Effect of Bovine $\alpha_1$ Interferon on Bovine Herpesvirus Type 1-induced Respiratory Disease

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SUMMARY

Treatment of calves with bovine recombinant $\alpha_1$ interferon prior to challenge with bovine herpesvirus type 1 increased the animals' ability to withstand a subsequent Pasteurella haemolytica challenge. The reduction in viral–bacterial synergy observed following interferon treatment did not appear to be due to a direct effect of the interferon on virus replication in the upper respiratory tract. Thus, even though interferon-treated animals shed slightly less virus from their nasal passages than did untreated animals, this reduction was not statistically significant. Furthermore, there was no difference in the level of intranasal interferon secreted by control or interferon-treated animals. These results suggest that interferon treatment does not affect the production of endogenous interferon. In contrast, a significant difference was observed between the number of days that control animals were sick, the levels of serum fibrinogen and the functional activity of polymorphonuclear neutrophilic granulocytes obtained from infected calves. These results suggest that bovine recombinant $\alpha_1$ interferon may have a greater immunomodulatory effect than a direct antiviral effect in this model. This is further supported by the observation that bovine herpesvirus type 1 is relatively resistant to the direct antiviral effect of bovine recombinant $\alpha_1$ interferon in vitro.

INTRODUCTION

Since the discovery of interferon (Isaacs & Lindenmann, 1957) and the demonstration that it could inhibit replication of a wide variety of viruses in vitro, considerable interest has been generated concerning its potential application as an antiviral agent in vivo (Arvin et al., 1976; Merigan, 1982; Merigan et al., 1968, 1973, 1978; De Clercq & De Somer, 1968; Douglas & Betts, 1974; Dziewanowska & Pestka, 1982). Although the quantities and purity of interferon were limited, various groups have clearly demonstrated that in at least some virus diseases, treatment with exogenous interferon has a beneficial effect. Whether this protective effect was due to the direct antiviral action of the interferon, its effects on the host's immunological defence mechanisms or to the presence of other lymphokines in these semi-purified interferon preparations remains to be clarified. Additional support for the beneficial effect of interferon in virus infections was obtained from studies where neutralization of endogenous interferon, which is produced as a result of virus infection, enhanced the severity of the virus infection (Fauconnier, 1971; Gresser et al., 1976a, b; Riviere et al., 1977).

Interferon can either enhance or depress the humoral and cellular immune response (Nakamura et al., 1984; Senik et al., 1979; Shalaby et al., 1984). Thus, it can diminish or enhance
the antibody response (Gresser, 1977; Johnson & Baron, 1976; Johnson et al., 1975) depending on the dose and timing of the interferon application relative to antigen stimulation. In virus infections, natural killer cells, cytotoxic T cells and macrophages are considered to be important in antiviral defences and the activity of all three cell types can be enhanced by interferon pretreatment (Brunda & Rosenbaum, 1984; Herberman et al., 1982; Bielefeldt Ohmann & Babiuk, 1984a; Babiuk & Rouse, 1978). In addition to the timing and dose of interferon used, the different interferon types (\(\alpha, \beta, \gamma\)) may vary in their immunoregulatory potential as well as in the mechanisms whereby they mediate their immunomodulatory effects (DeMaeyer & DeMaeyer-Guignard, 1982).

The bovine interferons, like human interferons (Sehgal, 1982), are classified into three types: alpha (\(\alpha\)), beta (\(\beta\)) and gamma (\(\gamma\)) on the basis of their antigen specificities, structure and gene organization. The genes are encoded by two distinct gene families (D. J. Capon, personal communication). The class I bovine (BoIFN-\(\alpha_1\)) gene family contains approximately 10 to 12 members whereas the class II bovine (BoIFN-\(\alpha_2\)) gene family contains 15 to 20 members (D. J. Capon, personal communication). A number of bovine \(\alpha, \beta\) and \(\gamma\) genes have now been cloned and used to obtain highly purified single proteins (Leung et al., 1984) for testing both in vivo and in vitro (Bielefeldt Ohmann & Babiuk, 1984a; Bielefeldt Ohmann et al., 1984).

