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SYNERGISTIC ACTIVITIES OF TYPE I (α, β) AND TYPE II (γ) MURINE INTERFERONS

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Type I (α, β) and type II (γ) murine interferons are able to potentiate each other with respect to the inhibition of encephalomyocarditis (EMC) virus and of herpes simplex virus type 1 (HSV-1) multiplication in a murine cell line (DBT).

Examination of two double-stranded RNA-dependent enzymes in DBT cells, the 2–5A synthetase and the 67,000 MW protein phosphokinase indicates that mixed interferon preparations act synergistically at least with respect to an increase in the activity of the former enzyme.

The results obtained with γ interferons of different origin and of different specific activity suggest that interferon itself, rather than the lymphokines present in the interferon preparations, is responsible for the synergistic effect.

interferons EMC virus Herpes simplex virus Synergism 2-5A synthetase protein kinase

INTRODUCTION

Interferons can be classified into three types, α, β and γ [23], which are distinguishable one from the other on the basis of their antigenic properties. Furthermore, while α and β interferons (type I) are produced in several kinds of cells following stimulation with viral or chemical inducers, γ interferon (type II) is probably produced by T lymphocytes as a response to an antigen or to lectins. Finally, α and β interferons are acid-stable while γ interferon is rapidly inactivated after pH 2 treatment.

With regard to their biological activities, differences have been also observed with respect to the sensitivity of cell lines [1, 2] and viruses [21] to various interferons. More recently, it has been shown that type I and type II interferons can potentiate each other in terms of their antiviral [8] and anticellular activity [9]. These observations seem to indicate that production of both types of interferons by the host may represent an advantageous mechanism of defense against viral infections and tumor

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invasions and thus bear some interest as far as the clinical application of interferon is concerned.

In our laboratory we have been studying some aspects of the interaction between type I and type II murine interferons. We have focused our attention on the role of interferon itself in the synergistic phenomenon and examined whether contaminants in type II interferon preparations, such as lymphokines, actually contribute to it. Furthermore, we have examined whether the potentiation of the antiviral effect obtained with mixed interferon preparations was accompanied by a concomitant increase in the level of two dsRNA-dependent enzymes, the pppA(2′p5′A)$_n$ synthetase (2–5A synthetase) and the protein kinase which is responsible for the phosphorylation of a 67,000 MW endogenous protein. These two enzymes appear to be involved, at least in some cells, in the mechanism of action of interferon [12].

MATERIALS AND METHODS

Cells

The DBT cell line (SR-CDF$_1$-DBT), which was originally derived from a brain tumor in mice [16], has been kindly provided by Dr. G. Obert (Laboratoire de Virologie, Faculté de Médecine, Strasbourg). Cells were passed weekly in Falcon bottles and grown in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal calf serum, 10% Bacto-Tryptose phosphate broth (Difco), penicillin (40 I.U./ml) and streptomycin (40 μg/ml). For infectivity studies (interferon and virus titration assays), 0.6×10^6 cells were seeded in Falcon Petri dishes (3.5 cm in diameter) in 2.5 ml of the above medium and incubated at 37°C in a 90% air–10% CO_2 atmosphere until confluency (48–72 h post-seeding). Monkey (Patas) kidney cells were obtained from BioMerieux. Cells were grown in Linbro tissue culture plates (1.5 cm in diameter) in Eagle's basal medium (EBM) with Earle's salts supplemented with 10% fetal calf serum and antibiotics (as above). Monolayers of human fibroblasts (MRC_5) in glass culture tubes (BioMerieux) were maintained in EBM medium (Earle's salts) supplemented with 2% calf serum and antibiotics. L-cell cultures were carried out in EMEM medium (Hanks' salts) containing 0.35% glucose, 0.295% Bacto-Tryptose phosphate broth and 6% newborn calf serum.

