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Levels of Interferon in Blood Serum and Toxicity Studies of Bacteria-Derived Bovine Alpha$_I$1 Interferon in Dairy Calves

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This paper reports information on the levels of interferon (IFN) in the blood serum of dairy calves given 10^6 U of bacteria-derived bovine alpha$_I$1 interferon per kg of body weight by intravenous (i.v.), intramuscular (i.m.), subcutaneous (s.c.), and intranasal (i.n.) routes. Highest levels (10,000 U/ml) in the vesicular stomatitis viral assay system were obtained after i.v. administration and occurred within 30 min of a dose; levels rapidly declined thereafter to a low of 200 to 300 U/ml by 24 h. Serum inhibitory activity against vesicular stomatitis virus in this range is sometimes found in normal dairy calves. Levels after i.m. and s.c. administration were similar: a plateau of 1,000 to 2,000 U/ml between 2 and 8 h after a treatment with a decline to 200 to 300 U/ml by 24 h. Serum IFN was not detected after i.n. dosing or in the control group given physiological buffered saline by the i.m. route. A transitory moderate febrile response, but no other clinical adverse effects, was noted after the first intramuscular dose of IFN, but not after subsequent i.m. doses. No clinical signs were noted after i.v., s.c., or i.n. dosing or in the control calves given physiological buffered saline intramuscularly. After i.v., s.c., and i.m. administration of IFN, leukopenia, neutropenia, and lymphocytopenia were observed; these were most prominent within the first 24 h after the initial dose of IFN.

Cattle and bovine cell cultures produce interferons (IFNs) in response to various inducers (1–5, 7, 8, 12–14, 16, 17, 19). The protective effects of the bovine IFNs against viral diseases in cattle have been difficult to assess, largely because of the scarcity of purified and well-defined IFNs in adequate concentration and amount to conduct appropriate trials in cattle. The recent cloning and expression of DNA encoding different IFNs (2, 9, 10, 12) has made it possible to compare the biological activities of various purified bovine IFN subtypes.

With the availability of purified bacterially derived bovine IFN-alpha$_I$1 (Bo IFN-α_I1), appropriate cattle trials against selected bovine viruses were possible. Before these infectivity studies, pharmacokinetic studies of this and other Bo IFNs in cattle were deemed necessary and essential.

The purposes of this study were (i) to determine IFN levels of bacterially derived Bo IFN-α_I1 in the blood serum of calves after intramuscular (i.m.), subcutaneous (s.c.), intravenous (i.v.), and intranasal (i.n.) administration; (ii) to determine any clinical signs of toxicity produced by the administration of Bo IFN-α_I1; and (iii) to determine the effects of Bo IFN-α_I1 on the blood cell profiles of calves.

MATERIALS AND METHODS

Bo IFN preparation. The Bo IFN-α_I1 was synthesized in *Escherichia coli* (2, 9, 10, 12) and was >94% pure as determined by polyacrylamide gel electrophoresis. The titers of IFN preparations and clinical serum samples were determined by virus-induced cytopathic effect inhibition assays in microtiter dishes by using Madin-Darby bovine kidney cells challenged with vesicular stomatitis virus. The Bo IFN titer was expressed in laboratory units based on internal laboratory standards. The activity of this IFN was 1×10^8 to 2×10^8 U/mg (3.3×10^7 U/ml). The IFN was maintained at 6°C in the laboratory.

Because previous reports (6, 13, 18) suggested that noncytopathic bovine virus diarrhea virus, often present in

