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Antiviral activity of interferon against transmissible gastroenteritis virus in cell culture and ligated intestinal segments in neonatal pigs

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ABSTRACT

Segments of jejunum in 5 to 6 days old piglets were surgically ligated, inoculated with transmissible gastroenteritis virus (TGEV) and 18 hours later the segments were fixed for histology or suspensions were prepared for plaque assay in swine testis (ST) cell cultures to determine the yield of virus. When the virulent Purdue strain of TGEV was used, villous atrophy was seen and TGEV antigen was demonstrated immunohistochemically in the villous enterocytes. The Miller M6 strain of virus produced less extensive lesions in the segments, but since it was titratable by plaque assay it was used in the subsequent yield reduction assays to determine the antiviral activity of interferon. When intestinal segments were inoculated simultaneously with either 3200 units of natural porcine interferon- α or up to 100000 units of recombinant human interferon- α_{2b} , and TGEV, there were no reductions in virus yield, although the same cytokines exerted an antiviral effect in ST cells treated in a similar way. However, virus yields were significantly reduced in intestinal segments in piglets treated parenterally with the synthetic interferon inducer polyinosinic: polycytidylic acid 6 hours before challenge of the segments with TGEV. There was also a trend for the antiviral effects of interferon induction before challenge to be augmented by the inclusion of interferon with the virus inoculum. It was concluded that interferon would be ineffective as a therapeutic for TGEV, although it might be useful prophylactically.

Key words: Transmissible gastroenteritis virus; Pig; Interferon; Antiviral activity

INTRODUCTION

Transmissible gastroenteritis virus (TGEV) is an enteric coronavirus associated with high mortality in preweaning pigs. Following oral exposure, viral replication occurs primarily in the mature villous epithelium of the jejunum and ileum (Hooper and Haelterman, 1969, Saif and Heckert, 1990). Lysis of infected cells leads to villous atrophy and crypt hyperplasia. Diarrhoea results from maldigestion, malabsorption, and loss of electrolytes and proteins from

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the damaged epithelium and leads to dehydration, nutritional deficiency, and electrolyte imbalances (Butler, et al., 1974).

Antiviral activity associated with TGEV infection was first described in intestinal washings in neonatal pigs (Pensaert et al., 1970). The activity was later shown to be due to alpha interferon (IFN- α) (La Bonnardière and Laude, 1981). The source of the interferon was unknown, but was thought to be the gut associated lymphoid tissue. More recently it has been shown that IFN- α induction is dependent on the TGEV transmembrane protein E1 (Charley and Laude, 1988, Laude et al., 1992), and that IFN- α is produced by lamina propria lymphocytes exposed to TGEV (Naidoo and Derbyshire, 1992). The role of the endogenous IFN produced early in the course of infection with TGEV is not currently understood. By virtue of its antiviral activity, it may restrict further cycles of viral replication in the intestinal epithelium, and the major objective of the present study was to investigate this possibility in a ligated intestinal segment model in which exogenous IFN- α could be injected into the intestinal segments concurrently with the infecting virus.

It has been clearly established that IFN- α can exert an antiviral role against TGEV when cell cultures or intestinal explants are pretreated with IFN before challenge with the virus (Derbyshire, 1989; Weingartl and Derbyshire, 1991). In the present study, cell cultures were treated simultaneously with IFN- α and TGEV, and since an antiviral effect was demonstrated, similar experiments were conducted in the ligated intestinal segment model. Since it was not possible to produce porcine (Po) IFN- α in vitro in concentrations comparable to those detectable in vivo (La Bonnardière and Laude, 1981), high concentrations of human recombinant IFN- α were used in some of these experiments.

Treatment of newborn piglets with a synthetic IFN inducer, polyinosinic: polycytidylic acid (poly I:C) complexed with poly-L-lysine and carboxymethylcellulose (poly ICLC), before oral exposure to virulent TGEV resulted in a delay in the onset of clinical signs (Loewen and Derbyshire, 1988a). Because of the timing of the poly ICLC administration in relation to exposure to virus, it is likely that the transient protective effect was due to the activation of natural killer cell activity in the treated piglets (Lesnick and Derbyshire, 1988). In the present study we utilized the ligated intestinal segment model for further observations on the antiviral effect of poly ICLC in the newborn piglet.

The initial experiments described in this paper relate to the development of the ligated intestinal segment model for TGEV, particularly to the selection of a suitable strain of the virus and an appropriate challenge dose to give consistent virus yields. This technique was used in preference to whole animals since it allowed the comparison of different treatments within one individual, and greatly reduced the number of animals required for the studies. The method has been widely used for the study of enteric bacterial (Gyles and

Barnum, 1967) and viral (Carpio, et al., 1981, Kirsten, et al., 1985, Deregt, et al., 1989) infections, but not for the study of TGEV.

