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Porcine Leukocyte Interferon and Antiviral Activity in Human Cells

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SUMMARY

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Interferon derived from a lower animal source, porcine leukocytes, is shown to have high biological activity in human cell cultures. This interferon shares some biochemical properties with human leukocyte interferon which had been shown previously to protect cells of animal origin. For example, when chromatographed on Phenyl-Sepharose CL-4B, both porcine and human leukocyte interferons bound and could be eluted with 50% ethylene glycol. Similarly, both porcine and human leukocyte interferons did not bind under physiological salt and pH conditions to concanavalin A-agarose. In contrast, the interferon produced by a variety of human and lower animal fibroblasts—which display much reduced cross-species activity—interact strongly with this lectin. Presumably, due to altered glycosylation, leukocyte interferons more easily express cross-species activity.

It seems that, generally, interferons elaborated by leukocytes are less restrictive in their species specificity than those elaborated by fibroblasts. Our findings suggest a possible structural homology between human leukocyte and porcine leukocyte interferons similar to those well recognized between various polypeptide hormones of animal and human origin.

INTRODUCTION

Since their discovery two decades ago (1), a hypothesis has been widely accepted that interferons are molecules with a distinct 'species-specific' barrier. Some exceptions have been reported, most notably the antiviral activity of human leukocyte interferon on cells of various lower animals (2–4). However, in general, such studies have not substantially evaluated, or revised, the original concept of 'species-specificity' of interferons. Two factors have contributed to the lack of challenge to this initial hypothesis. First, antiviral protection measurements in heterologous cells frequently give low, often variable, results. Second, no attempts have been made to isolate and characterize the cross-reacting species in their native form. Rather, one can infer that this particular biological property is widely perceived as a function of the entire

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population of interferon molecules present (2, 5).

As mentioned, interferons from human leukocytes do exhibit strong heterologous activity—sometimes even exceeding their measured activity in human cells (2–4). Recently, it has been noted that the human leukocyte interferon preparation is, in fact, a mixture: some interferon molecules are intact, while the majority (60%) seem to have undergone proteolytic cleavage (6). It is also possible that the molecular size heterogeneity of human leukocyte interferon is due to a lack of, or altered, glycosylation. The intact molecules are actually 5–8 fold more active in bovine cells than in human cells, whereas—when their peptide fragments (5000 MW) are dislodged—the heterologous and homologous activities become equal.[1] By contrast, human fibroblast interferon has much less expressed activity in heterologous cells, including bovine(2); indeed, when a search was done for possible cross-specific antiviral activity of human fibroblast interferon, purified (ca 10^8 units/mg protein) by concanavalin A-agarose chromatography (7), it measured at 0.1% or less in the same bovine cells which were highly sensitive to the human leukocyte interferon. Similarly, bovine fibroblast interferon gives quite low (2), and apparently variable (5), protection of human cells against viral challenge.

Interestingly, the majority of human leukocyte interferon molecules binds neither to concanavalin A-agarose nor to other immobilized lectins specific for various carbohydrate moieties (8); therefore, these molecules are presumed to lack certain carbohydrate residues, although their 'masked' status cannot be formally excluded.

We report herein the production of a novel interferon derived by induction of porcine leukocytes with Newcastle disease virus. This interferon exhibits potent activity in human cells and chromatographic similarity with human leukocyte interferon. Our data suggest a linkage between a biochemical property—the apparent absence (or 'masked' status) of carbohydrate moieties—and the ability to express the biological property of cross-species antiviral protection.

MATERIALS AND METHODS

Chemicals. Methyl α-D mannopyranoside was obtained from Sigma Chemical Co. Fluorescamine was purchased from Roche Diagnostics. Ethylene glycol was obtained from Baker Chemical Co. Plasmagel was obtained from Associated Biomedic Systems.

Adsorbents. Con A-Sepharose and Phenyl-Sepharose CL-4B were purchased from Pharmacia Fine Chemicals. Soybean, wheatgerm and Lotus lectins were obtained from Miles Laboratories and Castor bean lectin from PL Biochemicals.