TABLE 1. Experimental design

Group	Calf no. (wt [kg])	IFN treatment[a]			
		Substance	Route	Dose (ml)	Total U/treatment
A	1 (154.5)	PBS	i.m.	4.68	
	2 (147.7)	PBS	i.m.	4.48	
	3 (142.7)	PBS	i.m.	4.32	
B	4 (163.6)	IFN	i.m.	4.96	1.64×10^8
	5 (175.0)	IFN	i.m.	5.30	1.75×10^8
	6 (121.8)	IFN	i.m.	3.69	1.22×10^8
C	7 (92.2)	IFN	s.c.	2.82	0.93×10^8
	8 (102.7)	IFN	s.c.	3.11	1.03×10^8
	9 (89.5)	IFN	s.c.	2.71	0.90×10^8
D	10 (115.0)	IFN	i.n.	3.48	1.15×10^8
	11 (120.5)	IFN	i.n.	3.65	1.20×10^8
	12 (110.9)	IFN	i.n.	3.06	1.01×10^8
E	13 (115.0)	IFN	i.v.	3.48	1.15×10^8
	14 (95.5)	IFN	i.v.	2.89	0.96×10^8
	15 (89.5)	IFN	i.v.	2.71	0.90×10^8
F	16 (130.0)	PBS	i.m.	3.95	
G	17 (104.6)	IFN	i.m.	3.17	1.05×10^8
	18 (100.0)	IFN	i.m.	3.03	1.00×10^8

[a] Calves were treated daily with 10^6 U of Bo IFN-α_I1 (PH1259-52; activity, 3.3×10^7 U/ml) per kg of body weight. Groups B through E were treated on days 0, 1, 2, 3, and 4, and group G was treated on days 0, 1, and 2. Groups A and F were control groups which received PBS (Genentech, lot PH1259-1) instead of IFN.

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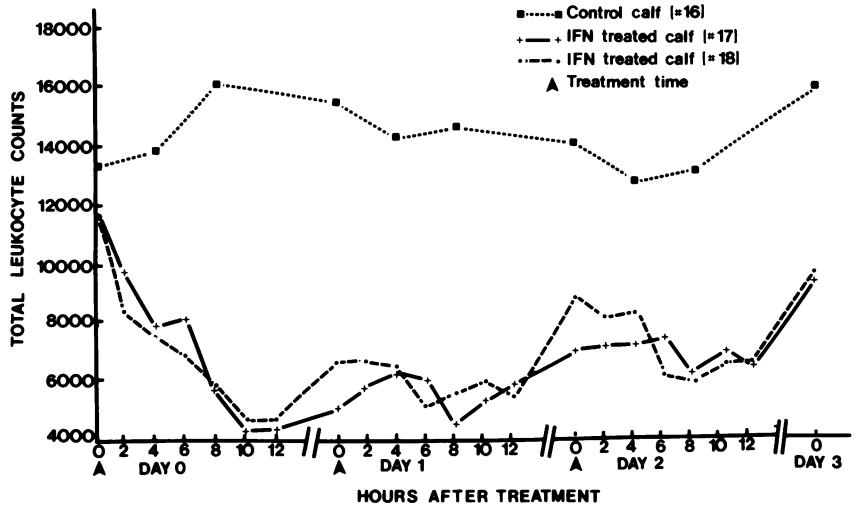

FIG. 1. Total leukocyte counts in calves treated i.m. with 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight.

cell lines or in fetal bovine serum, can affect the assay of IFN in bovine cell lines, the Bo IFN-$\alpha_1$1 was titrated on several bovine lines, some of which were free of bovine virus diarrhea virus. There was no difference in the IFN titer when calibrated against the appropriate standards.

Treatments. A total of 15 dairy calves (weight, 90 to 175 kg) were assembled from local dairy farms and divided into five groups, and each animal was placed in a separate isolation unit. The calves were observed for a 1- to 2-week period before the initiation of IFN treatment. During this acclimation period, base-line temperatures, total leukocyte counts, and differential leukocyte counts were established for each animal. Blood samples were drawn at −24, 0, 2, 4, 6, 8, 24, 72, 96, 120, 144, 168, and 336 h relative to the first injection of IFN administered by the i.m., s.c., or i.n. route. After i.v. IFN administration, blood was drawn at −24 h, 0, 20, 40, and 60 min, and also at 2, 24, 48, 72, 96, 120, 144, 168, and 336 h. All samples were taken just before the administration of IFN when the times coincided. A 1-ml sample of blood was allowed to clot on ice and was centrifuged under refrigeration. Next, the serum was harvested and stored at −20°C until assayed for IFN. Another sample of whole blood was placed in EDTA tubes for determination of total and differential leukocyte counts.

