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Ann. Inst. Pasteur/Virol.
1986, **137 E**, 171-180

BIOLOGICAL AND ANTIGENIC RELATIONSHIPS BETWEEN VIRUS-INDUCED PORCINE AND HUMAN INTERFERONS

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SUMMARY

Endogenous interferon (IFN) made by the newborn piglet in response to enteric coronavirus TGEV (transmissible gastroenteritis virus) infection was identified as leukocytic IFN (IFN-α). Indeed, the antiviral activity found in the serum and in the urine of infected piglets displayed the same main biological and antigenic properties as the IFN induced in influenza-infected pig leukocytes. It is therefore concluded that most, if not all, circulating IFN activity must be derived from lymphoid cells. Moreover, it was shown that a high degree of antigenic homology exists between porcine and human IFN-α: antibodies to HuIFN-α could efficiently neutralize PorIFN-α, and they were used for its purification by immunoaffinity.

KEY-WORDS: Interferon, Transmissible gastroenteritis, Influenza; Lymphoid cells, Swine, Man, Homology.

INTRODUCTION

In vivo studies on endogenous interferon (IFN) synthesis in response to various viral infections have produced an insight into the early steps of viral pathogenesis. Furthermore, the use of anti-interferon globulins has provided

Manuscrit reçu le 11 décembre 1985, accepté le 14 février 1986.

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Part of the present work was presented at the EEC Seminar on Adjuvants, interferon and non-specific immunity, held in Venice (Italy) April 27-28, 1983.

supportive evidence for the important role played by IFN in several diseases of the mouse [6, 7, 20]. There are several reasons to extend this kind of an approach to other animal models: firstly, it would be interesting to see how general this model is and secondly, several viral infections of interest for domestic animals and man have no equivalent in rodent species.

The model we are interested in is the piglet which, subsequent to infection with enteric coronavirus TGEV (transmissible gastroenteritis virus), presents the symptoms of severe diarrhoea and dies within a few days post-infection [3, 8]. We have previously shown that this infection is accompanied by an intense synthesis of type I or viral IFN [14]. Determining which species of IFN is made during the infection may help in understanding its cellular origin, and hence may explain some yet unrecognized aspects of the pathogenesis of coronavirus enteritis (Laude and La Bonnardière, to be published). Moreover, the identification of IFN species will help in raising specific antibodies, with a view to evaluating the eventual role played by IFN in the course of the disease.

In the present work, some of the main biological and antigenic characteristics of porcine endogenous IFN have been determined by comparison with porcine and human IFN-α.

MATERIALS AND METHODS

Cells and media.

We used the following cell types: the bovine MDBK cell line (Madin-Darby bovine kidney); the human MRC_5 cell line (from foetal lung), a gift of F. Le Fur (Institut Pasteur Production, Garches); sheep MM, a line of sheep fibroblasts, obtained in this laboratory by subcultivation of foetal lamb muscle; RPA, a strain of low-passaged pig kidney cells, obtained in this laboratory from an adult pig [15]; RPE, a low-passaged strain of embryonic pig kidney cells, also from this laboratory; RPTG and RPD, two epithelioid pig kidney cell lines also established in this laboratory. All these cells, as well as simian MA104 and mouse L_{929} cell lines, were grown in Eagle's minimal essential medium (MEM) plus 10% calf serum.

Origin of interferons and assays.

Human leukocyte IFN (HuIFN-α) was kindly provided to us by Institut Pasteur Production (Garches). It was semi-purified according to Cantell and Hirvonen [4]. Its specific activity was 10^6 IU/mg as assayed in MDBK cells.

Bov = bovine.
Hu = human.
IFN = interferon.
MDBK = Madin-Darby bovine kidney (cell line).
MEM = Eagle's minimal essential medium.
NDV = Newcastle disease virus.
NI = neutralization index.
PBS = phosphate-buffered saline.
Por = porcine.
SDS = sodium dodecyl sulphate.
TGEV = transmissible gastroenteritis virus.
VSV = vesicular stomatitis virus.

