In Vitro Protective Effect of Bacteria-Derived Bovine Alpha Interferon I1 against Selected Bovine Viruses

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We used bacteria-derived bovine alpha-interferon I1 (Bo IFN-αI1) to study its antiviral effect in a bovine turbinate cell line on bovine diarrhea virus, infectious bovine rhinotracheitis virus, parainfluenza 3 virus, and pseudorabies virus. We based our study upon replicate tests for each strain by using a block titration system with various concentrations of Bo IFN-αI1 against various concentrations of virus. The data were compiled in two-axis tables (replicate × concentration) and were statistically analyzed by the Spearman-Kärber method. An increase in the concentration of Bo IFN-αI1 enhanced its protective effect against every test virus strain. Bo IFN-αI1 had a marked in vitro effect on the bovine diarrhea viral strains. It demonstrated less protection against the pseudorabies and parainfluenza 3 viruses. Its effectiveness against the two infectious bovine rhinotracheitis viral strains was lesser and of a low order.

MATERIALS AND METHODS

Source of bacteria-derived Bo IFN-αI1. Bo IFN-αI1 was synthesized in Escherichia coli (3) and was >95% pure as determined by polyacrylamide gel electrophoresis. Interferon titers 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-αI1 titer was expressed in laboratory units based on internal laboratory standards used by Genentech, Inc., scientists. The specific activity of the batch of Bo IFN-αI1 used in these experiments was 3.5 × 107 U/ml as determined at Genentech, Inc. The stock was stored at 6°C in our laboratory until required for test purposes.

Virus stocks. We used six virus strains in these experiments. (i) IBR-Cooper virus was obtained from Donald L. Croghan at the National Veterinary Service Laboratory, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa. Our stock pool virus (lot no. 81-3) had a titer of 105.5 TCID50/0.1 ml. (ii) IBR-Cornell virus was isolated from a natural case at the New York State College of Veterinary Medicine. The viral stock titer was 106.3 TCID50/0.1 ml. (iii) Bovine virus diarrhea (BVD)-Singer virus was received from the National Animal Disease Laboratory Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa. Our stock pool virus (PI FBK1 5541) had a titer of 106 TCID50/0.1 ml. (iv) BVD-NADL virus was received from A. W. McClurkin of the National Animal Disease Laboratory. The viral titer was 105.5 TCID50/0.1 ml. (v) Parainfluenza type 3 (PI3)-SF4 virus originally was obtained from Robert Reisinger, Agricultural Research Service, U.S. Department of Agriculture. The stock virus titer was 105.5 TCID50/0.1 ml. (vi) Pseudorabies (PR) virus had a titer of 106 TCID50/0.1 ml and was obtained from an original isolate from a case at Cornell University.

Source of cell line. A bovine turbinate cell line (BT12) was obtained from A. W. McClurkin, National Animal Disease Laboratory, and was used in all experiments. This line is free of noncytopathic BVD virus.

Method of cell culture. Monolayers of the bovine turbinate cell line were grown on Corning 48-well plates at 36°C in an incubator containing 5% CO2. The medium used was Eagle minimal essential medium with 0.5% lactalbumin hydrolysate, 1% antibiotic-antimycotic, 2% of 7.5% NaHCO3, and 10% fetal bovine serum. Cell sheets were confluent in 4 to 5 days and were maintained with medium containing 2% fetal bovine serum. The cultures were rinsed once with basic culture medium without any serum before addition of Bo IFN-αI1 and virus. Cell line passage levels ranged between 12 and 38.

Test procedure. We used a block titration method with various concentrations of virus ranging from 104 to 106 TCID50/0.1 ml against concentrations of Bo IFN-αI1 from 103 to 105/0.1 ml and the appropriate virus and cell controls. The block titration configuration pattern is shown in Fig. 1.

After 4 to 5 days, monolayers were formed in 48-well plates; the growth medium was discarded, and cells were washed with basic culture medium without any serum. To each well, 0.1 ml of Bo IFN-αI1 was added first, followed by 0.1 ml of virus. At the same time, the virus control dilutions were placed in the appropriate wells. The plates were agitated every 15 min for 45 min at 37°C. Then, maintenance
medium with 2% fetal bovine serum was added to Bo IFN-α1, virus mixture wells, as well as to the Bo IFN-α1 and virus control wells. This absorption method was initially compared with the pretreatment method with the same virus. In the pretreatment method, 2 ml of Bo IFN-α1 was placed in a Falcon tube, followed by the addition of 2 ml of virus in appropriate dilutions. The tubes were agitated every 15 min at room temperature for 45 min. We placed 0.2 ml of Bo IFN-α1 virus mixture on the cells to allow absorption for 15 min at 37°C and then added the maintenance medium with 2% fetal bovine serum to each well. The plates were sealed in pouch bags to protect against evaporation. At 4 and 7 days post inoculation, the plates were examined with a dissecting microscope for cytopathic effect. The results at either time were comparable, so the 4-day procedure was used for all of the tests.

**Defining degree of Bo IFN-α1 protection.** At a given dose level of Bo IFN-α1, the endpoint of protection (PD90) was defined to be that point in a virus dilution series at which 90% of the inoculated wells remained negative. The PD90 increased with increasing concentrations of Bo IFN-α1, producing dose-response curves of consistent form in replicate tests with the same stock virus. The virus solution was titrated in each replicate test, and the resulting minor shifts in the TCID50 between tests were adjusted by applying a corresponding additive correction to all of the PD90 values in a test.