Interferons

Interferon titers were determined by reduction of the cytopathic effect (CPE) in L cells [11] and are reported as international reference units (I.U.) per ml or per mg protein. Type I (α, β) interferon, a mixture of α and β types according to Yamamoto and Kawade [27], was produced in L cells infected with Newcastle disease virus (NDV) and further purified to a specific activity of 10^7 I.U./mg as described previously [4]. Two kinds of γ interferons were used. One γ interferon was obtained by in vitro stimulation of spleen lymphocytes with phytohemagglutinin (PHA). Such interferon (γPHA) was

purified through ammonium sulfate precipitation and chromatography on Blue Sepharose to a specific activity of 10^5 I.U./mg [26]. The second γ interferon preparation was obtained by intravenous injection of 1 mg of bovine tuberculin into mice which have been previously (21 days) sensitized with *Mycobacterium bovis* (strain BCG) [15, 28]. Crude sera, which were collected 4 h after tuberculin injection, were used in most of the experiments as source of γ interferon (approximately 2000–4000 I.U./ml, specific activity around 10^2 I.U./mg). We will refer to such interferon preparation as γBCG. Inactivation of γBCG was carried out in acid (pH 2) buffer [11]. In some experiments, we made use of a more purified γBCG (10^4 I.U./mg) which was obtained from crude sera by chromatography on Cibacron-Blue F_3-GA-poly-trisacrylamide (IBF France) [22]. Human γ interferon (huγPHA) was prepared as described [5].

Viruses

Preparations of encephalomyocarditis (EMC) virus, herpes simplex virus type 1 (HSV-1) and vesicular stomatitis virus (VSV) were obtained by freezing and thawing of infected DBT, MRC_5 and L cells, respectively.

Antiviral studies

Confluent DBT cell cultures (approximately 4×10^6 cells in 3.5 cm diameter Petri dishes) were treated with different dilutions of the interferon preparations for 18 h, washed twice with PBS and 2 ml EMEM medium, containing 2% fetal calf serum, were suspension in PBS containing 50 μg/ml of DEAE Dextran (Pharmacia). Culturing of cells, virus adsorption and growth were all carried out at 37°C in a 90% air–10% CO_2 atmosphere. After an adsorption period of 1 h, the virus inoculum was removed, cultures were washed twice with PBS and 2 ml of EMEM medium, containing 2% fetal calf serum, were added. After an incubation of 24 h the entire cultures (cells together with their medium) were frozen and thawed twice, centrifuged at $400 \times g$ for 10 min at +4°C and the virus yield was determined by plaque assays. A minimum of two DBT cultures, which were pooled after the first thawing, were used for each assay corresponding to a given amount of interferon. In the case of EMC virus, the plaque assay was also carried out in DBT cells, according to a method which has been described for mouse hepatitis virus (MHV_3) [24].

In the case of the determination of HSV-1 yield in DBT cells, the plaque assays were carried out on monkey kidney cell monolayers using EBM medium supplemented with 0.7% carboxymethylcellulose and 2% calf serum. Forty-eight hours after virus inoculation, the plaques were visualized by fixing with formaldehyde and staining with crystal violet [19]. The results, which were expressed as average virus yield (for three or four determinations) ± the standard error of the mean (S.E.M.), were evaluated statistically by analysis of their variances.

Enzymatic studies

Control and interferon-treated DBT cells in 150 mm plates were washed with PBS and then lysed by addition of 0.5% Nonidet P40 (NP40) in 10 mM Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, pH 7.6, containing 10 mM KCl, 2 mM Mg acetate, 7 mM 2-mercaptoethanol. After 5 min the extract was collected, KCl and glycerol were added to obtain a concentration of 50 mM and 20%, respectively [14]. 0.3 ml poly(I) : poly(C)-Sepharose equilibrated in Hepes buffer–glycerol (10 mM Hepes pH 7.6, 50 mM KCl, 15 mM Mg acetate, 7 mM 2-mercaptoethanol and 20% glycerol) were mixed with 2 mg of cell extract proteins in a 5 ml polystyrene tube. Thirty minutes at room temperature, with occasional gentle mixing, were sufficient for binding the 2–5A synthetase and the protein kinase to the poly(I) : poly(C)-Sepharose. After elimination of unbound proteins by centrifugation, the poly(I) : poly(C)-Sepharose-bound enzymes were separated into two equal aliquots (0.15 ml), one for the assay of the 2–5A synthetase and the other for the assay of the 67,000 MW polypeptide kinase.

