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THE ROLE OF CELL MEMBRANE IN THE ANTIVIRAL EFFECT OF INTERFERON

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The mechanism of interferon action in human fibroblasts has been studied by use of both antisera to human fibroblast interferon and the antisera to the surface of human fibroblast cell. The anti-interferon serum completely neutralized the antiviral effect of human fibroblast interferon. Interferon antiserum prevented the intracellular antiviral state from developing when added to the medium of the cells in which interferon synthesis had already been induced by poly (I · C). This suggests that development of the antiviral state involves interferon interaction with the external part of the producing cell. Treatment with the serum directed against the surface of human fibroblast cells failed to inhibit the antiviral activity of human interferon in these cells.

In addition, the effect of gangliosides on the antiviral activity of human interferon was studied and it was found that human interferon binds to gangliosides and that this interaction leads to inactivation of the antiviral effect of interferon. Pretreatment of human fibroblasts with gangliosides had no effect on the sensitivity of these cells to exogenous interferon.

INTRODUCTION

The mechanism by which interferon induces an antiviral state in cells is not completely understood. It has not been shown whether for the manifestation of the effect, contact of interferon with the sensitive cells is sufficient or whether intracellular uptake of the interferon is required. The existence of an interferon-specific membrane receptor site has been postulated (1, 2). It was further suggested that the receptor is probably a protein molecule, since the binding of interferon can be destroyed by mild trypsin treatment. However, recently it was shown that mouse interferon binds to gangliosides (3), thus suggesting that the ganglioside also may be part of cell surface interferon receptors.

In the present work, the mechanism of interferon action had been studied using antisera to both human fibroblast interferon and to the surface of human fibroblast cells. In addition, the effect of gangliosides on the antiviral effect of human fibroblast interferon has been examined. The results indicate that for the development of the antiviral state, interferon has to interact with the external part of cellular membrane and that ganglioside may be contained in interferon cell surface receptors.

METHODS

Cells and Virus

Human foreskin diploid fibroblast cells (HFC), passages 9–15, were used in all studies (4). Vesicular stomatitis virus (VSV), New Jersey serotype, was propagated in DEAE-dextran (10 μg/ml) treated (mouse) L cells infected at low multiplicity and harvested 24 hr later when the titer was 10^9 plaque-forming units (pfu/ml).

Assays for Antiviral Activity

HFC were incubated for various lengths of time with a solution of poly (I · C) (100 μg/ml) or interferon sample of given dilution (18 hr at 37°C) in maintenance medium. Resistance to virus infection and interferon titers were measured colorimetrically, employing VSV as challenge virus (5, 6). The activity is given in research reference units, using NIH standard human interferon as a reference. The antiviral effect of poly(I · C) on a single step infection was determined by virus yield at 15 hr postinfection.

Antisera

Antibody to human fibroblast interferon prepared in the rabbit was a gift of Dr. K. Paucker of the Medical College of Pennsylvania. A 1:3,000 dilution of this serum neutralized 12 units of human fibroblast interferon. The γ-globulin fraction of sheep antisera to partially purified human leukocyte interferon was a gift from Dr. C.B. Anfinsen of the National Institute of Health; a 1:100 dilution of this fraction completely neutralized 100 units of human leukocyte interferon, but was without any effect on human fibroblast interferon.

Antisera to cell surface of HFC were prepared by injecting 10^8 cells into the footpad of the rabbit. Antibody titer was determined by using the chromium release (^{51}Cr) cytotoxicity test (7); a 1:64 dilution of this serum increased radioactive chromium release by 35% as compared to the control serum.

Ganglioside Interferon Interaction

Cells were treated with mixtures of interferon and ganglioside (preincubated at 37°C for 1 hr) and the antiviral activity was assayed 18 hr later (3). To test the effect of gangliosides on cells, cell monolayers were incubated for 1 hr at 37°C with varying concentrations of ganglioside in phosphate buffered saline (8). The cellular monolayers were then washed and incubated for 30 min with tissue culture medium (supplemented with 2% fetal bovine serum) to remove any extraneous unbound ganglioside before testing for sensitivity to interferon.