The experimental design is presented in Table 1. The three calves in group A served as controls and received only physiological buffered salt (PBS) solution (Genentech PBS lot PH1259-1) by the i.m. route; groups B, C, D, and E (each with three calves) received Bo IFN-$\alpha_1$1 (10^6 U/kg of body weight) by i.m., s.c., i.n., and i.v. routes, respectively, at 0, 1, 2, 3, and 4 days. Another experiment involving three calves was done to establish IFN levels in the blood serum at frequent intervals by taking samples from two calves at 2-h intervals for 24 h after i.m. treatment with 10^6 U of IFN per

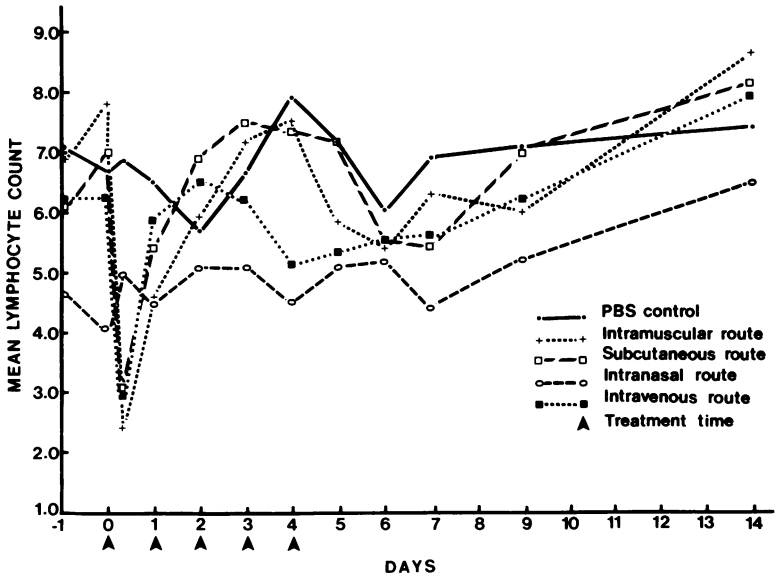

FIG. 2. Mean lymphocyte counts of calves (three per group) treated with 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight by various routes of administration.

kg of body weight (Table 1). One control calf given PBS was handled in the same way. In this short-term experiment, the three calves were treated at 0, 1, and 2 days.

RESULTS

Throughout the course of the cattle experiments, no adverse effects were noted clinically after the administration of 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight after five daily injections each by the i.m., i.v., i.n., or s.c. route. The injections did not result in any significant reaction after five daily treatments with IFN. However, four of five calves treated i.m. developed a moderate febrile response within 8 h of the first injection. All temperatures returned to normal before the second injection, at 24 h, and the response did not reoccur after subsequent injections. None of the other calves developed a febrile reaction or other signs of illness. The control group given PBS had normal temperatures and no signs of illness.

The results of the total and differential leukocyte counts for all groups including the control group are given in Fig. 1, 2, and 3. Bo IFN-$\alpha_1$1 at a dose of 10^6 U/kg of body weight by i.m., s.c., and i.v. routes resulted in transient leukopenia, neutropenia, and lymphocytopenia after first injection. Subsequent injections had less effect, and counts gradually returned to pretreatment levels after the last treatment on day 4. In another study in which frequent early blood samples were taken, two calves (17 and 18) given 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight (i.m.) had depressed leukocyte counts by 2 h after injection with a gradual decline through hour 10 (see Fig. 5). Subsequent IFN injections did not decrease the counts further. The i.n. administration of Bo IFN-$\alpha_1$1 or i.m. injection of PBS had no effects on total or differential leukocyte counts.

The levels of Bo IFN-$\alpha_1$1 in the blood serum at various intervals after three to five daily Bo IFN-$\alpha_1$1 treatments (10^6 U/kg of body weight) are given in Fig. 4. Based upon our experimental data, there were increased IFN levels in three calves 20 min after the first treatment by the i.v. route, reaching 10^4 U/ml, then dropping to $10^{3.15}$ U/ml by hour 1