MATERIALS AND METHODS

Experimental animals

Sows from a specific-pathogen free herd of commercial swine were farrowed in isolation. The herd was determined to be TGEV and porcine respiratory coronavirus negative by virus neutralization serology. Piglets were given 100 mg of iron dextran (Ironol 100, Sanofi Animal Health, Victoriaville, Quebec) at 2 days of age. At 5–6 days of age piglets were removed from the sow in preparation for experimental surgery. All procedures were carried out under Canadian Council of Animal Care guidelines.

Virus strains and cell cultures

Virulent Purdue strain TGEV was propagated and collected from infected pigs by the method of Ristic, et al. (1965).

The Miller M6 strain TGEV obtained from Dr. L. Saif, Ohio State University, was propagated and assayed using a continuous swine testis (ST) cell line (McClurkin and Norman, 1966). The virus was plaque purified three times and passed seven times on ST cells (Welch and Saif, 1988). It was assayed by plaque formation on ST cells. Monolayer cultures grown in 24 well plates were inoculated with 0.2 ml of virus serially diluted in Eagle's minimum essential medium (EMEM). After adsorption for one hour, the inoculum was removed and the wells overlaid with 1 ml 0.6% agarose in EMEM with 5% neonatal calf serum (NNCS). Plates were fixed with 10% phosphate buffered formalin and stained with crystal violet after 24 hours.

Interferons and interferon assays

Natural porcine leucocyte IFN (PoIFN- α) was produced as described by Weingartl and Derbyshire (1990). Venous blood from weaned pigs was collected into sodium heparin and mixed with equal volumes of Hanks' balanced salt solution (HBSS). Leucocytes were separated by centrifugation through Ficoll-Paque (Pharmacia LKB Biotechnology Inc., Piscataway, New Jersey). After washing in HBSS, 1×10^7 cells/ml were suspended in RPMI-1640 medium containing 20% foetal bovine serum and Hepes buffer and incubated with shaking at 125 rpm. After 18 hours the leucocyte culture was inoculated with 4000 haemagglutinating units of Newcastle disease virus (La Sota strain). Twenty-four hours post-infection the leucocytes were pelleted by centrifugation and the supernatant treated with 0.1 ml 1 mM dithiothreitol (Sigma Chemical, St. Louis, Missouri) per millilitre. Virus was removed by ultracentrifugation at 60000 g for 60 min. The supernatant was stored at -20°C .

Human recombinant interferon α_{-2a} (Hu rec IFN- α_{2a}) was obtained from

a commercial source (Roferon, Hoffman – La Roche). Antiviral activity was determined as described below and in our system, 10000 international units were equivalent to 6400 laboratory units.

Interferon was assayed by plaque reduction. Samples to be assayed were serially diluted in EMEM containing 2% NNCS and 0.4 ml applied to each well of a 24 well plate containing a monolayer of Madin-Darby bovine kidney cells. After 18 hours the sample was removed and the plates treated with 40–60 plaque forming units (pfu) of vesicular stomatitis virus (Indiana strain). Following 60 min adsorption the inoculum was removed and wells overlaid with 1 ml 0.9% gum tragacanth (Sigma Chemical) in EMEM and 5% NNCS. Forty-eight hours post infection the cells were fixed with phosphate-buffered formalin and stained with crystal violet. IFN titres were determined as the reciprocal of the highest dilution which resulted in a 50% reduction in the number of plaques.

Polyinosinic:polycytidylic acid complexed with poly-L-lysine and carboxymethylcellulose (poly ICLC) was prepared as described by Levy, et al. (1975) as modified by Loewen and Derbyshire (1988b). Polyinosinic: polycytidylic acid (poly I:C, Sigma Chemical) was reannealed by heating at 71 °C for 60 min then added to a mixture containing equal volumes poly-L-lysine (3 mg/ml, Sigma Chemical) in normal saline and carboxymethyl cellulose (1%, Sigma Chemical) to yield a final concentration of 1 mg/ml poly I:C. Piglets were inoculated intravenously with 0.5 mg/kg of poly I:C.

In vitro yield reduction assays

The protocol has been previously described (Derbyshire, 1989). Four day old monolayer cultures of ST cells in 24 well plates were treated for 18 hours with Hu rec IFN- α_{2a} diluted in EMEM containing 5% NNCS. After washing with phosphate buffered saline (PBS), the cells were treated with 20 or 100 pfu/well of Miller M6 TGEV, representing multiplicities of infection of 5×10^{-5} and 2.5×10^{-4} . Following adsorption for 1 hour, the inoculum was removed and replaced with EMEM with 5% NNCS with or without IFN. Supernatants were collected for virus assay 12, 18, or 24 hours later.

