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Interferon in Nasal Secretions and Sera of Calves After Intranasal Administration of Avirulent Infectious Bovine Rhinotracheitis Virus: Association of Interferon in Nasal Secretions with Early Resistance to Challenge with Virulent Virus

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Calves which had received avirulent infectious bovine rhinotracheitis virus (AV-IBR) by intranasal (IN) administration developed detectable levels of interferon (IF) in nasal secretions as early as 40 hr later. Peak titers (1:640) of IF appeared in secretions 72 to 96 hr after administration of virus, and titers of 1:80 to 1:320 were maintained through the 8th day. Lower titers (1:5 to 1:10) of IF were detected in sera obtained on the 4th to 8th days after administration of virus. Peak titers of IF in respiratory tract secretions were accompanied by a 100- to > 1,000-fold reduction in the levels of AV-IBR present in the secretions. Serum antibody was not detected prior to the 8th day after administration of AV-IBR. Calves which received AV-IBR by the IN route 72 or 96 hr earlier were refractory to challenge with virulent infectious bovine rhinotracheitis virus (IBR), whereas calves receiving AV-IBR 18 or 40 hr earlier became clinically ill following challenge. The temporal association between appearance of IF in respiratory tract secretions and onset of protection against challenge suggests a cause and effect relationship. No IF was detected in either nasal secretions or sera of calves receiving modified IBR virus by intramuscular injection. Following subsequent IN challenge of these calves, IF was detected in nasal secretions as early as 24 hr postchallenge and was maintained at titers of 1:40 to 1:80 for approximately 4 days, even in the absence of virus recovery. Greater ease of local IF induction with IBR virus in calves previously sensitized with that virus is suggested.

The elaboration of interferon (IF) by cells in response to virus infection is considered a major factor of the host defense mechanism, particularly during the course of primary infections. Studies related to induction, synthesis, properties, and mechanism of action of IF have been reviewed (3, 4, 11, 21, 35, 37). Studies on the in vitro induction of IF by calf kidney cells infected with several different viruses and the in vivo induction of circulating IF in the bovine by viral and nonviral inducers have been reported (9, 19, 29-32, 34). These studies have not provided information on the role of IF in either preventing or influencing the outcome of viral infections in the bovine.

Investigations were undertaken in our laboratory to evaluate factors involved in early onset of protection against infectious bovine rhinotracheitis virus (IBR) challenge following intranasal (IN) administration of an avirulent strain of IBR (AV-IBR). It was discovered that high levels of IF developed in nasal secretions after AV-IBR administration and that the time of first appearance coincided with the time resistance to challenge with virulent virus became evident. Subsequent studies were conducted to evaluate the relationships between virus and IF levels in nasal secretions and sera of calves after IN or intramuscular

(IM) administration of modified strains of IBR and subsequent to challenge.

MATERIALS AND METHODS

Experimental animals. Angus-Hereford cross-bred calves 6 to 10 months of age and devoid of serum neutralizing (SN) antibody against IBR were obtained from a single source. They were maintained in isolation for 3 weeks and were then placed as experimental groups into Rockefeller-type isolation units at least 7 days preceeding exposure.

Virus. The AV-IBR used for IN administration had been developed by 54 serial passages in monolayer cultures of bovine kidney (BK) cells followed by 18 serial passages in monolayer cultures of rabbit kidney cells. This material was administered by IN instillation as a 2.0 ml dose containing a $\log_{10}$ 5.7 to 6.2 median tissue culture infective dose ($TCID_{50}$).

Virulent challenge IBR (C-IBR) was the Cooper strain which had been passaged 12 times in BK cell cultures. It was administered by IN instillation in a 2.0-ml volume containing $\log_{10}$ 6.0 to 6.4 $TCID_{50}$.

The modified strain of IBR used for IM inoculation of calves in experiment B had been passaged 54 times in BK cell cultures and was administered as a 2.0-ml dose containing $\log_{10}$ 5.7 $TCID_{50}$.

Collection and processing of serum. Blood obtained by jugular venipuncture was allowed to clot for 1 hr at 25 C and to stand overnight at 4 C prior to removal of serum. Samples to be used for assay of SN antibody were heat inactivated at 56 C for 30 min. All sera were stored at −20 C until tested.

