Activity of Mouse Interferon in Human Cells (35796)

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Interferon action is usually species specific (1a). To be active in human cells, interferons must be derived either from human or from monkey cells (2–4). However, crude mouse brain interferon was reported to be active in HeLa cells (5). Other investigators did not find activity of mouse interferon in human cells (6–8). In our experiments with crude and purified mouse L-cell interferon, a very low but consistent activity was noticed in human amnion cells. To prove that this heterologous activity was interferon specific, some of its properties were studied in more detail.

Materials and Methods. Mouse interferon was induced in L-929 cell monolayers with Newcastle disease virus strain Victoria (NDV) in serum-free medium at a multiplicity of 10 plaque-forming units/cell by the method of Younger *et al.* (9). Crude interferon was kept at pH 2 and 4° for 3 to 5 days to inactivate virus. Interferon was purified by concentration with 0.02 *M* zinc acetate at pH 7.2 (10), dissolving the precipitate in cold 0.1 *M* HCl and adsorption of interferon on SE-Sephadex C-25 at pH 2.5. After washing at pH 6.0 with 0.1 *M* phosphate buffer containing 0.005 *M* EDTA, interferon was eluted at pH 8.0 with 0.1 *M* phosphate buffer containing 1 *M* KCl. Eluates were concentrated to a small volume with aquacide I (Calbiochem) and kept at −20°. Mouse interferon was further purified by filtration over DEAE-cellulose in 0.01 *M* phosphate, pH 7.5 (11). The filtrate was adjusted to pH 5.5 and interferon was absorbed onto CM-Sephadex C-25 (7). The column was washed with 0.1 *M* phosphate, pH 6.0, and interferon was eluted with 0.1 *M* phosphate, pH 8.0 (12). The eluate was concentrated to a small volume and kept at −20°.

Human leukocyte interferon was prepared with Sendai virus by the method of Lee (13). It was kept at pH 2 and 4° for 2 days, neutralized, and ultracentrifuged at 75,000*g* for 90 min.

Gel filtration. A column 85 × 2.5 cm of Sephadex G-100 (140–400 mesh) was used. Elution buffer was phosphate buffered physiological saline, pH 7.3 (PBS). Chromatography was performed at 4° at a flow rate of 10 ml/hr. Dextran blue 2000, bovine serum albumin, ovalbumin, and horse heart cytochrome *c* were used for molecular weight calibration.

Interferon titers were determined by the plaque reduction method using monolayers of mouse L-929 cells (Flow Laboratories, Irvine, Scotland), the U-line of human amnion cells (U-cells, kindly supplied by K. Cantell, University of Helsinki) and human foreskin fibroblasts (HF) in 60-mm plastic petri dishes (Falcon). Foreskins were from children up to 4 years of age and cell cultures used at passage levels 4 to 10. Maintenance medium was Eagle's MEM supplemented with 2% inactivated calf serum and 2.95 g/liter of Difco tryptose phosphate. Monolayers were treated for 24 hr with 1 ml of interferon dilutions and interferon removed before challenge. Vesicular stomatitis virus strain Indiana (VSV) was used for challenge. Plaquing medium was maintenance medium with 0.8% Methocel (Mc 4000 cP, Fluka, Buchs, Switzerland). The U-cell monolayers were alternatively also challenged with arbovirus A strain Middelburg (VM). Plaques were formed in maintenance medium with

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TABLE I. Interferon Titers[a] Obtained in Different Test Systems.

Test system	Mouse interferon			Human interferon
	Crude 12,000 U/mg[b]	Purified SE-Sephadex 440,000 U/mg	Final product 3,360,000 U/mg	Crude 180 U/mg[c]
L-929/VSV	1500	1,130,000	2,040,000	5
U-cells/VM	30	14,000	73,000	70
U-cells/VSV	40	1500	Not done	20
HF/VSV	<20	250	Not done	320

[a] Dilutions resulting in 50% plaque reduction.
[b] Specific activities determined in L-929/VSV test.
[c] Specific activities determined in HF/VSV test.

0.75% agarose (L'Indistrie Biologique Francaise, S.A., Gennevilliers, France). Cell monolayers were stained with gentian violet 48 hr after challenge. Agarose was removed before staining after fixation of monolayers with 5% trichloroacetic acid.

