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Cyclic GMP levels in interferon treated cells

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Summary

Various types of normal and neoplastic lymphoid cells in culture respond to interferon with elevated cyclic GMP levels. Human pre-T and natural killer cells are more sensitive to this effect than B lymphocytes. Cultured neurones and erythroid cells also show the effect. However, fibroblasts from different origin, although responsive to the antiviral effect of interferon, do not elevate their levels of cyclic GMP. Only fibroblasts from patients with Down's syndrome which are trisomic for chromosome 21 and more sensitive than their normal disomic counterparts to the antiviral action of interferon exhibit enhanced cyclic GMP levels following interferon treatment. These results suggest that an increase in cyclic GMP does not mediate the principal biologic actions of interferon. Further, the inability of certain cell types to increase their cyclic GMP content in response to interferon may reflect differences in their degree of interferon sensitivity rather than an inherent inability to respond to this action of interferon.

cyclic GMP; interferon

Introduction

Interferons constitute a group of closely related proteins which exert multiple effects on cells including the establishment of an antiviral state, inhibition of cell multiplication, modifications of the cell surface, and modulation of specialized cellular functions [9,10]. The initial step in interferon action had been shown recently to be binding of the protein to a specific high affinity cell surface receptor [2], a property characteristic of other biologically active substances such as peptide hormones and neurotransmitters [11]. We have recently shown that again in common with certain glycopeptide hormones, interferon induces a very rapid and marked increase in the

intracellular concentration of guanosine 3'5'-cyclic monophosphate (cyclic GMP) in mouse leukemia L1210 cells [18,19,21] and in mouse splenic lymphocytes [22]. We also found that the induction of cyclic GMP is closely related to the biologic action of interferon suggesting that this cyclic nucleotide may play a role in the development of the diverse effects of interferon on cells [19]. However, in more recent studies we established that an increase in the intracellular concentration of cyclic GMP is not necessary for the development of the antiviral state or the inhibition of cell multiplication in mouse leukemia L1210 cells [19], or for the enhancement of natural killer (NK) cell toxicity [22]. In confirmation of this, we report here that an early increase in the intracellular concentration of cyclic GMP is not a prerequisite for interferon action in various interferon sensitive cells.

Materials and Methods

Cell cultures

Mononuclear cells were isolated from leucocyte enriched buffy coats by Ficoll-Radioselectan density gradient centrifugation. The few remaining erythrocytes were lysed by hypotonic shock. The resulting cell suspension was designated as 'normal lymphocytes'.

Natural killer (NK) cells were enriched from such a cell population by filtration through a nylon wool column and by purification of the remaining non-adherent cells on a discontinuous Percoll density gradient as previously described [22]. A morphologically homogeneous population of large granular lymphocytes was isolated which was highly enriched for NK cell activity when tested in a 4 h chromium release assay using K562 cells as targets [22].

Peripheral blood cells from untreated patients with neoplasia were obtained by courtesy of Dr. Claude Jasmin, ICIG, Villejuif, France. Mononuclear cells were isolated as described above.

The mouse embryonal carcinoma cell lines, PCC4 and PCD3 [3], mouse 10T1/2 cells [6,7] and human secondary fibroblasts from patients with Down's syndrome [13] were cultivated as monolayers as previously described. Secondary chick fibroblasts were cultivated in Eagle's minimal essential medium (MEM) with 10% inactivated foetal calf serum [16]. Mouse C243 cells were cultivated either in monolayer culture in Eagle's MEM with 10% inactivated calf serum or in suspension culture in Joklik modified MEM with 10% inactivated calf serum as previously described [20]. Explants of cerebral cortex from newborn kittens were cultivated as previously described [8]. Friend leukemia cells (clone 745 obtained from Dr. C. Friend (New York) and clone 3C18 obtained from Dr. E. Affabris (Rome)) were cultivated in suspension culture as previously described [5].

Interferon preparations

Human interferon prepared from the human lymphoblastoid cell line Namalwa and purified by affinity chromatography to a specific activity of 2×10^8 ref. U/mg protein was a gift from Dr. K.E. Mogensen. This interferon was electrophoretically pure when

subjected to electrophoresis in polyacrylamide gels containing SDS. Mouse interferon was prepared from suspension cultures of mouse sarcoma C243-3 cells inoculated with Newcastle disease virus [20] and purified to electrophoretic homogeneity (specific activity of 0.5–1.0 × 10^9 ref. U/mg protein) as previously described [2]. Chick interferon prepared from secondary chick fibroblasts and purified to a specific activity of 10^4 ref. U/mg protein on SP-Sephadex was a gift from Dr. G. Bodo.

