Heterologous Activity of Monkey Interferons (37650)

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From the time of its early characterization, the action of interferon has been observed to be genus or family specific (1). Interferon generally exerts its strongest antiviral activity on homologous cells, considerably less activity on cells of closely related species (2–4) and rarely manifests strong activity on distantly related genera (5). Examples of the latter case are the protection of rabbit cells (6, 7) and rat cells (7) by certain types of human interferon. During the development of a method to determine the genus of origin of cell cultures (8), another major exception has been discovered. Monkey interferon protected rat cells and monkey cells while rat interferon protected only rat cells. The present paper documents this observation and characterizes the protective properties of monkey interferon on rat cells as being the same as those of interferon.

Materials and Methods. Cells. BN strain rat embryo cells (BNRE), African Green monkey kidney (AGMK) primary cultures and continuous Fischer rat cultures, F1706, F1853, and F2643 were obtained from Microbiological Associates, Inc. (Bethesda, Md.). Cells were grown on Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 2 mmole glutamine, 1 mmole non-essential amino acids, 100 units penicillin and 100 μg/ml streptomycin. Cells were maintained on the same medium but with 2% FBS.

Interferon preparation. Monkey interferon was prepared in primary AGMK monolayers in 32 oz bottles. Viruses used to induce interferon were Chikungunya virus ($10^{7.3}$ PFU/ml), Kunz strain of influenza A ($10^{6.0}$ EID_{50}/ml) and Newcastle Disease virus (NDV) ($10^{7.0}$ PFU/ml). In all cases, 5 ml of the virus dilution containing the indicated concentration of virus was absorbed to the AGMK cells for 1 hr at 37°. The virus was then decanted and the cells fed with maintenance medium. After 24 hr at 37° the supernatants were harvested and treated at pH 2 at 4° overnight for Chikungunya virus and Kunz virus induced interferon and for 5 days for NDV induced interferons.

Interferon assay. Dilutions of the interferons were made in maintenance medium and allowed to react with the test cells for 24 hr at 37°. After this time the tubes were decanted, washed once with Earle's balanced salt solution (EBSS), and then 0.2 ml of the challenge virus was applied for 1 hr at 37°. Tubes were then washed 3 times with 2 ml of EBSS and refed with 0.5 ml of maintenance medium without serum when Sindbis virus was the challenge virus or with 1 ml of maintenance medium when VSV was the challenge virus. Cytopathogenic effect (CPE) was read at 24 hr. To determine inhibition of yield of Sindbis virus hemagglutinin (HA), duplicate tubes were frozen, thawed once and pooled. HA was done according to the method of Clarke and Casals (9).

In the determination of temperature stability, the interferon was incubated for 1 hr in a water bath at the appropriate temperature. The heat-treated interferon was assayed as above.

To determine the effect of trypsin, 200 μg/ml recrystallized trypsin or diluent was added to 100 units of interferon, incubated at 37° for 1 hr, after which time the trypsin was inactivated by 400 μg/ml soybean tryp-

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TABLE I. Protection of Rat Cells Against VSV and Sindbis Virus by Rat or Monkey Interferon.

	VSV CPE[a]			Sindbis virus				
				CPE			Reduction of HA yield (log_{10})[b]	
	Interferons			Interferons			Interferons	
Rat cells	Rat[c]	Monkey[d]	None	Rat	Monkey	None	Rat	Monkey
F1853 p43	1[e]	2+	3	1+	1+	3+	—[f]	—
F1853 p67	1+	1+	3+	1	1	3	—	—
F1706 p111	—	—	—	—	—		1.5	1.5
F2643 p101	—	—	—	±	1+	2	2.1	0.3
BNRE p0	1	1	4	1	2	4	—	—

[a] CPE: 4 = 100% destruction of cell monolayer, 3 = 75% destruction, 2 = 50% destruction, 1 = 25% destruction, 0 = Cell monolayer intact, and + = Intermediate value.

[b] 0.5 log reduction in virus yield is significant.

[c] 25 units/ml.

[d] 5 units/ml.

[e] Underlined figures are significantly different from controls.

