Vol. 110, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1983 Pages 155-160

MICROINJECTED INTERFERON DOES NOT PROMOTE AN ANTIVIRAL RESPONSE IN HELA CELLS

Georges Huez [1], Michèle Silhol [2] and Bernard Lebleu [2]

(1) Département de Biologie Moléculaire, Université Libre de Bruxelles, 67, rue des Chevaux, 1640 Rhode St-Genese, Belgium

(2) Laboratoire de Biochimie des Protéines, ERA CNRS 482, Université de Montpellier II, place E. Bataillon, 34060 Montpellier Cedex, France

Received September 20, 1982

Human fibroblast interferon (Hu IFNβ) was directly introduced with glass micropipets into the cytoplasm of Hela cells. Such an injection of more than 10^4 molecules per cell failed to induce any antiviral state when challenged with vesicular stomatitis virus (VSV).
These findings are discussed in relation to the possible role of internalization in the mechanism of antiviral action of interferon.

There is now direct evidence that in the first step of its interaction with the cells, interferon binds to plasma membrane specific receptors, as demonstrated in murine (1) and human (2) cells. This leads to early modifications e.g. an inhibition of capping of conA receptors (3) or a transient increase in cGMP intracellular concentration (4), as well as more slowly developing effects which require cell nucleus activation e.g. the development of an antiviral state (5, 6).

There is a paucity of experimental data concerning the mechanism of information transfer from the cell surface to the nucleus. The intracellular concentrations of cGMP (4) and cAMP (7, 8) increase significantly upon interferon treatment but this is probably irrelevant (4, 9, 10) at least to the development of an IFN-induced antiviral state.

The internalization of IFN itself or of a fragment of the molecule, as documented for several polypeptide hormones (11), might be another way of transferring a signal downstream from the receptor. The immobi-

0006-291X/83/010155-06$01.50/0

lization of mouse IFN on Sepharose beads (12) does not impair its antiviral activity which would argue against the necessity of its penetration into the cell. However, leakage of the ligand from its support cannot be eliminated in such experiments. Evidence for (Baglioni, personal communication) and against (13) internalization of purified ^{125}I labelled IFNs have been provided recently. Primary amines, which are known inhibitors of receptor-mediated endocytosis, have been tested recently for their effects on IFN-induced antiviral action (14, 15). If however dansyl cadaverin or ammonium chloride are effective in inhibiting the development of an antiviral state, methylamine and the transglutaminase inhibitor bacitracin are not.

An alternative approach to this problem consists in the deliberate introduction of IFNs in cells. Human IFN has been successfully incorporated into different forms of liposomes (16, 17) and yet able to induce an antiviral state (18) without prior disruption of the liposome structure. Unfortunately however, the mode of interaction of liposomes-associated material with cells is insufficiently clear to draw definitive conclusions on the way IFN acts in these cases.

We examine here whether the direct microinjection with micropipets of human fibroblast interferon (Hu IFNβ) in the Hela cells allows the development of an antiviral state.

Materials and Methods

Materials

Human fibroblast interferon (Hu IFNβ) was obtained from Dr. A. Billiau (Rega Institute, University of Leuven). It was partially purified to a specific activity of 10^6 units/mg protein from poly (rI) poly (rC) superinduced human fibroblasts as described by Heine et al. (19).
Hu IFNβ antiserum was also kindly provided by Dr. A. Billiau and used at a concentration sufficient to neutralize 10 times the amount of interferon present in the incubation medium.

Vesicular stomatitis virus (Indiana strain) was obtained from Dr. J. Content (Pasteur Institute, Brussels).

Cells cultures

For maintenance, Hela cells were grown in monolayers in RPMI 1640 medium supplemented with 10 % (v/v) fetal calf serum.
For microinjection experiments, Hela cells were grown on small pieces of glass (2 mm^2) so that approximately 200 cells attach to each glass fragment (20).

Cell microinjection

Cells microinjections were performed according to the method originally described by Graessmann (21). A volume of 510^{-10} ml (average value) was injected into the cytoplasm of each recipient cells with glass micropipets having a diameter at the tip of about 0,5 to 1 µm. Injections were monitored under a phase contrast microscope (total magnification of 320) (22).

Assay of interferon antiviral activity

Cells were infected with VSV at a multiplicity of 20-50 for 1 h at 37° in RPMI medium supplemented with 2 % FCS. The unadsorbed virus was carefully removed by 3 washings with RPMI containing 10 % FCS. The number of plaque forming units was determined 15 hours later according to published procedures (23).

Results and Discussion

As mentioned above contradictory experimental data have been provided concerning the penetration of interferons in cells which in any case could be irrelevant to its biological activity. Direct microinjection experiments of the IFNβ in Hela cells with micropipets have thus been performed and their effect on the replication of VSV has been examined. The results of representative experiments have been summarized in Table 1.

It has proven difficult to prevent completely cell leakage during the process of microinjection as well as during their subsequent incubation

Table 1.

Effect of Hu IFNβ microinjection on the production of VSV by Hela cells.

Expt.n°	Cell treatment	Virus yield (pfu/200 cells)
1	CONT	1.0×10^4
	IFN out	0
	IFN out + IFN antiserum	8.4×10^3
	IFN in	7.8×10^3
2	CONT	3.5×10^4
	IFN out	0
	IFN in	6.3×10^4
3	CONT	1.8×10^3
	IFN out	-
	IFN in	2.1×10^3

Hela cells grown on 2 mm^2 glass pieces were microinjected with 5×10^{-10} ml each of a solution of Hu IFNβ as described in Material and Methods. Uninjected cells were incubated in parallel in the absence (CONT) or presence (IFN out) of 100u/ml of Hu IFNβ. When added (IFN in and IFN out + anti IFN) human interferon antiserum was added at a concentration sufficient to neutralize interferon in the incubation medium. After 10 (expt. 2) or 15 (expts. 1 and 3) hours incubation, the cells were challenged with VSV and the yield of virus was determined as described in Material and Methods.