Cyclic GMP determination

For cells in suspension, 1 ml aliquots of cell suspension were extracted with 1 ml of 12% trichloroacetic acid for 15 min at 0°C and centrifuged. For cells grown in monolayer, the culture medium was poured off and the monolayer rapidly covered with 1 ml of ice-cold 6% trichloroacetic acid. The cell sheet was then removed with a rubber policeman. The trichloroacetic acid supernatant was then extracted twice with 10 volumes of water saturated ether and the neutralized sample was assayed for cyclic GMP content using a radioimmune assay (Amersham, U.K.). Recovery (70–80%) was monitored with 1 nCi of [^{3}H]cyclic GMP and the specificity of the test was checked with 3'5'-cyclic nucleotide phosphodiesterase as previously described [14]. The trichloroacetic acid insoluble material was dissolved in 0.6 N NaOH and the protein content was determined by the method of Lowry et al. [12] using bovine serum albumin as a standard. Results are expressed in pmol per 10^6 cells or per mg protein.

Results

Cyclic GMP responses in lymphoid cells

As shown in Fig. 1, electrophoretically pure human interferon caused increases in cyclic GMP levels in a morphologically homogeneous population of large granular human lymphocytes enriched for NK cell activity. In contrast, interferon treatment did not affect cyclic GMP levels of normal human peripheral lymphocytes even though the cells were able to respond to other inducers of cyclic GMP such as sodium azide.

Peripheral blood mononuclear cells from untreated patients with various types of leukemia were also tested for their cyclic GMP levels following interferon treatment. No increase in cyclic GMP was observed when cells from patients with chronic lymphocytic leukemia (CLL) were treated with interferon, even though these cells showed elevated levels of cyclic GMP when treated with sodium azide (Fig. 1). The cell populations were rich in B lymphocytes [4]. Similar results were obtained when populations of peripheral cells from patients with acute myelogenous leukemia (AML), which contained mainly myeloblasts were treated with interferon (Fig. 1), although these cells were highly sensitive to the antiviral action of interferon when challenged with vesicular stomatitis virus. In contrast, interferon induced a marked increase in cyclic GMP in peripheral cells from patients with acute lymphocytic leukemia (ALL) where most of the cells are pre-T lymphoblasts. The maximum response, which was of the same magnitude as that induced by 10 mM NaN_3, was observed 5 min after the addition of interferon.

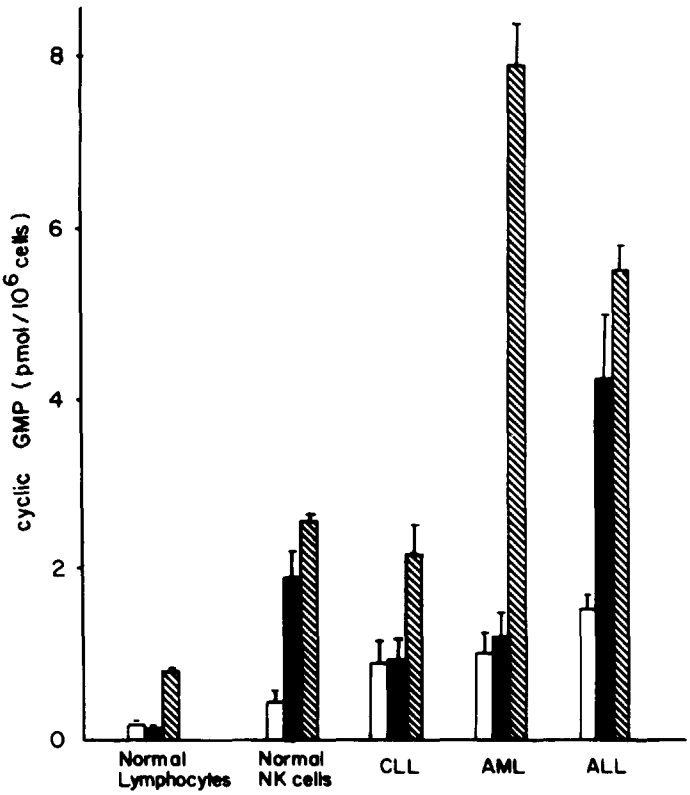

Fig. 1. The effect of interferon and sodium azide on the intracellular concentration of cyclic GMP in normal and leukemic human lymphoid cells. Cells isolated from fresh heparinized blood were resuspended in RPMI 1640 medium with 5% foetal calf serum at a concentration of 10^7 cells/ml and either left untreated (□) or treated with either 10^4 units/ml of electrophoretically pure human interferon (■) or 10 mM sodium azide (▨). The values shown in the figure are peak values obtained 5 min after treatment. Each bar represents the mean ± S.E. Two cases of either normal peripheral lymphocytes or natural killer cells were examined. Four patients with CLL were studied, in which 60–95% of the cells were B lymphocytes and 2–8% T cells. Three preparations of AML with 95–100% myeloblasts were examined. One patient with ALL was tested twice, which contained 95–100% pre-T lymphoblasts.

