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Antiviral and Antigenic Properties of Recombinant Porcine Interferon Gamma

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ABSTRACT

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Recombinant porcine interferon gamma (rPoIFNγ) induced a dose-dependent inhibition of the cytopathic effect produced by vesicular stomatitis virus (VSV) challenge of both homologous and heterologous (bovine) cell lines. In addition, an antiviral effect of rPoIFNγ was demonstrable against the coronavirus transmissible gastroenteritis virus (TGEV) infection of porcine epithelial cells and of pulmonary macrophages. A rabbit anti-PoIFNγ antiserum was prepared and shown to specifically neutralize the antiviral effects of natural and recombinant porcine IFNγ preparations. This antiserum could also neutralize recombinant bovine IFNγ but not recombinant human IFNγ. These results suggest antigenic homology of porcine and bovine IFNγ but antigenic differences between these molecules and human IFNγ.

INTRODUCTION

Interferon γ (IFNγ), produced by activated T lymphocytes, can exert antiviral activity and a number of immunomodulatory effects such as enhancement of NK- and T cell-mediated cytotoxicity, B cell differentiation, surface antigens expression and macrophage activation (reviewed by Trinchieri and Perussia, 1985).

Although extensively studied in rodents and man, IFNγ has only recently received increasing attention in domestic animals. Adequate evaluation of IFNγ for its potential use against viral diseases or as an immunomodulator was difficult mainly because of the paucity of purified material. Cloning and expression of DNA encoding bovine and porcine IFNs (Capon et al., 1985; Ceretti et al., 1986; Lefevre and La Bonnardière, 1986), however, has enabled evaluation of their biological activities, particularly in the bovine species (Babiuk et al.,

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1985; Bielefeldt Ohmann and Babiuk, 1985, 1986; Czarniecki et al., 1986). Briefly, recombinant bovine IFNγ (rBoIFNγ) was shown to inhibit viral replication, to exert antiproliferative effects in vitro (Czarniecki et al., 1986) and to modulate neutrophil and lymphocyte functions both in vitro and in vivo (Bielefeldt Ohmann and Babiuk, 1985, 1986).

In contrast, very little is presently known about porcine IFNγ: supernatants of PHA-stimulated porcine blood mononuclear cells were found to afford antiviral protection to ovine cells challenged with vesicular stomatitis virus (VSV) (Yilma, 1983). Sauvagnac (1987) described the production of IFNγ and the synthesis of IFNγ-specific messenger RNA by porcine lymphocytes pretreated by a phorbol ester prior to induction by PHA. There is indirect evidence that IFNγ may be responsible for the stimulatory effects of supernatants from PHA-induced porcine lymphocytes on newborn pig NK activity (Charley and Fradelizi, 1987).

The gene coding for porcine IFNγ was cloned and sequenced by Genentech Inc. (U.S.A.). *E. coli*-derived rPoIFNγ was shown to contain 166 residues, for a molecular weight of 31.6 kD, as expected for a dimer (unpublished data from Genentech, U.S.A.).

The present report describes the effects of recombinant porcine IFNγ (rPoIFNγ) in vitro on multiplication of VSV in bovine and porcine cells as well as of coronavirus transmissible gastroenteritis virus (TGEV) in porcine kidney cells and pulmonary macrophages. A polyclonal anti-porcine IFNγ antiserum was also used to delineate the antigenic relationships between porcine, bovine and human IFNγ with respect to their biological activity.

MATERIALS AND METHODS

Cells and media

Cell lines of bovine (Madin-Darby bovine kidney, MDBK), porcine (PD5 and RPTG pig kidney cells) and human (Wish amnion cells) origin were used. All cell lines were cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS).

Porcine alveolar macrophages were obtained by lung washings of exsanguinated animals (Charley et al., 1983), kept frozen in liquid nitrogen and thawed before culture.