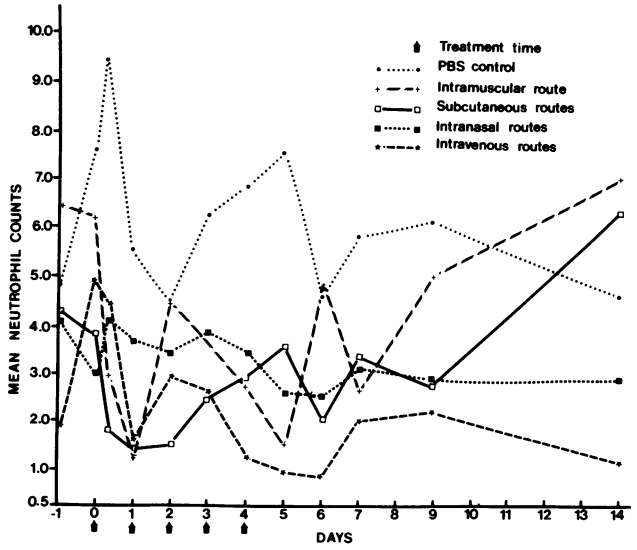

FIG. 3. Mean neutrophil counts of calves (three per group) treated with 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight by various routes of administration.

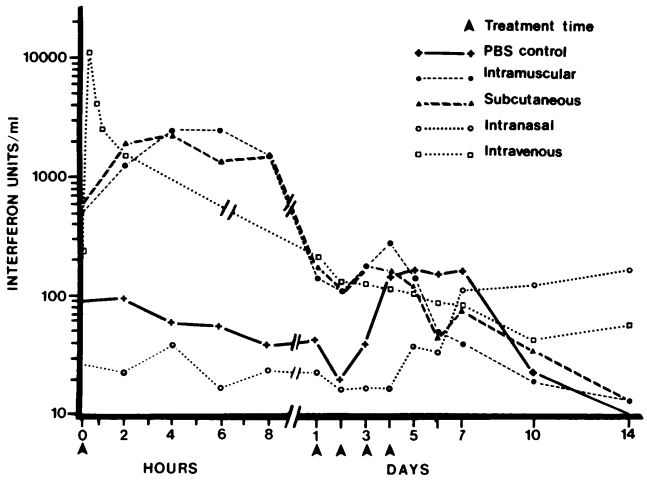

FIG. 4. The mean IFN levels in blood serum in calves (three per group) at various intervals after five treatments at 24-h intervals by various routes of inoculation with 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight.

and to $10^{3.05}$ by hour 2. At 24 h, the mean IFN level for this group was $10^{2.1}$ U/ml. Later samples taken at 24-h intervals showed no change in IFN levels, indicating no apparent accumulation of IFN after four daily treatments. Clearly, i.v. administration of IFN resulted in high initial serum concentrations, but this cleared rapidly. The experimental data from groups B and C (i.m. and s.c. treatment) were quite similar. IFN levels in the serum in both groups at 2, 4, 6, and 8 h were greater than 10^3 U/ml and were quite constant, but at 24, 48, 72, 96, and 120 h, they were slightly greater than 10^2 U/ml. The levels decreased with increasing time. These two groups received treatments with 10^6 U of Bo IFN-$\alpha_1$1 per kg at 0, 24, 48, 72, and 96 h. Group D calves that were treated i.n. at 0, 24, 48, 72, and 96 h with the same concentration of bovine IFN had no appreciable increase in serum IFN at 0, 2, 4, 6, 8, 24, 48, 72, 96, or 120 h (Fig. 4). Group A (control) calves given PBS i.m. at 0, 24, 48, 72, and 96 h had no increase in serum IFN levels (Fig. 4).

In a second experiment, two calves (no. 17 and 18) were given 10^6 U of Bo IFN-$\alpha_1$1 per kg i.m. on days 0, 1, and 2,

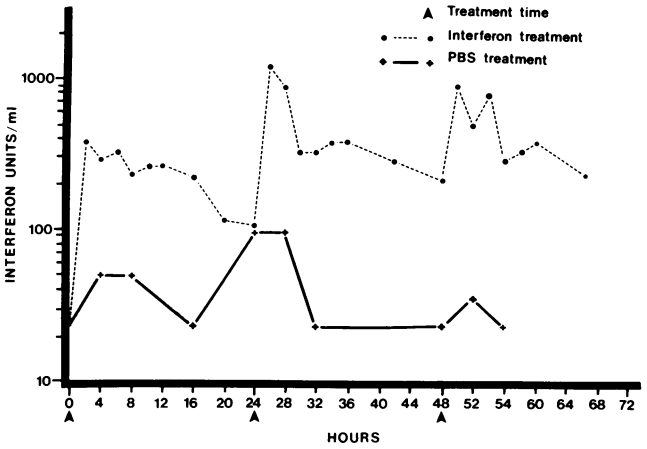

FIG. 5. Mean IFN titers in blood serum of two calves treated daily for 3 days by i.m. injections with 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight compared with a single control calf treated with PBS.

