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Inhibition of feline leukemia virus replication by human leukocyte interferon

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The replication of feline leukemia virus (FeLV) is inhibited by treatment of cat cell cultures with crude human leukocyte interferon (HuIFN-α) as evidenced by titration of the infectious progeny. The inhibition can be demonstrated in three different cell lines in which the production of hemagglutinin by encephalomyocarditis (EMC) virus, and plaque formation by vesicular stomatitis virus (VSV) are also inhibited by the HuIFN-α. The dose dependency of the inhibition of EMC virus by the HuIFN-α is similar to that obtained with feline interferon in each of the three cell lines. VSV and EMC virus are less than 10 times more sensitive than FeLV to the inhibitory action of HuIFN-α if responses to a single interferon treatment are compared for each of the viruses tested in the most sensitive cell line, FEA. The interferon effect on FeLV is more pronounced when it is added within one day after the inoculation of the cells rather than applied before cell infection. The induction of focus formation by FeLV can also be inhibited by HuIFN-α in cat cells (CCC-81) which contain the murine sarcoma virus genome.

feline leukemia virus; human interferon; cat cell lines; FeLV; interferon; leukemia virus

Experimental section

Feline leukemia virus (FeLV) is a horizontally transmitted virus of outbred cat species which may induce a variety of diseases including several types of neoplasms [7]. For these reasons the virus provides a useful model for the study of inducible malignancies which resemble those occurring in man, and for evaluation of potential modes of therapy, such as interferon administration. One study suggested that this retrovirus might be much less sensitive to the antiviral effects of interferon than other types of viruses [9]. The interferon effect on FeLV was determined by measuring the reduction in accumulation of virus-specific antigens within infected cells, which may not be the most sensitive method of detecting the inhibition of the viral replication processes by interferon and predicting the possible consequence of interferon treatment on the progress of the FeLV infectious process in vivo. The present work

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was initiated to evaluate the effect of interferon on the production of infectious virus after inoculation of cat cells with FeLV.

Sensitivity of feline cells to interferon

Three cat cell lines (CCC-81 [1], FEA [6], and FLF [2]) were found to be sensitive to the antiviral action of virus-induced [5] feline interferons produced by fibroblastoid (CCC-81) or lymphoblastoid (F422 [8], and FL74 [10]) cell lines and to Sendai virus induced crude human leukocyte interferon (HuIFN-α, from Dr. Cantell) as measured by reduction in yield of EMC virus hemagglutinin (EMC HA-YR) [3]. The dose response patterns for the two species of interferon were similar in a given cell line. FEA and FLF cells were similar to each other in their sensitivity, and were about 10 times more sensitive than the CCC-81 cell line in their response to interferon of either species. HuIFN-α and samples of interferon from three cat cell lines were titrated in three lines of cat cells using the EMC HA-YR method. HuIFN-α inhibited virus replication in cat cell cultures about 10 times more than any of the feline interferon samples tested. Three tests of a laboratory reference crude HuIFN-α with the FEA cell line using the EMC HA-YR method measured 85 000 U/ml, which is comparable to the sensitivity of the A549 human cell line using the same interferon. Previously the A549 cell line was found to measure 1.8 times the assigned activity of 20 000 reference U/ml for the human leukocyte standard G-023-901-527, National Institutes of Health; this cell line also detected a geometric mean titer of 55 000 U/ml for the laboratory reference HuIFN-α in 84 determinations [4].

Inhibition of FeLV by interferon

The sensitivity of FeLV (Subgroup A of feline leukemia virus produced by F422 cells [8]) to inhibition by interferon was tested in the three cat cell lines, along with VSV and EMC viruses (Table 1). A comparison of their responses is made by determining a titer for a single sample of HuIFN-α using inhibition of VSV plaque formation, yield of infectious FeLV or of EMC HA in each of the three different cell lines. Yields of FeLV were determined by assay of focus formation resulting from the rescue of the murine sarcoma virus (MSV) genome from transformed CCC-81 cells [1]; titers are expressed as focus-forming units/ml (ffu/ml). EMC virus and VSV were 3 to 50 times more sensitive than FeLV to inhibition by HuIFN-α depending on the cell and interferon treatment tested. A single application of interferon for one day before virus inoculation was used with VSV and EMC viruses; interferon was applied the day before inoculation and again after the attachment period with FeLV. In general, exposure of cultures to interferon was terminated when the medium was removed prior to inoculation with virus or to take samples for determining virus yield. In the FEA and FLF cells, EMC was about 7 to 13 times more sensitive than FeLV, and VSV was about 3 to 8 times more sensitive than FeLV.

One or two treatments of CCC-81 cells with HuIFN-α within two days after inoculation with FeLV (day 0) reduced the number of foci of transformed cells detectable two weeks after virus inoculation to 30 to 60% of the number observed in untreated infected plates (average count of 60 foci/plate).

