INTERFERON-SEPHAROSE: INDUCTION OF THE ANTIVIRAL STATE

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SUMMARY: Mouse (L cell) interferon has been covalently attached to Sepharose beads. The interferon-Sepharose beads induce resistance in L cells to virus replication. It is concluded that interferon induces resistance to viral replication from the cell surface only without entry into the cell.

Interferon induces in animal cells resistance to a wide variety of viruses; just how interferon stimulates this resistance, how it develops, and how it is manifest, however, is unclear. The induction of resistance could be initiated by either of the following: (1) entry of the interferon molecule into the cell with subsequent activation of the resistance mechanism from within the cell or (2) binding of interferon to a specific receptor on the cell surface and subsequent induction of resistance without entry into the cell. The latter mechanism has been proposed for insulin (1) in isolated fat cells and ACTH (2) in adrenal cells. In this paper we report the covalent attachment of L cell interferon to Sepharose beads and the induction of resistance to MM virus in L cells by the interferon-Sepharose complex. While this manuscript was in process for publication, analogous results and conclusions were reported (3).

MATERIALS AND METHODS: Mouse L cells were grown in Eagles minimum essential medium (monolayer) and in Eagles suspension culture medium, Joklik modified; both contained 7% fetal calf serum. MM virus was replicated in L cells and titrated by plaque assay under 1% agar. Interferon was assayed by the inhibition of MM induced cytopathic effects on L cells using the semi-micro technique (4).

Interferon from L cells (sp. act.-2 x 10^6 reference units/mg protein) was reacted with cyanogen bromide activated Sepharose 4B beads (CNBr activated Sepharose 4B was purchased from Pharmacia Fine Chemicals) according to published methods (5). The Sepharose beads were washed prior to use with 1 mM HCl. One gram (dry weight) of beads was mixed with 4 x 10^6 units of interferon (2 mg) in 3 ml of 0.1 M sodium bicarbonate buffer, pH 8.0, containing 0.5 M NaCl. The suspension in a 10 ml screw-cap tube was rotated for 18 hr at 4°. The beads were washed once with 0.1 M sodium bicarbonate containing 0.5 M NaCl. Unreacted groups were blocked with 1 M ethanolamine (50 ml of 1 M ethanolamine, 2 hr, 4°, gentle stirring). The beads were then washed 5 times each (alternating) with 0.1 M sodium acetate, pH 4.5, containing 1 M NaCl and 0.1 M sodium borate, pH 8.0, containing 1 M NaCl. Finally, the beads were washed 2 times with phosphate buffered saline (PBS) and stored at 4° in 20 ml of PBS. Control preparations consisted of Sepharose 4B beads (not activated) that were mixed with interferon then washed extensively as described above or activated beads mixed with bovine serum

Table 1

Induction Of Resistance to MM Virus Replication
In L Cells By Interferon-Sepharose

Transfer No.	MM Virus, PFU/ml x 10^5	
	Control	Interferon-Sepharose
1	3000	4
2	2000	2
3	2000	4
6	1000	3

100 mg of interferon-Sepharose was mixed with 20 ml of L cells in suspension culture. After 24 hr at 37° the cells were separated from the beads and 20 ml of new cells were added. This sequence was repeated 5 times so that the original interferon-Sepharose induced resistance in 6 different cultures of cells. Control cultures contained 5 mg/ml of BSA-Sepharose. Soluble interfer could not be detected in the medium at any time while the cells were in contact with the interferon-Sepharose.

albumin (BSA-Sepharose) and treated and washed as with interferon-Sepharose. Neither preparation caused a reduction in MM virus yield in L cells.

Soluble interferon could not be detected (1 unit in the assay is equivalent to 6 reference units) in the PBS supernatant even after a month of storage at 4°. Experiments to test the induction of resistance by the interferon-Sepharose complex were performed as follows: (1) a 20 ml culture of L cells at 30 x 10^4 cells/ml containing 1-5 mg/ml of interferon-Sepharose (concentration

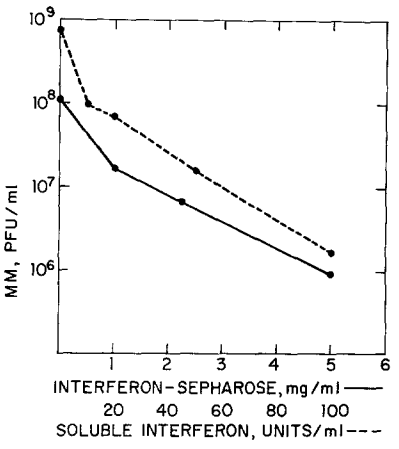

Fig. 1. The effect of concentration of soluble interferon and interferon-Sepharose on reduction of MM virus yield in L cells. Experiments were performed as described in Methods.

of Sepharose only) was stirred in suspension for 18 hr at 37°; (2) the beads were separated from the cells by settling for 15 min; (3) the supernatant cells were plated in plastic culture dishes and after 2 hr at 37° the monolayers were washed to remove remaining beads then infected with MM virus at a multiplicity of 1; (4) after 24 hr the medium (2 ml) from the infected monolayer was assayed for virus.

RESULTS AND DISCUSSION: The data in Table 1 show that the interferon-Sepharose complex is effective in inducing resistance to MM virus replication in L cells. The same interferon-Sepharose beads caused reduction in virus yields by 500 fold after the sixth transfer to new cells. The extent of resistance that is developed by a cell is dependent on the concentration of interferon The data in Fig. 1 show that development of resistance in L cells as measured by reduction in virus yield is dependent on the

concentration of interferon-Sepharose. With this preparation 5 mg/ml of interferon-Sepharose beads will cause a reduction in virus yield similar to a concentration of soluble interferon of 50-100 units/ml (Fig. 1). No soluble interferon was detected in the medium at any time. These data show (1) that interferon has been attached to Sepharose 4B beads, (2) that the interferon-Sepharose complex induces resistance in L cells, and that the complex is stable and can be used serially with 6 separate cultures of cells without loss in activity.

It is concluded that interferon retains its biological activity after covalent attachment to Sepharose beads and is able to induce the antiviral state in L cells from the cell surface. A slow, undetectable release of interferon from the beads in soluble form which enters the cell and activates the resistance mechanism cannot be ruled out but seems unlikely. Uptake of the beads is unlikely also since the smallest beads (40 μ) are 4 to 5 times larger than a single cell.

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