Surgical protocol

Piglets were removed from the sow and deprived of food and water 6 hours prior to surgery. Anaesthesia was induced and maintained with halothane (Fluothane, Ayerst Laboratories, Montreal) using a Bain circuit. Midline laparotomies were performed and up to 14 five cm long segments of the mid-jejunum were isolated by ligation, with 2–3 cm intervening segments. The 5 cm segments were injected intralumenally with a total 1 ml volume of virus (one injection) and in some experiments IFN (a second injection), both diluted in PBS containing 500 Kallikrein units of aprotinin (Sigma Chemical), a protease inhibitor and penicillin (400 units/ml), streptomycin (0.4 mg/

ml) and gentamicin (0.05 mg/ml). Control inoculations consisted of this PBS diluent except in the experiment involving natural Po IFN- α , in which case a mock-induced leucocyte supernatant was used as a control inoculum. The incision was then closed in 2 layers and the animal allowed to recover from anaesthesia. Flunixin meglumine (1 mg/kg, Banamine, Schering-Plough) was administered to maintain analgesia post-operatively and water was provided. The piglets remained alert and active, with no clinical evidence of dehydration.

Sample collection and processing

Eighteen hours after infection the animals were again anaesthetised. Intestinal samples collected for histological examination and immunostaining were placed immediately in 10% phosphate buffered formalin.

For virus assay, the ligated segments were removed and placed in 5 ml EMEM containing antibiotics and frozen at -70°C . After thawing, the intestinal contents and mucosae were scraped into the media, vortexed and re-frozen. The samples were then thawed, mixed and clarified by centrifugation at 1000 g at 4°C for 20 min and stored at -70°C until assayed.

The piglets were euthanized by barbiturate overdose after the above samples were collected (Euthansol, Schering-Plough).

Immunohistochemistry

Intestinal sections were fixed in 10% phosphate buffered formalin for 24 hours, then transferred to 70% ethanol prior to embedding in paraffin. Slides were stained using a streptavidin immunoperoxidase technique (Dimension Laboratories, Mississauga, Ontario). Briefly, dewaxed and rehydrated sections were treated with protease at 37°C for 10 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Normal rabbit blocking serum (20%) was applied for 20 min, and without rinsing, mouse anti-TGEV monoclonal antibodies or non-specific mouse monoclonal antibodies as control were applied for 120 min. After rinsing, biotinylated rabbit anti-mouse serum was applied for 10 min. Binding was detected using a streptavidin/peroxidase conjugate for 5 min, followed by chromogen solution containing aminoethylcarbazole and hydrogen peroxide. Slides were then counterstained briefly with haematoxylin.

RESULTS

Histology and immunochemical staining for TGEV of intestinal segments

A single piglet was used in a preliminary experiment in which ligated segments were inoculated with dilutions of the virulent pig-passaged Purdue strain of TGEV ranging from 10^0 to 10^{-5} , or with the PBS control inoculum. The histological changes were consistent with naturally occurring TGEV infection, including cytoplasmic vacuolation, enterocyte enlargement and ne-

crisis, and villous atrophy. With the 10^{-2} dilution, the length of the villi was reduced by approximately 30% in comparison to the control segments, in which no histological abnormalities were seen. The extent of the changes occurred was dose dependent, and somewhat variable along the length of an individual segment. Immunohistochemistry revealed intense specific staining of TGEV antigen in villous enterocytes and also in luminal debris (Fig. 1). Non-specific staining occurred primarily in the muscularis mucosae, around blood vessels, and in erythrocytes. Such non-specific staining was readily distinguished from viral antigen on the basis of its location and its colour intensity. Virus was not isolated from the infected or control segments.

The Miller M6 strain of TGEV was inoculated at concentrations of 10^3 , 10^4 , and 10^5 pfu into two ligated segments in each of three piglets. One segment in each piglet was used for histology and one for virus isolation. There were three control-inoculated segments in each piglet. The virus produced less marked histological changes than the Purdue strain, but specific immunostaining of TGEV antigen was seen in the villous enterocytes (Fig. 2). No changes were seen in the control segments, from which no virus was isolated. The yields of the virus obtained from the infected segments are shown in Table 1.