RESULTS AND DISCUSSION

Effect of Anti-Interferon Serum

Antisera to human fibroblast interferon were used to examine the correlation between the antiviral effect of poly(I · C) and the induction of interferon. After induction, cells were incubated for an additional 6 hr in maintenance medium containing a sufficient

amount of anti-interferon serum to neutralize any interferon produced and released into the medium (Table I). When poly(I · C)-induced extracellular interferon was neutralized by the presence of antisera to human fibroblast interferon, the antiviral state was not induced. Sera from nonimmunized animals were without any effect, as was a control serum prepared against medium from uninduced cells. Additionally, the anti-interferon sera did not inhibit virus replication in HFC. The sheep antiserum (γ-globulin fraction) to human leukocyte interferon (which partially binds human fibroblast interferon but does not neutralize its antiviral activity), when studied under identical conditions, was without any effect (Table II). In addition, the antiserum to human fibroblast interferon did not inhibit the induction of the antiviral state of poly(I · C) in mouse L cells.

For the inhibitory effect to be achieved, the interferon antiserum had to be present in the medium from the time when interferon starts to be released into the medium. Pretreatment of HFC with the antihuman interferon serum for 1 hr before or during exposure of the cells to poly(I · C) affected neither interferon production nor the appearance of the antiviral state.

The next question we asked was whether anti-interferon antiserum prevented the establishment of the antiviral state of the interferon-producing cells, or whether it only blocked the spread of interferon from the small population of cells producing interferon to nonproducers (9). To test the fraction of interferon-producing cells after poly(I · C) treatment in the population of HFC, we measured the number of cells in antiviral state on a single cell basis. Under conditions where the spread of interferon should be minimal (1 hr after poly(I · C) induction when no detectable interferon was in the medium), 88% of the cells were in the antiviral state (Table III). Six hr postinduction, when a substantial amount of extracellular interferon was produced, 98% of the cells were found to be in the antiviral state. Thus, in the HFC system, a minimum of 88% of the cells were producing interferon. When the induced interferon was neutralized by antiserum, cells which produced interferon did not develop the antiviral state. The effect appears to be specific for interferon since antiserum directed against the surface of HFC did not inhibit the establishment of the antiviral state. In addition, serum which reacted with poly(I · C) was without any effect when added after interferon induction was initiated.

This finding may have further implications for the mechanism of interferon action. The possibility that antiserum would neutralize interferon at a site internal to the cellular membrane cannot be completely eliminated. However, it is generally believed that antibodies do not enter living cells. Thus, our results lead us to suggest that in order to induce the antiviral state, interferon has to be associated with the external part of the cellular membrane, even in the cell in which it is produced. It was recently suggested that for biological activity, penetration of interferon was not required (10).

Effect of Antisera Directed against the Cell Surface of HFC

Antiserum to HFC contained antibodies for surface components of these cells, as shown by membrane immunofluorescence (indirect method using fluorescein-labeled goat antirabbit globulin). The antiserum was also cytotoxic to HFC in the presence of guinea pig complement, as measured by isotope release from cells previously labeled with radioactive chromium.

The treatment of HFC with anti-HFC serum (1 : 10 dilution) either at 37°C or 4°C

TABLE I. Inhibition of the Antiviral Activity of Poly(I · C) by Antiserum to Human Fibroblast Interferon

Poly(I · C)	Incubation medium	VSV yield (pfu/ml)	Interferon (units/ml)	Antibody titer
–	MEM	1.5×10^7	<4	–
–	Interferon antiserum	1.4×10^7	<4	200
+	MEM	6.2×10^3	384	–
+	Interferon antiserum	5.6×10^6	<4	10
+	Serum*	9.6×10^3	384	–

Human fibroblast cells were treated with poly(I · C) (100 μg/ml) for 15 min at 37°C, poly(I · C) was removed, and cells were incubated in MEM containing rabbit anti-interferon serum for 6 hr. Medium was collected and assayed for interferon and antibody titer; cells were washed and infected with VSV for virus yield assay.

*Serum from nonimmunized rabbits or chickens injected with medium from uninduced cells.

TABLE II. Lack of the Effect of Antisera to Human Leukocyte Interferon on the Antiviral Activity of Poly(I · C) in Human Fibroblast Cells

Poly(I · C)	Incubation medium	VSV yield (pfu/ml)	Interferon (units/ml)
–	MEM	1.2×10^7	<4
–	Interferon antiserum	2.4×10^7	<4
+	MEM	1.2×10^4	192
+	Interferon antiserum	1.8×10^4	192

HFC were treated with a solution of poly(I · C) (100 μg/ml) for 15 min, washed, and incubated in MEM containing 200 units of human leukocyte interferon antiserum for an additional 6 hr.

