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# Antiviral action of interferon-alpha against porcine transmissible gastroenteritis virus

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## Abstract

Swine testis (ST) cell cultures were treated with various doses of recombinant human interferon-alpha 2a (IFN), and assayed for 2',5' oligoadenylate synthetase (2–5 A synthetase) activity. Treatment with 100 or 1000 units/ml of IFN resulted in increased 2–5 A synthetase activity, but there was no significant response to 1 unit/ml of IFN. Titres of porcine transmissible gastroenteritis virus (TGEV) were reduced between 6 and 15 hours post-infection in ST cells treated with 1000 or 2500 units/ml of IFN. Polyacrylamide gel electrophoresis of lysates of TGEV-infected ST cells, and of lysates immunoprecipitated with anti-TGEV antibodies, revealed that the synthesis of the N and S proteins of TGEV was reduced in cells treated with 100 or 1000 units/ml of IFN. Viral RNA production, as determined with a probe which hybridized to the S gene of TGEV, was found to be reduced in ST cells treated with 1000 units/ml of IFN, but not in cells treated with 100 units/ml. It was concluded that, in IFN-treated ST cells, TGEV protein production may be decreased in the absence of reduced viral RNA production, and that 2–5 A synthetase may not be a significant factor in the antiviral activity of IFN against TGEV.

*Keywords:* Interferon; Transmissible gastroenteritis virus; Viral proteins; Viral RNA; Antiviral activity

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## 1. Introduction

It is well established that the replication of transmissible gastroenteritis virus (TGEV), a porcine enteric coronavirus, may be inhibited by treatment with interferon (IFN)-alpha. Thus natural porcine IFN-alpha was found to reduce the yields of TGEV in swine testis (ST) cells and in porcine enterocytes (Weingartl and Derbyshire, 1991). Similarly, an antiviral effect of recombinant human IFN-alpha was demonstrated against TGEV in ST cells by Jordan and Derbyshire (1994). Information is lacking, however, on the mechanism of the antiviral action of IFN-alpha against TGEV and other coronaviruses.

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For many viruses, the most consistent characteristic of the IFN-induced antiviral state is a reduction in viral protein synthesis. Interferons induce a number of cellular proteins (Sen and Ransohoff, 1993), including 2',5' oligoadenylate synthetase (2–5 A synthetase), which has been studied extensively and shown to be a major contributor to the IFN-induced antiviral state, at least in relation to picornavirus infections (Samuel, 1988). The antiviral effects of 2–5 A synthetase depend upon its ability to catalyse the synthesis of 2–5 A oligoadenylates, which activate a latent endonuclease which can degrade viral messenger RNA and therefore inhibit viral protein synthesis. Recombinant bovine IFN-alpha induced a dose-dependent increase in 2–5 A synthetase in ST cells, and infection of neonatal pigs with TGEV caused a significant increase in enterocyte 2–5 A synthetase activity (Bosworth et al., 1989). The role of 2–5 A synthetase in the antiviral activity of IFN-alpha against coronaviruses is unknown.

The objective of the present study was to investigate 2–5 A synthetase activity and the synthesis of viral proteins and RNA in the antiviral state induced by IFN against TGEV. ST cells were treated with various concentrations of human recombinant IFN-alpha 2, which has been shown to be closely related antigenically to porcine leukocyte IFN (Karayianni-Vasconcelos et al., 1993) and to have an antiviral effect against TGEV in ST cells (Jordan and Derbyshire, 1994). The treated cells were monitored for 2–5 A synthetase activity, and, after infection with TGEV, the stage of the infectious cycle inhibited by IFN was determined. Viral protein production in IFN treated ST cells was examined by radiolabelling and immunoprecipitation, and viral RNA production was examined by probing lysates of infected cells with a radiolabelled TGEV S gene fragment.

## 2. Materials and methods

### 2.1. Interferon and interferon assays

Human recombinant IFN-alpha-2a was obtained from a commercial source (Roferon, Hoffman-La Roche). Antiviral activity was determined by plaque reduction assays on Madin-Darby bovine kidney cells challenged with vesicular stomatitis virus as previously described (Loewen and Derbyshire, 1986).