Endogenous porcine IFN (PorIFN-endo.) was collected as previously described from the blood [14] and also from the urine of newborn piglets infected at birth with TGE virus. All samples were cleared of residual virus before IFN assay by centrifugation at 10^5 *g* in an air-driven ultracentrifuge (Airfuge, Beckman).

Porcine leukocyte IFN (PorIFN-α) was obtained *in vitro* by infection of pig peripheral blood leukocytes with influenza virus (A/Philippines-H_3N_2).

Bovine « fibroblast » IFN (BovIFN-β/α) was produced in NDV-infected primary calf kidney cells according to a published procedure [13].

Unless specified, all IFN species were assayed on MDBK cells which were challenged with vesicular stomatitis virus (VSV) as previously described [14]. A laboratory standard bovine IFN was included in each assay. As no bovine or porcine IFN references are available, our laboratory standard was calibrated in MDBK cells with human international reference IFN B69/19 (NIH, Bethesda, USA). Its titre was then 1,000 IU/ml.

Origin of sera and seroneutralization test.

Sheep anti HuIFN-α antisera n° 654 and 166 were kindly provided to us by C. Chany (INSERM, Paris) and by P. Adamowicz (Institut Pasteur Production), respectively. Rabbit anti HuIFN-β serum was a gift of E. Van Damme (Rega Institute, Leuven, Belgium). Mouse anti HuIFN-α serum was obtained in this laboratory in 2 month-old BALB/c mice after 4 intravenous injections of liposome-entrapped IFN. In brief, the IFN solution to be encapsulated was mixed with a dried mixture of L-α-phosphatidylcholine, cholesterol and dicetylphosphate (respective molar ratios: 7, 2, 1). After complete swelling and resuspension of phospholipids, the mixture was submitted to a 1-min sonication using the probe of an « MSE » sonicator. Liposomes were always prepared freshly before injection.

Neutralization of antiviral activity by various antisera was assayed by a « constant antibody » method in which fixed dilutions of antiserum were applied to MDBK cells in microtitration plates just before serial 3-fold dilution of IFN. For each serum dilution, a neutralization index NI was determined, such as NI = $\log_3$ (IFN titre + antiserum) − $\log_3$ (control IFN titre). This simple procedure was adopted after it was shown to yield the same antibody titres as with preincubation (1h, 37°C) of the IFN-antibody mixture. Y. Kawade and Y. Watanabe recently presented theoretical and experimental arguments in favour of this method [12].

Gel filtration columns.

IFN was extensively dialysed against phosphate-buffered saline (PBS) and passed through a column of acrylamide-agarose ACA54 (LKB, Orsay, France) equilibrated with PBS made to 1M NaCl and 20% ethylene glycol [10].

Immunoaffinity column.

Highly adsorbed polyclonal, anti-HuIFN rabbit immunoglobulins (serum n° KB 1263) were covalently attached to a sepharose-4B matrix and used for purification of PorIFN-endo as described for HuIFN-α [1]: in brief, the column was equilibrated, loaded and washed in 0.3M NaCl, 0.1M acetate, pH 7.2. Elution was then carried out in 0.3M NaCl, 0.1M acetic-citric acid, pH 2.4. Eluted fractions were immediately made to 0.1% in sodium dodecyl sulphate (SDS).

RESULTS

I. – Cross-species antiviral activity of porcine, bovine and human interferons.

Four different IFN preparations from porcine, bovine and human species were assayed in parallel on 8 different cell lines or strains belonging to 6 mammalian species (table I). Despite some variations in susceptibility from one assay to another, some clear features came out: 1) the activity spectra of PorIFN-endo and PorIFN-α bore a close resemblance; 2) by comparison with HuIFN-α, both PorIFN were characterized by a much lower activity on simian cells (0.1 % to 0.3 % of titre on MDBK cells); 3) as a confirmation of our previous results [**14**], PorIFN were paradoxically less active on homologous porcine cells. Finally, unlike BovIFN-β/α, PorIFN and HuIFN elicited a weak but significant antiviral activity on mouse L cells (1-5 %).