Collection and processing of nasal secretions. Nasal secretions for IF or antibody evaluation, or both, and for quantitation of virus were obtained by use of tampons inserted carefully into the ventral meatus of the nasal passage where they were allowed to remain for approximately 1 hr. Secretions were immediately expressed into sterile tubes, clarified by centrifugation, and frozen at −60 C until tested. Sediments remaining after centrifugation were tested for presence of blood with Hematest (Ames Company, Elkhart, Ind.) tablets. Samples used for antibody assay were first heat-inactivated. Nasal secretions used for quantitative assay of virus content were inoculated into BK tubes within 1 hr of collection by procedures described below.

Virus isolation procedures. Specimens of nasal secretions for routine virus isolation procedures were obtained with cotton swabs which were immediately submerged and expressed into 3.0 ml of Hanks balanced salt solution (BSS) supplemented with 0.5% gelatin and containing 1,000 units of penicillin, 500 μg of streptomycin and 10 μg of amphotericin B per ml. Each of five BK tubes per specimen was inoculated with 0.2 ml of inoculum and incubated at 36 C. If no cytopathic effect (CPE) was observed within 7 days, specimens were subcultured into five additional BK tubes and incubated for 7 days. All pre-exposure nasal specimens were additionally tested for the presence of parainfluenza type 3 virus by means of the hemadsorption test (33) applied on the 7th day of subculture, and for noncytopathic bovine virus diarrhea virus by means of the viral interference test applied to a second set of inoculated BK tubes on the 3rd day of subculture (15).

For determining virus titers in nasal secretions after exposure and challenge in experiment B, serial 10-fold dilutions were prepared in Earle's BSS. Each of three BK tubes were inoculated with 0.2 ml of each dilution and incubated at 36 C for 5 days before final readings were made. End points ($TCID_{50}$) were calculated by the Reed-Muench method (28).

Antibody determinations. Sera and nasal secretions were assayed for IBR neutralizing antibody by standard SN procedures, testing twofold dilutions of serum or secretion against a constant amount (100 to 300 $TCID_{50}$) of virus. Each of three BK tubes was inoculated with 0.2 ml of serum (or secretion)–virus mixture at each dilution and was incubated for 5 days at 36 C before final readings were made. Median neutralization end points of final dilutions were calculated by the Karber method (23). Sera obtained on early days (4 to 10) after initial exposure of calves in experiment A were tested also by the neutralization-index and plaque reduction techniques in an additional effort to detect low levels of antibody.

Interferon assay. Sera and nasal secretions from experiment A calves were tested for IF activity on secondary BK cell monolayers in tissue culture microplates (Linbro Chemical Co., New Haven, Conn.) incubated in an atmosphere of 5% CO_2. Growth medium for BK cells was Eagle basal medium (BME) supplemented with 10% fetal bovine serum. To inactivate IBR present in nasal secretions, these were diluted 1:5 in BME, the *p*H of which had been lowered to 1.8 with 10 N HCl. The resulting mixture had a *p*H of 2.0 to 2.3 and was held at 4 C for 24 hr. The *p*H of this mixture was then raised to 7.2 to 7.4 by diluting 1:2 with BME to which 1 N NaOH had been added to elevate the *p*H to 9.0 to 9.2. Serial twofold dilutions of test material were prepared in BME supplemented with 2% fetal bovine serum, and 0.2 ml of each dilution was added to each of four microplate wells containing BK monolayers. Plates were incubated overnight at 36 C, after which time test material was removed by aspiration, and monolayers were washed twice with Earle's BSS. Cultures were challenged with 100 to 500 $TCID_{50}$ of vesicular stomatitis virus (VSV) contained in 0.2 ml of BME with 2% fetal bovine serum. Experiment B assays were conducted in tube cultures of BK cells, employing two tubes per twofold dilution since duplicate testing had shown this method to yield more reproducible end points than the microplate system. For this procedure, 1.0-ml volumes of test material or VSV challenge material were used. Otherwise the same procedures described above were followed. Tests were read when CPE of VSV control wells or tubes was complete (24–36 hr). End points were read as the highest dilution of test material providing at least 80% inhibition of CPE.

Leukocyte and differential counts. Blood specimens for leukocyte (WBC) and differential determinations were collected in ethylenediaminetetraacetic acid vacuum tubes (Becton-Dickinson, Rutherford, N.J.). Two WBC counts were made on each sample, and results were averaged. Thin blood smears were prepared within 2 hr after the collection of blood. These were rapidly

air-dried and were then stored for subsequent staining and differential counting.