Recombinant IFNs

Recombinant porcine (lot no. 4648-58) and bovine (lot no. 3229-38) IFNγ were provided by Ciba Geigy Ltd. (Basel, Switzerland). Specific activities were $5–10 \times 10^6$ antiviral units/mg for rPoIFNγ and 2×10^6 units/mg for rBoIFNγ.

Recombinant human (Hu) IFNγ with a titre of 10^7 units/mg (code ARN 3010) was purchased from Amersham (U.K.).

Natural IFNs

Porcine IFNα, obtained by in vitro infection of porcine lymphocytes with influenza virus (La Bonnardière et al., 1986), was kindly provided by C. la Bonnardière (Thiverval-Grignon, France). The source of porcine IFNβ was supernatants of TGEV-infected PD5 cells. Natural porcine IFNγ was prepared by the method developed by Sauvagnac (1987): briefly, porcine blood mononuclear cells isolated by Ficoll density centrifugation (Charley et al., 1985) were cultured at a concentration of 5×10^6 cells/ml in RPMI 1640 plus 10% v/v FCS for 3 h at 37°C in the presence of 10 ng/ml phorbol-myristate-acetate (no. P8139 from Sigma, St Louis, U.S.A.); PHA-P (no. 3.110.56, Difco, Michigan, U.S.A.) was then added to a final dilution of 1/700 and the cultures incubated for 20 h at 37°C.

IFN assays

Titrations were performed in microtitre plates (Falcon 3072, Becton Dickinson, Oxnard, U.S.A.). Serial dilutions of IFNs were assayed on monolayers of MDBK, RPTG or Wish cells challenged, 24 h after IFN treatment, with 50 plaque-forming units (PFU) of VSV per well (La Bonnardière and Laude, 1981) and on PD5 cells challenged with cell-adapted Purdue 115 strain of TGEV (Laude, 1981) at multiplicities of infection shown in the Results. IFN titres were expressed as the maximal dilution giving a total protection of cell monolayers against the viral challenge.

PoIFNγ was also assayed on porcine alveolar macrophages infected with TGEV; 3×10^5 cells per well were cultured in microtitre plates in RPMI 1640 plus 20% v/v FCS for 24 h at 37°C, treated at 37°C for 24 h with fresh medium containing various concentrations of rPoIFNγ, and then challenged with TGEV (10^3 PFU per well in RPMI 1640 plus 5% v/v normal calf serum) for 48 h at 38°C. The macrophage monolayers were then stained with neutral red as described before (Laude et al., 1984): a 10^{-4} dilution of neutral red was added for 30 min at 37°C, monolayers were then rinsed twice and treated with 100 μl/well of 90% v/v ethanol. Plates were read spectrophotometrically at 449 nm on a Titertek Multiskan ELISA reader (Flow Laboratories). Antiviral activity was expressed as percent protection by IFN, calculated from the ratio: (OD_{449} of infected wells/OD_{449} of control wells) $\times 100$.

Anti-IFN antisera and seroneutralization test

Sheep anti-human IFNα, goat anti-human IFNγ and rabbit anti-bovine IFNγ antisera were kindly provided by P. Adamovicz, S. Stefanos (Paris, France)

and Ciba Geigy Ltd. (Basel, Switzerland). Rabbit anti-rPoIFNγ antiserum (no. 652) was prepared as follows: the primary immunization was an injection of 16 μg rPoIFNγ in complete Freund's adjuvant into a popliteal lymph node (Sigel et al., 1983) and monthly subcutaneous booster injections of 32 μg rPoIFNγ in incomplete Freund's adjuvant. IFN seroneutralization was assayed as described by La Bonnardière et al. (1986); dilutions of antiserum were added to the cell monolayers in microtitre plates prior to serial dilutions of IFN, and for each serum dilution a neutralization index (NI) was determined as follows:

$$NI = \log_3 (\text{IFN titre with antiserum}) - \log_3 (\text{control IFN titre}) .$$