TABLE 1

Comparison of interferon sensitivities of three viruses in three cat cell lines by determining the titer of a single crude human leukocyte interferon sample

Cell line	Interferon titer, single observation or geometric mean U/ml[a] (number of titrations)			
	VSV	FeLV[b]		EMC
		Day 2	Day 3	
CCC-81	4 400 (2)	NT	650	11 000 (10)
FLF	51 000 (2)	6 800	10 000	60 000 (2)
FEA	33 500 (2)	12 000	6 400	85 000 (3)

[a] The titer of the interferon is expressed as the reciprocal of the dilution which reduces the yield of EMC virus hemagglutinin or FeLV focus-forming units by 0.5 log, or reduces the number of VSV plaques to 50% relative to that obtained with the untreated control culture.

[b] Virus yields were measured in fluids removed either day 2 or day 3 after inoculation. Interferon was applied to the cells 1 day before, and immediately after the attachment period for the FeLV.

The inhibition of FeLV replication by interferon treatment was tested in all three cells using a variety of schedules for the addition of HuIFN-α, which was then left in the culture medium until addition of virus, or removal of sample fluid (Table 2). Instead of reporting the yield of FeLV as a measure of relative sensitivity of FeLV to the antiviral effect of interferon, the comparison of FeLV response in the different cells under various conditions was made by determining titers of the HuIFN-α sample used for all treatments. No correction factor was used to account for the variable number of interferon additions made in the various treatment schedules, because the time in the growth cycle of the virus in a particular cell at which interferon is added appeared to be more important than the number of additions made. There was little inhibition of FeLV by the HuIFN-α in CCC-81; the apparent interferon titers ranged from 370 to 800 U/ml, when cells were treated 2 to 4 times with HuIFN-α, beginning one day before inoculation of FeLV. Both FEA and FLF cells were more sensitive to interferon, and apparent titers of the HuIFN-α ranged from 190 to 15 000 U/ml. There was an obvious increase in the antiviral effect, if the addition of interferon was delayed until after the inoculation of cells with FeLV; the greatest level of inhibition of infectious virus yield occurred about 2 to 3 days after the last addition of interferon. Apparent titers of HuIFN-α ranged from 5 200 to 15 000 U/ml when interferon was added after viral inoculation and virus yield was measured on day 3 after inoculation. The inhibitory effect of two treatments appeared to be slightly greater than a single treatment of FLF cells. No striking increase in inhibitory effect was obtained by a second treatment of FEA cells either one day before or one day after the application of HuIFN-α at the conclusion of the inoculation process.

The replication of FeLV in CCC-81 cells is rather slow, with a plateau of infectious virus reached about 3 to 4 days after inoculation of a very sparse monolayer of rapidly growing cells with approximately 0.1 ffu/cell (Fig. 1). More rapid replication of FeLV

TABLE 2

Inhibition of FeLV replication in cat cells by human leukocyte interferon

Cell line	Interferon treatments given on days:				Antiviral effect of interferon (U/ml)[a] measured at the indicated time after FeLV inoculation (day 0)	
	-1	0	+1	+2	Day 2	Day 3
CCC-81	+	+			NT	650
	+	+	+		NT	800
	+		+		NT	370
	+	+	+	+	NT	560
FLF	+				440	190
	+	+			6 800	10 000
		+			3 600	5 200
		+	+		8 400	9 500
FEA Exp. I	+				2 000	270
	+	+			12 000	6 400
		+			12 000	6 700
Exp. II		+			2 500	5 700
		+	+		4 400	6 100
			+		1 900	15 000

[a] The titer of interferon is expressed as the reciprocal of the dilution which reduced the yield of FeLV focus-forming units by 0.5 $\log_{10}$. No adjustment was introduced for the calculation of titers in the groups which received multiple additions of interferon.

was observed in the FEA and FLF cell cultures, with a plateau level of virus yield obtained at about 2 days after inoculation for both cells. Data for FLF cells were similar to results with FEA cells (data not shown). Similar amounts of virus were measured in culture fluids from a particular cell type whether or not the cell layers were washed immediately after inoculation with FeLV; the yield from FLF cells was about 0.5 $\log_{10}$ higher than from FEA cells. Both of the cells supporting more rapid viral replication were more sensitive to the antiviral action of HuIFN-α measured by FeLV response (Table 2) as well as by the response of VSV and EMC viruses (Table 1). In view of these observations, it is possible that the apparent interferon sensitivity of FeLV replication in CCC-81 cells might be greater if the virus samples were taken as the virus plateau was approached, e.g. on day 4 or 5 (Table 2).

Figure 2 shows the dose-response relationship of interferon inhibition of FeLV. The change in yield of FeLV (log ffu) was roughly proportional to the magnitude of change in the interferon dilution in all three cell lines. Endpoints were interpolated at the 0.5 $\log_{10}$ reduction in yield of FeLV on lines projected by linear regression using three or more adjacent points which produced the best correlation with linearity (as indicated by the extent of the line drawn).