Bovine respiratory disease, an economically important disease, is caused by the interaction between a variety of viruses and bacteria (Babiuk & Acres, 1984). Since management conditions are such that infection with a variety of viruses often occurs before immunity to vaccination has developed, interest has been directed at controlling this disease by other means. Crude bovine interferon has been shown to be effective in protecting animals against at least a few of the virus agents involved in initiating bovine respiratory disease (Cummins & Rosenquist, 1980). Therefore, the bovine respiratory disease model involving viral–bacterial interactions appeared to be an excellent one for testing the efficacy of cloned bovine interferon in cattle. The present communication is a description of these findings.

**METHODS**

*Animals.* Range-bred, healthy Hereford calves aged 6 to 10 months and weighing 150 to 250 kg were used throughout the studies. All animals were seronegative for bovine herpesvirus type 1 (BHV-1) and *Pasteurella haemolytica* and neither of these agents was isolated from the animals prior to challenge. The calves were housed either in isolation or in an outdoor pen with no contact with other animals. In both cases they were fed alfalfa pellets and water *ad libitum.*

*Virus and bacteria.* BHV-1 strain 108 was cultured in Georgia bovine kidney (GBK) cells as described previously (Babiuk et al., 1975). Following infection of confluent monolayers at a m.o.i. of 1, cultures exhibited extensive cytopathology within 36 h, at which time they were harvested, titrated and stored at \(-70^\circ\)C until used. This preparation had an infectivity of 10 p.f.u./ml and was used undiluted for aerosol challenge. Vesicular stomatitis virus (VSV) for interferon assays was also prepared in GBK cells as described previously (Babiuk & Rouse, 1976). *P. haemolytica* (biotype A, serotype 1) was grown in brain heart infusion (BHI) broth containing 5% horse serum. For challenge, the bacterial culture was always in the exponential phase of growth (6 h culture) at the time it was used as an aerosol and had a titre of \(10^9\) to \(2 \times 10^9\) colony-forming units per ml (c.f.u./ml) (Yates et al., 1982).

*Antiviral effect of interferon* in vitro. Bovine \(\alpha_1\) interferon was synthesized in *Escherichia coli* and was \(\geq99\%\) pure as determined by polyacrylamide gel electrophoresis. The antiviral activity of bovine recombinant interferon \(\alpha_1\) was assayed as described previously (Bielefeldt Ohmann et al., 1984). Briefly, confluent monolayers were pretreated for 18 h with various concentrations of interferon and then infected with BHV-1 or VSV (m.o.i. 0.01). Forty-eight h later the cultures were harvested and titrated for the amount of virus present.

*Experimental procedures.* Calves were bought from a ranch, transported to the research facility and rested for at least 2 days. They were then subjected to a thorough clinical examination including recording of temperatures and of leukocyte cell numbers and functional activity. Analysis of the microbial flora of the nasal passages was also conducted to ensure that there had been no recent infection with pathogens. At the start of the experiment, calves were randomly divided into groups (5 to 10 animals/group) and were either treated intranasally with interferon (10 mg/animal) or with a placebo 48 h prior to being challenged individually by exposure to an aerosol of BHV-1 virus, followed 4 days later with *P. haemolytica*. In each case the aerosol was generated by a DeVilbis nebulizer, Model 65 (DeVilbis, Barry, Ontario, Canada). Treatment was for 5 min with each microbe as described previously (Bielefeldt Ohmann & Babiuk, 1985).

*Clinical evaluation.* The clinical evaluations were performed at the same time each day by two independent investigators who were uninformed about the specific treatment of the individual animals. The parameters...
evaluated included depression, appetite, fever, conjunctivitis, rhinitis, mouth breathing, tracheitis, pneumonia and dehydration. In each case a score of 0 was assigned to healthy animals. Clinical scores of 1 to 4 were assigned to sick animals as follows: 4, severe; 3, marked; 2, moderate; 1, mild. Total clinical scores for each animal are the sums of scores for each parameter.

Haematology. Blood specimens for leukocyte and differential determinations, as well as fibrinogen determination, were collected in heparinized vacutainer tubes (Becton-Dickinson) and processed within 2 h of collection. Serum was collected for analysis of serum interferon in the standard manner.