Interferon preparations. Leukocytes were isolated, using plasmagel 20% v/v, essentially according to the procedure of Pidot *et al.* (9). Porcine leukocyte interferon was prepared by infecting leukocytes with five plaque-forming units of Newcastle disease virus. After an hour of virus adsorption, the leukocyte concentration was adjusted to 5×10^6 cells/ml in RPMI 1640 medium (Gibco, Grand Island, N. Y.) containing 5% heat-inactivated fetal calf serum. The leukocytes were gently stirred during a 24 hr incubation at 37° in an atmosphere of 10% CO_2 and 90% air. At the end of the incubation period, the cells were pelleted ($1500 \times g$ for 15 min) and the supernatant containing interferon was adjusted to pH 2.0 using 1 N HCl to inactivate the inducer virus. A day later, the preparation was readjusted to pH 7.0 prior to assay for antiviral activity. Control experiments were done to confirm that the virus was fully inactivated by this procedure. In a few cases, instead of using plasmagel, the red blood cells were lysed with a chilled solution of 0.83% ammonium chloride; the remaining leukocytes were collected by centrifugation and used to prepare interferon essentially according to the procedure described above.

Cells and interferon assays. Human fibroblast cells (GM 2504), trisomic in chromosome 21 which encodes for interferon sensitivity (10, 11) were used. These cells were selected because they were less subject to fluctuation in interferon assay dur-

ing serial cell passage (11) (10–25th passages were used). Similarly, primary bovine embryonic kidney cells were selected for assay of porcine interferon on the basis of greater sensitivity. Porcine leukocyte interferon was 5–10 fold more active on GM 2504 cells than on primary porcine cells. All cells were maintained in Eagle's medium containing nonessential amino acids, glycine and supplemented with 10% fetal calf serum. During interferon assays, the maintenance medium contained 2% fetal calf serum. Cells were grown at 37° in a humidified atmosphere containing 10% CO_2 and 90% air.

Interferon assays were performed by a modification (12) of the colorimetric technique of Finter (13) using vesicular stomatitis virus as the challenge virus (multiplicity of infection of 0.2 plaque-forming units per cell). Titers were generally expressed as the geometric mean of two or more assays.

Protein concentration was measured by the fluorometric assay (14) using bovine serum albumin as a standard.

RESULTS

Table 1 demonstrates the measurements of various preparations of porcine leukocyte interferon in human and animal cells. Antiviral activities were frequently several fold higher when measured in the human cells. Results similar to those of Table 1 were obtained with over 10 different preparations of porcine leukocyte interferon.

Porcine leukocyte interferon, chromatographed on the hydrophobic ligand Phenyl-Sepharose CL-4B, is indistinguishable from human leukocyte interferon. As can be gleaned from the structure of this ligand, any potential for electrostatic interaction is ruled out because the benzine ring is immobilized to the agarose matrix via an ether linkage; hence no positive charge is introduced. Porcine leukocyte interferon (Fig. 1) is tightly bound to Phenyl-Sepharose CL-4B and requires 50% ethylene glycol for its displacement. Over 90% of the other proteins are unretained and overall recovery of porcine interferon activity is nearly complete. One can see that the activity profile of porcine leukocyte interferon in human cells closely tracks its activity in the animal cells. In separate experiments, both human and bovine fibroblast interferons were also retained, thus indicating the high intrinsic affinity of each of the four interferons, under physiologic solvent conditions, for this aromatic ligand.

Figure 2 shows that porcine leukocyte

TABLE 1

Cross-species antiviral activity of preparations of porcine leukocyte interferon

Preparations	Interferon activity (units/ml)	
	Bovine	Human
1	160	500
2	420	850
3	210	810
4	110	800
5	240	2,000