These data not only confirm the wide range of antiviral activities of these different IFN, and particularly of HuIFN-α, but also argue in favour of a close similarity between TGEV-induced (endogenous) and leukocyte-derived porcine IFN.

TABLE I. — **Antiviral activities of human, porcine and bovine IFN on various mammalian cells.**

Species	Cell line	HuIFN-α	PorIFN-endo	PorIFN-α	BovIFN-β/α
Bovine	MDBK	100	100	100	100
Human	MRC_5	44-100(*)	60	25-100	40
Simian	Ma104	5-11	0.1-0.2	0.1-0.3	2-11
Sheep	MM	100-130	100-300	100-170	30-100
Porcine	RPA	44-100	12-19	14-24	25-100
	RPE	19-30	2	ND	100
	RPD	4	0.7	ND	ND
Murine	L_{929}	2	1.2	5	< 0.2

Activities expressed as per cent of activity in MDBK cells.
(*) The extreme values are given whenever the data have been obtained in two or three experiments.
ND = not determined.

II. – Porcine interferons are recognized by anti-HuIFN-α antibodies.

As shown in table II, when different PorIFN preparations were assayed in MDBK cells in the presence of three anti-HuIFN-α sera, they were all neutralized to a large extent. This was not the case for BovIFN-β/α, nor was it the case for PorIFN incubated in the presence of anti-HuIFN-β immunoglobulins.

TABLE II. — **Neutralization indexes of various anti-HuIFN sera against PorIFN and BovIFN assayed in MDBK cells.**

IFN	Sera			
	anti-HuIFN-α			anti-HuIFN-β
	Sheep 654 (1/1000)	Sheep 166 (1/1000)	Mouse (1/100)	Goat (1/500)
Hu-α	− 4.25	> − 6	− 4	− 0,5
Hu-β	− 2.25(*)	ND	ND	> − 5
Por-endo	− 3	− 3.75	− 2.5	0
Por-α	− 3	− 5	ND	ND
Bov-β/α	0	ND	− 0.25	0

Neutralization index (NI) is calculated as shown in «Materials and Methods».
(*) Probably represents the fraction of IFN-α in the preparation of IFN-β.
ND = not determined.

Although the two PorIFN were differentiated by serum-166 antibodies (NI = − 3.75 against IFN-endo, − 5 against IFN-α), these results suggest that TGEV-induced IFN is mostly composed of alpha-like interferon.

We compared the neutralization index of various dilutions of antiserum 654 against HuIFN-α, PorIFN-endo (serum and urine) and BovIFN-β/α (fig. 1). The results show that (1) IFN from urine and serum of piglets were identically cross-neutralized by anti-HuIFN globulins; in this respect, it is noteworthy that heterologous neutralization was about 1/10 that of homologous neutralization; 2) there was a striking parallelism in the neutralization curves between Por- and HuIFN, which suggests a similar affinity of at least a fraction of antibodies for human and porcine molecules; 3) there was no detectable neutralization of BovIFN. It should be pointed out that PorIFN was almost entirely neutralized at the 1/50 dilution of antiserum (5 $\log_3$ out of 6 initially); therefore, if any PorIFN fraction were antigenically distinct, it would not amount to more than 1/700 of total activity.

III. – Polyclonal anti-HuIFN-α globulins for the purification of PorIFN.

The above results suggested that porcine endogenous IFN, consisting of very crude preparations (with a specific activity in the range of 10^3 to 10^4 IU/mg of protein), could be purified using available columns of anti-HuIFN-α globulins [1].