### 2.2. 2–5 A synthetase production in ST cells

ST cells (McClurkin and Norman, 1966) were grown to confluence in Nunclon six well tissue culture dishes (Nunc) in Eagle's minimum essential medium (EMEM) supplemented with 5% neonatal calf serum (NNCS). The cells were then treated for 18 hours with 0, 1, 100, or 1000 units of IFN diluted in EMEM with 5% NNCS. The activity of 2–5 A synthetase in the treated cells was assayed as described by Bielefeldt-Ohmann et al. (1989). Briefly, the ST cells were pelleted and lysed, and the cell extracts were incubated with poly (rI):(rC)-agarose beads. The beads were then incubated with the reaction mixture containing [<sup>32</sup>P] labelled ATP. After termination of the reaction with alkaline phosphatase, the beads were pelleted and the supernatant applied to acid alumina columns. The phosphatase-resistant 2–5 A oligoadenylate was eluted from the columns, and radionuclide incorporation

was enumerated by liquid scintillation counting. Means and standard deviations of counts per min per  $2 \times 10^6$  cells were calculated, and differences between mean yields were analysed using the *d* value following Fisher-Behren's distribution (Campbell, 1989).

### 2.3. TGEV growth curve in IFN treated ST cells

ST cells were grown to confluence in Nunclon six well tissue culture dishes and treated with 0, 1000, or 2500 units/ml of IFN. After 18 hours, the cells were infected with the Miller M6 strain of TGEV (Welch and Saif, 1988), obtained from Dr. L.J. Saif, Ohio State University, at a multiplicity of infection (m.o.i.) of 0.5. After adsorption for 60 min, the inoculum was removed, the cells washed with phosphate buffered saline (PBS), and replaced with 3 ml/well of EMEM containing 5% NNCS and Hepes buffer. Supernatants were collected at intervals and subsequently assayed for virus by plaque formation in ST cells. Means and standard deviations of virus yields expressed as  $\log_{10}$  PFU/ml were calculated, and differences between mean yields were analysed using the *d* value following Fisher-Behren's distribution (Campbell, 1989).

### 2.4. Radiolabelling of TGEV proteins

The effects of IFN treatment on TGEV protein production were examined by radiolabelling of the proteins produced during infection in ST cells. Four-day-old monolayers of ST cells grown in six well Nunclon tissue culture dishes were treated with 3 ml/well of EMEM with 2% NNCS containing 0, 100, or 1000 units/ml IFN (two wells per plate). After 18 hours, the cells were washed twice with PBS and inoculated with 1 ml/well of Miller M6 TGEV in EMEM at a m.o.i. of 5, or EMEM alone, and the virus was allowed to adsorb for 60 min. The inoculum was then removed, the plates were washed with PBS, and EMEM containing 2% NNCS was added to the plates. Radiolabelling was done at 3–6, 6–9, and 9–12 hours post-infection (h.p.i.) with 100  $\mu\text{Ci/ml}$  [ $^{35}\text{S}$ ] methionine (1143 Ci/mmol, ICN Biomedicals Canada Ltd) following a 1 hour starvation period in methionine-free EMEM supplemented with 2% methionine-free NNCS. The labelled cells were washed in PBS and lysed in 200  $\mu\text{l}$  lysis buffer (150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulphate (SDS), 10 mM Tris HCl, pH 7.2). The lysates were stored at  $-70^\circ\text{C}$ . Culture supernatants were collected and assayed for virus concentration by plaque formation.

### 2.5. Immunoprecipitation of TGEV proteins

One hundred  $\mu\text{l}$  of radiolabelled cell lysates were incubated at room temperature for 60 min with 25  $\mu\text{l}$  of polyclonal anti-TGEV porcine antiserum, raised in pigs infected by oral dosing with the Ambico vaccine strain of TGEV and subsequent intramuscular injection of the attenuated Purdue strain of the virus, kindly provided by Dr. Tamás Tuboly, University of Guelph, and 30  $\mu\text{l}$  of a 10% suspension of formalin-fixed *Staphylococcus aureus* (Pansorbin<sup>®</sup> Cells, Calbiochem Corp.). The complexes were pelleted and washed sequentially in wash buffers A (1 M NaCl, 1% sodium deoxycholate, 1% Triton X-100, 1% SDS, 10mM Tris HCl, pH 7.2), B (1M NaCl, 0.01 M Tris HCl, 0.1% NP-40, pH 7.2) and C

