Antiviral Effects of Bovine Interferons on Bovine Respiratory Tract Viruses

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The antiviral effects of bovine interferons on the replication of bovine respiratory tract viruses were studied. Bovine turbinate monolayer cultures were treated with bovine interferons and challenged with several bovine herpesvirus 1 strains, bovine viral diarrhea virus, parainfluenza type 3 virus, goat respiratory syncytial virus, bovine respiratory syncytial virus, bovine adenovirus type 7, or vesicular stomatitis virus. Treatment with bovine interferons reduced viral yield for each of these viruses as compared with that of control cultures.

Several viruses representing different families and, in some instances, multiple serotypes for each virus have been associated with bovine respiratory tract diseases (44). These viruses include: bovine herpesvirus type 1 (BHV-1), also known as infectious bovine rhinotracheitis virus; bovine viral diarrhea virus (BVDV); parainfluenza type 3 virus (PI-3V); bovine respiratory syncytial virus (BRSV); bovine adenoviruses (BAV); DN-599 and FTC 2 bovine herpesviruses; rhinoviruses; influenza virus; and enteroviruses. Licensed vaccines are available in the United States for only BHV-1, PI-3V, and BVDV. At present there are no licensed antiviral drugs for use in food-producing animals.

Interferon (IFN) has been detected in the serum and nasal and vaginal secretions of cattle inoculated with BHV-1 (1, 10-12, 26, 29, 48, 49, 52, 53, 57). In addition, cattle inoculated intranasally with BAV-3, PI-3V, or a bovine rhinovirus had nasal secretion IFN postchallenge (10-12, 39). IFN in serum was detected in calves aerosolized with BRSV (15). A temporal relationship between IFN in respiratory tract secretions and onset of protection against virulent BHV-1 challenge has been reported (53). However, another study indicated that nasal IFN did not protect calves against virulent BHV-1 challenge (49). Calves with IFN in their nasal secretion subsequent to inoculation with a vaccinal strain of BHV-1 had decreased viral yield in nasal secretions for BAV-3, PI-3V, and a bovine rhinovirus compared with the viral yield of calves not receiving vaccinal BHV-1 (10-12, 39).

The results of several studies suggest that bovine IFN may protect against infection or at least reduce the clinical severity of infection with certain viruses. In vivo, there may be other host defense mechanisms which afford protection besides or in combination with IFN. Thus, it would be advantageous to test the protective role of IFN in an in vitro system. In vitro systems permit screening of compounds for possible further in vivo testing, detection of optimal IFN concentrations required for antiviral activity, and determination of the relative sensitivity of various viruses to antiviral effects.

This study reports on antiviral effects of bovine IFN when

applied to bovine turbinate monolayer cultures which were challenged with bovine respiratory tract viruses.

MATERIALS AND METHODS

Cell cultures. Monolayer cultures used included: bovine turbinate (BT) (19), ovine fetal turbinate (18), bovine dermal (obtained from Cell Culture Laboratory, Naval Biomedical Research Laboratory, Oakland, Calif.), and bovine fetal kidney (BFK) (10). The growth and maintenance media have been previously described (19). Cultures were incubated at 37°C in a humidified incubator.

Viruses. Stock BHV-1 included: Cooper strain (18), Los Angeles strain (19), Colorado I vaccinal (23), nasal vaccinal strain-A (23, 53), nasal vaccinal strain-B (26), BRD 37 (21), BRD 38 (21), and (8) 81B-3101, isolated from nasal secretions of an adult bull with respiratory tract disease (Louisiana State University Veterinary Medical Teaching Hospital and Clinics, Baton Rouge). These viruses were grown and processed as described previously (19). BHV-1 were grown in BT cells and titrated by plaque assay in bovine dermal cultures (19). Titers were expressed as PFU/0.5 ml.

The other stock viruses included: BVDV, Singer strain (18); PI-3V, SF-4 strain (18); BRSV (375 strains) (35); goat respiratory syncytial virus (GRSV) (18); BAV-7 (18); and vesicular stomatitis virus, Indiana strain (VSV) (23). BVDV, PI-3V, and BAV-7 were grown in BT cells (18) and titrated in 96-well microtiter cell cultures (6, 18). BRSV and GRSV were grown in ovine fetal turbinate cells (18) and titrated in 96-well microtiter cell cultures (6, 18). Median tissue culture 50% infective doses (TCID₅₀) per 0.025 ml were determined by the Spearman-Kärber method (6). VSV was grown, processed, and titrated by plaque assay as described previously (19).