Nasal secretions. Nasal secretions for interferon and virus titration were collected as described previously (Todd et al., 1972). Briefly, a tampon was inserted into the ventral meatus of the nasal cavity. After approximately 45 min the tampon was removed and the secretions were collected by squeezing the tampon. Virus and interferon levels were determined on GBK cells as described previously (Babiuk & Rouse, 1976). Samples for interferon assay were stored frozen at -20 °C until titrated.

Necropsy. Post-mortem examinations were done on animals that died or were killed during the experiments. The nasal passages, larynx, trachea, and lungs were examined and photographed. Viral and bacterial lesions were recorded. The extent of pneumonia was assessed by a numerical method developed by Thomson et al. (1975). The pneumatic lesions in each lung lobe (except for the accessory lobe) were graded from 0 to 5 according to the amount of tissue involved. Total scores for seven lung lobes ranged from 0 to a theoretical maximum of 35 if the entire lung was affected.

Leukocyte isolation and functional analysis. Venous blood was drawn into syringes containing citrate/dextrose. The blood was centrifuged at 1000 g for 20 min, the buffy coat was collected and the peripheral blood mononuclear leukocytes (PBL) were isolated on Ficoll-Hypaque as described previously (Bielefeldt Ohmann & Babiuk, 1985). The polymorphonuclear neutrophils (PMN) were isolated from the original pellet by lysis of the erythrocytes as described previously (Bielefeldt Ohmann & Babiuk, 1984b).

Migration of PMNs was assayed in agarose plates as described previously (Bielefeldt Ohmann & Babiuk, 1984a). Migration was measured after 2 h using zymosan-activated serum as a chemoattractant and Hanks' balanced salt solution (HBSS) as a control. Oxygen metabolism of PMNs was measured by the nitro blue tetrazolium (NBT) reduction assay as described elsewhere (Bielefeldt Ohmann & Babiuk, 1985).

Lectin-induced lymphocyte proliferation was measured as described elsewhere (Filion et al., 1983). Triplicate cultures were stimulated with either concanavalin A (Con A, 5 μg/ml) or phytohaemagglutinin (PHA, 5 μg/ml). Purified human interleukin-2 (IL-2) was added to replicate cultures at a final concentration of 10 units/ml. Cultivation was for 72 h, with [Me-3H]thymidine added during the last 12 to 16 h.

IL-2 generation by PBL was determined as described previously. Briefly, 1 × 10^7 cells/ml were cultured in modified Iscove’s medium (Baker & Knoblock, 1982a) with Con A (Calbiochem-Behring) at a final concentration of 5 μg/ml. The amount of IL-2 generated was quantified with an IL-2-dependent bovine lymphoblastoid cell line (Baker & Knoblock 1982b).

Statistical analysis. All quantitative parameters of test groups were compared to those of the placebo-treated group using a two-way, one-tailed Student’s t-test assuming equal variance of the populations. Statistical comparison of mortality in treatment groups with control groups was performed using a Fischer permutation analysis.

RESULTS

Clinical response of calves challenged with BHV-1

Prior to challenge, all animals were healthy and had a normal rectal temperature. However, following challenge the rectal temperature rose in all animals within 48 h post-infection, regardless of whether they were untreated or treated with interferon. Temperatures continued to increase until days 4 to 6 (Fig. 1a, b, c). In animals that were subsequently infected with P. haemolytica, temperatures remained elevated for longer periods in the untreated group than in the interferon-treated group (Fig. 1a, b). In contrast, in these animals which were infected with BHV-1 only the temperature responses of both interferon-treated and untreated groups returned to normal at approximately the same time (Fig. 1c). Since interferon-treated BHV-1/P. haemolytica-infected calves appeared to respond more like those which received the BHV-1 challenge alone, it appeared that interferon treatment had a greater effect in preventing the subsequent colonization by Pasteurella than in preventing BHV-1 infection.

Results from the three different experiments depicted in Fig. 1 demonstrate that interferon-treated animals generally had a smaller rise in temperature which was of shorter duration. There were some variations between experiments which might be related to the age and genetic background of the animals.
In addition to temperature responses a variety of other parameters of respiratory disease were assessed. Fig. 2 illustrates the results of one representative experiment where clinical signs were evaluated. The signs examined included depression, appetite, fever, conjunctivitis, rhinitis, mouth breathing, tracheitis, dehydration and pneumonia. Interferon-treated animals were never as sick as those untreated, and returned to normal or a state of subclinical disease much earlier.