RESULTS

Antiviral activities of recombinant porcine IFNγ

The antiviral activity of rPoIFNγ was evaluated in homologous and heterologous (bovine) cell systems infected by either VSV, the model pathogen for IFN assays, or coronavirus TGEV, a major pathogen for pigs. PoIFNγ proved not to be strictly species-specific since it caused a dose-dependent inhibition of VSV-induced cytopathic effects in bovine MDBK cells (Table 1). In the same assay system rBoIFNγ showed high antiviral activity (higher than rPoIFNγ) whereas rHuIFNγ was inactive (Table 1). Antiviral activity of rPoIFNγ was also observed on homologous (porcine) RPTG cells challenged with VSV (Table 1). rPoIFNγ, assayed on porcine (PD5) cells challenged by TGEV, was found to have a protective effect against up to 10^3 PFU/well (Fig. 1). With TGEV titres greater than 10^4 PFU/well, no effect of the rPoIFNγ was observed. In contrast, natural porcine IFNα could protect cells against as high a titre as 10^5 PFU/well of TGEV (Fig. 1).

TABLE 1

Antiviral activities of rPoIFNγ, rBoIFNγ and rHuIFNγ on bovine and porcine cells challenged with vesicular stomatitis virus

Cell lines	rIFNγ	Dosage (μg/ml)	IFN titre
Bovine MDBK	Porcine	160	9[a]
		10	5
		1	2.5
		0.1	1
Bovine MDBK	Bovine	1	7
Bovine MDBK	Human	10	<1
Porcine RPTG	Porcine	160	9

[a] $\log_3$ of the highest IFN dilution giving total protection.

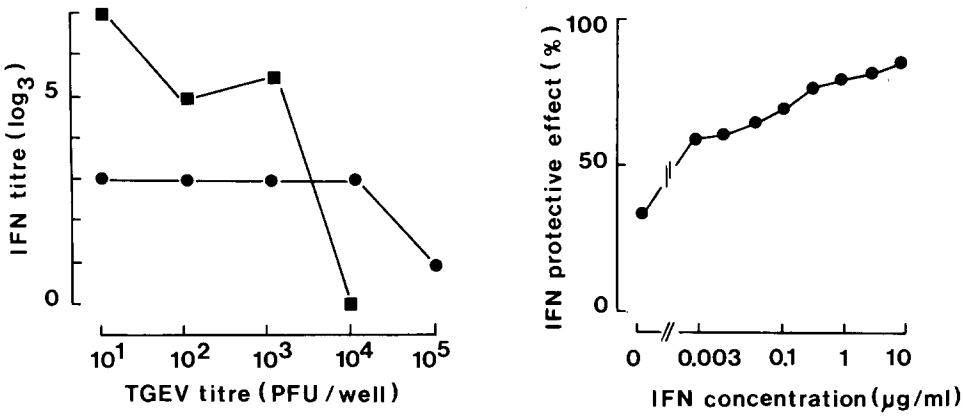

Fig. 1. Antiviral activities of porcine recombinant IFNγ (5×10^4 units/ml, ■) and natural IFNα (10^3 units/ml, ●) on PD5 cells challenged with TGEV: influence of the titre of virus challenge on the titre of IFN. IFN titres are expressed as the $\log_3$ of the highest protective dilution (representative of four experiments).

Fig. 2. Antiviral activity of rPoIFNγ relative to concentration, on pulmonary macrophages challenged with TGEV. The % IFN protective effect is expressed as [OD_{449} (infected cells)/OD_{449} (control cells)] $\times$100: see Materials and Methods.

Since TGEV can also replicate in pulmonary macrophages (Laude et al., 1984), the antiviral effect of rPoIFNγ was also assayed in this cell system. Fig. 2 shows that a dose-dependent protective effect was obtained when porcine pulmonary macrophages were incubated with rPoIFNγ prior to TGEV challenge. Antiviral activity was obtained with rPoIFNγ doses as low as 3 ng/ml.