**Statistical methods.** The data for each stock virus were first analyzed separately as a replicated experiment in a complete block design. A pair of plates (Fig. 1) incubated together constituted a “block” or “replicate” within which four concentrations (10, 10², 10³, and 10⁴ U/ml) of Bo IFN-α1 constituted the “treatments” in a “block by treatment” analysis of variance of PD90 values calculated by the Spearman-Kärber method (1). These within-strain analyses of variance of the (computable) log₁₀ PD90 values produced a pooled error mean square of 0.014 (df = 42) for statistical tests of dose response to Bo IFN-α1. A one-sided least significant difference was calculated for each virus strain and was plotted on a vertical bar (Fig. 2); any response increment which exceeds this difference is statistically significant at the 5% level. One exception occurred where this statistical approach could provide only a lower-bound estimate of the PD90; in the case of BVD-NADL virus, the highest Bo IFN-α1 dose produced complete protection at all six virus dose levels in every experiment.

**RESULTS**

Graphic interpretation of data. The in vitro protective effect of bacteria-derived Bo IFN-α1 against two strains of BVD virus, two strains of IBR virus, one strain of PR virus, and the SF4 strain of bovine PI3 virus is presented in Fig. 2. There was an obvious dose-protective Bo IFN-α1 response in the cell culture trials against all virus strains.

As evidenced in replicate tests, the Singer (r = 4) and NADL (r = 6) strains of BVD virus were equally and markedly susceptible to Bo IFN-α1 in the bovine turbinate cell culture system. There was some biological activity against these two strains at a concentration of 10 U/ml with approximately log₁₀ TCID₅₀ of the NADL strain and log₁₀ TCID₅₀ of the Singer strain being inactivated. With 10-fold increases of Bo IFN-α1, a corresponding amount of virus was inactivated. The greatest activity occurred at a Bo IFN-α1 concentration of 10⁴ U/ml with inactivation of log₂ TCID₅₀ of the Singer strain and log₆ TCID₅₀ of the NADL strain.

Bo IFN-α1 had a protective effect against PR virus as demonstrated in six replicate tests. At a concentration of 10 U/ml, approximately log₁₀ TCID₅₀ of virus was inactivated. At concentrations of 10³ and 10⁴ U/ml, there was a slight increase in viral inactivity, and at 10⁵ U/ml, approximately log₁₀ TCID₅₀ of virus was inactivated.

Bo IFN-α1 also showed biological activity against the SF strain of PI3 virus. The order of viral inactivity in six replicate tests was in essence 1 log lower than that observed with PR virus, as a Bo IFN-α1 concentration of 10⁴ U/ml inhibited slightly more than 2 log of PI3 virus.

The Bo IFN-α1 had the least inhibitory effect on two strains of IBR virus at Bo IFN-α1 levels of 10⁴ U/ml. In seven replicate tests with the Cornell strain, 10⁴ U of Bo IFN-α1 per ml inhibited slightly more than 1 log of virus. In three replicate tests with the Cooper strain, the same con-

![FIG. 1. Block titration method, using PI3 data from one replicate test.](image-url)
The availability of purified preparations of bacteria-derived bovine interferons affords investigators an opportunity to study the effects of various classes of bovine interferon produced by recombinant technology both in vitro and in vivo. The production of appreciable amounts of highly concentrated, relatively pure, stable interferons has permitted investigators to design experiments heretofore impossible.

In our search of the literature, we were unable to locate any study where bacteria-derived Bo IFN-α11 was tested for its in vitro protective effect against various bovine viruses. The use of the block titration configuration with appropriate virus and cell controls in a sufficient number of replicate tests provided an excellent method to analyze the data by statistical means. Although it is common practice to pretreat cell cultures with interferon overnight, we decided to analyze protection against bovine viruses by pretreatment with Bo IFN-α11 followed immediately by introduction of virus. As the data indicate, there was marked viral inhibition against two strains of BVD virus; thus, it is a sensitive test procedure. There was no evidence of additive protection by interference when Bo IFN-α11 was introduced into our system 2 h in advance of BVD, IBR, or PI3 viruses.

In the case of two economically important viruses, BVD and IBR, two different strains were employed in the tests. Although the Singer and Holmes strains of BVD virus cross-protect in reciprocal cattle challenge tests, there is a considerable titer difference in the reciprocal serum neutralization test. Thus, representatives of two serological groups for this virus were evaluated. The Bo IFN-α11 provided a marked protective effect against each strain of BVD virus in our bovine turbinate cell culture test system. At the other end of the protective spectrum, both the Cornell and Cooper strains of IBR virus were affected only to a minor degree by the Bo IFN-α11. Bo IFN-α11 had a greater protective effect against PI3 virus that was slightly greater than the observable effect on IBR virus. These in vitro experiments provide encouragement to perform experimental and field trials to evaluate the usefulness of Bo IFN-α11 in the control of these virus diseases in cattle. It also would be interesting to combine some other bovine interferon classes with Bo IFN-α11 to ascertain whether higher levels of protection can be achieved in in vitro and in vivo experiments.

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LITERATURE CITED