(0.1 M NaCl, 1 Mm EDTA, 0.01 M Tris HCl, 0.1% NP-40, 0.3% SDS, pH 7.2) and then resuspended in 100  $\mu$ l electrophoresis sample buffer (ESB, 0.0625 M Tris-HCL pH 6.8, 1% SDS, 10% glycerol, 2%  $\beta$ -mercaptoethanol, 0.001% bromophenol blue). After boiling for 3 min the cell debris was removed by centrifugation and the supernatant was stored at  $-70^{\circ}\text{C}$ .

## 2.6. Polyacrylamide gel electrophoresis

Whole cell lysates and immunoprecipitates were examined by SDS-polyacrylamide gel electrophoresis (PAGE) using the method of Laemmli (1970). Samples were diluted in ESB and separated by electrophoresis at 100 volts on 10% polyacrylamide gels containing 0.1% SDS. The gels were fixed and treated with an autoradiography enhancer (EN<sup>3</sup>HANCE, Du Pont Canada, Inc.) according to the manufacturer's instructions, and exposed to Kodak X-OMAT AR or RP radiographic film.

## 2.7. Preparation of radiolabelled cDNA probe for TGEV S protein gene

The baculovirus transfer vector, pVL1393, containing a cDNA copy of the 1.6 kb 5' end of the gene encoding the S protein of the attenuated Purdue 115 strain of TGEV (Tuboly et al., 1994) was obtained from Dr. Tamás Tuboly, University of Guelph. The 1.6 kb fragment was removed from the vector by digestion with *Bam*H1 and *Eco*R1 restriction endonucleases, and separated by electrophoresis on a 0.8% agarose gel. The fragment was extracted from the agarose and radiolabelled with 50  $\mu$ Ci [<sup>32</sup>P]dCTP (3000 Ci/mmol, ICN Biomedicals Canada Ltd) using the Random Primers Labelling System (BRL Life Technologies Inc.).

## 2.8. Extraction of RNA and DNA-RNA hybridization

Five-day-old monolayers of ST cells were treated for 18 hours with 100 or 1000 units/ml of IFN, and infected with the Miller M6 strain of TGEV at a m.o.i. of 1. At 1, 3, 6, 9, and 12 hours post-infection the culture supernatants were collected for virus assay by plaque formation, and lysates from the infected cells to be used for RNA hybridization were prepared as follows. The cells were washed twice with cold PBS, scraped into 1 ml of PBS, and pelleted by centrifugation for 15 seconds. The pellet was resuspended in 45  $\mu$ l of TE buffer pH 7.2 and 5  $\mu$ l of 5% Nonidet P-40 (Sigma Chemical Co.). The cells were placed on ice for 5 min and the debris was pelleted for 3 min. The supernatant, containing cellular and viral RNA, was separated into two aliquots and stored at  $-70^{\circ}\text{C}$ .

Supernatants were blotted on to nylon membranes (S&S Nytran, Schleicher and Schuell) using a slot-blot apparatus (Schleicher and Schuell Mini Fold II Slot-Blot System). RNA collected above (10  $\mu$ l) was denatured by mixing with 190  $\mu$ l of  $6\times$  SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0), 7.4% formaldehyde in TE (pH 7.2) and heating to  $60^{\circ}\text{C}$  for 15 min. Fifty  $\mu$ l of this RNA were loaded into each well of a slot blot apparatus while under vacuum. The blot was rinsed in  $5\times$  SSC, air dried, baked under vacuum at  $80^{\circ}\text{C}$  for 1 hour, and then probed with the [<sup>32</sup>P] labelled 1.6 kb S gene cDNA for 24 hours at  $42^{\circ}\text{C}$  in the

presence of 50% formamide (Fisher Scientific) as described by Sambrook et al. (1989). The blot was then exposed for 6 days to Kodak X-OMAT AR radiographic film at  $-70^{\circ}\text{C}$ .

### 3. Results

#### 3.1. Effect of IFN on 2–5 A synthetase production in ST cells

The results are shown in Table 1. A basal level of 2–5 A synthetase activity was found in the untreated cells. Treatment with 100 units/ml and 1000 units/ml of IFN resulted in a significant increase in 2–5 A synthetase activity relative to untreated cells ( $P \leq 0.05$ ), but the increase in 2–5 A synthetase activity in response to 1 unit/ml of IFN was not significant ( $P > 0.05$ ).