To demonstrate better the type of variability that was observed within experiments, the results of one experiment are presented in Table 1. This experiment demonstrates that animals began to die within 2 days after bacterial challenge. Based on the scoring system, a clinical score of $\geq 10$ was set to indicate severe disease, and $< 10$ a moderately mild infection. Thus, a value of 10 indicated an animal that would be considered sick enough to be placed in a sick pen for individual treatment under feedlot conditions. A breakdown of total sick days per group in two different experiments is summarized in Table 2. Two experiments are presented to demonstrate the range of responses. In both experiments, control animals had a significantly higher number of sick days than did interferon-treated animals. In experiment 1, interferon had a dramatic effect in reducing clinical disease, the untreated animals being sick for an average of 4-3 days against 0.5 days for interferon-treated animals. Furthermore, the clinical scores for untreated animals were twice those for treated animals. In experiment 2 the beneficial effect was less convincing but significant differences at $P < 0.05$ were still observed. Thus, interferon treatment provided a beneficial protective effect against challenge. In 14 other experiments the effect was in between these two extremes. In an attempt to confirm that clinical examination was a true reflection of the extent of respiratory tract infection and pathology, animals that
Table 1. *Clinical scores of individual animals treated with interferon (10 mg) or with a placebo*

<table>
<thead>
<tr>
<th>Placebo-treated</th>
<th>Clinical score* on</th>
<th>Group</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 4</td>
<td>Day 5</td>
<td>Day 6</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>7</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>6</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>3</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>2</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>8</td>
<td>12</td>
<td>12</td>
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<tr>
<td>17</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>7</td>
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<tr>
<td>20</td>
<td>2</td>
<td>6</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Sample size</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean</td>
<td>1.700</td>
<td>5.000</td>
<td>10.100</td>
<td>12.100</td>
</tr>
</tbody>
</table>

* Values represent the total points an animal received when scored with respect to extent of disease. Parameters include depression, appetite, fever, conjunctivitis, apathy, rhinitis, tracheitis, mouth breathing, etc. as described in Methods. The higher the score, the sicker the animal. Maximum score possible was 36.

Table 2. *Analysis of the number of sick days following BHV-1/P. haemolytica challenge*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of sick days*</th>
<th>Average clinical score</th>
<th>Average no. of sick days/animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. 1</td>
<td>Expt. 2</td>
<td>Expt. 1</td>
</tr>
<tr>
<td>Control</td>
<td>43</td>
<td>54</td>
<td>11.5</td>
</tr>
<tr>
<td>Interferon-treated</td>
<td>3</td>
<td>28</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* Animals were examined daily and their clinical scores were recorded. A day on which clinical score was 10 or more was considered a sick day. Clinical scores are arrived at as described in Methods. Total clinical scores for each animal are the sums of the scores for each parameter of evaluation. Values represent the mean of 10 animals/experiment. Values represent the number of recordings by two observers that were ≥10. Differences between control and treated animals are significant at *P* < 0.05 in both experiments.

survived experiment 2 were killed at the end of the observation period (day 9 after BHV-1 challenge) and the entire respiratory tract was assessed for pathological changes. Table 3 illustrates that approximately twice as much lung tissue was pneumonic in untreated animals than in interferon-treated animals. Furthermore, four of ten treated animals had completely cleared the virus and bacteria by this time. These animals were considered clinically normal. In contrast, only one untreated animal returned to normal.
Fig. 3. Serum fibrinogen levels in BHV-1/P. haemolytica-infected animals. Ten animals per group were treated with interferon or untreated. Results are the mean for each group.

Fig. 4. Interferon titres in animals challenged with BHV-1. (a) Serum interferon titres; (b) intranasal secretion interferon titres. Results are the mean of ten animals/group. Serum interferon levels were not significantly different on any day, whereas nasal interferon levels were significantly increased on days 2 to 9 and 3 to 9 in interferon-treated and placebo-treated groups respectively.