As a preliminary purification step, endogenous IFN (from urine and serum) were run through an «Ultrogel Aca54» column. Figure 2 (A and B) shows the elution patterns of the two porcine IFN. In both cases, the antiviral activity assayed in MDBK cells eluted as a broad peak with a modal molecular

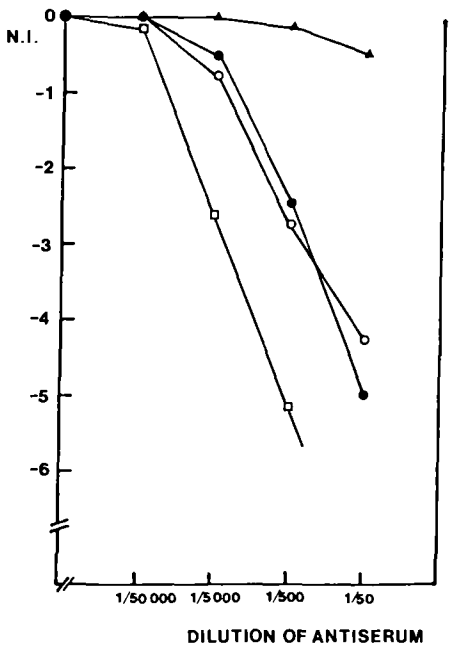

FIG. 1. – *Neutralization of porcine, bovine and human IFN by various concentrations of sheep serum 654.*

Four IFN preparations were assayed in MDBK cells in the absence or in the presence of the indicated dilutions of anti-Hu-α-IFN serum in the conditions described in «Materials and Methods».
▲ = BovIFN-β/α; ○ = urinary PorIFN-endo; ● = seric PorIFN endo; □ = HuIFN-α.

weight of around 18,000 daltons. The small peak of antiviral activity which eluted at fractions around 30-35 most probably corresponds to IFN bound to larger proteins, in particular to serum albumin. Both IFN activities were recovered with a 90% yield and with a 30-fold purification factor.

Fractions (5 ml) with IFN activities were pooled independently for urinary and seric IFN. Each pool was thus loaded on a column of anti-HuIFN-α globulins (KB163) and eluted at pH 2.4. The patterns of absorption-elution shown in figure 3 demonstrate that a major fraction of input IFN could be retained by the antibodies and eluted at acidic pH. The yield was 80% for urinary IFN and 70% for seric IFN, and the purification factor was 100-fold.

DISCUSSION

Two kinds of PorIFN have been compared, namely, endogenous IFN collected from TGEV-induced piglets, on the one hand, and leukocytic IFN (IFN-α) produced by influenza-infected pig leukocytes on the other. The rela-

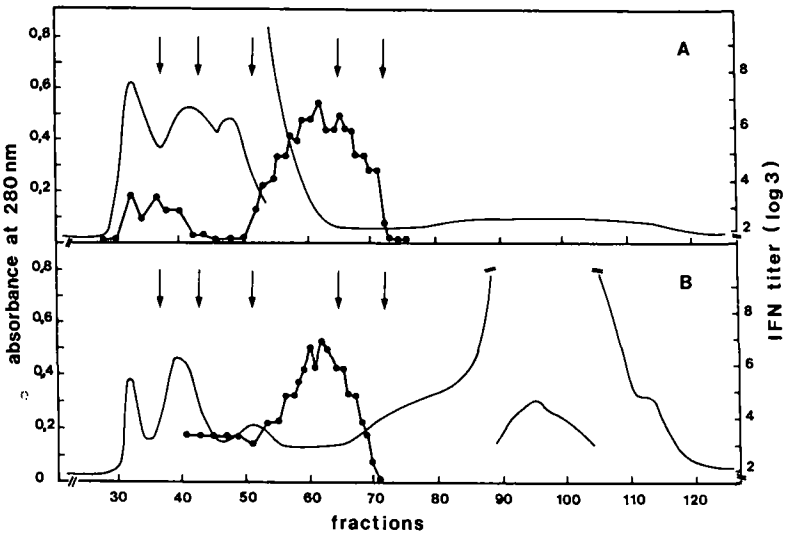

FIG. 2. – *Gel filtration of crude concentrated porcine urinary (A) and seric (B) IFN on «Ultrogel Aca54».*