For the 2–5A synthetase assay, the poly(I) : poly(C)-Sepharose-bound enzyme was diluted to 750 μl by addition of 3 μCi [^{3}H]adenosine triphosphate (ATP) (3 mM) in Hepes buffer–glycerol. After incubation at 30°C for 17 h, the enzyme–poly(I) : poly(C)-Sepharose complex was removed by centrifugation (1000 × *g* for 10 min) and the supernatant solution was heated at 95°C for 10 min. The ^{3}H-labelled 2–5A was purified by DEAE-cellulose chromatography [14] and its radioactivity measured by liquid scintillation counting.

The 2–5A synthetase levels were determined from the amount of adenosine monophosphate (AMP) present in the 2–5A synthesized in 17 h at 30°C in the reaction mixture (750 μl containing the enzyme derived from 1 mg cell proteins). The results were expressed in concentration (μM) of AMP. For the phosphorylation of the 67,000 MW polypeptide, the kinase–poly(I) : poly(C)-Sepharose fractions (150 μl) were incubated for 60 min at 30°C with 10 μM of [γ-^{32}P]ATP (90 Ci/mmol) in Hepes buffer–glycerol. All samples were then heated at 95°C for 10 min in electrophoresis sample buffer (3.3% sodium dodecyl sulfate (SDS), 100 mM Tris-HCl, pH 6.8, 100 mM 2-mercaptoethanol, 25% glycerol and 0.016% bromophenol blue) and supernatants equivalent to 0.5 mg cell extract were applied to the gel. Polyacrylamide slab gel electrophoresis and autoradiography were carried out as described [14]. The intensity of the 67 K band was evaluated from the surface area (cm^2) of the peak, by scanning the photografic negative (Helena Laboratories Scanner, Texas).

RESULTS

Synergistic antiviral activity of mixed interferon preparations

Fleischmann et al. [8] have shown that, in the presence of both (α, β) and (γ) interferons, the multiplication of Mengo virus in L cells was inhibited to a higher extent than

what could have been expected from the mere sum of their activities. Fig. 1 indicates that a similar phenomenon occurs also in DBT cells infected with EMC virus. The DBT cell line itself is characterized by a poor sensitivity to interferon. We have previously reported [29] that DBT cells, as compared to L cells, are approximately 80–160 times less sensitive to all the interferon types that have been used in the present study.

The degree of inhibition of EMC virus yield which is achieved in the presence of a mixture of 20 I.U. of (α, β) and 5 I.U. of γBCG interferon (total: 25 I.U.) corresponds to approximately 56 I.U. of each single interferon type. Comparable results were obtained by mixing 20 I.U. of (α, β) and 10 I.U. of γBCG interferon, in which case an activity of approximately 74 I.U. of interferon is obtained. Fig. 2 indicates that such a potentiation phenomenon occurs also in the case of HSV-1, thus implying that viruses other than Mengo virus and EMC virus are synergistically affected by mixed interferon preparations. It should be noted, however, that in our experiments the magnitude of the synergistic effect is lower than that described by others [8]. The observed antiviral activity of mixed interferon preparations was equal to that obtained with approximately twice the total interferon units.

The experiment described in Fig. 1 was carried out with crude serum as source of interferon γ. Potentiation of the antiviral effect was also observed when interferon preparations of higher purity, such as γBCG (10^4 I.U./mg) or γPHA (10^5 I.U./mg) were