Table 3. Lung scores of calves infected with BHV-1 and P. haemolytica

<table>
<thead>
<tr>
<th>Lung scores</th>
<th>Control</th>
<th>Interferon-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean (0)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>&lt;25% involvement (1-9)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>25%-50% involvement (10-18)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>&gt;50% involvement (&gt;19)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Mean lung score</td>
<td>13.7†</td>
<td>7.4‡</td>
</tr>
</tbody>
</table>

* Number of animals that had the specific lung score at the end of the experiment. These animals represent expt. 2 presented in Table 2.
† P < 0.05.

A further reflection of the extent of pasteurellosis is the serum fibrinogen content (Garry, 1984). Untreated animals exhibited a significantly higher rise in serum fibrinogen than did interferon-treated animals (Fig. 3). Thus, interferon-treated animals were considered not only by a subjective double blind clinical examination to withstand a BHV-1/P. haemolytica challenge better, but also by quantitative differences in serum parameters and lung involvement. Generally, the higher the serum fibrinogen level the greater the lung involvement.

Virus excretion by BHV-1-infected animals

In an attempt to explain why interferon-treated animals were not as severely affected by the virus–bacterial challenge, we assessed the effect of interferon on virus shedding from the nasal
Intranasal interferon levels in healthy calves exposed to interferon intranasally (10 mg/calf) at 0 h and 24 h. Interferon was measured by bioassay (●) and by radioimmunoassay (▲). The radioimmunoassay used rabbit anti-bovine α1 interferon as the capture antibody and 125I-labelled mouse monoclonal antibody against bovine α1 interferon.

Fig. 6. Fluctuations in leukocyte numbers of BHV-1-infected animals. Interferon-treated (●) and untreated (▲) animals assayed were tested during the course of the observation period. Values are the mean of five animals/group. WBC, White blood cells.

passages. Virus shedding could be detected as early as 24 h after challenge (Fig. 1 d). Within 48 h of challenge all the animals shed virus regardless of whether they had been treated with interferon or not. In most cases the quantity of virus present in nasal secretion increased until 4 to 6 days post-infection and then gradually returned to undetectable levels by day 10 or 11.

In one experiment there was a delay in virus excretion (Fig. 1 f) by interferon-treated animals but by day 4 post-infection, virus titres were similar to those in untreated calves. A delay in virus excretion has previously also been shown with cytomegalovirus following interferon treatment (Cheeseman et al., 1979).

**Interferon levels in serum and nasal secretions**

Nasal and serum interferon levels were measured daily to determine whether differences occurred between treatment groups. Interferon levels present in nasal secretions closely paralleled the pattern of virus shedding. Thus, no interferon was detectable for the first day but rapidly increased thereafter to reach peak levels by day 4. These high levels were maintained until day 8 and then began to drop to baseline levels by day 10 or 11 (Fig. 4b). There were no differences in interferon levels in treated or untreated animals, suggesting that interferon pretreatment did not alter the animals' ability to produce endogenous interferon. The interferon present in nasal secretions was presumed to be virus-induced rather than a carry-over from the interferon treatment since baseline levels of interferon were present at the time of virus infection. Thus, even though high levels of interferon were present at 4 h after application, these
levels fell so that by 24 h post-application, nasal interferon levels had returned to baseline (Fig. 5). If a second application was given, a similar pattern occurred. Despite these very high levels of interferon in the nasal secretions, there was no significant increase \((P > 0.05)\) of interferon in the blood of infected animals at any time regardless of whether or not they were treated with interferon (Fig. 4a). Thus, it appears that interferon either does not enter the blood or, if it does, it rapidly becomes cell-associated and not detectable in the serum. Furthermore, even though BHV-1 can itself induce interferon (Fig. 4) and is presumably spread systemically this does not result in elevated serum interferon activity.

**Susceptibility of BHV-1 to bovine interferon**

Even though high levels of interferon were present in the nasal secretions of BHV-1-infected animals, they still shed virus. Therefore, we attempted to determine whether BHV-1 was susceptible to interferon. Table 4 illustrates that whereas VSV was inhibited >99% at a concentration as low as 10 units/ml, 100 times that concentration did not inhibit BHV-1 replication to the same extent.