#### 3.2. Replication of TGEV in IFN-treated ST cells

The virus yields are given in Table 2. At 3 h.p.i., virus titres in supernatants were similar in both IFN-treated and virus control wells. From 6 to 15 h.p.i., the virus titres in the IFN-treated cells were significantly lower ( $P \leq 0.05$ ) than in the untreated cells, with reductions of approximately  $1.5\text{--}2.0 \log_{10}$  pfu/ml. There was no appreciable difference in virus titres between cells treated with 1000 or 2500 units/ml IFN.

#### 3.3. Effects of IFN on TGEV protein production

The virus yields in the radiolabelling experiments (Table 3) were always significantly lower ( $P \leq 0.05$ ) in the cultures treated with IFN than in the untreated cultures. The reduction in virus yield associated with IFN treatment was greatest in the 3–6 hour period, with yield reductions of  $0.53$  and  $2.21 \log_{10}$  pfu/ml in the cells treated with 100 or 1000 units/ml of IFN. In the 6–9 hour period yield reductions remained at  $0.57 \log_{10}$  pfu/ml in the cells treated with 100 units/ml IFN, but they declined to  $1.17 \log_{10}$  pfu/ml at 1000 units/ml IFN. In the 9–12 hour labelling period, reductions in virus yield had further declined to  $0.50$  and  $0.62 \log_{10}$  pfu/ml in the cells treated with 100 and 1000 units/ml IFN respectively. PAGE of whole cell lysates (Fig. 1) showed differences between cell control,

Table 1  
2–5 A synthetase activity in ST cells treated with IFN

Sample $n = 3$	Counts per min/ $2 \times 10^6$ cells	
	Mean	Std. dev.
Negative control	1298	58.5
0 units/ml	47 066	20 282
1 units/ml	76 041	38 434
100 units/ml	173 770	25 661
1000 units/ml	204 302	37 817

Table 2

Virus yields in supernatants of ST cell cultures ( $n = 3$ ) treated with IFN and infected with TGEV at a m.o.i. of 0.5

Hours post-infection	IFN units/ml	Virus yield ( $\log_{10}$ pfu/ml)	
		Mean	Std. dev.
3	0	2.66	0.15
	1000	2.32	0.15
	2500	2.41	0.28
6	0	4.15	0.08
	1000	2.53	0.20
	2500	2.42	0.20
9	0	5.05	0.09
	1000	3.69	0.13
	2500	3.23	0.13
12	0	5.47	0.17
	1000	4.46	0.26
	2500	4.46	0.16
15	0	5.83	0.19
	1000	4.44	0.12
	2500	4.57	0.09

virus control and IFN treated cells. In the virus control lanes (B, F, and J) a protein of slightly more than 46 kDa, probably the N protein of TGEV, was seen. The intensity of this band increased from the 3–6 to the 9–12 hour labelling periods. In the cells treated with 100 units/ml of IFN this band was visible at 3–6 and 6–9 hours (lanes C and G), and became evident in the cells treated with both 100 and 1000 units/ml of IFN at the 9–12 hours labelling period (lanes K and L). It was never as intense in IFN-treated cells as in virus control cells. The band was not detected in cell control samples (lanes A, E, and I).

PAGE of immunoprecipitates of the radiolabelled samples is shown in Fig. 2. A protein of approximately 200 kDa was evident in the virus control lanes (B, F, and J). This band

Table 3

Virus yields in culture supernatants ( $n = 3$ ) following radiolabelling at intervals post-infection with TGEV at a m.o.i. of 5 in ST cells treated for 18 hours with IFN

Hours post-infection	IFN units/ml	Virus yield ( $\log_{10}$ pfu/ml)	
		Mean	Std. dev.
3–6	0	5.35	0.14
	100	4.72	0.09
	1000	3.07	0.14
6–9	0	6.17	0.12
	100	5.60	0.03
	1000	5.10	0.09
9–12	0	5.72	0.02
	100	5.22	0.10
	1000	5.10	0.01

