PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 253-255 (1978)

Cholera Toxin Inhibits Antiviral and Growth Inhibitory Activities of Human Interferon (40032)

MIKLOS DEGRÉ

Capt. W. Wilhelmsens Institute of Bacteriology, University of Oslo, Rikshospitalet, Oslo 1, Norway

Recent studies indicate that binding of interferon to ganglioside or ganglioside-like oligosacharide surface receptors is a necessary first step for the establishment of antiviral activity (1-3). Cholera toxin and the glycoprotein hormones thyreotropin (TSH) and human chorionic gonadotropin (hCG) bind to gangliosides with similar but slightly different carbohydrate specificity. The monosialoganglioside G_{M1} seems to be one specific receptor for cholera toxin. The ganglioside binds cholera toxin and blocks its action (4). Both cholera toxin and the glycoprotein hormones inhibit the induction of antiviral state of interferon, supporting the notion of common binding sites on the cell membrane (1, 5).

Besides its classical antiviral effect interferons are known to influence numerous cellular functions, fe. cell growth, phagocytic activity, antigen expression, etc. commonly described as nonantiviral activities. The cell growth inhibitory activity is probably the most important and certainly the most thoroughly studied of these activities. It seems reasonable to believe that reaction with surface membrane receptors might be a common step in development of the various effects of interferons. We, therefore, examined whether cholera toxin could inhibit the growth inhibitory activity of interferon and correlated it to the inhibition of antiviral activity. The present communication shows that the two different activities are inhibited by the addition of cholera toxin to a comparable extent. Since most of the earlier studies were performed on murine cells with murine interferon we extended the phenomenon to include human cells and human interferon.

Materials and methods. Cells. The human amnion U-cell line was originally obtained from Dr K. Cantell, Helsinki. The U cells were grown in Eagle's Minimal essential medium (MEM) with addition of 10% calf serum, and maintained in MEM with 5 or 2% calf serum.

Reagents. Cholera enterotoxin was purchased from Schwarz-Mann. A 10^{-5} *M* stock solution was prepared in Tris buffer and kept at $-20°$. Further dilutions were prepared in MEM immediately before use.

Interferon. Partially purified human leukocyte interferon was a gift from Dr K. Cantell, Helsinki. It was assayed by an infectivity inhibition microtest (6) employing U cells and Vesicular stomatitis virus (VSV). The titers are expressed in international standard reference units (69/19).

Antiviral activity was also assayed by the effect on virus yield. One day old U cells, about 10^5 cells per tube were treated with interferon and with cholera toxin as described in the results section. After incubation overnight the cells were washed and infected with 10^3 TCID of VSV. After 18 hr incubation the cultures were frozen and thawed and assayed for infectious virus yields in U cells. Cytopathic effect (CPE) was read after 72 hr incubation. The titers are given as the highest dilution showing CPE in half of the tubes determined by the method of Reed and Muench.

Assay of cell growth inhibitory activity. Culture tubes were seeded with 10^5 U cells in 1 ml of MEM with 2% calf serum, containing cholera toxin and interferon as indicated in the results section. The cultures were incubated at 37° in 5% CO_2 atmosphere. After 72 hr the monolayers were treated with trypsin-versene and the cells were counted in a hemocytometer. Each value given in the results represents the mean of at least five parallel cultures.

Results. The data in Fig. 1 show that interferon dose-dependently reduced the yield of VSV produced in U cells. When 10^{-9} or 10^{-7} *M* cholera toxin was added to the cells together with interferon the antiviral activity was reduced. With 50 units of

0037-9727/78/1572-0253$01.00/0

interferon there was a significant reduction of interferon effect with both 10^{-9} and 10^{-7} *M* toxin. Addition of 10^{-7} *M* cholera toxin also reduced the antiviral titer of an interferon preparation tested in an infectivity inhibition microtest (Table I). This effect was more pronounced if the cells were pretreated with the toxin for 2 hr prior to addition of interferon. If toxin was added 2 hr after interferon no effect was observed.