IFN. IFN used in this study included bluetongue virus serotype 10 (BTV) induced in Georgia bovine kidney (GBK) monolayers (20), BTV-10 induced in BFK monolayers (J. M. Cummins, Texas A & M Research Center, Amarillo), and three bovine nasal secretion IFN (A, B, and C; J. M. Cummins) (11). IFN assays were performed by a plaque reduction method in bovine dermal and BFK cells with VSV as the challenge virus (10, 19). IFN titers (units per milliliter) were expressed as the reciprocal of the dilution that reduced the number of VSV plaques by 50%. These samples were characterized as IFN by previously described methods (11, 20). Due to insufficient nasal secretion IFN concentrations,

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different lots of nasal secretion IFN were used in the present study for various experiments.

Yield reduction for antiviral activity. Preformed BT monolayers in 24-well tissue culture plates were treated with IFN at 37°C 18- to 24-h before viral challenge. IFN dilutions were made in maintenance medium plus 5% goat serum, and then 1.0 ml was added to each well. Virus controls (not receiving IFN) were included in each experiment. After the 18- to 24-h IFN pretreatment, wells were rinsed with minimal essential medium, and each respective virus was added in 0.5-ml amounts. The virus was adsorbed at 37°C for 1 h. After the 1-h adsorption period, wells received maintenance medium (1.0 ml) with 5% goat serum. In certain experiments, to determine the effects of IFN after viral challenge, IFN pretreatment medium (1.0) was returned to wells. Due to the low titers of available IFN preparations, there was not sufficient fresh IFN available for IFN posttreatment. For BHV-1 yield reduction studies, cultures and fluids were collected 24 h (for a single replication cycle) after viral challenge and frozen at -70°C until assayed for infectivity. For the other viruses, cultures and fluids were frozen when there was a 4+ cytopathic effect (CPE) (75% or greater CPE). Thus for these viruses there were multiple replication cycles. Viral infectivity was titrated in 96-well microtiter cell cultures (6). Ten-fold dilutions were made (10^{-1} was the lowest dilution tested), and four wells were used per dilution. Assays for virus controls and IFN treatment were performed concurrently for each virus in a particular experiment. All assays for viral infectivity were performed in ovine fetal turbinate cells, except those for BAV-7 which were performed in BT cells. Microtitrations were observed daily, and the final reading for CPE was made 7 days after inoculation. Viral yields were expressed as $\log_{10}$ TCID₅₀ per 0.025 ml. If CPE was not evident in any of the four wells or was evident in only one well of the 10^{-1} dilution, this was recorded as <1.00 of $\log_{10}$ TCID₅₀ per 0.025 ml.

RESULTS

In vitro sensitivity of BHV-1 strains, PI-3V, BRSV, GRSV, BVDV, BAV-7, and VSV to bovine IFN. BT cells incubated with IFN 18- to 24-h before challenge with 10^2 or 10^3 PFU of BHV-1 strains had yield reductions in the 24-h collection compared with cultures not receiving IFN (control cultures) (Table 1). In several instances there were lower levels of infectivity in cultures receiving IFN in the postchallenge media. All eight BHV-1 strains tested in this study were susceptible to the antiviral effects of IFN. BT cultures treated with bovine IFN before challenge with PI-3V, BRSV, GRSV, BVDV, BAV-7, or VSV had yield reductions compared with viral yields of control cultures (Tables 2 through 7).

DISCUSSION

There have been several previous studies indicating that various bovine viruses were susceptible to IFN. However, each of these studies usually examined antiviral activity against only one virus. In addition, IFN concentrations, sources of IFN, culture conditions, and infectivity assays may have differed between these studies. The results described for the present study indicate that exogenously applied bovine IFN samples have an antiviral effect in BT cells and also that several viruses are susceptible to the antiviral effects of these IFN. All viruses in this study were sensitive to IFN, including eight BHV-1 strains, PI-3V, BRSV, GRSV, BVDV, BAV-7, and VSV. VSV was utilized

as a control because of its sensitivity to various IFN. BHV-1, PI-3V, BRSV, BVDV, and BAV-7 have all been associated with bovine respiratory tract diseases.