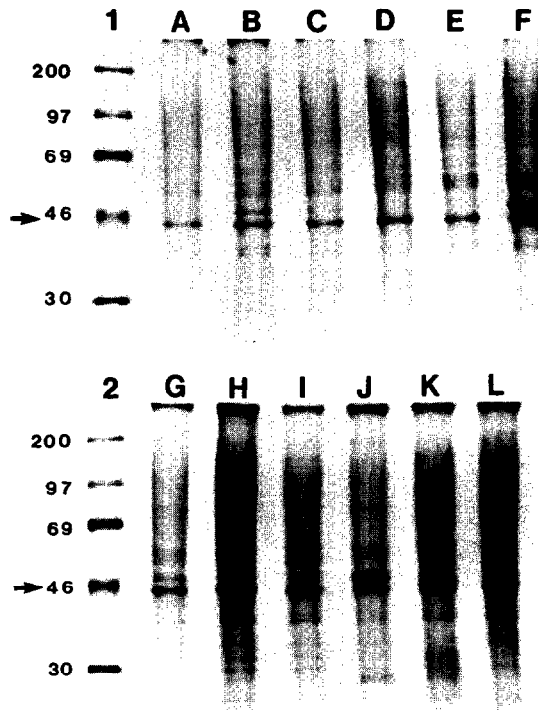


Fig. 1. Protein radiolabelling of ST cells treated with IFN and subsequently infected with TGEV at a m.o.i. of 5. Whole cell lysates were separated by electrophoresis on a 10% SDS-polyacrylamide gel. Lanes 1 and 2, molecular weight markers. Lanes A, B, C and D, labelling period 3–6 h.p.i.; cell control (lane A), virus control (lane B), 100 units/ml IFN (lane C), 1000 units/ml IFN, (lane D). Lanes E, F, G and H, labelling period 6–9 h.p.i.; cell control (lane E), virus control (lane F), 100 units/ml IFN (lane G), 1000 units/ml IFN (lane H). Lanes I, J, K and L labelling period 9–12 h.p.i.; cell control (lane I), virus control (lane J), 100 units/ml IFN (lane K), 1000 units/ml IFN (lane L). Arrow indicates TGEV N protein.

increased in intensity in the later radiolabelling periods. It was also present, though at much lower intensity, in the IFN-treated cells (lanes C, D, G, H, K, and L) and the intensity of the band decreased with increasing IFN concentration. The band was not present in the cell controls (lanes A, E, and I). A 46 kDa protein, probably the N protein, was very faintly visible in lanes K and L.

#### 3.4. Effects of IFN on TGEV RNA production

The virus yields in the supernatants of the cultures used for RNA extraction are shown in Table 4. At 1 and 3 h.p.i., virus yields in the virus controls and the IFN treated cells were approximately the same. By 6 h.p.i, virus titres had increased by similar amounts in both the virus control and the cells treated with 100 units/ml of IFN. Virus titres continued to increase to 12 h.p.i. In samples collected at 6, 9, and 12 hours however, virus yields in the cells treated with 1000 units/ml of IFN were approximately  $1 \log_{10}$  pfu/ml less than in the virus controls or in the cells treated with 100 units/ml of IFN.

The results of RNA-DNA hybridization are shown in Fig. 3. Virus specific RNA was detected at 3 h.p.i. in all samples. The intensity of hybridization increased from 3 to 12