Experimental designs and procedures: experiment A. It had been previously determined that calves which had been exposed to AV-IBR by the IN route were resistant to IN challenge with C-IBR administered 21 to 28 days later. Experiment A was designed to determine how soon, after IN administration of AV-IBR, resistance to challenge could be detected and to evaluate factors involved in early protection should such be observed.

Twenty-eight calves were placed in seven isolated groups of four, numbered I to VII. Group I calves served as negative controls, and group VII served as challenge controls, calves in the latter group receiving C-IBR by the IN route on day 0. Groups II to VI were given AV-IBR by the IN route (hereafter referred to as vaccination) on day 0. Calves in groups III to VI were challenged with C-IBR at 96, 72, 40, and 18 hr, respectively, following vaccination, whereas calves in group II served as vaccinate controls.

Calves were examined for clinical signs of illness, body temperatures were recorded, and WBC determinations were made twice daily for 5 days preceding day 0 and for 14 days following. Nasal swab specimens for virus isolation studies were collected from all calves at the time they were first placed in isolation, on the day preceeding day 0, and daily thereafter for 16 days.

Sera and nasal secretions for IF evaluation were obtained on day 0 (pretest) and at 18, 40, 72, 96, 120, and 144 hr postvaccination (or challenge) from calves in groups II (vaccinate controls) and VII (challenge controls). Sera for antibody evaluations were obtained from all 28 calves on days 0, 4, 5, 6, 8, 10, 14, and 21. Blood smears for calves in groups II and VII were prepared daily for 5 days before and 14 days after day 0.

Experiment B. A second study was conducted (i) to compare IF titers of serum and nasal secretions with levels of virus present in the nasal secretions at various intervals following IN exposure of calves to AV-IBR, (ii) to compare responses of calves vaccinated IN with those of calves vaccinated IM, and (iii) to evaluate responses in both groups of calves after subsequent IBR challenge.

Four IBR-susceptible calves were placed in two isolated groups designated A and B. Calves in group A received AV-IBR by the IN route on day 0, whereas the two calves in group B were given BK-passage IBR by IM injection. All four calves were challenged with C-IBR 19 days later. Nasal swab specimens for virus isolation attempts along with sera and nasal secretions for antibody and IF assays were obtained prior to vaccination. Sera and nasal secretions for IF, antibody or virus assays were obtained, and body temperatures were recorded every 12 hr for the first 4 days postvaccination (PV), and then at 24-hr intervals through day 14. WBC determinations were made daily throughout the period. After challenge with C-IBR on day 19, the above procedures were conducted at 24-hr intervals for 10 days, followed by collection of sera and nasal secretions on day 14.

Nasal secretions obtained at 24-hr intervals after each exposure and whole-blood samples taken on days 6 and 7 PV were assayed for virus content. All sera and secretions obtained on days 0 to 14 PV and on days 0 to 10 postchallenge (PC) were assayed for IF activity. Antibody assays were conducted on selected samples as indicated under results.

RESULTS

Experiment A. No clinical signs of disease were observed during the pretest period. No virus isolations were made from nasal specimens obtained at the time of grouping or on the day preceeding day 0.

The average body temperature for all animals for 4 days preceeding day 0 was 101.7 F (ca. 38.7 C). Group I (negative control) calves maintained this average throughout the 14-day test period. Daily mean body temperature patterns for all calves in each of groups II to IV were similar but were markedly different from patterns of calves in groups V to VII (Fig. 1). Mean days of fever (> 102.7 F; ca. 39.3 C) per calf for groups II through VII were 1.25, 2.0, 0.75, 4.25, 6.0, and 6.25, respectively, revealing a pronounced difference between calves either not challenged or chal-