(not shown), before or simultaneously with interferon, did not inhibit the action of human fibroblast interferon in these cells (Table IV). The results may indicate either that the antibodies to the receptor site were not present in our serum (receptor site may form only a very small part of the cell surface proteins), or that, since our preparation of antisera contained a mixture of antibodies against the cell surface, the lack of effect could be caused by strong antigen competition.

Effect of Gangliosides

Preincubation of human fibroblast interferon with a mixture of purified gangliosides inhibited the antiviral effect of interferon (Table V). Under the same conditions, the di-

TABLE III. The Antiviral Effect of Poly (I · C) Assayed by Infectious Centers at Different Times Postinduction

Poly (I · C)	Treatment	Number of infectious centers 1 hr	6 hr
+	0	28	6
+	Interferon antiserum	158	105
—	0	230	237
—	Interferon antiserum	250	249

HFC were treated with poly (I·C)(100 μg/ml) for 15 min; poly (I · C) was removed, and cells were washed and incubated in MEM containing rabbit anti-interferon serum for an additional hour. The cells were then infected with VSV (moi 10), and 2 hr later trypsinized; 1,000 cells were plated over mouse L cells; plaques were read 24 hr later.

TABLE IV. Effect of Antisera Directed against the Cell Surface of Human Fibroblast Cells

Treatment	Interferon (units/ml)	VSV yield (pfu/ml)
Interferon	96	3.6×10^4
Antiserum → interferon	96	2.8×10^4
Antiserum + interferon	96	3.4×10^4

HFC were treated with antiserum (1 : 10 dilution) against HFC surfaces either before exposure to interferon (1 hr at 37°C), or with antisera and interferon simultaneously (6 hr at 37°C).

TABLE V. Effect of Gangliosides on the Antiviral Activity of Human Interferon

Interferon (units/ml)	Gangliosides	VSV yield (pfu/ml)
0	0	$2–7.8 \times 10^7$
1,000	0	6.8×10^3
1,000	Mixture	4.6×10^7
1,000	GT_1	1.2×10^7
1,000	GD_{1a}	5.0×10^7
1,000	GM_1	3.0×10^7
1,000	GM_2	6.8×10^7

Human interferon was incubated with gangliosides (500 μg/ml) for 1 hr at 37°C in phosphate buffer before being applied on human fibroblast cells; antiviral activity was assayed by VSV yield 18 hr later.

rect mixing of VSV with gangliosides before application to the cells or the preincubation of human fibroblast cells with gangliosides did not affect VSV replication in these cells as measured by virus yield. When human fibroblast interferon was preincubated with the individual purified gangliosides, it was found that all gangliosides tested were able to inactivate the exogenous interferon.

Direct evidence for the ganglioside-interferon interaction was obtained by the chromatography of interferon on a column of agarose (8), containing covalently attached gangliosides; human interferon was retained on these columns and could be eluated with 50% ethylene glycol and 0.1 M α-methyl-D-mannopyranoside.

These results indicate that gangliosides bind to human interferon and that the interferon-ganglioside complex is not biologically active. Furthermore, these results also suggest that in the interaction of exogenous interferon with the cell surface, gangliosides may play a role.

It was previously shown that gangliosides can be incorporated into the cell membrane of mouse cells (8). If the ganglioside is a part of the interferon receptor on the cell membrane, then the preincubation of human cells with gangliosides may increase the sensitivity of these cells to exogenous interferon. However, the preincubation of human fibroblast cells with interferon did not alter the sensitivity of these cells to exogenous interferon. The observed lack of stimulation suggests that in cells which are sensitive to interferon, sensitivity cannot be measurably improved by the addition of exogenous gangliosides. However, in an interferon-insensitive and ganglioside-deficient mouse cell line (11, 12), the sensitivity to interferon was increased by preincubation of the cells with gangliosides (Vengris et al., unpublished observations).

Thus, these results suggest that exogenous interferon interacts with a ganglioside containing receptor on the cell surface before the onset of the antiviral state. It is assumed that the recognition for interferon species specificity occurs at the cellular membrane. However, the fact that gangliosides are able to interact and inactivate both human and mouse interferon (3) suggests that this interaction may be common for different interferons, thus indicating that for the specificity of interferon recognition, additional factors may be involved.

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