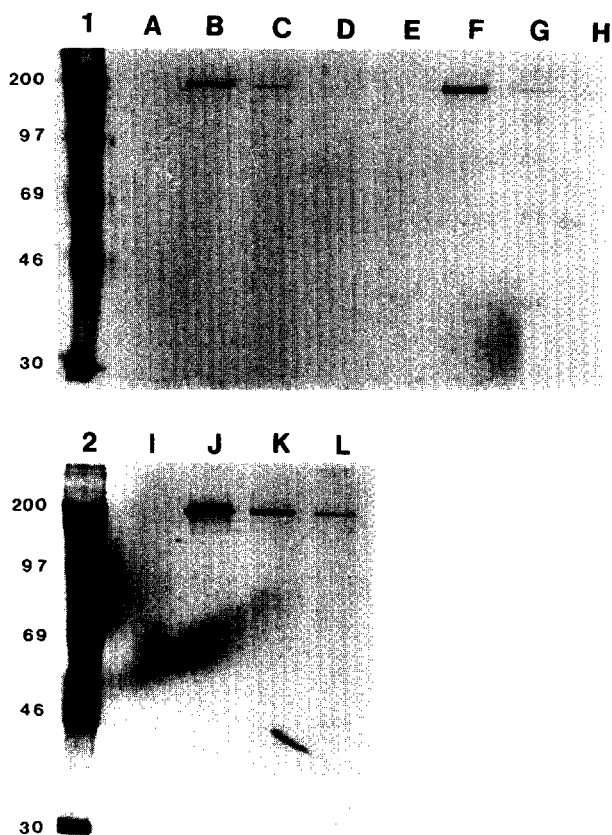


Fig. 2. Protein radiolabelling of ST cells treated with IFN and subsequently infected with TGEV at a m.o.i. of 5. Whole cell lysates were immunoprecipitated by antiserum to TGEV and separated by electrophoresis on a 10% SDS-polyacrylamide gel. Lanes 1 and 2, molecular weight markers. Lanes A, B, C and D, labelling period 3–6 h.p.i.; cell control (lane A), virus control (lane B), 100 units/ml IFN (lane C), 1000 units, (lane D). Lanes E, F, G and H, labelling period 6–9 h.p.i.; cell control (lane E), virus control (lane F), 100 units/ml IFN (lane G), 1000 units/ml IFN (lane H). Lanes I, J, K and L labelling period 9–12 h.p.i.; cell control (lane I), virus control (lane J), 100 units/ml IFN (lane K), 1000 units/ml IFN (lane L).

Table 4

Virus yields in supernatants of ST cell cultures treated with IFN and subsequently infected with TGEV at a m.o.i. of 1.0, and used in determination of TGEV RNA production

Hours post-infection	Virus yields ( $\log_{10}$ pfu/ml)		
	IFN (units/ml)		
	0	100	1000
1	2.74	2.65	2.44
3	2.35	2.48	2.57
6	6.74	6.72	5.92
9	7.30	7.09	6.21
12	7.52	7.30	6.49

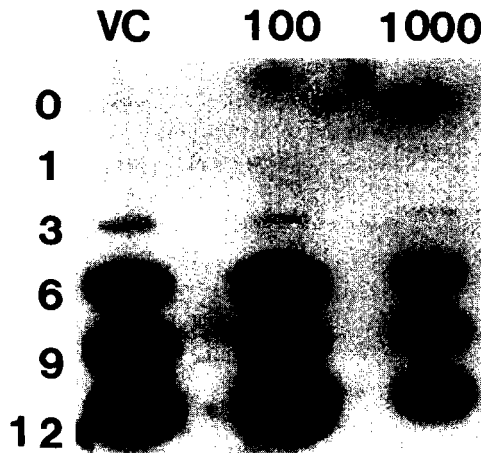


Fig. 3. Detection of TGEV RNA in ST cells treated with IFN and subsequently infected with TGEV at a m.o.i. of 1.0. ST cells were treated with 0 units/ml IFN (virus control, VC), 100 units/ml IFN (100) or 1000 units/ml IFN (1000). Samples were collected 0, 1, 3, 6, 9, and 12 h.p.i., blotted onto nylon and subsequently probed with a radiolabelled cDNA of the 5' 1.6 kb of the TGEV gene encoding the S protein.

h.p.i. The intensity of the hybridization appeared to be similar in the virus controls and in the cells treated with 100 units/ml of IFN. In the cells treated with 1000 units/ml of IFN, the intensity of hybridization at all time periods was less than that in the virus controls or in the cells treated with 100 units/ml of IFN.

#### 4. Discussion

In these studies, treatment of ST cells with recombinant human IFN- $\alpha$  was shown to increase the activity of 2–5 A synthetase in the cells. The ability of a heterologous IFN to induce 2–5 A synthetase in porcine cells was demonstrated previously, when Bosworth and MacLachlan (1990) noted similar effects with recombinant bovine IFN- $\alpha$  in primary swine testis and kidney cells.