A 100 cm-long column (K 25/100, Pharmacia) was packed at 4°C with «Ultrogel Aca54» in column buffer (1M NaCl in PBS pH 7.2, 25% ethylene glycol). IFN were cleared of residual TGE virus by ultracentrifugation at $10^5 \times g$ (1 h, 4°C), extensively dialysed against distilled water, then lyophilised. Before gel filtration, urinary IFN and seric IFN were dissolved in 5 ml of column buffer. Size of fractions, 5 ml. Flow rate, 40 ml/h. Arrows indicate the position of MW marker proteins: 67, 45, 25, 17.8 and 12.4 Kd.
—— = absorbance.
●—● = IFN titre.

tedness of the two preparations was clearly established, considering both their spectrum of antiviral activity in a panel of mammalian cells (table I) and their neutralization by the same antisera (table II and fig. 1). Actually, we could find at least one antiserum (sheep n° 166) which allowed a discrimination between PorIFN-endo and PorIFN-α (table II), which could suggest the presence of a minor non-alpha IFN fraction in the serum of infected piglets. However, we conclude that most circulating IFN which is synthesized following TGEV infection is alpha-like. This may have implications as to the source of this IFN *in vivo,* in view of the assumed strict enterotropism of TGEV. Present results support other data from this laboratory showing that, among lymphoid cells, at least alveolar macrophages are clearly a target for TGEV multiplication and a source of IFN synthesis **[16]**.

Now comparing PorIFN and HuIFN, we wish to underline two main features.

First, it is possible to clearly distinguish IFN from the two species by their antiviral activity in different cells when challenged with VSV. Simian cells, in particular, discriminate to a good extent between human and porcine species,

with a much better receptivity to HuIFN. As previously published, PorIFN are clearly active in human MRC_5 cells [5], but a paradoxical result shown in table I is the remarkably low activity of PorIFN in pig kidney cells (12-19 % of activity in MDBK cells). These results also confirm the wide range of susceptibility of ovine cells to various mammalian IFN, as previously shown in this laboratory [14] and by others using sheep plexus choroid cells [21].

Second, the antigenic relationship between HuIFN and PorIFN is interesting in several respects: it allowed us to use anti-HuIFN-α globulins for the purification of porcine endogenous IFN (fig. 3) and, since that time, of PorIFN-α (unpublished results). The extensive binding of PorIFN-α onto a column of anti-HuIFN-α monoclonal antibody LO22 has been reported by K. Berg [2], thus confirming the antigenic resemblance of human and por-