In the present study there was, in almost all instances, less viral infectivity in virus-infected cultures treated with IFN than in control cultures. However, the method of assay for infectivity, $\log_{10}$ dilutions with four wells per dilution, may not have quantitated the significant differences, due to the potential error of 0.5 to 1.5 $\log_{10}$ for this assay. In the present study, each virus had individual assays in which there were 1.5 or greater $\log_{10}$ reductions. It is possible that these differences would have been better demonstrated if the plaque reduction method had been used. Also, the antiviral effects of IFN might have been more evident if, after IFN treatment and after viral challenge, collections had been made after single-replication cycles rather than after multiple-replication cycles.

In the present study, antiviral activity was evident for several days after IFN was removed, thus permitting multiple-replication cycles for these viruses. However, in other studies the antiviral state decayed rapidly in cell cultures after IFN removal (5, 33). Yet in another study, the antiviral state persisted for at least 72 h after removal of IFN (28). Several studies have shown that different viruses exhibit different sensitivities to IFN-induced resistance in the same cell when measured by single- and multiple-cycle assays (24, 51, 56). Hallum et al. (27) have shown that in the same cell the antiviral state is lost at different rates for different viruses. Similarly in the present study, there may have been varied responses due to the differences associated with the several viruses used.

There have been several reports of in vitro susceptibility in bovine cells to bovine IFN for BHV-1, PI-3V, BVDV, and a bovine rhinovirus. Antiviral activity against BHV-1 was demonstrated in BFK cultures by bovine serum IFN (47); BHV-1 induced IFN from bovine fetal spleen cultures, bovine peripheral blood leukocytes and bovine alveolar macrophages; and phytohemagglutinin induced peripheral blood leukocyte IFN (23). Supernatant fluids from BHV-1-infected bovine alveolar macrophages (centrifuged to remove BHV-1) interfered with BHV-1 infection of alveolar macrophage cultures (17). Bovine alveolar macrophage IFN reduced BHV-1 yield in tracheal explant cultures derived from adult cattle (22), and BHV-1 antigen-stimulated lymphocyte culture supernatant caused a transitory yield reduction in bovine fetal tracheal organ cultures (8). Bovine immune IFN reduced BHV-1 yields in GBK monolayers (4). PI-3V-induced BFK culture IFN had antiviral activity against PI-3V in BFK cultures (46), and PI-3V was inhibited by IFN induced by PI-3V in bovine embryonic kidney cells (54). BVDV was sensitive to the antiviral effects of Newcastle disease virus-induced calf kidney IFN (14). Calf kidney IFN protected calf tracheal organ cultures against a bovine rhinovirus (50).

Not all studies have indicated that PI-3V and BHV-1 were sensitive to IFN under the experimental conditions used (49, 54). PI-3V was inhibited by Newcastle disease virus- and influenza virus-induced IFN in chicken embryo fibroblast cells, but PI-3V was not inhibited by IFN produced in bovine embryonic kidney cells induced by BHV-1, Newcastle disease virus, or influenza virus (54). Also in that study, BHV-1 was not inhibited by IFN produced by bovine embryonic kidney or chicken embryo fibroblasts exposed to BHV-1, Newcastle disease virus, PI-3V, or influenza virus (54). In another study, nasal secretion IFN from calves vaccinated with a commercial intranasal BHV-1 vaccine was added to

bovine embryonic kidney cell cultures before challenge with various viruses (49). PI-3V, BVDV, and BAV-1 and -3 infections were inhibited or prevented, as indicated by the lack of viral CPE or hemadsorption, when the cultures were treated with IFN. Cell cultures pretreated with IFN and exposed to BHV-1 strains (Cooper and three local strains) and the bovine rhinovirus did not show inhibition of CPE for each virus. In that study, the amount of IFN used for pretreatment (units per volume of culture fluids), the amount of virus added to each culture, and the observation interval after viral challenge for viral CPE or hemadsorption were not indicated. It is possible that the difference in results of the above study and those presently reported may be explained by variations in the amount of IFN applied, the

amount of viral challenge, collection time, and the assay for viral activity (infectivity versus CPE or hemadsorption). It must also be pointed out that many studies mentioned above had not indicated the particular culture conditions and assays used.