Results. Table I shows the titers of crude and purified L-cell interferon in mouse and human cells. For comparison, the results obtained with crude human leukocyte interferon are also given. In crude mouse interferon preparations, a very low activity could be found in human U-cells only and no activity was detected in HF-cells. In the purified samples, a definite activity was always observed in the U-cell/Middelburg virus (VM) test system. Heterologous titers varied, but were usually 1–5% of those found in the homologous system. When U-cells were challenged with VSV, the heterologous titers of mouse interferon were found to be lower than those observed after challenge with Middleburg virus (VM). The test system human foreskin fibroblasts (HF)/VSV, which has been used by others to quantitate human interferon activity (14, 15), was the least sensitive for mouse L-cell interferon. This is in agreement with the results from Merigan *et al.* (7), who could not demonstrate activity of mouse L-cell interferon in this test system. The low sensitivity of the HF/VSV test system for the active component of mouse interferon is in contrast to that for human leukocyte interferon. Preparations of human leukocyte interferon always gave higher titers in this system when compared to those obtained from the U-cell/VSV or U-cell/VM test systems.

TABLE II. Influence of Heat on Mouse Interferon Activity.[a]

	Interferon titers in test system	
	U-cells/VM	L-929/VSV
Not heated	3400	66,000
1 hr, 56°	1800	6000
2 hr, 56°	450	900

[a] Heated at 0.51 mg of protein/ml in PBS.

Table II shows the influence of heat on the activity of SE-Sephadex purified mouse interferon in mouse and human cells. Both activities were sensitive to heating at 56°. Whereas the homologous activity could be almost completely destroyed within 2 hr, the heterologous activity in U-cells seemed to be somewhat more resistant to heating.

TABLE III. Influence of Time of Incubation Before Virus Challenge on Antiviral Activity of Mouse Interferon.

Time at 37° (hr)	Interferon titers in test system	
	U-cells/VM	L-929/VSV
2	450	8000
6	1000	23,000
24	2800	140,000

Table III demonstrates the influence of time of incubation before virus challenge on antiviral activity of SE-Sephadex purified mouse interferon in mouse and human cells. In both cell systems, antiviral activities in-