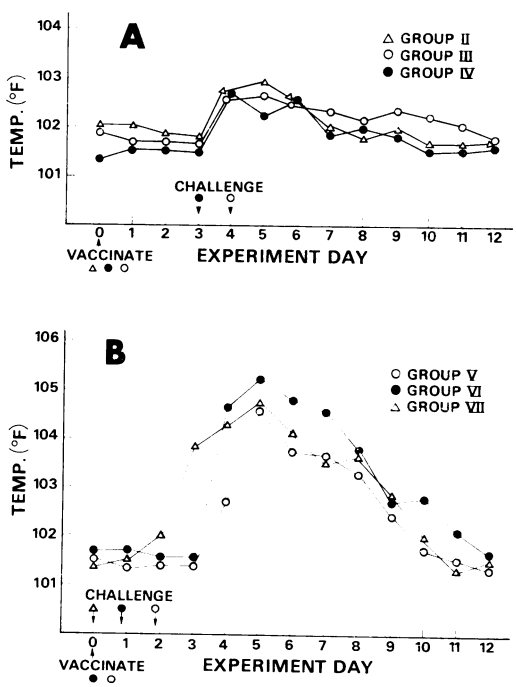

FIG. 1. *Mean temperature responses of groups of calves after AV-IBR intranasal vaccination or C-IBR challenge, or both. (A) Group II: AV-IBR day 0, not challenged; group III: AV-IBR day 0, challenged 96 hr later; group IV: AV-IBR day 0, challenged 72 hr later. (B) Group V: AV-IBR day 0, challenged 40 hr later; group VI: AV-IBR day 0, challenged 18 hr later; group VII: no AV-IBR, challenged day 0.*

lenged at 72 or 96 hr PV (groups II to IV) and those challenged at 0, 18, 40 hr PV (groups V to VII).

Changes in WBC levels were not dramatic; however, counts were rather uniformly suppressed on days 5 to 12 in calves which were challenged. Differential counts of calves in groups II and VII were essentially unchanged throughout the period.

No clinical signs of illness were observed in any of the calves in groups I and II. A slight increase in serous nasal discharge was noted on days 2 to 6 in animals of groups II to IV. Calves in groups III and IV remained bright and alert, although two calves in each group developed mild hyperpnea for 2 to 3 days.

In contrast, all calves in groups V, VI, and VII developed pronounced clinical signs of IBR, including high fever, moderate to severe depression, profuse mucous to mucopurulent nasal discharge, inflammation and erosion of the nasal mucosa and external nares, lacrimation, and dyspnea. These signs began to appear on the 3rd day after challenge for each group. Calves in group V had an abbreviated illness, with an average of 2 fewer febrile days than challenge controls.

TABLE 1. *Recovery of IBR virus from nasal secretions*[a]

Group no.	AV-IBR (day 0)[b]	Time (hr) of challenge (Post-day 0)	Duration (days) of IBR virus recovery (range)	Mean days of virus recovery per calf
I	No	Not challenged	0	0
II	Yes	Not challenged	11	11.0
III	Yes	96	11–13	12.5
IV	Yes	72	12–13	12.25
V	Yes	40	12–14	12.75
VI	Yes	18	12–15	13.25
VII	No	Challenged day 0	12–14	13.25

[a] Abbreviations: IBR, infectious bovine rhinotracheitis; AV-IBR, avirulent strain of IBR virus.
[b] Yes or no indicate whether or not AV-IBR was administered on day 0.

Virus recovery data are summarized in Table 1. No virus isolations were made from calves in group I. IBR was recovered from each of the remaining 24 calves for at least 11 days. The mean days of virus recovery for groups II to VII ranged from 11.0 to 13.25.

Results of antibody evaluations are summarized in Table 2. No SN antibody to IBR was detected prior to day 8, irrespective of the assay method employed. Low levels of antibody were present in sera from four of the 24 exposed calves on day 8 and in sera of all exposed calves on day 10. Titers were at maximal values by day 14. Patterns and magnitude of antibody responses were similar in all exposed groups. No IBR antibody could be detected in sera obtained on days 14 and 21 from negative control calves.

Low titers (1:10 to 1:20) of IF were detected in nasal secretions obtained at 40 hr from three of the calves in groups II and VII. Secretions obtained at 72 hr from seven calves had IF titers of 1:80 to 1:160. Maximal titers (1:320 to 1:640) were present in secretions obtained at 96 hr and were maintained through 144 hr. Low levels (1:5 to 1:20) of serum IF were detected in one of the eight calves at 40 hr, in six of eight calves at 72 hr, and in all calves at 96, 120, and 144 hr.