[f] Not done.

sin inhibitor and added to the cell cultures.

The metabolic inhibitors actinomycin D and cycloheximide were mixed with selected concentrations of interferon and reacted with the cell cultures for 6 hr, then washed off with EBSS and the cells challenged with virus. On AGMK cells, 200 μg of Actinomycin D and 10 μg cycloheximide were used. On rat cells 2 μg/ml actinomycin D and 10 μg/ml cycloheximide were used. These concentrations of actinomycin D and cycloheximide had previously been shown to inhibit cellular RNA and protein synthesis respectively by more than 90% (10).

Results. Protection of rat cells of varied strain and passage. The observation of cross-protection of continuous passage Fischer rat cells by monkey interferon (8) has been extended to include both primary and secondary rat cultures as well as cells of different rat strains (Table I). Five units of monkey interferon protected rat cells somewhat less effectively than did 25 units of rat interferon. The transformed Fisher rat line (F2643) which is less sensitive to rat interferon than the other rat lines (8) was protected by 25 or 5 units of rat interferon and by 50 but not 5 units of monkey interferon.

Characterization of the antiviral activity. Several tests were performed to compare the antiviral effects of these preparations with those of interferon.

Thermal inactivation. Figure 1 displays the parallelism in heat inactivation of protection of monkey and rat cells by a Kunz virus preparation induced in monkey cells. The parallel kinetics of inactivation suggests identity of protective substance in both systems. The displacement of the curve indicated that the preparation was somewhat more active in homologous monkey cells than in rat cells.

Trypsin. As measured by both CPE and by reduction in HA titer (Table II), trypsin almost completely eliminated the antiviral factor when tested in either monkey or rat cells, suggesting that the inhibitor is protein in nature.

Metabolic inhibitors. Actinomycin D, a potent inhibitor of mRNA synthesis, completely blocked the antiviral effect (Table III). On the other hand, full antiviral activity was maintained in both cell species when treated with cycloheximide, a translational inhibitor.

The properties of the antiviral activity are summarized in Table IV. In addition to the characteristics discussed above, the preparation was stable at pH 2 for 5 days. It was

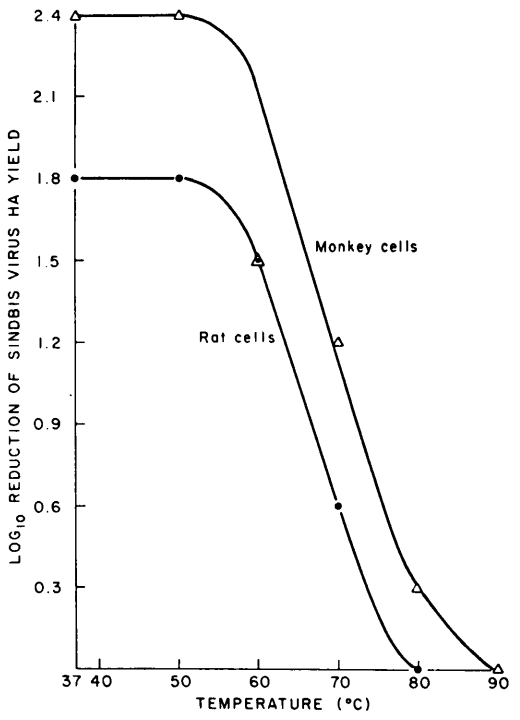

FIG. 1. Heat inactivation of monkey interferon as assayed on monkey and rat cells.

effective against both a type A arbovirus (Sindbis virus) and a rhabdovirus (VSV). The antiviral activity was cell bound since it was not removed by repeated washing. It is therefore clear that the action and properties of this factor were similar in both monkey and rat cells indicating that the same material is acting in both cells. The similarities to known interferons will be discussed below.

Comparative sensitivity of monkey and rat cells to monkey interferons of different origin. Monkey interferons induced by three different viruses all confer protection on rat cells as well as monkey cells (Table V). In each case, however, monkey cells were afforded greater protection than rat cells. It appears that monkey cells are approximately four times more sensitive to their homologous interferons than are rat cells.