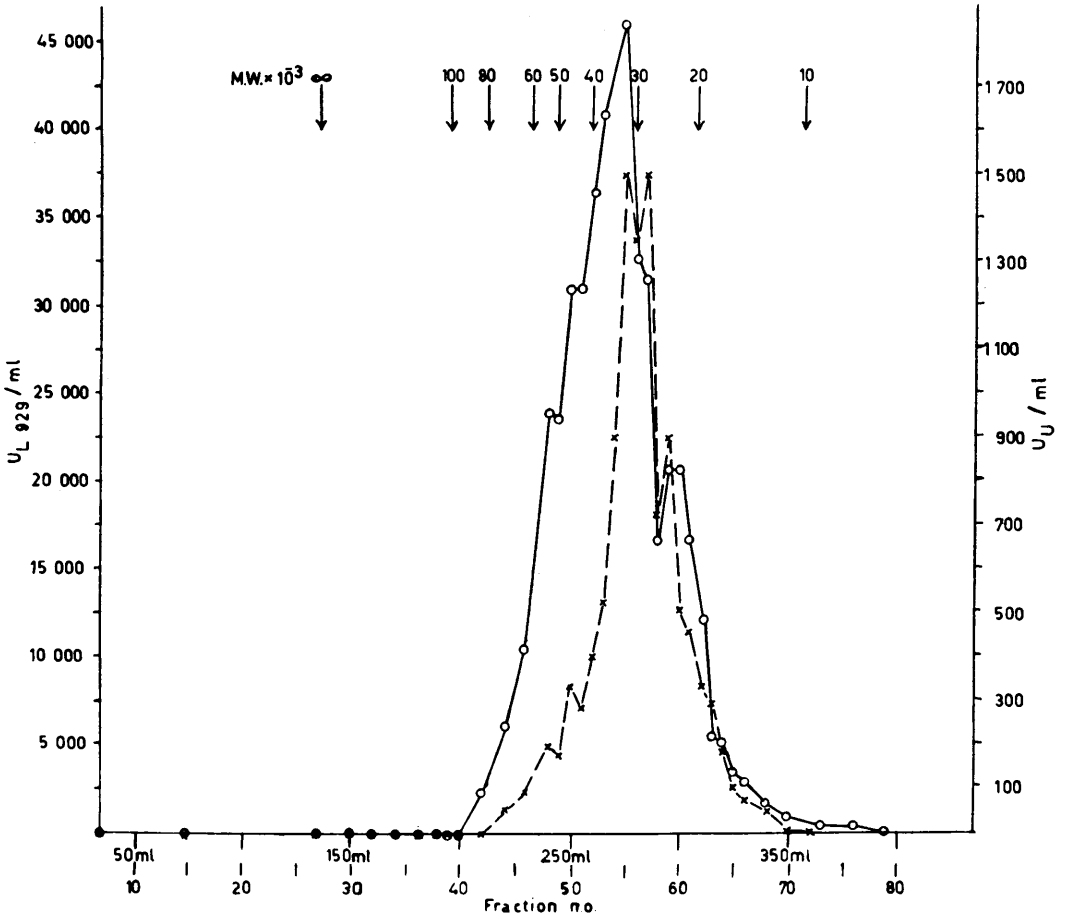

FIG. 1. Gel filtration of mouse L-cell interferon on Sephadex G-100 (4,500,000 units, 23 mg of protein): (abscissa) ml column eluate (fraction number); (ordinate, right) units/ml obtained in human U-cells challenged with Middleburg virus (X- -); (left) units/ml in mouse L-929 cells challenged with vesicular stomatitis virus (O—).

creased with longer exposure time of cells to interferon before challenge. Since it is known that Newcastle disease virus (NDV), which was used as interferon inducer, is only slowly inactivated at pH 2, there seemed to be a possibility that the heterologous activity observed was due to incompletely inactivated NDV still present in even the highly purified mouse interferon preparations. Therefore, the influence of several different NDV-antisera on antiviral activity was studied. All antisera tested showed a strong neutralizing effect when included in a NDV plaque-test on chick embryo fibroblast monolayers. However, neither the homologous nor the heterologous activity in U-cells of purified mouse interferon was influenced when preparations were incubated with NDV antisera prior to testing. The antiviral activity in human U-cells was therefore not due to incompletely inactivated NDV contaminating our interferon preparations.

Figure 1 gives the result of a gel filtration experiment using mouse interferon purified by SE-Sephadex. As expected, mouse interferon was eluted as a peak corresponding to a molecular weight between 30,000 and 40,000 daltons (1b) when tested in mouse cells. When eluates were tested in the U-cell/VM system, a peak in the same molecular weight region was obtained. This peak was slightly displaced to the low molecular weight side of

the peak obtained in the homologous test system.

Discussion. These results demonstrate that mouse L-cell interferon produced with NDV is to a small extent also active in human amnion cells. This activity is not reduced upon purification. Since the purified material is still not homogeneous, the heterologous activity in human cells must not necessarily be related to the interferon component itself. However, the criteria used in this study to characterize it further could not distinguish it from the interferon component. Even the molecular weight was found to be in the range expected for mouse interferon. It is therefore very tempting to speculate that this activity is in fact due to the mouse interferon component. The displacement of the activity peak towards lower molecular weights in comparison to the homologous activity and the small differences found in heat stability between homologous and heterologous activity might reflect heterogeneity of mouse L-cell interferon.

It is noteworthy that human, rabbit, and mouse interferons seem to share common antigens (16). Furthermore, activity of human interferon in rabbit cells has been demonstrated and a variable proportion of homospecific and interspecific interferon components been postulated (17). Our results would thus seem to support this concept by suggesting that a small fraction of mouse L-cell interferon is also active in human amnion cells. Nevertheless, the possibility that this heterologous activity is not interferon specific can still not completely be excluded.

Summary. Highly purified preparations of mouse L-cell interferon exhibited a small but consistent antiviral activity in human amnion cells (U-line) challenged with arbovirus A strain Middelburg. This heterologous activity was variable, but titers usually ranged from 1 to 5% of those found in homologous mouse L-cells challenged with vesicular stomatitis virus.

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