Hu rec IFN- α_{2a} treatment of ST cells

The data in Table 2 indicate that in ST cell culture Miller M6 TGEV was sensitive to treatment with Hu rec IFN- α_{2a} . Virus yield reduction was evident with all doses of IFN used, all treatment regimens and in harvests at 12, 18,

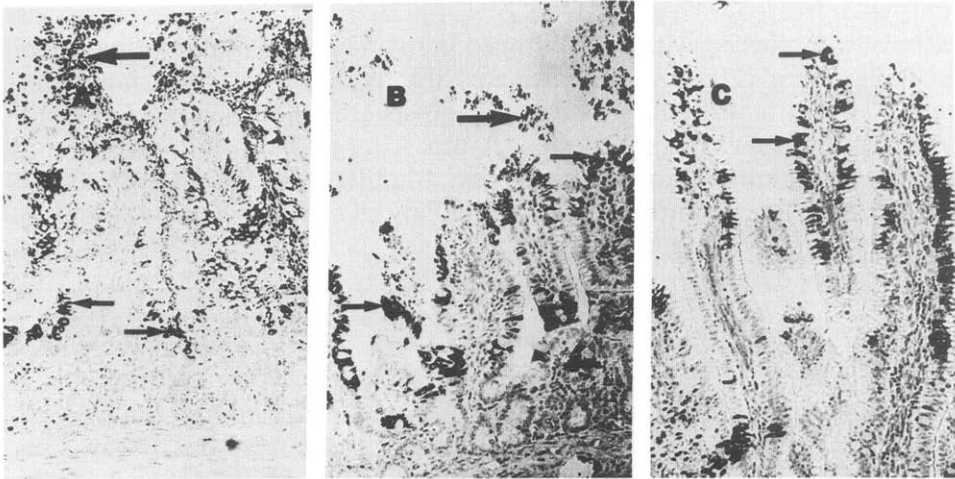


Fig. 1. Specific immunostaining of TGEV in a histological section of an intestinal segment treated (A) 10^{-1} , (B) 10^{-2} , and (C) 10^{-3} dilutions of Purdue strain TGEV. Specific staining of viral antigen in villi (small arrow) and luminal debris (large arrow) (haematoxylin, $\times 400$).

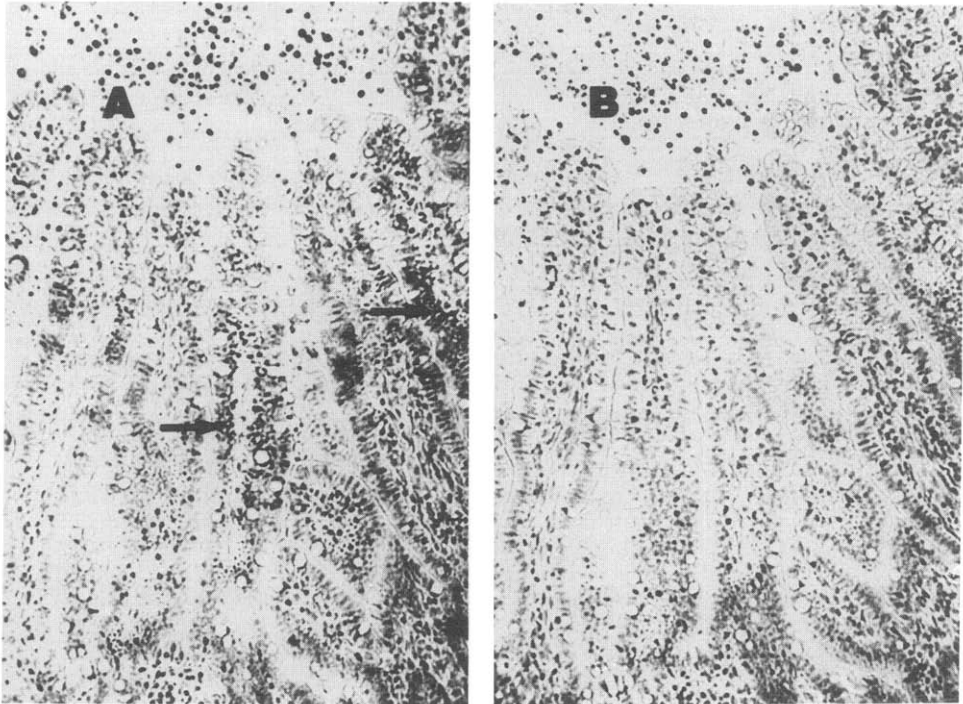


Fig. 2. Specific immunostaining of TGEV in a histological section of an intestinal segment treated with 10^5 pfu of Miller M6 strain TGEV. (A) Immunostaining using anti-TGEV mAb showing viral antigen in villi (arrow). (B) Immunostaining using negative control sera (haematoxylin, $\times 400$).