Nasal secretions obtained from one calf at 40 through 144 hr were pooled for IF characterization studies. The viral inhibitory titer of the pool was found to be 1:160/0.2 ml. This activity was retained following dialysis against KCl-HCl buffer at *p*H 2.0 (48 hr at 4 C), was inactivated by pepsin

TABLE 2. *Serum-neutralizing antibody responses of calves*[a]

Group no.	AV-IBR[b] (day 0)	Time (hr) of challenge (post-day 0)	No. of calves with antibody on post-0 day and geometric mean titer for each group[c]									
			0		6		8		10		14	
I	No	Not challenged	0/4	0	0/4	0	0/4	0	0/4	0	0/4	0
II	Yes	Not challenged	0/4	0	0/4	0	1/4	<1	4/4	3	4/4	36
III	Yes	96	0/4	0	0/4	0	0/4	0	4/4	2	4/4	28
IV	Yes	72	0/4	0	0/4	0	0/4	0	4/4	4	4/4	36
V	Yes	40	0/4	0	0/4	0	0/4	0	4/4	3	4/4	58
VI	Yes	18	0/4	0	0/4	0	0/4	0	4/4	3	4/4	44
VII	No	Challenged day 0	0/4	0	0/4	0	3/4	<2	4/4	6	4/4	28

[a] Abbreviations: IBR, infectious bovine rhinotracheitis; AV-IBR, avirulent strain of IBR virus.
[b] Yes or no indicate whether or not AV-IBR was administered on day 0.
[c] Number of calves with antibody/number of calves challenged. Titers expressed as reciprocals of 50% end point of final dilutions.

(0.25% at *p*H 2.1, 24 hr at 37 C) but not by ribonuclease (10 μg/ml, 20 hr at 37 C), was inhibited by simultaneous treatment of BK cell cultures with actinomycin D (0.5 μg/ml), was inhibitory to VSV on BK cells but not on RK-13 cells, and had no direct antiviral effect when incubated with VSV.

Because nasal secretions which possessed IF-like activity also contained *p*H-inactivated IBR, the possibility existed that this virus was inducing de novo synthesis of interferon in the BK cells during the overnight incubation period. To explore this possibility, $\log_{10}$ 5.7 $TCID_{50}$/ml of AV-IBR was added to prevaccination nasal secretions which had shown no virus-inhibiting activity. This mixture was subjected to *p*H 2.2 for 24 hr at 4 C as described above, resulting in complete inactivation of virus. After restoration of the *p*H to 7.2, the material was tested for virus-inhibiting activity. No inhibition of VSV was observed, indicating that inactivated IBR present in secretions did not induce interferon synthesis in the BK cell cultures.

Experiment B. No clinical signs of illness were observed in any of the four calves either PV or PC. Slight elevations in temperature were noted PV in group A calves on days 4 to 6 and in group B calves on days 2 to 4. No elevations occurred PC. Variations in WBC levels were not significant.

Levels of virus present in secretions, levels of IF detected in sera and secretions, and levels of SN antibody detected during the PV and PC periods are shown in Fig. 2. IBR was present in secretions of group A calves for 8 to 9 days PV, maximal titers (> $10^{6.4}$ $TCID_{50}$/ml) being reached on day 3. Virus was not recovered from whole-blood specimens obtained on PV days 6 and 7. Moderate titers (1:80 to 1:160) of IF in secretions were detected at 60 and 72 hr PV, with peak titers of 1:320 to 1:640 having developed at 96 hr. Titers of 1:160 to 1:320 were maintained through day 7, titers of 1:80 to 1:160 were present on day 8, and no detectable IF activity was detected in secretions on day 9. IF was present at titers of 1:5 to 1:10 in sera from both calves on PV days 4 to 8. Serum antibody was present in one calf on day 8 and in both calves on day 10, reaching a mean titer of 1:36 by day 19. Antibody (1:2 to 1:8) was present in nasal secretions from both of these calves on PV day 14. After challenge, no virus was recovered from secretions and no IF was detected in either secretions or sera. There was a gradual increase of serum antibody titer PC, the mean titer on PC day 14 being 1:125.