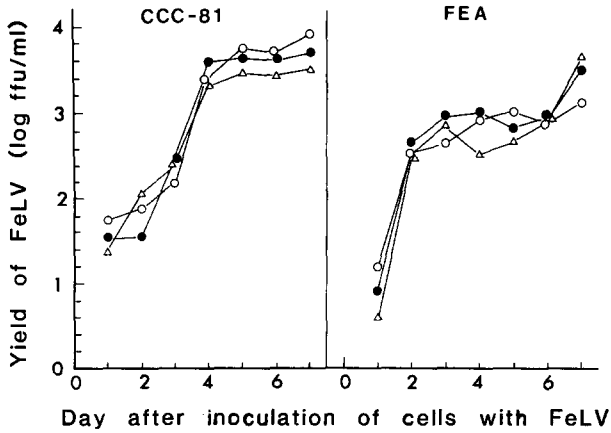

Fig. 1. Replication of FeLV in feline cell lines. Cell monolayers were initiated with 1.5 ml of 2.5 $\times 10^5$ cells/ml in a 3-cm well. The next day growth medium was removed and FeLV (4.7 $\log_{10}$ ffu) was inoculated in 0.5 ml of medium containing polybrene (8 μg/ml); after 1 h adsorption, 1.5 ml of medium were added (○) or the inoculum was removed, cells were washed and 2 ml of medium were added (●, △). Samples collected from the unwashed cultures immediately after addition of medium had about 3.6 log ffu/ml; a sample from a washed culture of CCC-81 cells had 1.9 log ffu/ml). Samples were taken at daily intervals by pooling 0.5 ml aliquots from duplicate cultures; either a 1-ml sample (○, ●) or the entire supernatant fluid was removed (△), and fresh medium was added to replace that removed.

We conclude that interferon decreases the yield of infectious FeLV from infected cat cells. The antiviral activity of HuIFN-α is also expressed as a reduction in direct FeLV induced focus formation resulting from the rescue of the MSV genome from the

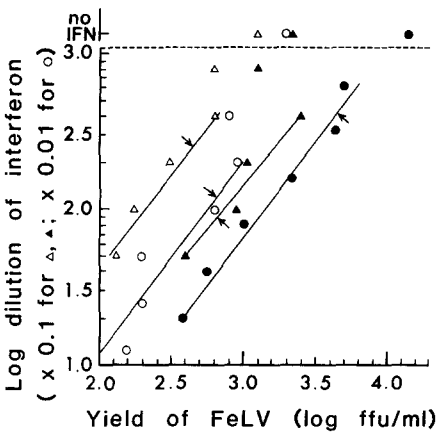

Fig. 2. Inhibition of FeLV replication by human leukocyte interferon in feline cell lines. Interferon was added to obtain the indicated dilution in the culture medium one day before and immediately after monolayer inoculation for FEA (○) and CCC-81 (●) cells; but only after the inoculation of cell monolayers for FLF (▲) and FEA (△) cells. Interferon was not removed from the cultures. Virus yields were measured in samples of supernatant fluids collected on day 2 from FEA and FLF cells, and on day 4 from CCC-81 cells. Yields obtained in cultures not treated with interferon are shown at the top. The arrows (→) indicate the endpoint, or interferon titer (U/ml), at 0.5 log below the virus yield in untreated controls.

transformed CCC-81 cell line. The fact that this process can be inhibited as late as 1 to 2 days after inoculation of the cell layer suggests that either interferon may inhibit the induction of pseudotype MSV replication and the associated cellular changes may be inhibited to some extent, or that the continued presence of interferon may inhibit secondary FeLV production which would otherwise induce some foci. But the latter possibility is not supported by observed titration patterns [1].

The antiviral action of HuIFN-α on FeLV replication is greatest when it is applied after inoculation of virus; as evidenced in virus production measured 2 to 4 days after inoculation of cells with virus.

Although optimal conditions have not been established for determining the relative interferon sensitivity of the three viruses studied, comparisons which can be made of results obtained with single interferon treatments in the FEA cell line suggest that EMC and VSV are less than 10-fold more sensitive than FeLV. Therefore, FeLV resembles most viruses in being subject to control by interferon. The interferon sensitivity observed for FeLV in these experiments appears to be greater than stated in a previous report that VSV is 1 000 times as sensitive as FeLV [9]. The different findings may depend partly upon both the cell cultures used, and the methods for detection of virus replication in the two systems. The use of crude human interferon in this study could not account for the relative responses of the three viruses since the effect of the interferon is upon the cell, not upon the virus, and the inhibitory effect on all three viruses was detected in three different cell lines with somewhat different sensitivities to either human or feline interferons.

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