TABLE I

Virus yields 18 hours post infection of ligated intestinal segments with 10^3 , 10^4 , or 10^5 pfu of Miller M6 strain TGEV

Virus dose (pfu/segment)	Animal I.D. #	Virus yield (\log_{10} pfu/ml)	Mean	Std. dev.
10^3	555	5.72	6.18	0.52
	557	6.07		
	561	6.74		
10^4	555	5.94	5.96	0.08
	557	6.05		
	561	5.89		
10^5	555	6.05	6.76	0.89
	557	6.47		
	561	7.77		

TABLE 2

Virus yield reduction 12, 18, and 24 hours post infection following treatment of St cells pre and/or post infection with 500, 1000, 2500, or 5000 units/well Hu rec IFN- α_{2a} and subsequent challenge with 20 or 100 pfu/well Miller M6 TGEV

Virus dose (pfu/well)	IFN dose (units/well)	Treatment (s) (pre and/or post infection)	Yield reduction (\log_{10} pfu/ml) Time post infection (h)		
			12	18	24
20	500	Pre	NT*	1.62	NT
		Pre/post	NT	2.44	NT
		Post	NT	0.86	NT
100	500	Pre	NT	1.28	NT
		Pre/post	NT	1.99	NT
		Post	NT	0.93	NT
20	1000	Pre	1.18	1.70	2.08
		Pre/post	1.34	2.40	3.18
		Post	0.51	1.22	1.73
100	1000	Pre	1.24	1.80	1.56
		Pre/post	1.57	2.63	2.12
		Post	0.37	1.09	0.92
20	2500	Pre	NT	2.58	NT
		Pre/post	NT	3.46	NT
		Post	NT	1.59	NT
100	2500	Pre	NT	2.64	NT
		Pre/post	NT	3.52	NT
		post	NT	1.56	NT
20	5000	Pre	NT	2.75	NT
		Pre/post	NT	3.45	NT
		Post	NT	1.41	NT
100	5000	Pre	NT	2.64	NT
		Pre/post	NT	5.35	NT
		Post	NT	2.32	NT

*Not tested.

and 24 hours. The magnitude of this yield reduction increased with IFN concentration, and the reduction in yield was always greatest in cells treated with IFN before and after infection, although some reductions in yield occurred when IFN was applied to the cells only post-infection.

Simultaneous treatment of intestinal segments with Miller M6 TGEV and interferon

In this experiment (Table 3), 3200 units of Po IFN- α were inoculated into four ligated segments in each of three piglets and the same number of loops received the control inoculum. For each piglet, the inoculum for two of the IFN and control segments contained 10^3 pfu of Miller M6 TGEV, and 10^4 pfu for the other two segments. There was an additional control segment that was

TABLE 3

Virus yields following treatment of ligated intestinal segments simultaneously with either 3,200 units of Po leucocyte IFN or 10,000 or 100,000 units of Hu rec IFN- α_{2a} and 10^3 or 10^4 pfu of Miller M6 TGEV

Virus dose (pfu)	IFN dose (units)	Virus yields (\log_{10} pfu/ml)		No. of samples
		mean	std. dev.	
10^3	control	5.68	1.00	6
10^3	Po IFN 3200	5.78	0.95	6
10^4	control	6.24	1.40	6
10^4	Po IFN 3200	6.38	0.84	6
10^3	control	5.83	0.63	6
10^3	Hu IFN 10,000	5.54	0.66	6
10^3	Hu IFN 100,000	5.51	0.38	5

not infected with TGEV. In each of a further three piglets, two segments were inoculated with 10000 units of Hu IFN, 100000 units of Hu IFN, or with control inoculum, mixed in each case with 10^3 pfu of Miller M6 TGEV. In each piglet, two additional control segments were not infected with TGEV. No virus was recovered from any of the uninfected control segments. As shown in Table 3, no significant reduction in virus yield was evident by Student's *t*-test when intestinal segments were treated simultaneously with PoIFN- α and TGEV, or with higher concentrations of Hu rec IFN- α_{2a} and virus.

Interferon induction with poly ICLC

Two piglets were inoculated with poly ICLC and two were inoculated with a control solution containing poly-L-lysine and carboxymethylcellulose, but lacking poly I:C. Poly ICLC treatment resulted in the appearance of circulating IFN 6 hours post-treatment (Table 4). This level was greatly decreased by 25 hours post-treatment. The control solution did not induce circulating IFN at 6 hours. Poly ICLC treatment was also associated with a transient leucopaenia involving both segmented neutrophils and lymphocytes at 6 hours and persisting in segmented neutrophils at 25 hours. There was no evidence of a systemic IFN response to the inoculation of intestinal segments with TGEV. This may be due to the localized nature of this infection, the relative avirulence of the Miller M6 strain, or may indicate that this strain is a poor inducer of IFN.