No virus was recovered from nasal secretions of group B calves during the 14-day PV period nor from whole-blood specimens obtained on PV days 6 and 7. Likewise, no IF was detected in either secretions of sera and no antibody was detected in secretions taken on PV day 14. Serum antibody was first detected in both of these calves on PV day 8, having reached a mean titer of 1:12 by day 19. After challenge, IBR was recovered (days 3 to 5) from secretions of only one of these two calves. IF was present, however, in secretions of both calves following challenge, being first detected 24 hr PC, reaching peak titers (1:80) on days 3 and 4, and becoming subdetectable by day 6. No serum IF was detected PC. Levels of serum antibody in both calves increased abruptly between PC days 6 and 7, reaching a mean titer of 1:145 on day 7. Antibody (1:8) was detected in nasal secretions of one of these two calves (the same calf from which virus was recovered PC) on PC day 14.

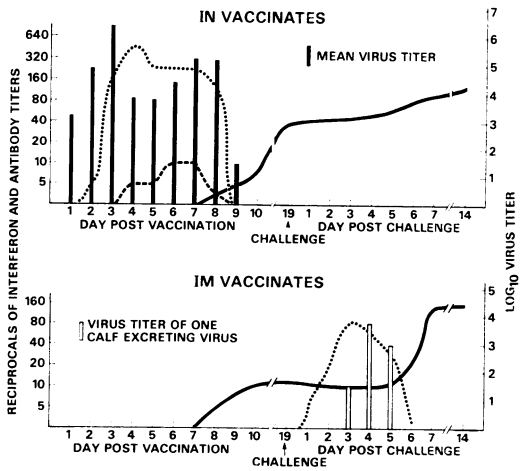

FIG. 2. *Mean titer of interferon in serum* (——) *and nasal secretions* (· · ·), *mean titers of serum-neutralizing antibody* (—), *and titers of IBR in nasal secretions after intranasal (IN) or intramuscular (IM) vaccination of calves, and subsequent to challenge with virulent IBR.*

DISCUSSION

Calves vaccinated IN with AV-IBR 72 or 96 hr prior to challenge with virulent IBR virus did not develop clinical disease, whereas administration of AV-IBR to calves 18 or 40 hr prior to challenge had little observable effect in reducing the severity of illness. The development of resistance to challenge at some point between 40 and 72 hr was associated temporally with the appearance of appreciable levels of IF in nasal secretions and is suggestive of a cause and effect relationship. This suggestion was strengthened by the correlation, observed in the second study, between the development of peak titers of IF in secretions and the significant reduction in amounts of virus present

in secretions, indicating suppression of vaccine virus replication. Such IF-mediated suppression would be expected also to prevent or retard establishment of infection by challenge IBR virus administered at that time. A similar temporal relationship between the experimental induction of circulating IF and protection against Theiler's virus challenge in mice has been reported (2). It was concluded that the observed protection resulted from suppression of virus replication in the target organ. It has also been observed that mice challenged intracerebrally with herpes simplex virus (HSV) after subcutaneous inoculation of HSV manifested a biphasic, early (days 0–6) and late (day 21) resistance to challenge (6). The early resistance was shown to be nonspecific since significant protection against encephalomyocarditis virus challenge was also observed, whereas late protection was afforded against only HSV.

Factors other than IF which might have been responsible in the present studies for the early resistance to challenge include the early production of virus-specific antibody and cellular immunity. It seems quite unlikely that production of specific antibody was responsible for the early protection observed. The appearance of antibody at 40 to 72 hr would have been expected to result in cessation of virus excretion within a relatively short time, assuming that a constantly increasing amount of antibody would be synthesized following first appearance. Cessation of virus excretion did not occur, however, in any of the calves in experiment A until after the 11th day. At that time there was indeed a temporal association between the first appearance of detectable antibody (days 8 to 10) and cessation of virus excretion (days 12 to 14). This association was also evident in group A calves of experiment B. The protective effect of antibody was not evident, therefore, until about the 9th day post vaccination. Since cell-mediated immunity was not evaluated in these studies, the role of this factor in conferring early protection cannot be determined.

The results of experiment B revealed that extensive replication of IBR was required before detectable levels of IF appeared in respiratory tract secretions. The data indicate also that continued replication of virus, probably within newly infected cells, promoted continued synthesis of IF which in turn exerted a suppressive effect on virus replication. Studies conducted subsequent to those reported here have shown that levels of IF in secretions may persist as long as 14 days in calves which continue to excrete IBR for that period of time. Similar relationships between virus replication and IF production have been reported from studies with influenza virus in chick embryos and with Eastern encephalitis virus in chick embryo and mouse L cells in vitro (36).