Generally, herpesviruses have been considered to be less sensitive to IFN when compared with other viruses, including VSV (32). The results of the present study indicated that BHV-1 strains tested were sensitive to IFN when 100 to 400 U of IFN were used, 10^2 and 10^3 PFU were used as challenge virus, and collections were made at 24 h postchallenge. Examples of other herpesviruses affecting other animal hosts and sensitive to IFN in *in vitro* studies include: pseudorabies virus (11, 55), herpes simplex viruses (2, 13, 30, 31, 34, 36,

TABLE 1. Effect of bovine IFN on replication of BHV-1 strains

BHV-1 strain	IFN		Virus yield ^a after viral challenge ^b					
	Stock	IFN concn applied (U/ml)	10 ² PFU			10 ³ PFU		
			Virus control ^c	IFN pretreatment only ^d	IFN pre- and post-treatment ^e	Virus control ^c	IFN pretreatment only ^d	IFN pre- and post-treatment ^e
Cooper	BTV-BFK	100	2.75	1.50	<1.00	4.25	2.00	2.75
		200	4.25	1.50	<1.00	4.50	2.50	1.00
		400	4.25	1.00	<1.00	4.50	1.75	<1.00
	Nasal (C)	100	2.75	2.00	1.75	4.25	3.50	3.50
Los Angeles	BTV-BFK	100	3.25	1.50	2.00	3.75	2.25	1.75
		200	3.75	1.75	<1.00	5.25	2.50	1.00
		400	3.75	1.75	<1.00	5.25	3.00	<1.00
	Nasal (C)	100	3.25	2.50	2.25	3.75	2.50	2.50
BRD 37	BTV-BFK	100	3.00	1.25	1.25	4.25	2.25	2.00
		200	3.25	1.75	<1.00	4.75	2.25	1.50
		400	3.25	1.50	<1.00	4.75	2.50	1.25
	Nasal (C)	100	3.00	1.75	2.25	4.25	2.50	2.75
BRD 38	BTV-BFK	100	3.50	1.25	<1.00	4.50	1.75	2.75
		200	5.25	3.25	2.25	4.00	3.00	1.75
		400	5.25	2.75	2.50	4.00	2.25	1.25
	Nasal (C)	100	3.50	2.25	2.25	4.50	3.25	4.00
81B-3101	BTV-BFK	100	1.75	<1.00	<1.00	3.50	1.50	1.50
		200	4.75	2.50	1.75	4.00	1.75	1.00
		400	4.75	1.50	1.25	4.00	1.25	1.00
	Nasal (C)	100	1.75	1.75	<1.00	3.50	2.50	2.50
CVS ^f	BTV-BFK	100	2.75	<1.00	<1.00	4.50	1.75	1.75
		200	4.00	1.25	<1.00	5.25	3.00	1.00
		400	4.00	<1.00	<1.00	5.25	1.75	<1.00
	Nasal (C)	100	2.75	2.00	1.75	4.50	3.75	2.75
Nasal vaccine (A)	BTV-BFK	100	2.50	<1.00	1.00	4.50	2.75	2.50
		200	3.75	1.25	<1.00	4.75	2.50	1.25
		400	3.75	<1.00	<1.00	4.75	1.50	1.00
	Nasal (C)	100	2.50	1.50	1.00	4.50	3.00	3.00
Nasal vaccine (B)	BTV-BFK	100	2.50	1.00	<1.00	3.50	1.25	1.25
		200	1.25	1.00	<1.00	3.50	1.00	<1.00
		400	1.25	<1.00	<1.00	3.50	<1.00	<1.00
	Nasal (C)	100	2.50	2.00	<1.00	3.50	3.00	1.75

^a Yield is expressed as log₁₀ TCID₅₀ per 0.025 ml of culture fluids. All culture fluids were from 24 h viral postchallenge collections.

^b Virus challenge per 0.5-ml culture well.

^c Cultures did not receive IFN.

^d BT cultures were treated with IFN 18 to 24 h before viral challenge. After virus adsorption, 1 ml of maintenance medium with 5% goat serum was added to each well.

^e Treatment was the same as that described in *d* except the IFN-containing medium in the pretreatment wells was returned to the wells for posttreatment.

^f CVS, Colorado I vaccinal.

TABLE 2. Effect of bovine IFN on replication of PI-3V

Expt ^a (postchallenge collection day)	IFN		Virus yield ^b	
	Stock	Concn applied (U/ml)	Virus control	IFN treated
I (4)	BTV-GBK	100 ^c	4.25	<1.00
	Nasal (A)	100 ^c	4.25	1.00
II (7)	BTV-GBK	100 ^d	4.50	<1.00
	Nasal (B)	100 ^d	4.50	<1.00
III (6)	BTV-BFK	100 ^c	4.25	<1.00
		100 ^d	4.25	<1.00
	Nasal (C)	100 ^c	4.25	1.00
		100 ^d	4.25	<1.00
IV (5)	BTV-BFK	200 ^c	4.50	<1.00
		200 ^d	4.50	<1.00
		400 ^c	4.50	<1.00
		400 ^d	4.50	<1.00

^a Viral challenge was 0.5 ml containing $10^{3.5}$ TCID₅₀ per 0.25 ml.