Cyclic GMP responses in non lymphoid cells

Secondary cultures of chick fibroblasts, mouse 10T1/2 cells, and Moloney sarcoma virus-transformed mouse C-243 cells all failed to produce elevated levels of cyclic GMP in response to interferon treatment (Table 1), even though these cells were sensitive to the antiviral action of interferon [6,7,16,20] and exhibited elevated levels of cyclic GMP in response to treatment with sodium azide. The lack of a cyclic GMP response following interferon treatment did not appear to be related to the fact that the cells were cultivated as monolayers since mouse C-243 cells failed to respond when cultivated either in monolayer or suspension culture (Table 1). However, fibroblasts from patients with Down's syndrome were found to exhibit elevated levels of cyclic GMP 5–10 min after the addition of interferon (Table 1 and Fig. 2).

TABLE 1

The effect of interferon on the intracellular concentration of cyclic GMP in non-lymphoid cells

Cell type	Interferon sensitivity (reference)	Interferon treatment	Cyclic GMP (pmol/mg protein)[a]		
			Control	Interferon	NaN_3 (10 mM)
Chick embryo fibroblasts	Sensitive (16)	Partially purified chick interferon	1.84 ± 0.13	2.02 ± 0.29	6.19 ± 2.33
Mouse 10T1/2 cells	Sensitive (6)	Electrophoretically pure mouse interferon	9.13 ± 0.03	6.06 ± 0.77	26.66 ± 14.16
Mouse C243 cells (monolayer)	Sensitive (20)	Partially purified mouse interferon	3.87 ± 0.14	3.57 ± 0.12	7.45 ± 0.83
Mouse C243 cells (suspension)	Sensitive (20)	Partially purified mouse interferon	0.99 ± 0.03[b]	1.24 ± 0.18[b]	10.9 ± 0.13[b]
Human fibroblasts from patients with Down's syndrome	Highly sensitive (13)	Electrophoretically pure human interferon	0.72 ± 0.07	2.02 ± 0.22	4.25 ± 0.34
Mouse embryonal-carcinoma cells, line PCC4	Resistant (3)	Partially purified mouse interferon	2.02 ± 0.05	1.64 ± 0.06	ND
Mouse teratocarcinoma cells, line PCD3	Resistant (3)	Partially purified mouse interferon	0.12 ± 0.02	0.09 ± 0.01	ND
Friend erythroleukemia cells, line 745 N	Sensitive (1)	Electrophoretically pure mouse interferon	0.29 ± 0.16[b]	0.81 ± 0.26[b]	0.90 ± 0.12[b]
Friend erythroleukemia cells clone 3C18 N	Resistant (1)	Electrophoretically pure mouse interferon	0.78 ± 0.30[b]	0.55 ± 0.17[b]	1.50 ± 0.33[b]
Cultured cat neurones	Sensitive (8)	Electrophoretically pure human interferon	1.46 ± 0.67	4.37 ± 0.35	ND

[a] The data are the values obtained either 1, 5 or 10 min after the addition of interferon and correspond to the peak values obtained in a particular experiment.
[b] Results expressed in pmol/10^6 cells.

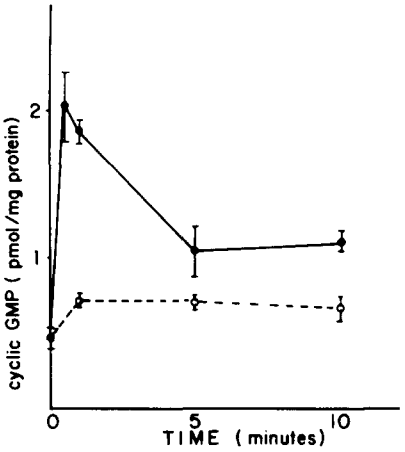

Fig. 2. The effect of interferon on the intracellular concentrations of cyclic GMP in human skin fibroblasts from a patient with Down's syndrome. Human fibroblasts trisomic for chromosome 21 (passage 26, approximately 2×10^6 cells per point) were treated with either 10^4 units/ml of electrophoretically pure human interferon in RPMI 1640 medium containing 0.5% foetal calf serum (●———●) or medium alone (○---○) for the times indicated.

It was of interest to determine whether the degree of differentiation can influence the ability of cells to respond with increased cyclic GMP levels. The undifferentiated embryonal carcinoma cell line PCC4 is resistant to a number of the biologic effects of interferon while the differentiated teratocarcinoma cell line PCD3 is fully interferon sensitive even though both cell lines possess functional interferon receptors [3]. No increase in cyclic GMP was observed when either PCD3 or PCC4 cells were treated with interferon (Table 1).