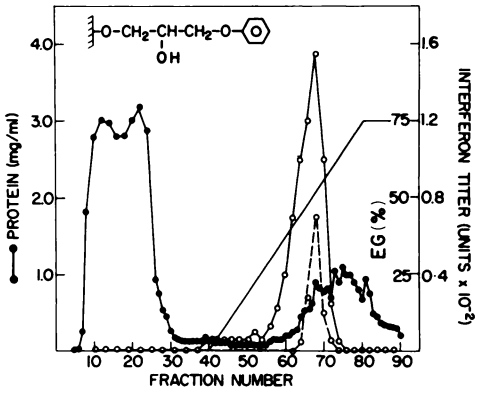

FIG. 1. *Cross-species antiviral activity of porcine leukocyte interferon on Phenyl Sepharose CL-4B*

A sample of an interferon preparation was dialyzed against 0.15 M sodium chloride in 0.02 M sodium phosphate, pH 7.4 ($NaCl/PO_4$) for 18 hr at 4°. The dialyzed interferon preparation was applied onto a column (0.9 × 5 cm) equilibrated with $NaCl/PO_4$. The flow rate of the column was adjusted to 30 ml/cm²/hr. The column was first washed with $NaCl/PO_4$ and then equilibrated with 0.02 M phosphate buffer, pH 7.4. A linear concentration gradient of ethylene glycol was then developed by mixing: A, 20 ml of 0.02 M phosphate buffer, pH 7.4; B, 20 ml of 75% ethylene glycol (vol/vol) in 0.08 M sodium phosphate buffer, pH 7.4. Finally, the column was further developed with the terminal solvent. The eluate from the column was divided 9:1 by a stream splitting device and 0.1 ml fractions to be used for interferon assays were collected into 0.9 ml of a 1% solution of bovine serum albumin in $NaCl/PO_4$. O——O, represents antiviral activity on human cells and O- - -O, antiviral activity on bovine cells.

interferon does not bind to concanavalin A-agarose and can be recovered in the breakthrough fractions, a chromatographic behavior which is identical with human leukocyte interferon (7, 8). Specifically, there are no traces of biological activity following development of the column with either 0.1 M methyl α-D-mannopyranoside (E_1) or 0.1 M methyl α-D-mannopyranoside and ethylene glycol (50% [vol/vol], E_2). Also, one notes that the elution profile of antiviral activity in human cells is coincident with that obtained in the animal cells.

To test for the presence of other sugar moieties, a set of additional lectin-agaroses was also examined (8). As seen in Table 2, all of the porcine leukocyte interferon, within experimental error, was unbound, thus indicating either the absence of these sugars or their inaccessibility to the various immobilized lectins.

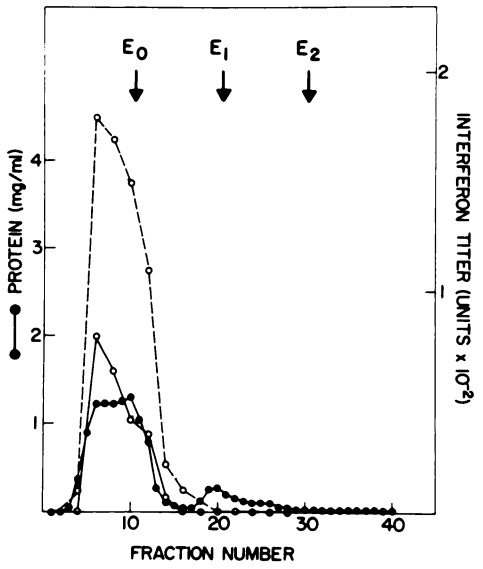

FIG. 2. *Cross-species antiviral activity of porcine leukocyte interferon chromatographed on concanavalin A-agarose*

A sample of an interferon preparation was dialyzed against 0.15 M sodium chloride in 0.02 M sodium phosphate buffer, pH 7.4 ($NaCl/PO_4$) for 18 hr at 4°. The dialyzed preparation was applied to a column (0.9 × 5 cm) equilibrated with $NaCl/PO_4$ (E_0). The flow rate of the column was adjusted to 30 ml/cm²/hr. Following a brief rinse with $NaCl/PO_4$, the column was developed first with 0.1 M α-methyl-D-mannopyranoside in $NaCl/PO_4$ (E_1) and then with 50% ethylene glycol in E_1 (E_2). O——O, antiviral activity on human cells, O- - -O, antiviral activity on bovine cells.