^b Yield is expressed as log₁₀ TCID₅₀ per 0.025 ml of culture fluids.

^c Cultures were treated with IFN 18 to 24 h before viral challenge. After virus adsorption, 1 ml of maintenance medium with 5% goat serum was added to each well.

^d Treatment was the same as that described in ^c except the IFN-containing medium in the pretreatment wells was returned to the wells for posttreatment.

38, 41, 43, 45), herpesvirus platyrrhine (13), herpesvirus saimiri (13), herpesvirus ateles (13), varicella zoster virus (3, 38, 41), Medical Lake macaque herpesvirus (41), and cytomegaloviruses (38, 42). Another bovid herpesvirus, bovine mammillitis virus (BHV-2), was susceptible to BHV-2-induced bovine IFN (37).

There does not appear to be strain variation of BHV-1 for

TABLE 3. Effect of bovine IFN on replication of BRSV

Expt ^a	IFN		Virus yield ^b	
	Stock	Concn applied (U/ml)	Virus control	IFN treated
I	BTV-GBK	100 ^c	3.75	<1.00
	Nasal (A)	100 ^d	3.75	3.00
II	BTV-GBK	100 ^d	4.50	<1.00
	Nasal (B)	100 ^d	4.50	<1.00
III	BTV-BFK	100 ^c	3.75	<1.00
		100 ^d	3.75	<1.00
	Nasal (C)	100 ^c	3.75	2.00
		100 ^d	3.75	1.00
IV	BTV-BFK	200 ^c	4.25	<1.00
		200 ^d	4.25	1.00
		400 ^c	4.25	1.00
		400 ^d	4.25	1.00

^a Yields were determined on postchallenge collection day 3. Viral challenge was 0.5 ml containing 10^3 TCID₅₀ per 0.025 ml.

^b Yield is expressed as log₁₀ TCID₅₀ per 0.025 ml of culture fluids.

^c Cultures were treated with IFN 18 to 24 h before viral challenge. After virus adsorption, 1 ml of maintenance medium with 5% goat serum was added to each well.

^d Treatment was the same as that described in ^c except the IFN-containing medium in the pretreatment wells was returned to the wells for posttreatment.

TABLE 4. Effect of bovine IFN on replication of GRSV

Expt ^a	IFN		Virus yield ^b	
	Stock	Concn applied (U/ml)	Virus control	IFN treated
I	BTV-GBK	100 ^c	2.00	<1.00
	Nasal (A)	100 ^d	2.00	<1.00
II	BTV-GBK	100 ^c	2.75	<1.00
		100 ^d	2.75	<1.00
III	Nasal (C)	100 ^c	2.75	1.50
		100 ^d	2.75	<1.00
IV	BTV-BFK	200 ^c	2.25	<1.00
		200 ^d	2.25	<1.00
		400 ^c	2.25	<1.00
		400 ^d	2.25	<1.00

^a Yields were determined on postchallenge collection day 4. Viral challenge was 0.5 ml containing 10^3 TCID₅₀ per 0.025 ml.

^b Yield is expressed as log₁₀ TCID₅₀ per 0.025 ml of culture fluids.

^c Cultures were treated with IFN 18 to 24 h before viral challenge. After virus adsorption, 1 ml of maintenance medium with 5% goat serum was added to each well.

^d Treatment was the same as that described in ^c except the IFN-containing medium in the pretreatment wells was returned to the wells for posttreatment.

IFN sensitivity as indicated in this study. Challenge viruses (Cooper and Los Angeles strains), isolates from healthy calves (BRD 37 and 38), an isolate from a clinically ill bovine (81B-3101), and three vaccinal strains (Colorado I vaccinal and nasal vaccinal A and B) were sensitive to the bovine IFN used in this study. Ten isolates of herpes simplex virus 1 and nine isolates of herpes simplex virus 2 were sensitive to three human IFN preparations (43); the clinical isolates did not appear to differ in sensitivity from that of the laboratory strains.