Infection of intestinal segments with TGEV in piglets treated with poly ICLC

In each of the two poly ICLC treated piglets and the two control piglets, four ligated segments were inoculated with 100000 units of Hu IFN, and four

TABLE 4

Serum antiviral (units/0.4 ml) and leucocyte numbers (10^9 cells/l) following intravenous treatment with poly ICLC or a control solution 6 hours prior to the inoculation of intestinal segments with 10^3 pfu Miller M6 TGEV and 10^5 units Hu rec IFN- α_{2a} or a control solution

Treatment	I.D. #	Parameter	Time post-treatment		
			Pre	6 hours	25 hours
poly ICLC	630	antiviral	10	320	0
		WBC ¹	9.1	5.8	3.6
		seg neut ²	5.64	1.84	0.61
	634	lym ³	2.46	0.38	0.68
		antiviral	10	320	20
		WBC	9.9	8.4	4.2
control	631	seg neut	5.31	3.11	1.39
		lym	2.33	0.88	1.71
		antiviral	0	10	0
	632	WBC	7.7	8.1	9.3
		seg neut	4.62	5.75	5.86
		lym	2.39	2.11	3.16
		antiviral	0	0	10
		WBC	9.3	9.1	9.8
		seg neut	5.12	5.10	1.47
		lym	2.98	2.55	3.04

¹WBC = total nucleated cell

²seg neut = segmented neutrophil

³lym = lymphocyte

TABLE 5

Virus yields following intravenous treatment with poly ICLC or a control solution 6 hours prior to the inoculation of intestinal segments with 10^3 pfu Miller M6 TGEV and 10^5 units Hu rec IFN- α_{2a} or a control solution

Intravenous treatment	Intestinal treatment	Virus yields (\log_{10} pfu/ml)		No. of samples
		mean	std. dev.	
poly ICLC	control	4.00	0.81	8
poly ICLC	IFN	3.55	0.48	8
control	control	6.45	0.60	8
control	IFN	6.21	0.73	8

segments were inoculated with a control solution. In each case the challenge included 10^3 pfu of Miller M6 TGEV. As shown in Table 5, there was a significant reduction in virus yield by Student's *t*-test ($P < 0.05$) from the intestinal segments in the piglets which had been treated with poly ICLC. There was also a trend for the effects of poly ICLC treatment to be augmented by

subsequent intraluminal IFN treatment, though this was not significant ($P < 0.1$). As in the earlier experiment, there was no significant reduction in virus yield from the IFN treated segments in the control piglets.

DISCUSSION

Previous experimenters have described the use of isolated intestinal loops for the study of TGEV pathogenesis, in which relatively large lengths of the small bowel were exteriorized (Pensaert, et al., 1970). While this technique is ideal for the study of the sequential events occurring during TGEV infection, it was not thought suitable for the purposes here, where the objective was to compare the effects of various treatments. The length of the small bowel in a 5 day old conventional piglet is approximately two metres; in our studies up to fourteen 5 cm segments were used, although 8–10 segments were found to be a more satisfactory maximum in an animal of this age.

The Miller M6 strain of TGEV was selected for use in our yield reduction assays because it could be readily titrated in cell culture, in contrast to the virulent Purdue virus. While less virulent than the Purdue strain, the Miller M6 strain was capable of replicating in the intestinal segments, in contrast to vaccine strains of TGEV which were evaluated in preliminary experiments (results not shown). Antigenic and nucleotide sequencing data have established the close relationship between porcine and human alpha interferons (La Bonnardière, et al., 1986; Lefèvre and La Bonnardière, 1986; Lefèvre, et al., 1990; Weingartl, 1989). Human interferon has been shown to have antiviral activity in non-human cells (Gresser, et al., 1974). Human interferon has also been shown to have activity against feline infectious peritonitis virus, a coronavirus closely related to TGEV (Weiss and Oostram-Ram, 1989). Oral treatment with human IFN has been reported to decrease rotavirus shedding in pigs (Lecce, et al., 1992). Previous reports have described the reduction in TGEV yield following treatment with both porcine and bovine IFN of both cell and intestinal explant cultures (Maclachlan and Anderson, 1986; Derbyshire, 1989; Weingartl and Derbyshire, 1990). Of particular interest in the present study was the demonstration that post-treatment of ST cell cultures after virus inoculation with a low multiplicity of virus would result in some decrease in subsequent virus yield. In the intestinal segment experiments, however, the simultaneous treatment of enterocytes with Po IFN- α or Hu rec IFN- α_{2a} and TGEV did not cause a decrease in virus yield. It is possible that the kinetics of viral replication and/or the kinetics of the IFN system differ in enterocytes relative to ST cells in culture such that viral replication is well underway by the time an effective antiviral state is induced. It is also possible that the IFN inoculum was being degraded by intestinal proteases, despite the inclusion of a protease inhibitor in the inoculum.