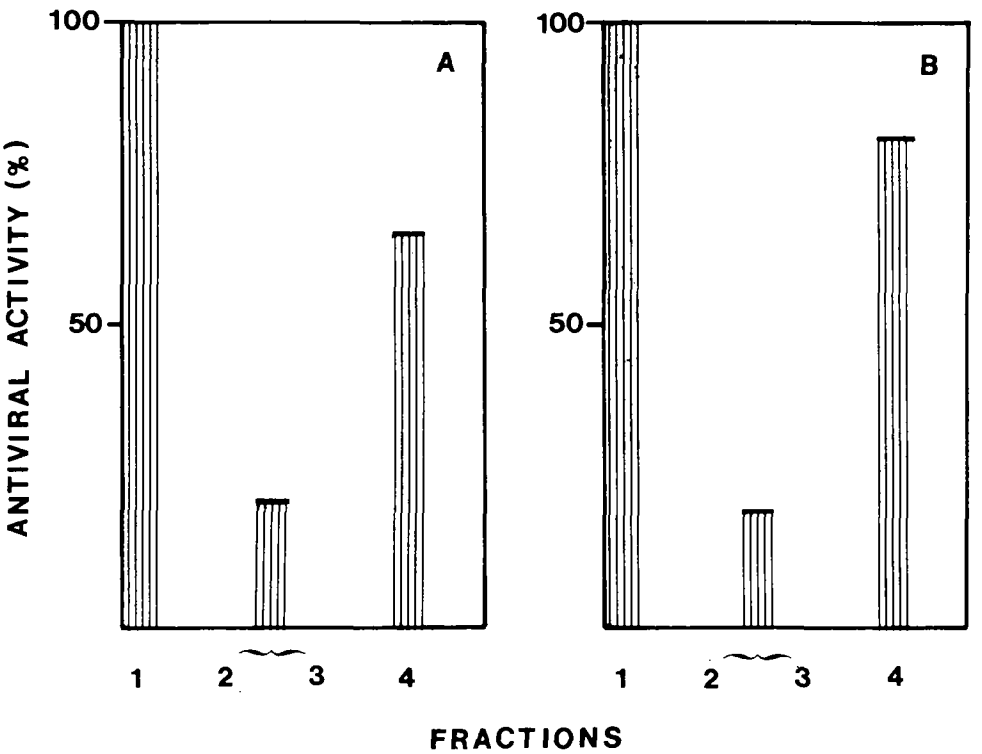

FIG. 3. – *Absorption-elution of porcine endogenous IFN on a column of anti-HuIFN-α globulins.*

The IFN activity eluted from the gel filtration column was directly loaded onto a 5-ml immunoaffinity column; then, washing and elution were performed as described in «Materials and Methods». Vertical bars represent antiviral activity (in % of input) in the different fractions, as follows: fraction 1 = input activity; fractions 2-3 = unbound activity (breakthrough + wash); fraction 4 = activity eluted at pH 2.4.
A = IFN from the blood; B = IFN from the urine.

cine molecules. Two other monoclonal antibodies against HuIFN-α, namely HBA [17] and NK2 [18], did not significantly bind PorIFN. This was not a surprise to us, because there must be at least some epitopes which are very dissimilar between porcine and human molecules. Finally, considering both biological and antigenic characteristics, PorIFN-α seems to share with the HuIFN-α family the highest known degree of relatedness. Murine IFN-α has been previously shown to be antigenically related to HuIFN-α [9, 11, 19], and also to be cross-reactive on human cells, but in both cases with a much lesser degree than PorIFN [11, 19]. Cloning and sequencing of novel animal IFN in addition to that of human and mouse species should aid in our understanding of structure-function relationships in the IFN molecular family. From this point of view, PorIFN should be regarded as one interesting basis of comparison.

ACKNOWLEDGMENTS

The authors wish to thank Ms C. de Vaureix for her skillful technical assistance; they are also indebted to K. Verstergaard for preparing and running the immunoaffinity columns.

RÉSUMÉ

COMPARAISON DES PROPRIÉTÉS BIOLOGIQUES ET ANTIGÉNIQUES DES INTERFÉRONS LEUCOCYTAIRES PORCIN ET HUMAIN

L'interféron (IFN) endogène synthétisé par le porcelet nouveau-né en réponse à l'infection par le virus de la gastro-entérite transmissible, a été identifié comme étant du type leucocytaire (IFN-α). L'activité antivirale détectée dans le sérum et l'urine des porcelets infectés présente en effet les mêmes propriétés biologiques et antigéniques que celles de l'IFN porcin produit en culture de leucocytes infectés par le virus grippal. Une origine essentiellement lymphoïde de l'IFN circulant est donc proposée. De plus, ce travail a mis en évidence une forte homologie antigénique entre l'IFN-α humain et l'IFN-α porcin: des anticorps dirigés contre l'IFN-α humain neutralisent très significativement l'IFN-α porcin et peuvent être utilisés par sa purification par immunoaffinité.

MOTS-CLÉS: Interféron, Gastro-entérite transmissible, Grippe; Cellules lymphoïdes, Porc, Homme, Homologie.

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