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Susceptibility of Feline Herpesvirus 1 and a Feline Calicivirus to Feline Interferon and Recombinant Human Leukocyte Interferons

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Feline lung monolayer cultures were treated with either a feline interferon (IFN) or one of two recombinant human α-IFNs and then challenged with feline herpesvirus 1 (FHV-1), feline calicivirus (F-9 strain), or vesicular stomatitis virus. Treatment with these IFNs reduced the viral yield for each of these three viruses as compared with that of control cultures. Vesicular stomatitis virus was more sensitive to each IFN than were FHV-1 or feline calicivirus F-9.

Feline herpesvirus 1 (FHV-1) and feline caliciviruses (FCV) are the major feline respiratory disease agents (4). Each is estimated to cause 40 to 45% of feline respiratory illness (10). Vaccines, including attenuated or killed FHV-1 and FCV strains, are available for controlling viral respiratory disease signs in feline infections with these viruses (4). The usefulness of antiviral drugs against FHV-1 and FCV infections in cats appears limited. In vitro ribavirin inhibits replication and cytopathogenicity of FCV strain 255 but has only slight effects on FHV-1 in a feline monolayer culture system (1). Orally administered ribavirin did not affect the clinical course of disease or viral excretions after aerosol exposure to FCV strain 255 (12).

There are few studies on the susceptibility of the common feline viruses to the antiviral action of interferon (IFN). This study reports on the effects of recombinant human IFN and a feline IFN when applied to feline monolayer cultures subsequently challenged with feline viruses.

Feline lung (FL) cells obtained from the American Type Culture Collection, Rockville, Md., were grown in culture medium containing Eagle minimum essential medium (Earle salts base) supplemented with 10% fetal bovine serum and 100 U of penicillin and 100 μg of streptomycin per ml and incubated at 37°C in a humidified CO_2 incubator. Maintenance medium was the same except that 2% fetal bovine serum was used.

FHV-1 C-27 strain (1), FCV F-9 strain (1), and vesicular stomatitis virus (VSV) Indiana strain (7) were grown and processed as described previously (5, 6) and titered by plaque assay in FL cells (5) with titers expressed as PFU/0.1 ml.

Recombinant human IFN, Hu-αA, and the hybrid Hu-αA/D (*Bgl*) were obtained from the Roche Institute of Molecular Biology, Nutley, N.J. (13). The feline IFN used in this study (NDV-FL) was prepared by incubating 2 ml of Newcastle Disease Virus suspension containing 2,560 HA units (8) on a confluent FL monolayer in a 75-cm^2 tissue culture flask. The virus was removed after a 1-h adsorption period; 20 ml of maintenance medium was then added, and the monolayer culture was incubated at 37°C for 48 h. The culture was frozen at −70°C and then thawed, after which the suspension was centrifuged to remove cellular debris. The supernatant fluid was dialyzed against pH 2.0 for 4 days, back dialyzed at pH 7.4 for 24 h, and ultracentrifuged at 105,000 × g for 1 h (7). The dialyzed and centrifuged samples were assayed for IFN as described below.

IFN assays were performed by a plaque reduction method in 24-well tissue culture plates containing confluent FL monolayers with VSV as the challenge virus (6). IFN titers (in units per milliliter) were expressed as the reciprocal of the dilution that reduced the number of VSV plaques by 50%.

Preformed FL monolayers in 24-well tissue culture plates were treated with IFN at 37°C for 18 to 24 h before viral challenge for the yield reduction study for antiviral activity. IFN dilutions were made in maintenance medium, and then 1.0 ml was added to each well (two wells per dilution). Virus controls (not receiving IFN) were included in each experiment. After the 18- to 24-h pretreatment with IFN, the IFN was removed and each virus added in 0.1-ml volumes and the virus adsorbed at 37°C for 1 h. After the 1-h adsorption, wells received 1.0 ml of maintenance medium containing the same IFN dilution as the IFN pretreatment. The cultures were then incubated at 37°C. Due to low titers of available IFN preparations, not all the same IFN concentrations were used. The cultures were then collected at 24 h after viral challenge and frozen at −70°C until assayed for infectivity. Viral infectivity was measured by plaque assay in 24-well plates using FL monolayers (5). Tenfold dilutions were made (undiluted [10^0] was the lowest dilution tested), and 0.1 ml of each dilution was added to the wells (two wells per dilution). After 1 h of viral adsorption, 1.0 ml of overlay medium was added to each well, and the plates were incubated at 37°C. The plates were fixed and stained at 48 to 96 h after inoculation. Viral yields were expressed as PFU per 0.1 ml. If PFU were not evident at 10^0 (undiluted), this was recorded as negative.