The effects of poly ICLC on circulating IFN levels and leucocyte values are

consistent with those previously described (Loewen and Derbyshire, 1986, Loewen and Derbyshire, 1988b). This present study demonstrated that in addition to the activation of natural killer cells, previously described by Lesnick and Derbyshire (1988) poly ICLC treatment will result in a decreased TGEV yield in enterocytes. This may be a factor in the delay in onset of clinical signs associated with poly ICLC treatment (Loewen and Derbyshire, 1988a). It is not certain whether the reduced virus yield in the intestinal segments of the poly-ICLC treated piglets resulted from a direct antiviral effect of the induced IFN on the enterocytes, or by lysis of enterocytes early in infection by NK cells, activated by the induced IFN. The latter mechanism seems less likely, since the intestinal segments were infected with TGEV well before NK activity would have peaked (Lesnick and Derbyshire, 1988). A more direct effect, through the induction of an antiviral state in the enterocytes, seems more likely. It has been shown that 2'-5' oligoadenylate synthetase is induced during TGEV infection, and is also produced in IFN treated porcine cell cultures (Bosworth, et al., 1989; Bosworth and MacLachlan, 1990). Whether this system is involved in the antiviral state against TGEV remains to be determined.

The fact that simultaneous IFN treatment did not decrease virus yields in the intestinal segments may preclude the use of IFN as treatment for TGEV infection. However, the reduction in virus yield following the administration of an IFN inducer suggests that IFN may be useful prophylactically.

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REFERENCES

- Bosworth B.T. and MacLachlan, N.J., 1990. Evaluation of age-related effects on the antiviral activity of interferon and induction of 2-5A synthetase activity in testicular cell cultures derived from swine of various ages. *Am J. Vet. Res.*, 51: 723-725.
- Bosworth, B.T., MacLachlan, N.J. and Johnston, M.I., 1989. Induction of the 2-5A system by interferon and transmissible gastroenteritis virus. *J. Interferon Res.*, 9: 731-739.
- Butler, D.G., Gall, D.G., Kelly, M.H. and Hamilton, J.R., 1974. Transmissible gastroenteritis. Mechanisms responsible for diarrhea in acute viral enteritis in piglets. *J. Clin. Invest.*, 53: 1335-1342.
- Carpio, M., Bellamy, J.E.C. and Babiuk, L.A., 1981. Comparative virulence of different rotavirus isolates. *Can. J. Comp. Med.*, 45:38-42

