum. The most acceptable explanation for the failure of PRV to induce interferon in porcine kidney (PK_{15}) cell cultures would be that the virus replication damaged cells prior to interferon synthesis. All the injected pigs showed a febrile response at different times post inoculation but no positive correlation between febrile response and serum interferon titers was observed.

The persistence of PRV-induced interferon was much longer than Poly I:C induced interferon. This can be explained by the fact that virus is able to replicate in swine and stimulates the interferon-producing cells for a longer period of time. Since Poly I:C is degraded in the body (11, 13) its interferon-inducing capacity is limited to the initial dose.

The enveloped, single-stranded RNA-containing viruses of the myxovirus group are considered to be good interferon inducers. In contrast to what one would expect swine influenza virus was found to be a very poor interferon inducer in pigs in this investigation. The poor interferon-inducing ability of SIV in pigs may be a characteristic of the virus strain. It has been reported that different myxovirus strains possess variable interferon-inducing abilities (8, 18).

The antiviral substance produced in pigs in response to these inducers possessed characteristics which justify the conclusion that this substance is interferon (10). In contrast to findings of Torlone *et al* (20) who found that swine interferon is active on primary calf kidney cells the Madin-Darby bovine kidney (MDBK) cell line used

in these experiments did not respond to swine interferon.

The potential prophylactic value of interferon has been recognized, especially when it has been produced as a result of stimulation of the host. Induction of endogenous interferon is also a very promising approach to antiviral therapy largely because of its lack of viral specificity (6, 10). The results of this investigation show that pigs can produce interferon in response to viral and synthetic inducers. This mechanism probably plays a significant role in antiviral defense in pigs as it does in other species of animals.

REFERENCES

1. **BARON, S.** Biological significance of interferon system. Archs Int. Med. **126**: 84-91. 1970.
2. **BUCKLER, C. E., H. G. DU BUY, M. L. JOHNSON and S. BARON.** Kinetics of serum interferon response in mice after single and multiple injections of Poly I:C. Proc. Soc. exp. Biol. Med. **136**: 394-398. 1971.
3. **FENJE, P. and B. POSTIC.** Protection of rabbits against experimental rabies by Poly I:C. Nature, Lond. **226**: 171-172. 1970.
4. **FIELD, A. K., A. A. TYTELL, G. P. LAMPSON and M. R. HILLEMAN.** Inducers of interferon and host resistance. Proc. Nat. Acad. Sci. **58**: 1004-1010. 1967.
5. **FIELD, A. K., C. W. YOUNG and I. H. KRADKOFF.** Induction of interferon in human subjects by Poly I:C. Proc. Soc. exp. Biol. Med. **136**. 1180-1186. 1971.
6. **HARRIS, M.** Interferon: Clinical application of molecular biology. Science **70**: 1068-1070. 1970.
7. **HILLEMAN, M. R.** Prospects for use of double-standed ribonucleic acid (Poly I:C) inducers in man. J. infect. Dis. **121**: 196-211. 1970.
8. **HO, M.** Role of infection in viral interference. Archs Int. Med. **110**: 653-659. 1962.
9. **HUHN, R. G., G. D. OSWEILER and W. P. SWITZER.** Application of the orbital sinus bleeding technique to swine. Lab. Anim. Care **19**: 403-405. 1969.
10. **LEVINE, S. and F. R. NICHOL.** Interferon inducers. BioScience **20**: 696-700. 1970.
11. **LEVY, H. B., F. RILEY and S. MARGOLIS.** Interferon and interferon inducers in the treatment of malignancies. J. gen. Physiol. **56**: 238-248. 1970.
12. **LINDSAY, H. L., P. W. TROWN, J. BRANDT and M. FORBES.** Pyrogenicity of Poly I:C in rabbits. Nature, Lond. **223**: 715-718. 1969.
13. **MERIGAN, T. C., E. DE CLERCQ and G. H. BAUSEK.** Nonviral interferon inducers. J. gen. Physiol. **56**: 57-75. 1970.
14. **MERIGAN, T. C. and M. S. FINKELSTEIN.** Interferon-stimulating and **in vivo** antiviral effect of various synthetic anionic polymers. Virology **35**: 363-374. 1968.
15. **PORTNOY, J. and T. C. MERIGAN.** The effect of interferon and interferon inducers on avian influenza. J. infect. Dis. **124**: 545-552. 1971.
16. **PUCK, T. T., S. J. CIECIURA and A. ROBINSON.** Genetics of somatic mammalian cells. J. exp. Med. **33**: 339-349. 1961.
17. **ROSENQUIST, R. D.** Polyriboinosinic-Polyribocytidylic acid-induced interferons in calves. Am. J. vet. Res. **32**: 35-39. 1971.
18. **SOLOVIEV, V. D. and N. R. GUTMAN.** Characteristics of Hong Kong influenza virus strains as interferon inducers. Vop. Virus **6**: 652-658. 1970.
19. **STEWART, W. E., W. D. SCOTT and S. E. SULKIN.** Relative sensitivities of viruses to different species of interferon. J. Virol. **4**: 147-153. 1969.
20. **TORLONE, V., F. TITOLI and L. GIALETTI.** Circulating interferon production in pigs infected with hog cholera virus. Life Sci. **4**: 1707-1713. 1965.
21. **VENGRIS, V. E. and C. L. MARÉ.** Swine interferon I: Induction in porcine cell cultures with viral and synthetic inducers. Can. J. comp. Med. **36**: 282-287. 1972.