FL cells incubated with IFN for 18 to 24 h before challenge had yield reductions for FHV-1, FCV, and VSV in the 24-h collections compared with cultures not receiving IFN (virus controls). Comparisons between different IFN, IFN concentrations, or amounts of viral challenge were not always possible due to the lack of high IFN titers in stock materials. VSV was the most sensitive to the antiviral effects of each IFN tested (Table 1) compared to FHV-1 and FCV-F9. With only 1,000 U of each IFN applied and the largest viral challenge (1,000 PFU), there was greater than a 4 log_{10} PFU reduction due to IFN treatment against VSV.

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TABLE 1. Inhibition of VSV, FHV-1, and FCV-F9 by preparations of feline and human IFN

IFN treatment	IFN concn applied (IFN U/ml)	Viral challenge (PFU)	Reduction in virus yield (log_{10})[a]		
			VSV	FHV-1	FCV-F9
NDV-FL	1,000	1,000	5.0	2.3	3.3
HuIFN-αA	1,000	100	5.6	1.4	2.1
	1,000	1,000	4.7	1.4	1.6
HuIFN-αA/D	10,000	100	6.1	3.1	4.3
	10,000	1,000	5.3	3.1	3.1
	1,000	100	5.6	3.0	3.9
	1,000	1,000	4.8	2.7	3.2

[a] Yield is expressed as PFU/0.1 ml of culture fluids, and the titer represents the mean of three individual experiments. The titers in cultures not receiving IFN were: (i) VSV, 1,000 PFU challenge ($10^{6.1}$) and 100 PFU challenge ($10^{6.1}$); (ii) FHV-1, 1,000 PFU challenge ($10^{4.8}$) and 100 PFU challenge ($10^{3.7}$); and (iii) FCV-F9, 1,000 PFU challenge ($10^{6.4}$) and 100 PFU challenge ($10^{6.3}$).

When the IFN concentration was the highest (10,000 U) and the viral challenge the lowest (100 PFU), the yield reductions were the greatest. This was true for each of the viruses tested. FHV-1 was also sensitive to each IFN (Table 1). There was at least a 1.4 log_{10} PFU reduction when each IFN was used in cultures challenged with FHV-1. The greatest reduction for FHV-1 (>3 log_{10} PFU) occurred with 10,000 U of HuIFN-αA/D with a viral challenge of 100 PFU. FCV-F9 was also sensitive to each IFN (Table 1). There was at least a 1.6 log_{10} PFU reduction for FCV-F9. Similar to that with VSV and FHV-1, the greatest reduction (>4 log_{10} PFU) occurred with 10,000 U of HuIFN-αA/D treatment and a viral challenge of 100 PFU. Of the two recombinant Hu-α IFNs, the hybrid HuIFN-αA/D had greater antiviral effects than HuIFN-αA. At the 1,000 IFN concentration and at both viral challenge concentrations (100 or 1,000 PFU), the viral yields for both FHV-1 and FCV-F9 were reduced to a greater extent by the HuIFN-αA/D treatment compared to the HuIFN-αA treatment.

There are relatively few reports on the activity of IFN against naturally occurring feline viruses. A feline IFN produced by Newcastle disease virus-exposed feline monolayer cultures inhibited feline leukemia virus (FeLV) replication in IFN-treated feline monolayer cultures (14). FeLV was inhibited by the treatment of feline cell lines with Sendai virus-induced crude HuIFN-α (9). Both natural HuIFN-α and several recombinant HuIFN-α, including hybrids, reduced FeLV production by F1F3 cells, a feline fibroblast line chronically infected with FeLV (15). In a recent report, a feline IFN (γ-like) produced by feline lymphocytes treated with *Staphylococcus* enterotoxin A inhibited FCV-F9 but not FHV-1 (3). However, the latter study utilized a plaque reduction assay instead of the yield reduction assay used in the present study. There were also differences in the sources of IFN used in these studies. Thus, different assay systems and IFN sources may explain the variation between our results and those of the previous study (3).

The results of the present study indicate that recombinant human IFN and a feline IFN conferred protection against FHV-1 and FCV, both naturally occurring viral pathogens in the cat. Thus, there may be potential for these IFN as antiviral agents used in prophylaxis or therapy against these infections. The use of such IFN takes on even greater importance since FeLV-infected cats often have FHV-1 or FCV infections (2). FeLV has several immunosuppressive properties including the inhibition of IFN production by feline lymphocytes treated with a mitogen (3). Thus, IFN treatment might protect cats against FeLV infection and FHV-1 and FCV infections in FeLV-immunosuppressed cats.

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