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Antiviral Activity in Interferon-Treated Bovine Tracheal Organ Cultures

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The effect of bovine interferon on the replication of infectious bovine rhinotracheitis virus and vesicular stomatitis virus in bovine tracheal organ cultures was studied. After treatment of tracheal organ cultures with interferon, inhibition of infectious bovine rhinotracheitis virus and vesicular stomatitis virus replication was observed. This tracheal organ system may be useful in determining the in vivo response to interferon for viral infections of the bovine respiratory tract.

Similar to the interferons of other species, bovine interferon (IF) has been investigated to determine its possible role in antiviral resistance, both in vivo and in vitro. Cattle as well as cell and organ cultures of bovine origin, derived from adults or fetuses, are capable of producing IF in response to various inducers (1, 2, 6, 7, 9, 10). Also, monolayer cultures of bovine origin and tracheal organ cultures derived from calves are sensitive to exogenous IF (1, 2, 6, 7, 9). The purpose of the present study was to determine if tracheal organ cultures derived from adult cattle were sensitive to the antiviral effects of interferon when challenged with infectious bovine rhinotracheitis virus (IBRV) or vesicular stomatitis virus (VSV).

Monolayer cultures of Crandell feline kidney cells were obtained from the Cell Culture Laboratory, Naval Biomedical Research Laboratory, Oakland, Calif., and bovine turbinate cells were from the U.S. Department of Agriculture Diagnostic Laboratory, Ames, Iowa. Bovine interferon was prepared from bovine alveolar macrophages (2). Viruses used were IBRV (Colorado I strain) and VSV (Indiana strain) (2). IF activity was assayed by plaque-forming unit reduction in bovine turbinate cultures in 60-mm dishes (Falcon Plastics, Oxnard, Calif.). The challenge virus was VSV. The interferon titer was expressed as the reciprocal of the dilution that inhibited 50% of the viral plaques.

Organ explants were prepared from tracheas of adult cattle and were obtained from a local abattoir (8). Six explants (each approximately 0.5 cm^2) were placed in each dish with the epithelial surface uppermost. Culture medium was Eagle minimum essential medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 25 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid buffer (United States Biochemical Corp., Cleveland, Ohio), 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.), and antibiotics (3). A 5-ml volume of medium was added to each dish. The cultures were incubated at 37°C in a humidified chamber. Interferon, 60 or 30 U, was added to each of three replicate dishes containing tracheal explants for 24 h prior to viral challenge. Virus control cultures (three replicate dishes) received fresh medium without IF. After IF treatment, the medium was removed and the cultures were challenged with VSV (10^3 plaque-forming units per ml) or IBRV (10^3 50% tissue culture infective dose per ml) in 1.0 ml of medium. After adsorption for 1 h at 37°C, the inoculum was removed, and each culture washed with sterile phosphate-buffered saline. The IF-treated cultures were refed with 5.0 ml of medium with IF, and virus control cultures were refed with medium devoid of IF. At 24 h after challenge, the fluids from the three replicate dishes for each treatment were pooled and stored at −70°C until assayed for infectivity. Treated cultures were refed with medium with IF, and virus control cultures were refed with medium free of IF. Culture fluids were collected 48 h after challenge. The IF-treated and virus control cultures for each experiment were derived from the same animal. Virus titers were determined on all samples by plaque assay in Crandell feline kidney cells.

Table 1 shows the effect of bovine IF on IBRV and VSV replication in IF-treated tracheal organ cultures. When either 60 or 30 U was applied to the tracheal explants for 24 h prior to challenge and added to postchallenge medium, the infectivity for IBRV and VSV was reduced when compared to the virus controls for both the 24- and 48-h collections.

The ability of tracheal organ cultures to be protected by IF against virus challenge has been

TABLE 1. *Effect of bovine interferon on IBRV and VSV replication yields in tracheal organ cultures*

Expt	IF applied (U)[a]	Collection (h)	IBRV yield[b]		VSV yield[b]	
			Control	IF treated	Control	IF treated
1	60	24	3.7	1.9	5.9	1.0
		48	6.4	4.2	4.4	—[c]
2	30	24	3.6	0.0	5.6	2.4
		48	6.0	2.9	3.0	—

[a] Interferon applied to cultures 24 h prior to viral challenge and interferon contained in postchallenge medium.

[b] Virus yields, expressed as $\log_{10}$ plaque-forming units per milliliter.

[c] —, No plaques detected in undiluted sample.

documented. In previous studies, calf kidney IF protected tracheal organ cultures against infection with parainfluenza 1 and a bovine rhinovirus (9). Also, human fetal tracheal organ cultures have been shown to be sensitive to the antiviral effects of IF (4).

This report describes the effect of exogenously applied IF on tracheal organ cultures. These data indicate that IBRV and VSV are susceptible to the antiviral action of interferon in tracheal organ explants derived from adult cattle. Thus, these results suggest that IF may function to protect the respiratory tract against viral infections in cattle.

Several viruses representing different virus classifications and possible multiple antigenic types for each virus have been associated with bovine respiratory tract diseases. These viruses include IBRV, adenoviruses, reoviruses, parainfluenza-3 virus, bovine viral diarrhea virus, DN 599 herpesvirus, FTC 2 herpesvirus, bovine respiratory syncytial virus, rhinoviruses, influenza virus, and enteroviruses (5). Of these viruses, IBRV, parainfluenza-3 virus, bovine viral diarrhea virus, and a bovine rhinovirus have been found to be susceptible to the antiviral effects of interferon (6, 9). The results of this study and prior studies indicate that tracheal organ cultures treated with IF and subsequently challenged with viruses may possibly be used to detect additional bovine viruses susceptible to IF.

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