### Table 4. Effect of bovine \(\alpha\) interferon on BHV-1 and VSV replication

<table>
<thead>
<tr>
<th>Interferon concn. (units)*</th>
<th>Virus yield†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VSV</td>
</tr>
<tr>
<td>0</td>
<td>(5 \times 10^6)</td>
</tr>
<tr>
<td>1</td>
<td>(2 \times 10^5)</td>
</tr>
<tr>
<td>10</td>
<td>(1 \times 10^3)</td>
</tr>
<tr>
<td>100</td>
<td>(4 \times 10^2)</td>
</tr>
<tr>
<td>1000</td>
<td>(1 \times 10^1)</td>
</tr>
</tbody>
</table>

* Units of interferon/ml: lot no. E9044A calibrated against a bovine interferon laboratory standard no. A. 1591-14.
† Virus yield expressed in p.f.u./ml.

Results from a representative experiment are depicted in Fig. 6. In both untreated and treated animals total white blood cell (WBC) and lymphocyte numbers decreased progressively throughout the observation period. Only on the 2nd day after infection was there a significant difference between these two parameters. No statistically significant differences in PMN numbers were observed between the groups on any day. Control and treatment groups exhibited a significant rise in monocyte count from the 5th to the 8th day after infection but were not statistically different from each other.

### Haematology

Leukocyte function

Since interferon did not significantly alter virus replication in the nasal passages, but did greatly enhance the animals' ability to withstand superinfection with \(P. haemolytica\), it is suggested that interferon may have had an effect on virus damage in the lower respiratory tract or on leukocyte functions, both of which influence secondary bacterial infection (Babiuk & Acres, 1984). In an attempt to test this, leukocytes were isolated from both interferon-treated and untreated animals at various times prior to and following BHV-1 infection. Fig. 7(a) illustrates that at 4 days after infection PMN migration is reduced in both interferon-treated and untreated animals. However, the PMNs from interferon-treated animals were almost twice as active as those isolated from untreated animals. Furthermore, by the 7th day post-infection, their activity returned to normal whereas those of untreated animals were still depressed. A similar effect was seen in the ability of lymphocytes to produce IL-2 (Fig. 8). In this case, following an initial drop in IL-2 production there was an increase, interferon-treated infected animals producing twice as much IL-2 as they did prior to infection. Although lymphocytes of untreated animals also produced more IL-2 by the 7th day post-infection, the activity was much lower than in interferon-treated animals. Oxygen metabolism of PMNs, as measured semi-
BoIFN-α effect on BHV-1 infection

Fig. 7. Effect of BHV-1 on PMN migration (a) and superoxide anion generation (b) in control or interferon-treated animals. Activity of all animals was tested prior to infection as well as at various times post-infection. Since there was no difference between the control and interferon-treated group prior to infection those results were pooled ('all animals'). Migratory difference represents the difference in migratory distance of cells towards a chemoattractant (zymosan-activated serum) and no chemoattractant as described in Methods.

Fig. 8. IL-2 generation by PBL from BHV-1-infected calves, interferon-treated (●) or untreated (△). Results are the mean of triplicate cultures from five animals in each group.

quantitatively by intracellular superoxide anion (O₂⁻) generation, was increased by 4 days following BHV-1 infection, in interferon-treated and untreated calves. However, by the 7th day post-infection this activity was nearly normal in interferon-treated calves, whereas O₂⁻ generation continued to increase in untreated animals (Fig. 7b).

DISCUSSION

The most interesting aspect of this study was that even though bovine interferon had a dramatic effect on the clinical signs, lung pathology and death of calves, it had only a marginal effect, if any, on the quantity of virus shed from the nasal passages (Fig. 1). These results are similar to those observed with herpes simplex virus, where excretion of virus was not affected by administration of interferon (Cheeseman et al., 1979). However, they do contrast with the results for man, where intranasal interferon treatment reduces rhinovirus shedding (Douglas & Betts, 1974; Scott et al., 1982). Reductions in the severity of clinical disease in man following treatment with interferon have also been reported to occur with varicella zoster virus (Merigan et al., 1978), cytomegalovirus (Emodi et al., 1976), herpes simplex virus-induced keratitis (Jones et al., 1976), hepatitis B virus (Desmyter et al., 1976) and rubella virus (Larsson et al., 1976). Interferon induced by intranasal inoculation of a virus or interferon inducers also appears to reduce replication of a second virus (Cummins & Rosenquist, 1980; Panusarn et al., 1974). There are also other reports in which only clinical signs were reduced with no effect on virus shedding. These results suggest that virus shedding may not be the best indicator of the efficacy of
interferon treatment and that there are differences between virus infections depending on the sites of replication and their relative sensitivity to interferon.