DISCUSSION

We have also observed[1] that interferons derived from horse and bovine leukocytes display antiviral activity in human cells (GM 2504) similar to that reported in Table 1 with porcine interferon. Since the porcine leukocytes were isolated by various techniques, including the use of either plasmagel or red cell lysis (by ammonium chloride) and induced by both viruses and synthetic polynucleotides (data not shown), the cross-species activity is not due to either the manner of leukocyte isolation or interferon induction.

One may observe that the relative activity in human versus animal cells in Fig. 2 is different from that illustrated in Fig. 1. We have observed that this is due to the biological variability within different preparations of porcine interferon. At present, we do not know whether porcine leukocyte interferon is, like human leukocyte interferon (6), a mixture of fully intact molecules and others which possess an inherent discontinuity in their polypeptide chain. In either case, this would not be expected to alter the elution profile on concanavalin A-agarose, by analogy to human leukocyte interferon, although it might prove to be a factor in the cross-species activity—as when different porcine interferon preparations are compared.

Overall, our current observations are consistent with the growing number of recent reports which, using various biochemical probes, now establish apparent homologies among certain mammalian interferons (15–18). These techniques include specific sites recognized by immobilized hydrocarbons (15), aromatic amino acids (16), the polyaromatic blue chromophore (17), and polynucleotides (18). Porcine leukocyte interferon thus seems to resemble, in its affinity for an aromatic hydrophobic probe, a group of interferons which now includes those of human, mouse and rabbit origin (15).

However, such biochemical similarities are in conflict with the generally low cross-

[1] Chadha, K. C. and W. A. Carter, unpublished observations.

TABLE 2

Chromatography of porcine leukocyte interferon on immobilized lectins

Lectin-agaroses	Sugar specificity	% Antiviral activity in breakthrough fraction	% Antiviral activity eluted with specific sugar
Castor bean lectin	D-galactose	70%	none
Lotus	L-fucose	50%	none
Soybean lectin	N-acetyl-galactosamine	75%	none
Wheatgerm lectin	N-acetyl-glucosamine	100%	none

reactivity observed when mammalian interferons are assayed in various heterologous cell systems. It is tempting to speculate that the nature or extent of glycosylation might be a factor which accounts for the wide variation in cross-species activity. For example, the low but consistent level of cross-species protection seen with interferon derived from certain fibroblastic cell strains, as bovine, may be accounted for by a small subpopulation of molecules which are in the breakthrough fraction on Concanavalin A-agarose.[2]

However, more importantly, one can observe that the extent of cross-species activity of an interferon can be correlated with the cell-type in which it is elaborated; namely, porcine leukocyte interferon, as well as human leukocyte interferon, show a remarkable extent of cross-species activity by comparison with their counterpart fibroblast interferons.

Current knowledge of various polypeptide hormones suggests that they may not have evolved for 'best fit' in the receptor of the same species (19). The receptor proteins thus appear to be under evolutionary constraints, possibly because of their other functions essential to the cell membrane. One can now advance the notion that interferons from various lower species may protect extensively human cells. Specifically, interferons derived from leukocytes—which appear to generate interferon molecules varying in their post-translational modification—may demonstrate relative ease in expression of their underlying homology.

An interesting two-gene model, as a source for variation in biological and antigenic activity of human interferons, has recently been advanced (20). Our current experiments now suggest another source for biological variation: namely, changes in the extent of post-transcriptional modification may vary the biological activity, thus simulating the production of novel molecular components.

Chany has advanced a model of interferon action based on a membrane receptor with distinct binding and activator sites (21). It is tempting to speculate that binding on heterologous cells might be modulated by the sugar prosthetic group in which case glycosylation would serve as a negative control element. However, further experiments are necessary to distinguish among various possibilities. Direct determination of the relative carbohydrate content of different interferons, as well selective inhibition of the glycosylation of interferons, will be valuable in further assessing the possible role of glycosylation in expression of antiviral activity on both homologous and heterologous cells.

[2] Miller, L., K. C. Chadha and W. A. Carter, unpublished observations.

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