We then examined cells derived from the erythroid series. A marked increase in cyclic GMP was observed following interferon treatment of the interferon sensitive Friend erythroleukemia cell line 745 whereas no increase in cyclic GMP occurred in the interferon resistant clone 3C18 (Table 1 and Fig. 3), although this resistant clone exhibited specific saturable interferon binding sites as well as the interferon sensitive one [1].

Finally, cultured cat neurones, the excitability of which is enhanced by human interferon [8], displayed a marked increase in the intracellular concentration of cyclic GMP in the presence of interferon (Table 1).

Discussion

We have shown that cultures of normal as well as various neoplastic lymphoid cells exhibit elevated levels of cyclic GMP when treated with interferon. Our results suggest that human normal natural killer cells are more sensitive than normal peripheral lymphocytes to this effect of interferon. Furthermore, peripheral mononuclear cells

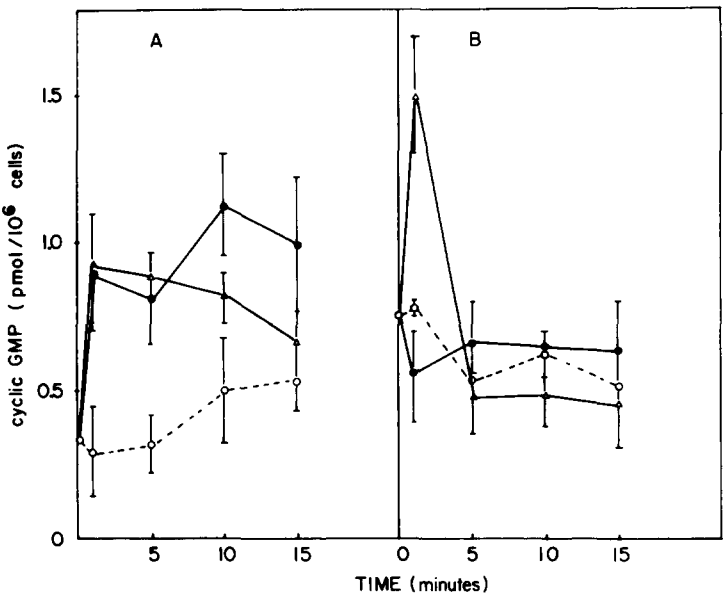

Fig. 3. The effect of interferon on cyclic GMP levels in Friend erythroleukemia cells. Friend cells (2×10^6 cells/ml) in RPMI 1640 medium with 5% foetal calf serum were treated with 10^4 units/ml of electrophoretically pure mouse interferon in phosphate buffered saline (PBS) (●———●); or an equal volume of PBS alone (○---○); or 10 mM sodium azide (△———△). (A) Interferon-sensitive 745 cells. (B) Interferon-resistant clone 3C18.

from patients with CLL which are predominantly B cells [4] were unresponsive, whereas peripheral cells from patients with ALL which are mainly pre-T cells showed a marked increase in cyclic GMP in response to interferon. However, we have shown that the highly interferon sensitive B cell derived Burkitt cell line Daudi does display a peak of cyclic GMP following interferon treatment [15].

The ability of cells to exhibit elevated levels of cyclic GMP in response to interferon is not confined to lymphoid cells but is also shared by fibroblasts, neurones and erythroid cells. Although a number of fibroblasts from different origins failed to respond to interferon, fibroblasts from patients with Down's syndrome did exhibit elevated levels of cyclic GMP following interferon treatment. These cells which are trisomic for chromosome 21 are more sensitive than their normal disomic counterparts to the action of interferon, since the gene(s) coding for the interferon receptor are carried on chromosome 21 [13]. We have also shown that clones of Daudi cells with reduced sensitivity to both the antiviral and antiproliferative actions of interferon fail to produce elevated levels of cyclic GMP in response to interferon, even though these cells bind interferon in a manner similar to the highly interferon sensitive parental Daudi cells [23].

Thus the results of experiments with Daudi cells and cells trisomic for chromosome 21 suggest that the inability of certain cell types such as B lymphocytes or normal fibroblasts to exhibit elevated levels of cyclic GMP in response to interferon treatment

may reflect differences in the degree of interferon sensitivity of different cells rather than an inherent inability of certain types of cells to respond to this action of interferon.

Although an increase in cyclic GMP does not appear to mediate the principal biologic actions of interferon in the systems that we have studied [17,19,22], an increase in cyclic GMP probably reflects rapid changes at the surface of interferon treated cells. The elucidation of the events leading to the increase in cyclic GMP in interferon treated cells should help towards an understanding of the mechanism of the action of interferon on cells.

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