Whether interferon had a direct antiviral effect in the lungs was not determined since the lower respiratory tract of cattle cannot be sampled on a regular basis.

It is possible that interferon acts indirectly by altering the reactivity of cells involved in curtailing virus replication and in preventing colonization by *P. haemolytica*. Previous reports have clearly shown that interferons have multiple immunomodulatory effects, functioning in an integrated manner with other aspects of the specific and non-specific defence mechanisms (DeMaeyer & DeMaeyer-Guignard, 1982).

In BHV-1 it has been proposed that the reason for enhanced bacterial colonization by *P. haemolytica* following BHV-1 infection is the fact that in addition to causing anatomical damage which is conducive to bacterial growth (Babiuk & Acres, 1984) it also suppresses a variety of leukocyte functions assumed to be important in clearing the bacteria (Filion *et al.*, 1983; Jakab, 1984). It is interesting to note that the maximum susceptibility to superinfection occurs at approximately 4 days after BHV-1 infection (Yates *et al.*, 1982) and correlates with maximum immunosuppression (Filion *et al.*, 1983). One of the functions that is reduced is PMN migration (Bielefeldt Ohmann & Babiuk, 1985; Fig. 7a). In the case of interferon-treated animals, this reduction in PMN migration was not as dramatic as in untreated animals and returned to normal quite rapidly (Fig. 7a). Thus, it is possible that interferon treatment can either prevent systemic virus replication and thereby reduce the level of PMN paralysis or alternatively it may activate PMNs. Activation of bovine PMNs has been shown to occur *in vitro* following treatment with bovine interferon (Bielefeldt Ohmann & Babiuk, 1984a). Therefore, it is proposed that in interferon-treated animals there is less immunosuppression, at least with regard to the PMN function which can then clear the bacteria before they become established and cause damage. The exact mechanism of activation is presently being investigated. The observation that PMNs from untreated calves continued to produce higher levels of O$_2$ late in the disease may reflect continued stimulation with bacterial products *in vivo* which aid in the immunopathology of the disease (Slaustron, 1982). In contrast, no effect of interferon treatment was seen on the lectin-induced proliferation of lymphocytes or on their responsiveness to IL-2. This is probably due to a selective depletion of responder cells as IL-2 was continually being produced and the production was even enhanced by interferon treatment (Fig. 8).

The observation that interferon-treated animals had lower clinical scores (Fig. 2) was substantiated at autopsy, as well as in the clinical pathology laboratory prior to death. Thus, interferon-treated animals had a lower incidence of fibrinous pneumonia than control animals. Similarly, fibrinogen levels, which are an objective measurement of the severity of inflammation (Garry, 1984), were also elevated in untreated calves (Fig. 3). Thus, although cattle were sacrificed for laboratory tests, this was not necessary to show the value of interferon treatment in controlling viral-bacterial synergistic infection.

These observations show that interferon treatment may be a useful procedure in modern feedlot practice. The optimum regime would require confirmation by field trials but interferon might best be given at the time of entry into feedlots. This is the time when animals are exposed to the viruses responsible for initiating the bovine respiratory disease complex (Rosenquist, 1984). Consequently, most respiratory disease occurs shortly after entry into feedlots and before adequate immunity is developed following vaccination at entry. Since BHV-1 is more resistant to interferon than many other viruses associated with the bovine respiratory disease complex, treatment might be even more beneficial with respect to these other virus infections.

Intranasal administration of interferon to cattle under our conditions had no adverse effects. Other investigators, using homologous interferon in other species have found that interferon could induce fever as well as alter haematological and immunological parameters (for reviews, see Cesario, 1983; Dziewanowska & Pestka, 1982). Reasons for this discrepancy could be the route of administration, dose and timing of interferon administration or the species.

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BoIFN-α, effect on BHV-1 infection

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REFERENCES


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