The number of cultured U cells increased from 1×10^5 to $2.8\text{–}5.1 \times 10^5$ after 3 days incubation. Growth of U cells was dose-dependently inhibited by interferon (Fig. 2 and Table II). Addition of 10^{-9} *M* toxin greatly reduced this effect. Higher concentrations than 10^{-7} *M* of the toxin were slightly inhibitory itself, but 10^{-8} *M* alone had no effect on the cell growth. The effect of cholera toxin on the interferon-produced growth inhibition was more pronounced if the cells were pretreated prior to addition of interferon, and less if added after the interferon (Table II).

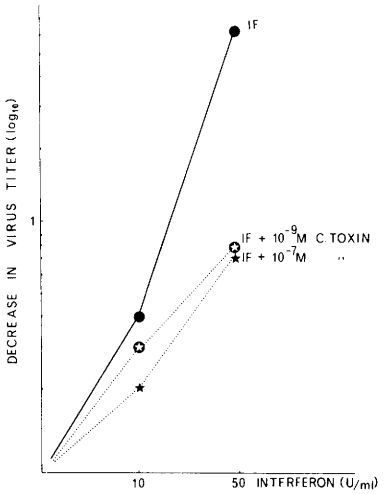

FIG. 1. Effect of cholera toxin on the antiviral activity of interferon. One day old U-cells, about 10^5 cells per tube, were treated with 10 or 50 units/ml of human leukocyte interferon with or without presence of 10^{-7} or 10^{-9} *M* cholera toxin or cholera toxin alone. The toxin was added together with interferon. After incubation overnight the cells were infected with 10^3 TCID of VSV. After 18 hr incubation the cultures were frozen and thawed and assayed for infectious virus yields.

TABLE I. EFFECT OF CHOLERA TOXIN ON INTERFERON TITER.[a]

10^{-7} *M* Cholera toxin	Interferon titer (log_{10} units)
0	2.85
Added together with interferon	2.45
Added 2 hr before interferon	1.65
Added 2 hr after interferon	2.85

[a] A sample of human leukocyte interferon was assayed by an infectivity inhibition microtest. Cholera toxin was added to the cells 2 hr before, at the same time or 2 hr after interferon and incubated overnight. The cells were then washed and infected with VSV. All cultures were examined for development of CPE after 3 days incubation.

Discussion. Cholera enterotoxin reduced two different activities of human interferon: induction of antiviral state and the cell growth inhibitory activity. The suggested mechanism of cholera toxin effect is an interaction with membrane surface receptors (1). Cholera toxin reacts with gangliosides, especially G_{m1} (4) and either directly or indirectly interferes with or blocks the interferon interaction with these structures (1). Alternative mechanism may be simply an alteration of the cell surface making the

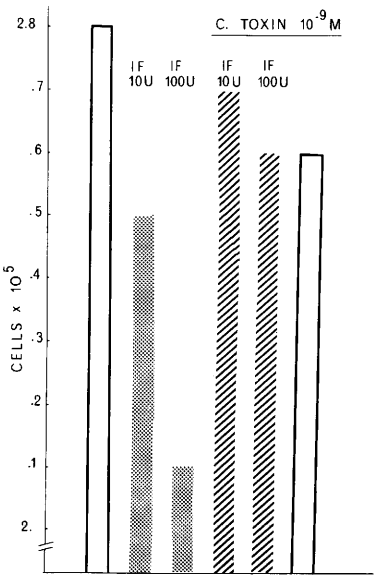

FIG. 2. Effect of cholera toxin on the cell growth inhibitory activity of interferon. U cells were seeded in Eagles MEM, about 10^5 cells per tube. Parallel tubes were added 10 or 50 units/ml of human leukocyte interferon, 10^{-9} *M* cholera toxin or interferon and toxin at the same time. After 3 days incubation the monolayers were trypsinized and the cells counted. The columns represent the mean of five tubes.

TABLE II. EFFECT OF INTERFERON AND CHOLERA TOXIN ON GROWTH OF U CELLS.[a]

Treatment of cells	Reduction of cell growth (% of control)
Interferon, 100 units	55.6 ± 8.6*
Interferon, 100 units + C. toxin, 10^{-9} *M*, same time	76.0 ± 6.8* [b]
Interferon, 100 units + C. toxin, 10^{-9} *M*, 2 hr later	59.0 ± 4.5** [c]
C. toxin, 10^{-9} *M* + Interferon, 100 units, 2 hr later	77.0 ± 3.6** [d b]
Interferon, 500 units	40.6 ± 4.0**
Interferon, 500 units + C. toxin, 10^{-8} *M*, same time	86.6 ± 7.6** [e]
C. toxin, 10^{-8} *M*	96
C. toxin, 10^{-9} *M*	98

[a] U cells were seeded in medium containing interferon, C. toxin or both. After 3 days incubation the monolayers were trypsinized and the cells counted.

