INTERFERON INHIBITS THE REDISTRIBUTION OF CELL SURFACE COMPONENTS*

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Recent evidence suggests that the pleiotropic effects of interferon are triggered by the rapid binding of interferon to cells, probably to a membrane receptor that contains, in part, ganglioside (1-5). A brief lag period has been observed between the initial binding of interferon to cells and the subsequent establishment of the anti-viral state. This lag probably corresponds to a series of cellular events initiated by interferon binding (6).

Interferon treatment of cells also results in modifications in the structure and function of cells (7). We have recently characterized the main features of the phenotype of interferon-treated human fibroblasts (8, 9). The phenotypic alterations include decreased rates of cell proliferation and cell movement; increased cell surface area, volume, and mass; altered organization and size of actin-containing cytoplasmic fibers; increased organization of cell surface fibronectin; and increased rigidity of the plasma membrane.

In this report, we examine the effects of interferon treatment on the lateral mobility of the cell surface receptors for the lectin concanavalin A (Con A). Study of the interaction of interferon with the cell surface may clarify the mechanism whereby interferon initiates a complex series of changes in cell structure and function.

Materials and Methods

Cells. HeLa-S3 cells were grown in suspension in the spinner modification of Eagle's minimum essential medium (spinner medium) supplemented with 4% fetal calf serum. The initial cell concentration was 2 × 10⁴ cells/ml. At 1 d after subculturing, human fibroblast interferon was added at concentrations varying from 10 to 640 U/ml; control cultures received no interferon.

Interferon. Two interferon preparations, produced in human fibroblasts that were induced with polyinosinic-polycytidylic acid, were used. A partially purified preparation, provided by Dr. W. Carter and Dr. J. Horoszewicz of Roswell Park Memorial Institute, Buffalo, N. Y., had a 2 × 10⁷ U/mg of protein sp act. It was purified by chromatography on Con A-agarose and phenyl-Sepharose (Pharmacia Fine Chemicals, Div of Pharmacia, Inc., Piscataway, N. J.) columns in tandem. A preparation of interferon purified to homogeneity (≥2 × 10⁸ U/mg protein sp act) was provided by Dr. E. Knight, Jr. of E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del. Interferon activity was assayed by a microtitration procedure with vesicular stomatitis virus as previously described (10), using the World Health Organization international...
human fibroblast reference standard (National Institutes of Health catalog number G-023-902-527) for comparison.

Results and Discussion

As is shown in Fig. 1 A and B, when either control or interferon-treated HeLa-S3 cells are incubated at 4°C with fluorescein-conjugated Con A (fl-Con A) (Calbiochem-FIG. 1. Effect of interferon treatment on the redistribution of Con A receptors. At 72 h after the beginning of treatment, aliquots of ~2 × 10⁷ cells were removed from control and interferon-treated (640 U/ml) cultures, pelleted, resuspended in 1 ml of spinner medium that contained 2 μg/ml of fl-Con A, and the cells maintained at 4°C for 45 min. The cells were pelleted and resuspended in 3 7% formaldehyde in phosphate-buffered saline (PBS) (A and B), or in spinner medium in which they were incubated at 37°C for 60-90 min before fixation in formaldehyde at room temperature for 10-20 min (C and D). The cells were then resuspended in 50 μl PBS and 50 μl twice-buffered glycerol-PBS (1 1 vol/vol) and examined in a Zeiss III RS photomicroscope equipped with epifluorescence and phase-contrast optics (Carl Zeiss, Inc., New York) (A) Control cells and (B) interferon-treated cells maintained at 4°C with fl-Con A, (C) Control cells and (D) interferon-treated cells warmed to 37°C. Fluorescence staining is shown at left, phase contrast is shown at right. Note that in control cells (C) the cap is in an extended portion of the cell. In interferon-treated cells, patched areas of fluorescence (arrows) are found in cells that remain essentially round. White line in A-C is 1 μm, white line in D is 1 μm.
FIG 2 Effect of interferon concentration on the redistribution of Con A receptors. HeLa-S3 cells were grown and treated with human fibroblast interferon at concentrations varying from 10 to 640 U/ml. At 48 h after the beginning of interferon treatment, aliquots of $2 \times 10^5$ to $5 \times 10^5$ cells were removed from control and interferon-treated cultures and the cells were processed for an examination of Con A-receptor distribution as described in the legend to Fig 1. Cells were examined for the fluorescence pattern of fl-Con A and scored for the presence of intense fluorescence at one pole of the cell (cap formation). At least 500 cells were scored for each point. At 72 h from the beginning of interferon treatment, a similar dose-response curve was obtained. Under the experimental conditions used, ~95% of the control HeLa-S3 cells showed capping of fl-Con A. The optimal concentration of fl-Con A is markedly dependent on the type of cell studied. The redistribution of Con A receptors is inhibited at high concentrations of fl-Con A. When control HeLa-S3 cells were incubated with fl-Con A at 50 µg/ml at 4°C, and then warmed to 37°C, <45% of the cells showed capping of fl-Con A. Data are expressed as follows:

$$\frac{\text{percent of interferon-treated cells capped}}{\text{percent of control cells capped}} \times 100$$

Behring Corp., American Hoechst Corp., San Diego, Calif.) before fixation with formalin, a diffuse pattern of fluorescence is observed over the entire cell surface.