(data not shown). This problem was circummented by the addition in the incubation medium of human interferon antiserum in excess.

As documented in Table 1, the introduction of the IFNβ in Hela cells cytoplasm does not provide any significant reduction in the amount of VSV produced whether the cells were challenged 10 or 15 hours after the onset of interferon treatment. Shorter periods of interferon treatment did not lead to any significant antiviral protection as well whether interferon was microinjected or added in the incubation medium (data not shown). Measurement of viral RNA synthesis has proven impossible to do on such low amounts of infected cells (data not shown).

If indeed pure Hu IFNβ has a specific activity of about 5×10^8 u/mg protein and a molecular weight of 20,000 daltons, 5×10^{-10} ml of a solution titrating 5×10^5 u/ml should contain about 1.5×10^4 molecules of interferon. This has to be compared with the number of specific IFN receptors estimated to be around a few thousands per cell at the most (25).
Our data thus suggest that interferon does not promote an antiviral activity upon entering cell cytoplasm as such.

It is however fully possible that binding to specific receptors induces modifications and/or cleavages of the IFN molecule whose further internalization would then initiate a biological response. Small peptides have been recently implicated in the mechanism of action of polypeptide hormones as for instance prolactin (26). It could be also that interferon needs to be directed to the proper cell comportment to be active although recent studies on the fate of radiolabelled purified mouse interferon do not support a binding-induced internalization of interferon followed by its degradation in lysosomes (25). Alternatively interferon delivery at the adequate concentration at the active site could be severely limited if its half-life is too short when microinjected in the cytoplasm. This would be particularly crucial if indeed the development of interferon responses and/or their maintenance requires its continuous presence at the cell surface (27 and Lebleu et al., unpublished data).

Acknowledgments

This study was supported by grants from INSERM and the "Fondation pour la Recherche Médicale" to B.L. and the Belgian State Contract "Actions Concertées". G.H. is Chercheur Qualifié of the Belgian Fonds National de la Recherche Scientifique.

References

1. Aguet, M. (1980) Nature (London) 284, 459-461.
2. Branca, A.A. and Balgioni, C. (1981) Nature (London) 294, 768-770.
3. Matsuyama, M. (1979) Exp. Cell Res. 124, 253-259.
4. Tovey, M.G., Rochette-Egly, C. and Castagna, M. (1979) Proc. Natl. Acad. Sci. USA 76, 3890-3893.
5. Taylor, J. (1964) Biochem. Biophys. Res. Comm. 14, 447-453.
6. Radke, K.L., Colby; C., Kates, J.R., Krider, M.M. and Prescott, D.M. (1974) J. Virol. 13, 623-645.

7. Meldolesi, M.F., Friedman, R.M. and Kohn, L.D. (1977) Biochem. Biophys. Res. Comm. 79, 239-246.
8. Goldberg, S.J., O'Shaghnessy, M.V. and Stenart, R.B. (1980) J. Gen. Virol. 48, 377-381.
9. Schneck, J., Rager-Zisman, B., Rosen, O.M. and Bloom, B.R. (1982) Proc. Natl. Acad. Sci. USA 79, 1879-1883.
10. Tovey, M.G. and Rochette-Egly, C. (1981) Virology 115, 272-281.
11. Goldstein, J.L., Anderson, R.G.W. and Brown, M.S. (1979) Nature (London) 279, 679-685.
12. Ankel, M., Chany, C., Galliot, B., Chevalier, M.J. and Robert, H. (1973) Proc. Natl. Acad. Sci. USA 70, 2360-2363.
13. Aguet, M. and Blanchard, B. (1981) Virology 115, 249-261.
14. Commoy-Chevalier, M.J., Robert-Galliot, B. and Chany, C. (1978) J. Gen. Virol. 41, 541-547.
15. Anderson, P., Tycko, B., Maxfield, F. and Vilček, J. (1982) Virology 117, 510-515.
16. Anderson, P. Vilček, J. and Weissmann, G. (1981) J. Infect. Immun. 31, 1099-1103.
17. Eppstein, D.A. and Stewart, W.E. (1981) J. Interferon Res. 1, 495-504.
18. Eppstein, D.A. and Stewart, W.C. (1982) J. Virol. 41, 575-582.
19. Heine, J.W., De Ley, M., Van Damme, J., Billiau, A. and De Somer, P. in "Regulatory Function of Interferon" (1980) Annals of the New York Academy of Sciences 350, 364-673.
20. Huez, G., Bruck, C. and Cleuter, Y. (1981) Proc. Natl. Acad. Sci. USA 78, 908-911.
21. Graessmann, A. and Graessmann, M. (1971) Hoppe Seyler's Z. Physical Chem. 352, 527-532.
22. Huez, G., Bruck, C., Portetelle, D. and Cleuter, Y. (1980) FEBS Letters 109, 37-42.
23. Stewart, W.E. II and Lockart, R.Z. (1970) J. Virol. 6, 795-799.
24. Knight, E.Jr (1976) Proc. Natl. Acad. Sci. USA 73, 520-523.
25. Aguet, M. and Blanchard, B. (1981) Virology 115, 249-261.
26. Teyssot, B., Houdeline, L.M. and Djiane, J. (1981) Proc. Natl. Acad. Sci. USA 81, 6729-6733.
27. Shulman, L. and Revel, M. (1980) Nature 288, 98-100.