IF detected in secretions of IN vaccinates was undoubtedly produced by cells of the respiratory tract since titers in secretions were over 100 times higher than those present in sera. Such concentration of IF at principal sites of virus replication has been reported for several viruses, including herpes simplex, varicella, vaccinia, influenza, and arboviruses (1, 10, 12, 20). The low levels of IF detected in sera may have originated from the respiratory tract or may have been produced by cells of the reticuloendothelial system as the result of low level viremia (13, 24). It has been previously reported that moderate levels of serum IF were produced and maintained for several days after intravenous (IV) or intravenous-intranasal (IV/IN) administration of IBR virus in large doses (31). Those data, in light of results reported here, indicate that the first appearance of serum IF probably resulted from induction by virus in the IV inoculum, whereas the continued presence of IF in the serum for several days may have been consequent to respiratory tract infection established following either IV or IV/IN administration of virus.

The above investigators also reported the detection of a low (1:3) titer of serum IF in one calf 2 days after IM administration of $10^{9.6}$ $TCID_{50}$ of IBR virus (31). In the present study, IM administration of $10^{5.7}$ $TCID_{50}$ IBR vaccine virus to group B calves (experiment B) failed to elicit detectable levels of IF in either their sera or respiratory tract secretions. Of special interest, however, was the appearance of IF in secretions of these calves following subsequent IN administration of IBR challenge virus. This response was different in several respects from the initial response of calves (group A) vaccinated by the IN route, in that IF appeared in secretions as early as 24 hr postexposure, appeared in the absence (in one calf) of virus recovery, and was present in lower titers and for a shorter time. It is apparent that both calves in the IM group became infected following challenge since virus was recovered on days 3 to 5 from one calf and both calves experienced characteristic secondary antibody responses. It would seem from these data that the local elaboration of IF was more easily induced following local infection with IBR in animals previously sensitized with that virus. Whether this was related to immune recognition as might be suggested by in vitro studies reported previously (14, 17, 39), or resulted from the release of preformed IF synthesized in response to the earlier IM exposure to IBR is not known. The data indicate, however, that local replication of virus was required for the secondary local IF response, since no IF was detected post-

challenge in secretions of IN vaccinated calves which apparently did not become infected following challenge.

IF has been detected in nasal washings of humans infected naturally or experimentally with rhinovirus, A2 influenza, and coxsackie type A viruses (7, 18, 22). In one study reported (22), the relationships between influenza virus shedding, presence of interferon in nasal washes and sera, and appearance of antibody were similar to those observed in the studies reported here. Clinical symptoms became less severe coincident with the appearance of peak titers of interferon in nasal wash specimens, and cessation of influenza virus shedding accompanied antibody increase.

Results of these studies may find direct, practical applications in reducing the incidence or severity, or both, of respiratory disease in cattle. Such applications include the use of intranasally administered avirulent IBR (i) to provide rapid protection against field challenge with IBR, (ii) to provide short-term protection against infection by viruses other than IBR which replicate primarily in the respiratory tract and which are sensitive to the antiviral action of IF, and (iii) to modify outbreaks of respiratory disease caused by IBR or other IF-sensitive viruses by administering the avirulent IBR to groups of cattle during the early stages of such outbreaks. The incidence of bovine respiratory disease, commonly called shipping fever, is usually high among groups of animals which have been transported from various sources and assembled in saleyards or feedlots. Such groups would potentially derive maximal benefit from administration of an effective IF inducer just prior to this rather predictable period of high risk. The presence of high levels of IF in secretions of the upper respiratory tract for 6 to 10 days should provide an effective antiviral barrier at this important portal of entry. Viruses other than IBR which have been shown to be associated with bovine respiratory disease include parainfluenza type 3 virus (38), bovine virus diarrhea virus (26), rhinoviruses (5), adenoviruses (8), reoviruses (25), and respiratory syncytial virus (27). Additionally, it has been shown that foot-and-mouth disease virus gains primary access to the body via the respiratory route (16). Field studies have been conducted to compare the incidence of respiratory disease in groups of feedlot cattle receiving AV-IBR by IN administration or IBR vaccine administered parenterally. Significantly less disease has occurred among the IN vaccinates. Data from these studies and from controlled studies conducted to demonstrate early resistance to heterologous virus challenge will be presented in a separate report (*in preparation*).

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