TABLE 5. Effect of bovine IFN on replication of BVDV

Expt ^a	IFN		Virus yield ^b	
	Stock	Concn applied (U/ml)	Virus control	IFN treated
I	BTV-GBK	100 ^c	3.25	<1.00
	Nasal (A)	100 ^c	3.25	2.75
II	BTV-BFK	100 ^d	4.25	<1.00
		100 ^d	4.25	3.50
III	BTV-BFK	200 ^c	4.25	3.75
		200 ^d	4.25	<1.00
		400 ^c	4.25	3.50
		400 ^d	4.25	<1.00

^a Yields were determined on postchallenge collection day 4. Viral challenge for each experiment was: I, 0.5 ml containing $10^{1.0}$ TCID₅₀ per 0.025 ml; II, 0.5 ml containing $10^{2.3}$ TCID₅₀ per 0.025 ml; and III, 0.5 ml containing $10^{3.6}$ TCID₅₀ per 0.025 ml.

^b Yield is expressed as log₁₀ TCID₅₀ per 0.025 ml of culture fluids.

^c Cultures were treated with IFN 18 to 24 h before viral challenge. After virus adsorption, 1 ml of maintenance medium with 5% goat serum was added to each well.

^d Treatment was the same as that described in ^c except the IFN-containing medium in the pretreatment wells was returned to the wells for posttreatment.

TABLE 6. Effect of bovine IFN on replication of BAV-7

Expt ^a (postchallenge collection day)	IFN		Virus yield ^b	
	Stock	Concn applied (U/ml)	Virus control	IFN treated
I (7)	BTV-GBK	100 ^c	5.50	2.75
	Nasal (B)	100 ^c	5.50	3.50
II (6)	BTV-BFK	100 ^c	3.75	1.25
		100 ^d	3.75	1.50
		100 ^c	3.75	1.50
		100 ^d	3.75	<1.00
III (6)	BTV-BFK	200 ^c	3.50	2.50
		200 ^d	3.50	1.50
		400 ^c	3.50	2.50
		400 ^d	3.50	<1.00

^a Viral challenge was 0.5 ml containing 10^{2.50} TCID₅₀ per 0.025 ml.

^b Yield is expressed as log₁₀ TCID₅₀ per 0.025 ml of culture fluids.

^c Cultures were treated with IFN 18 to 24 h before viral challenge. After virus adsorption, 1 ml of maintenance medium with 5% goat serum was added to each well.

^d Treatment was the same as that described in *c* except the IFN-containing medium in the pretreatment wells was returned to the wells for posttreatment.

Human respiratory syncytial virus, related antigenically to BRSV, has been reported to be sensitive to human IFN (7, 9, 25, 40) and polyinosinic acid-polycytidylic acid (29). In one study, differences in the antiviral activity of IFN produced by human macrophages exposed to different viruses, influenza or respiratory syncytial virus, were demonstrated (7). In that study, respiratory syncytial virus was more sensitive to respiratory syncytial virus-induced IFN activity than to influenza virus-induced IFN activity. In the present study,

TABLE 7. Effect of bovine IFN on replication of VSV

Expt ^a (postchallenge collection day)	IFN		Virus yield ^b	
	Stock	Concn applied (U/ml)	Virus control	IFN treated
I (1)	BTV-GBK	100 ^c	5.75	2.25
	Nasal (A)	100 ^c	5.75	2.50
II (2)	BTV-GBK	100 ^c	4.75	1.50
		100 ^d	4.50	<1.00
		100 ^c	4.50	<1.00
		100 ^d	4.50	<1.00
III (1)	BTV-BFK	100 ^c	5.25	3.5
	Nasal (C)	100 ^c	5.25	3.5
IV (1)	BTV-BFK	200 ^c	3.25	1.00
		400 ^c	3.25	1.25

^a Viral challenge for each experiment was: I, 0.5 ml containing 10⁶ PFU per 0.5 ml; II, III, and IV, 10^{4.8} PFU per 0.5 ml.

^b Yield is expressed as log₁₀ TCID₅₀ per 0.025 ml of culture fluids.

^c Cultures were treated with IFN 18 to 24 h before viral challenge. After virus adsorption, 1 ml of maintenance medium with 5% goat serum was added to each well.

^d Treatment was the same as that described in *c* except the IFN-containing medium in the pretreatment wells was returned to the wells for posttreatment.

BRSV and an antigenically related GRSV were sensitive to bovine IFN.

It is possible that in future studies, other culture conditions may permit greater yield reduction of various viruses. These conditions might include: (i) greater concentrations of one type of IFN; (ii) variations in concentration of challenge viruses; (iii) different types of characterized alpha, beta, and gamma IFN; (iv) potentiation of antiviral activity by mixed preparations of alpha, beta, or gamma IFN (16); and (v) presence of fresh IFN in the postchallenge medium.

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