In our studies in ST cells, the activity of 2–5 A synthetase was similar in cells treated with either 100 or 1000 units/ml of IFN, which suggests that maximum 2–5 A synthetase activity may be induced with relatively low amounts of IFN. However, there was a marked difference in the reduction in virus yield obtained with 100 units/ml versus 1000 units/ml of IFN. The experiments on the production of TGEV proteins and RNA in IFN treated cells indicated that RNA production was little affected by 100 units/ml of IFN, but decreased by 1000 units/ml of IFN, while protein production was decreased by both 100 and 1000 units/ml of IFN. The proposed mechanism of action of 2–5 A synthetase is through the activation of a latent ribonuclease which degrades viral RNA, particularly mRNA, and leads to decreased viral protein production (Samuel, 1991, Chebath and Revel, 1992, Sen and Ransohoff, 1993). Our results indicate that viral protein production may be decreased without apparent decreases in RNA production, and suggest that 2–5 A synthetase may not be a significant factor in the reduction in TGEV yield in IFN-treated ST cells.

The reductions in virus yield were lower than those reported previously for the same IFN preparation in ST cells (Jordan and Derbyshire, 1994), probably because a higher m.o.i. was used in the present study. In the protein radiolabelling experiment, the reduction in virus yield seemed to correspond to the reduction in protein yield. Examination of lysates of infected cells by PAGE revealed a protein of approximately 46 kDa, corresponding to the N protein of TGEV (Saif and Heckert, 1990). In addition, PAGE of immunoprecipitates from infected cells revealed a protein of approximately 200 kDa, corresponding to the S protein of TGEV (Saif and Heckert, 1990). The failure of the immunoprecipitation procedure to detect the TGEV N and M proteins was probably due to the use of an antiserum raised against heterologous strains of TGEV, since Vaughn and Paul (1993) found that antisera used to precipitate non-homologous strains of TGEV were efficient in precipitating the S protein, but not the N, or in some cases the M, protein of the virus.

The intensity of the protein bands obtained by PAGE of both cell lysates and immunoprecipitates indicated a reduction in virus protein production in IFN-treated cells. The extent of this reduction appeared to be related to the concentration of IFN, being greatest in cells treated with 1000 units/ml of IFN. This effect was consistent, in both whole cell lysates and in immunoprecipitated proteins, during all labelling periods, and was also consistent with virus yields in the corresponding culture supernatants.

Evidence for a decrease in viral RNA synthesis was found, at all times from 3 to 12 h.p.i., in cells treated with 1000 units/ml of IFN. The hybridizations were made with a probe capable of detecting both plus and minus sense RNA of the TGEV S gene. Therefore, both genomic RNA and TGEV S-gene mRNA, and their complementary minus strands, would be detected. The proportion of total RNA that is negative sense is probably very small (Sawicki and Sawicki 1986). At 3 h.p.i., it is likely that the primary RNA species is TGEV S-gene mRNA, and the ratio between genomic length RNA and mRNA would increase during the course of infection (Keck et al., 1988).

A reduction in virus protein production may arise from decreased mRNA production, or from a decrease in translation of these viral specific mRNAs. The present results indicate that TGEV protein production is more sensitive than RNA accumulation to the antiviral state induced by IFN; it is decreased by a lower concentration of IFN and is more significantly reduced than RNA accumulation. These results support the conclusion that decreased TGEV protein synthesis is not primarily due to a decrease in viral RNA accumulation, but rather results from a decrease in translation of these viral RNAs. This conclusion, and the data discussed above, suggest that the 2–5 A synthetase system does not play a significant role in the observed decrease in viral protein production. It is likely, therefore, that the IFN-induced antiviral state to TGEV is mediated primarily through another mechanism, perhaps the P1/eIF-2 kinase system, which is known to inhibit translation (Samuel, 1991).

The decreased accumulation of viral RNA observed in our experiment may be due to decreased transcription, or to increased destruction of these transcripts. The latter mechanism may be mediated by the 2–5 A synthetase system through the activation of ribonuclease. However, it has been shown that ongoing protein synthesis is necessary for coronaviral RNA synthesis (Mahy et al., 1983, Sawicki and Sawicki, 1986). Decreased production of the viral RNA-dependent RNA polymerase may reduce coronaviral RNA synthesis. Therefore, the reduction in viral protein synthesis may also be involved in the decreased accumulation of viral RNA.

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