Antigenic properties of recombinant porcine IFNγ

Production of rabbit polyclonal anti-rPoIFNγ antisera was achieved by inoculation of purified rPoIFNγ preparations into popliteal lymph nodes followed by subcutaneous booster injections. The sera obtained 3 weeks after the booster injection had a neutralizing titre which could not be enhanced by subsequent injections, and remained constant for at least 6 months without reimmunization. Preimmune sera were devoid of significant neutralizing activity.

The antiserum neutralized 98% of rPoIFNγ activity when diluted 100-fold, and more than 99% at a 10-fold dilution. This antiserum was specific for PoIFNγ since it showed little or no neutralizing activity against natural porcine IFNα and β (Fig. 3a). The antiserum neutralized the antiviral activity of both rPoIFNγ and that present in supernatants of porcine lymphocytes stimulated by PMA and PHA (Fig. 3a). This demonstrated that the antiviral activity in the latter was probably due to IFNγ.

The antigenic relationship with respect to biological activity, between por-

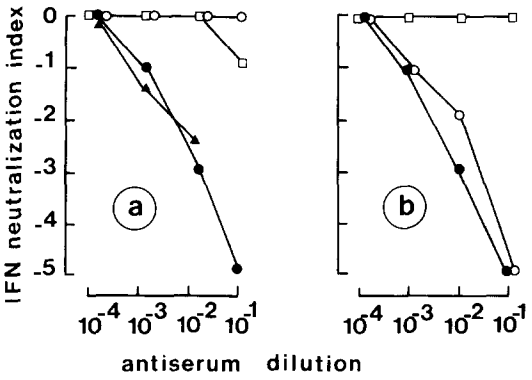

Fig. 3. IFN neutralization by rabbit anti-rPoIFNγ antiserum: dilutions of the antiserum were added to MDBK cells prior to IFN assay. The neutralization index is defined in Materials and Methods.
(a): rPoIFNγ (●), natural PoIFNγ (▲), PoIFNα (□), PoIFNβ (○).
(b): rPoIFNγ (●), rBoIFNγ (○), rHuIFNγ (□).

TABLE 2

Neutralizing activity of anti-human IFNγ and anti-bovine IFNγ antisera on homologous and heterologous IFNγ preparations

Specificity	IFN	Cells	Neutralization index[a]		
			antiserum dilution		
			10^{-4}	10^{-3}	10^{-2}
Anti-HuIFNγ	rHuIFNγ	Wish	0	−1	−3
	rPoIFNγ	MDBK	0	0	0
	rBoIFNγ	MDBK	0	0	0
Anti-BoIFNγ	rBoIFNγ	MDBK	−1	−3	−4
	rPoIFNγ	MDBK	0	0	0
	rHuIFNγ	Wish	0	0	−1

[a]See Materials and Methods.

cine, bovine and human rIFNγ was investigated by comparing the neutralizing activity of three antisera tested against each IFN preparation. The rabbit anti-rPoIFNγ antiserum neutralized both porcine and bovine IFNγ with equal efficacy but did not have any such activity against rHuIFNγ (Fig. 3b). In contrast, anti-human and anti-bovine IFNγ antisera could only neutralize homologous preparations (Table 2).

DISCUSSION

Recombinant porcine IFNγ was shown to protect homologous as well as heterologous (bovine) cells against VSV-induced cytopathic effects. Although

IFNγ is generally considered species-specific, the fact that rPoIFNγ was active on MDBK cells clearly indicates that certain cross-reactivities can exist. This would confirm in part, the report of Yilma (1983), who demonstrated that crude preparations of bovine, caprine, equine and porcine IFNγ had antiviral activity on heterologous (ovine choroid plexus) cells. Furthermore, rHuIFNγ can act upon porcine lymphocytes by stimulating their natural killing (NK) activity (Charley, unpublished data, 1988). Cross-species reactivities have also been described for IFNα (La Bonnardière and Laude, 1981; Yilma, 1983) and for IFNβ (Czarniecki et al., 1986). We have also shown that rHuIFNβ, although devoid of antiviral activity on porcine cells, could activate porcine NK (Charley, unpublished results, 1988).