Discussion. The present findings indicate another major exception to the "species", perhaps more accurately "genus", specificity of interferon. Monkey interferon was observed to manifest substantial antiviral activity on rat cells. The antiviral activity of monkey interferon on rat cells displayed the major criteria for interferon induced inhibition of virus replication (11). The apparent inability of the protein synthesis inhibitor, cycloheximide, to prevent the induction of antiviral activity by interferon in both monkey and rat cells is in agreement with previous observations that after removal of cycloheximide, preformed messenger RNA for the hypothesized antiviral protein is rapidly translated and results in the development of the antiviral state (12). The cross activity of monkey interferon onto rat cells seems fairly general in that monkey interferon (induced in culture by several inducers) was active in a variety of primary and continuous rat cultures.

It is of interest that the major heterologous crossings of interferon have been the activity of primate interferons on rodent-like species (6, 7). This may reflect a phylogenetic relationship between primates and rodents since both are thought to be descended from primitive insectivores (13). A test of this possible relationship would be to determine the heterologous activity of primate and rodent interferons on insectivore cells (*e.g.*, tree

TABLE II. Effect of Trypsin on the Protective Action of Monkey Interferon on Rat and Monkey Cells.

Treatment	AGMK cells		Rat cells	
	CPE	Reduction of Sindbis virus HA yield (log_{10})	CPE	Reduction of Sindbis virus HA yield (log_{10})
Virus control	3	—	3+	—
Interferon	0	>2.1	2	>2.1
Interferon + trypsin	4	0.3	4	0.6
Trypsin control	4	0.3	4	0

TABLE III. Effect of Metabolic Inhibitors on the Activity of Monkey Interferons on Rat and Monkey Cells.

	% Reduction of Sindbis virus HA yield			
	AGMK cells		Rat cells	
	Kunz interferon	Chikungunya interferon	Kunz interferon	Chikungunya interferon
Actinomycin D[a] + interferon	75[b]	50[b]	0[b]	0[b]
Cycloheximide[c] + interferon	99	>88	75	75
Interferon	>99	>88	75	75

[a] 200 μg/ml on AGMK cells and 2 μg/ml on rat cells.
[b] Differences from interferon treated cells significant at $p < 0.05$.
[c] 10 μg/ml.

shrews). Another possible explanation is that major heterologous activity of interferons is more common than currently believed but has only been shown for the rodents and primates because they have been the most extensively studied groups of animals. It is important to note that the cross activity of interferon is mainly in one direction—*i.e.*, primate interferon acting on rodent cells and not the reverse (8).

Summary. Interferon produced by cells of a given genus generally exerts antiviral activity best in cells of the same genus, partially in cells of closely related genera and rarely in distantly related genera. During a study of cell typing by interferons, it was observed that a preparation of monkey interferon as well as rat interferon strongly protected rat cells against vesicular stomatitis virus and Sindbis virus while human, mouse, and rabbit interferons provided no protection. Monkey interferons stimulated by several different viruses in African green monkey kidney cells protected several strains of rat cells. This heterologous antiviral activity was found to be (1) stable at pH 2, (2) inactivated by trypsin, (3) cell bound, (4) inhibited by actinomycin D but not by cycloheximide, (5) active against unrelated viruses, and (6) inactive on mouse and rabbit cells. The thermal lability curves were parallel when antiviral activity was assayed in homologous monkey cells or heterologous rat cells. The properties of the heterologously active antiviral material coincided with those of interferon.

TABLE IV. Summary of Properties of Monkey Interferon in Rat and Monkey Cells.

Property	Test cell	
	AGMK	Rat
Temperature of 50% inactivation	~60°	~60°
Trypsin inactivation	yes	yes
Action blocked by actinomycin D	yes	yes
Action blocked by cycloheximide	no	no
pH stability	yes	yes
Cell bound effect	yes	yes
Effective against unrelated viruses	yes	yes

TABLE V. Comparative Titer of 3 Different Monkey Interferons in Monkey and Rat Cells.

Interferon inducer	Interferon titer (units/ml)	
	AGMK cells	Rat cells
NDV	500	80
Chikungunya	350	80
Kunz	250	150

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