[b] $P < 0.01$ compared with 100 units interferon, by *t* test.

[c] $P < 0.01$ compared with 100 units interferon + C. toxin same time.

[d] $P < 0.02$ compared with 100 units interferon + C. toxin 2 hr later.

[e] $P < 0.01$ compared with 500 units interferon.

* Mean of five experiments ± SD.

** Mean of three experiments ± SD.

membrane less receptible for interferon.

One has to consider the possible role of cyclic nucleotides. It has been suggested that cyclic AMP acts as a second messenger to substances which interact with the cell surface from without, fe. insulin and other hormones. Also interferon acts on the cell surface possibly without entering the cell (7–9), and it influences intracellular cAMP levels (10). Cholera toxin increases the accumulation of cAMP intracellularly (11). However, cAMP seems to have an enhancing effect on antiviral activity (12, 13) and usually an inhibiting effect on cell growth (14), the opposite of the observed effect of cholera toxin. It is therefore not very likely that the observed effect of cholera toxin is mediated by cAMP.

The effects on antiviral state and on cell growth were obtained by the same concentrations of the cholera toxin, and they had comparable kinetics suggesting that the step inhibited by cholera toxin is the same or related for the two different activities. Furthermore that cholera toxin blocks an early phase of the interferon action expressed both as antiviral activity and cell growth inhibitory activity. Finally the present findings, obtained with human cells and human interferon show that the effect of cholera toxin is not restricted to the murine system.

Summary. Cholera enterotoxin reduced the antiviral activity and the cell growth inhibitory activity of human leukocyte interferon. Both effects were more pronounced if the cells were treated with toxin before interferon and less if toxin was added after interferon.

The excellent technical assistance of Mrs Solveig Beck and Mrs Zdenka Krajcova is gratefully acknowledged.

1. Friedman, R. M., and Kohn, L. D., Biochem. Biophys. Res. Commun. **70,** 1078 (1976).
2. Besancon, F., and Ankel, H., Nature (London) **250,** 782 (1974).
3. Besancon, F., and Ankel, H., Nature (London) **252,** 478 (1974).
4. van Heyningen, W. E., Nature (London) **249,** 415 (1974).
5. Kohn, L. D., Friedman, R. M., Holmes, J. M., and Lee, G., Proc. Nat. Acad. Sci. U.S.A. **73,** 3695 (1976).
6. Dahl, H., and Degré, M., Acta Pathol. Microbiol. Scand. Sect. B. **80,** 863 (1972).
7. Buckler, C. E., Baron, S., and Levy, H. B., Science **152,** 80 (1966).
8. Vengris, V. E., Stollar, B., and Pitha, P. M., Virology **65,** 410 (1975).
9. Ankel, H., Chany, C., Galliot, B., Chevalier, M. J., and Robert, M., Proc. Nat. Acad. Sci. U.S.A. **70,** 2360 (1973).
10. Mécs, I., Kásler, A., Lózsa, A., and Kovács, G., Acta Microbiol. Acad. Sci. Hung. **21,** 265 (1974).
11. Pierce, N. F., Greenough, W. B., and Carpenter, C. C. J. Jr., Bact. Rev. **35,** 1 (1971).
12. Friedman, R. M., and Pastan, I., Biochem. Biophys. Res. Commun. **36,** 735 (1969).
13. Allen, L. B., Eagle, N. C., Huffman, D. A., Shuman, D. A., Meyer, R. B. Jr., and Sidwell, R. W., Proc. Soc. Exp. Biol. Med. **146,** 580 (1974).
14. Pastan, G. S., Johnson, W. B., and Anderson, W. B., Ann. Rev. Biochem. **44,** 491 (1975).

Received September 23, 1977. P.S.E.B.M. 1978, Vol. 157.