If control cells are incubated with fl-Con A at 4°C, washed with fresh medium, and then incubated at 37°C for 60-90 min, the diffuse surface fluorescence redistributes and becomes concentrated at one pole of the cell forming a cap, as illustrated in Fig. 1 C. Electron microscopic observation of cells incubated with ferritin-conjugated Con A has indicated that most of the Con A is bound to the surface and little is internalized after 60-90 min at 37°C.

In contrast to the findings obtained with control cells, incubation of interferon-treated HeLa-S3 cells at 37°C after fl-Con A pretreatment at 4°C results in a redistribution of Con A receptors into a pattern of patched regions of surface fluorescence (Fig. 1 D). At an interferon concentration of 640 U/ml, cap formation is largely inhibited even when incubation at 37°C after fl-Con A pretreatment is extended to 180 min. The extent of inhibition of cap formation is dependent upon the interferon dose used (Fig. 2). The approximately exponential dose-response relationship for inhibition of capping is similar to that obtained for the inhibition of cell proliferation and of virus multiplication by interferon.

Mouse and chick interferons were not effective in the inhibition of cap formation, suggesting that interferon itself was the species-specific agent responsible for the observed effect.

2 Recently, Matsuyama (Matsuyama, M. 1979 Action of interferon on cell membrane of mouse lymphocytes. Exp Cell Res 124:253.) has reported that mouse interferon inhibited capping of certain surface components on mouse lymphocytes, which react with anti-lymphocyte serum or lectins. Only those surface receptors whose capping was enhanced by pretreatment of lymphocytes with colchicine were affected.
Fig. 3 shows the time-course of development of inhibition of lateral movement of Con A receptors in interferon-treated (640 U/ml) cells. Interferon treatment for only 3 h is sufficient to cause a 15% reduction in capping. A maximal inhibition of ~85% is reached within 36 h from the beginning of treatment. Two preparations of fibroblast interferon with different specific activities, one of which was purified to homogeneity, gave closely similar results. We have shown previously that human fibroblast interferon (640 U/ml) causes a 60% reduction in the rate of proliferation of HeLa-S3 cells, and that this effect is manifest within 24 h from the beginning of treatment (8).

It has been demonstrated in several cell culture systems that Con A binding to membrane receptors occurs rapidly at 4°C (11–13). When cells are then warmed to 37°C, the receptors, which are cross-linked by Con A, are rapidly redistributed within the plane of the membrane to form: first, patchy regions containing multiple receptor-Con A complexes, and ultimately, large aggregates in the form of a cap at one pole of the cell (13, 14). The redistribution of the cross-linked Con A receptors is sensitive to cytochalasin B (11, 15), which impairs the function of submembranous microfilaments (16, 17).

In previous studies (9) we have reported that treatment of human fibroblasts in culture with human fibroblast interferon (640 U/ml) causes not only a 55–60% reduction in the rate of cell proliferation, but also causes a 70% decrease in the rate of cell movement in 48 h. In other laboratories (16, 18–22), a close relationship has been established between the organization of submembranous actin-containing microfilaments and several aspects of cell locomotion in fibroblasts. The kinetics of the interferon-induced inhibition of lateral mobility of cell surface receptors (cf. Fig. 3) and of cell movement (9) are strikingly similar. Because these motile activities are intimately associated with the cellular contractile machinery, an attractive hypothesis
to explain the interferon-induced inhibition of lateral mobility of cell surface receptors and of cell movement is that such inhibition results from an alteration in the state of microfilaments. We have demonstrated that interferon treatment of human fibroblasts markedly alters the organization of cytoplasmic microfilaments (9). Very large and numerous microfilament bundles are formed spanning the length of enlarged cells (9). Experiments are in progress to determine whether interferon treatment alters the organization of microfilaments in HeLa-S3 cells, and if so, how the alterations may be related to the inhibition of cell proliferation and of the lateral mobility of cell surface receptors.

There is also evidence that interferon treatment of HeLa-S3 cells and human fibroblasts increases the rigidity of the plasma membrane lipid bilayer. This could be a contributing factor in the inhibition of the lateral mobility of lectin receptors as well as of cell movement in interferon-treated cells.

Interferon treatment of cells elicits a coordinated response that involves the cell membrane with its associated proteins and also the cytoplasmic microfilaments. Further work is needed to distinguish clearly between the initial effects of interferon on cells and those that represent phenotypic changes that are secondary to the decreased cycling activity of treated cells.

Summary

Interferon treatment impairs the ability of cells to redistribute cell surface receptors for concanavalin A (Con A). The effect of interferon becomes evident within 3–6 h and is maximal within 36–48 h. Highly purified human fibroblast interferon (>2 × 10^6 U/mg of protein sp act; concentration: 640 U/ml) caused ~85% inhibition of capping of fluorescein-conjugated Con A in interferon-sensitive HeLa-S3 cells at 36 h from the beginning of treatment.

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