Nevertheless, it still remained necessary to relate the antiviral activity of rPoIFNγ to the porcine situation. With this in mind, we were able to demonstrate that rPoIFNγ affords significant protection against TGEV challenge in vitro. Coronavirus TGEV induces acute and fatal diarrheas in young piglets (Haelterman, 1972) and replicates in two different cell populations: enterocytes (Haelterman, 1972) and pulmonary macrophages (Laude et al., 1984). We show that rPoIFNγ is able to protect both epithelial cells and alveolar macrophages from destruction by the virus (Figs. 1 and 2). This antiviral activity of rPoIFNγ in TGEV-infected porcine epithelial cells is highly dependent upon the multiplicity of infection of the virus, in contrast to the activity of the porcine IFNα preparation. The differences observed between IFNα and IFNγ preparations may reflect different modes of action on porcine epithelial cells. In addition, it is probable that the non-recombinant IFNα preparation contained a number of different forms of interferons and it is conceivable that the differences in antiviral activity observed between the IFNγ and IFNα preparations may be due to possible synergies between different interferon subtypes within the non-recombinant preparations. Certainly, different interferons appear to have different receptors on cells (for example, see Branca and Baglioni, 1981) and synergies have been seen between human IFNα and IFNγ (Weigent et al., 1983; Seow and Thong, 1986).

The antigenic relationship of the active sites of porcine IFNα, IFNβ and IFNγ, and of rPoIFNγ, rBoIFNγ and rHuIFNγ was studied using antisera raised against IFNγ of the porcine, bovine and human species. Anti-rPoIFNγ could neutralize the antiviral activity of rPoIFNγ and that present in the supernatants of PHA-activated porcine mononuclear cells, but had no such activity against non-recombinant porcine IFNα or IFNβ preparations. This supports the work done with other animal systems (for example, Branca and Baglioni, 1981) in demonstrating that in the porcine species the active site of, and probably the receptor for, IFNγ is different from IFNα and IFNβ.

Seroneutralization experiments conducted with anti-rPoIFNγ antiserum indicated that a high degree of antigenic homology exists between the active sites

of porcine and bovine IFNγ whereas human IFNγ was immunologically different. Experiments with anti-HuIFNγ antiserum confirmed the absence of antigenic cross-reactivity between human and porcine IFNγ. Conversely, antiserum to BoIFNγ did not neutralize either HuIFNγ or PoIFNγ: this suggests the existence of immunodominant cross-reactive epitopes on rPoIFNγ which would induce the production of antibodies, whereas these determinants would not be immunogenic on rBoIFNγ. Closer similarity between PoIFNγ and BoIFNγ than with HuIFNγ can also be seen in the degree of the sequence homologies between these three IFN molecules: rPoIFNγ/rBoIFNγ, 76%; rPoIFNγ/rHuIFNγ, 59%; rBoIFNγ/rHuIFNγ, 61% (McCracken, Genentech, U.S.A., personal communication, 1987). In addition, antigenic cross reactivity of lymphokines from different species has been found with other molecules. PoIFNα (La Bonnardière et al., 1986) and porcine IL1 (Saktvala et al., 1985) were found to have antigenic homologies with their human counterparts whereas no antigenic relationship was observed between human and porcine IL2 (Charley et al., 1985).

Following this initial description of several antiviral properties and of the antigenic characterization of rPoIFNγ, a better understanding and appreciation of this compound as an immunomodulator in vitro and in immature or immunocompromised animals may now be obtained.

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