TABLE 2. Total leukocyte counts in calves treated i.m. with 10^6 U of Bo IFN-$\alpha_1$1 per kg of body weight[a]

Day and group	Calf no.	Total leukocyte count at postinjection h:						
		0	2	4	6	8	10	12
0								
Control	16	13,419		13,894		16,013		
IFN	17	11,799	9,775	7,930	8,101	5,753	4,376	4,328
IFN	18	11,824	8,430	7,696	6,904	5,848	4,648	4,755
1								
Control	16	15,484		14,236		14,639		
IFN	17	5,074	5,836	6,361	6,096	4,597	5,453	5,974
IFN	18	6,680	6,655	6,424	5,248	5,675	6,019	5,506
2								
Control	16	14,198		12,841		13,120		
IFN	17	7,007	7,221	7,300	7,596	6,367	7,023	6,589
IFN	18	8,985	8,298	8,413	6,377	6,043	6,664	6,596
3								
Control	16	16,007						
IFN	17	9,405						
IFN	18	9,607						

[a] Bo IFN-$\alpha_1$1 treatment was 10^6 U/kg per day i.m. at hour 0 and days 0, 1, and 2.

and one calf (no. 16) was treated with PBS and served as a control animal (Table 2). By taking blood serum samples at more frequent intervals during the first 2 days, it was established that increased levels of IFN occurred during the first 16 h after each 24-h IFN treatment. If any peak occurred, it was evident at 2 to 6 h (Fig. 5). There was a marked and clear difference between the detectable serum IFN levels of the two i.m.-treated calves and the PBS control calf.

DISCUSSION

To obtain adequate information about the levels of IFN in blood serum and the toxicity of Bo IFN-$\alpha_1$1, we deliberately selected the maximum dosage of IFN that would be used in calves under field conditions. Based upon our data, it was clear that this amount of IFN in normal calves caused no adverse effects of any significance. There was a transient febrile response in a high percentage of calves treated i.m. This response occurred after the first injection and not after subsequent injections. There was also a transient leukopenia that was observed 8 h after the first parenteral injection, which was less apparent after four successive treatments at 24-h intervals. In studies designed to evaluate the potential of Bo IFN-$\alpha_1$1 for prevention or therapeutic effect on infectious disease agents, further studies of this phenomenon may be desirable to better understand its significance, particularly as it relates to some viral agents which are capable of producing leukopenia. Apparently, human alpha A IFN causes a more severe reaction in dairy calves, since the reaction in IFN-treated calves persisted throughout the treatment period (15). Recombinant alpha A IFN (IFLrA and IFN-C) used in patients with advanced cancer caused symptoms including fever, chills, myalgias, headache, fatigue, and reversible leukopenia and granulocytopenia (11).

Significant IFN levels in the serum of calves were observed after treatment with Bo IFN-$\alpha_1$1 by the i.m., i.v., or s.c. route, but no IFN could be detected in the serum after i.n. administration. After i.v. injection, the IFN levels in serum were quite high but were cleared within a relatively short time. By i.m. and s.c. routes, the levels in serum were lower but persisted for at least 8 h. An additional experiment after i.m. treatments at 24-h intervals showed that the levels in serum remained at a relatively high and significant level for approximately 16 h after each treatment in contrast to those in the control calves (Fig. 5). It would be interesting to know the ultimate fate of both the IFN that is cleared from the blood stream and the IFN that is introduced by nasal administration. This could have relevance, since it relates to the pathogenesis of infectious agents for which this IFN might be applied in prevention or treatment.

These studies were the prelude to a determination of the beneficial effects that Bo IFN-$\alpha_1$1 might have on the experimental diseases produced by infectious bovine rhinotracheitis virus and bovine virus diarrhea virus of cattle. The results will be reported in separate papers.

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