- Charley, B. and Laude, H., 1988. Induction of alpha interferon by transmissible gastroenteritis coronavirus: role of transmembrane glycoprotein E1. *J. Virol.*, 62: 8-11.
- Derbyshire, J.B., 1989. The interferon sensitivity of selected porcine viruses. *Can. J. Vet. Res.*, 53: 52-55.
- Deregt, D., Gifford, G.A., Ijaz, M.K., Watts, T.C., Gilchrist, J.E., Haines, D.M. and Babiuk, L.A., 1989. Monoclonal antibodies to bovine coronavirus glycoproteins E2 and E3: demonstration of in vivo virus-neutralizing activity. *J. Gen. Virol.*, 70: 993-998.
- Gresser, I., Bandu, M-T., Brouty-Rouge, D. and Tovey, M., 1974. Pronounced antiviral activity of human interferon on bovine and porcine cells. *Nature*, 251: 543-545.
- Gyles, C.L., and Barnum, D.A., 1967. *Escherichia coli* in ligated segments of pig intestine. *J. Pathol.*, 29: 189-194.
- Hooper, B.E. and Haelterman, E.O., 1969. Lesions of the gastrointestinal tract in pigs infected with transmissible gastroenteritis. *Can. J. Comp. Med.*, 33: 28-31.
- Kirsten, C.G., Clare, D.A., and Lecce, J.G., 1985. Development of resistance of enterocytes to rotavirus in neonatal, agammaglobulinemic piglets. *J. Virol.*, 55: 567-573.
- La Bonnardière, C., and Laude, H., 1981. High interferon titre in newborn pig intestine during experimentally induced viral enteritis. *Infect. Immun.* 32: 28-31.
- La Bonnardière, C., Laude, H. and Berg, K., 1986. Biological and antigenic relationships between virus-induced porcine and human interferons. *Ann. Inst. Pasteur/Virol.*, 137E: 171-180.
- Laude, H., Gelfi, J., Lavenant, L., and Charley, B., 1992. Single amino acid changes in the viral glycoprotein M affect the induction of alpha interferon by the coronavirus transmissible gastroenteritis virus. *J. Virol.*, 66: 743-749.
- Lecce, J.G., Cummins, J.M., and Richards, A.B., 1990. Treatment of rotavirus infection in neonate and weanling pigs using natural human interferon alpha. *Mol., Biother.*, 2: 211-216.
- Lefèvre, F. and La Bonnardière, C., 1986. Molecular cloning and sequencing of a gene encoding biologically active porcine α -interferon. *J. Interferon Res.*, 6: 349-360.
- Lefèvre, F., Mege, D., L'Haridon, R., Bernard, S., de Vaureix, C. and La Bonnardière, C., 1990. Contribution of molecular biology to the study of the porcine interferon system. *Vet. Microbiol.*, 23: 245-257.
- Lesnick, C.E., and Derbyshire, J.B., 1988. Activation of natural killer cells in newborn piglets by interferon induction. *Vet. Immunol.*, 18: 109-117.
- Levy, H.B., Baer, G., Baron, S., Buckler, C.E., Gibbs, C.J., Iadarola, M.J., London, W.T. and Rice, J., 1975. A modified polyribinosinic-polycytidylic acid complex that induces interferon in primates. *J. Infect. Dis.*, 132: 434-439.
- Loewen, K.G., and Derbyshire, J.B., 1986. Interferon induction with polyinosinic: polycytidylic acid in the newborn piglet. *Can. J. Vet. Res.*, 50: 232-237
- Loewen, K.G., and Derbyshire, J.B., 1988a. The effect of interferon induction in parturient sows and newborn piglets on resistance to transmissible gastroenteritis. *Can. J. Vet. Res.*, 52: 149-153.
- Loewen, K.G., and Derbyshire, J.B., 1988b. Interferon induction in piglets with poly I:C acid complexed with poly-L-lysine and carboxymethylcellulose. *Res. Vet. Sci.*, 44: 132-133.
- McClurkin, A.W., and Norman, J.O., 1966. Studies on the transmissible gastroenteritis of swine. II. Selected characteristics of a cytopathogenic virus common to five isolates from transmissible gastroenteritis. *Can. J. Comp. Med. Vet. Sci.*, 30: 190-198.
- Maclachlan, N.J. and Anderson, K.P., 1986. Effect of recombinant DNA-derived bovine α -1 interferon on transmissible gastroenteritis virus infection in swine. *Am. J. Vet. Res.*, 47: 1149-1152.
- Naidoo, D. and Derbyshire, J.B., 1992. Interferon induction in porcine leucocytes with transmissible gastroenteritis virus. *Vet. Microbiol.*, 30: 317-327.

- Pensaert, M., Haelterman, E.O., and Burnstein, T., 1970. Transmissible gastroenteritis of swine: virus intestinal cell interactions. I. *Archiv. Ges. Virusforsch.*, 31: 321-334.
- Ristic, M., Sibinovic, S. and Alberts, J.O., 1965. Electron microscopy and ether sensitivity of transmissible gastroenteritis virus of swine. *Am. J. Vet. Res.*, 26: 609-616.
- Saif, L.J., and Heckert, R.A., 1990. Enteric Coronaviruses. In: L.J. Saif and K.W. Theil (Editors), *Viral Diarrheas of Man and Animals*. CRC Press Inc., Boca Raton, Fla.
- Weingartl, H.M., 1989. The induction, partial purification and characterization of porcine interferons alpha and beta. M.Sc. Thesis. University of Guelph
- Weingartl, H., and Derbyshire, J.B., 1990. The induction and characterization of natural porcine interferons alpha and beta. *Can. J. Vet. Res.*, 54: 349-354.
- Weingartl, H., and Derbyshire, J.B., 1991. Antiviral activity against transmissible gastroenteritis virus, and cytotoxicity, of natural porcine interferons alpha and beta. *Can. J. Vet. Res.*, 55: 143-149.
- Weiss, R.C. and Oostram-Ram, T., 1989. Inhibitory effects of ribivarin alone or combined with human alpha interferon on feline infectious peritonitis virus replication in vitro. *Vet. Microbiol.*, 20: 255-265.
- Welch, S-K W., and Saif, L.J., 1988. Monoclonal antibodies to a virulent strain of transmissible gastroenteritis virus: comparison of reactivity with virulent and attenuated strains. *Arch. Virol.*, 101:221-235.