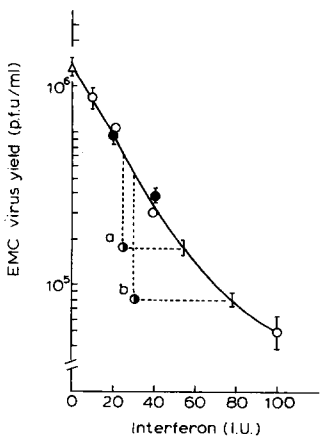

Fig. 1. Synergistic anti-EMC virus activity of (α, β) and γBCG interferons. DBT cells, treated for 18 h with different amounts of (α, β) or γ interferons, either alone or in combination, were then challenged with EMC virus at 6×10^4 p.f.u./plate. Virus yield (5.4×10^6 p.f.u./plate in untreated cultures) was determined as described in Materials and Methods. Vertical bars show S.E.M of three determinations. No interferon (△); (α, β) interferon alone (○); γBCG interferon alone (●); combination of (α, β) and γBCG interferons (◑). Comparison among interferon treatments with respect to their inhibitory activity on virus yield: *a* (20 I.U. α, β + 5 I.U. γBCG) versus 20 I.U. (α, β) or 20 I.U. γBCG alone: $P < 0.001$; *a* versus 40 I.U. (α, β) or 40 I.U. γBCG: not significant. *b* (20 I.U. α, β + 10 I.U. γBCG) versus 40 I.U. (α, β): $P < 0.01$; *b* versus 40 I.U. γBCG: $0.02 < P < 0.03$; *b* versus 100 I.U. (α, β): not significant.

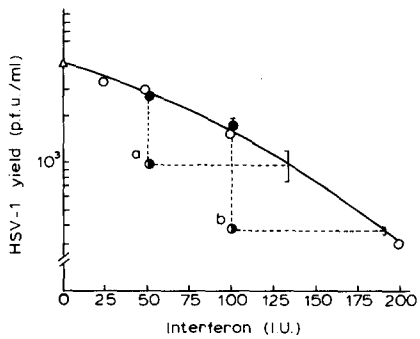

Fig. 2. Synergistic anti-HSV-1 activity of (α, β) and γBCG interferons. DBT cells, treated for 18 h with different amounts of (α, β) or γ interferon, either alone or in combination, were then challenged with HSV-1 at 10^2 p.f.u./plate. Virus yield (4.5×10^3 p.f.u./plate in untreated cultures) was determined as described in Materials and Methods. Vertical bars show S.E.M. of three determinations. No interferon (△); (α, β) interferon alone (○); γBCG interferon alone (●); combination of (α, β) and γBCG interferon (◑). Comparison among interferon preparations with respect to their inhibitory activity on virus yield: *a* (25 I.U. α, β + 25 I.U. γBCG) versus 50 I.U. of either (α, β) or γBCG interferon: $P < 0.001$. *b* (50 I.U. α, β + 50 I.U. γBCG) versus 100 I.U. of either (α, β) or γBCG interferon: $P < 0.001$.

mixed with (α, β) interferon (Table 1, Expts. 1 and 2). In both experiments the virus yield obtained in the presence of both 10 I.U. of (α, β) and 10 I.U. of γ interferon was lower than that obtained when cells were treated with 20 I.U. of (α, β) interferon alone ($P < 0.001$). No synergistic activity against EMC virus was observed by mixing γBCG and

TABLE 1

Multiplication of EMC virus in control and in interferon-treated DBT cells

Experiment No.	Interferon type (I.U./ml)	Virus yield ± S.E.M. ($\times 10^{-3}$)	Inhibition of virus yield (fold)
1	0	4133 ± 33.3	0
	(α, β) (10)	1866 ± 33.3	2.22
	(α, β) (20)	1110 ± 10	3.72
	(α, β) (100)	35.3 ± 5	116.99
	(α, β) (1000)	3.37 ± 0.7	1226
	γBCG (10)[a]	2033 ± 88	2.03
	(α, β) (10) + γBCG (10)[a]	96.7 ± 8.8	42.74
2	0	4700 ± 173	0
	(α, β) (10)	2500 ± 173	1.88
	(α, β) (20)	933 ± 177	5.05
	(α, β) (100)	163 ± 39	28.83
	γPHA (10)	2600 ± 100	1.81
	(α, β) (10) + γPHA (10)	157 ± 59	29.94

[a] γBCG specific activity: 10^4 I.U./mg.

γPHA interferons, in which case the antiviral effect corresponded to the sum of their separate activities. Similarly, serum from untreated mice, lacking interferon, were unable to potentiate the antiviral activity (α, β) interferon (results not shown). It could be argued that the purity of γ interferon preparations was not high enough to exclude the possibility that substances other than interferon itself, could play a role in the synergistic effect. In an attempt to exclude such a possibility we examined the behavior of human γ interferon (huγPHA) and pH 2-inactivated murine γBCG (γBCGpH2) for their ability to potentiate (α, β) interferon. The rational was that, if hypothetical contaminants (e.g. lymphokines) active with respect to the synergistic effect really did exist, they could eventually be differentiated from interferon itself by: 1) the lack of species specificity displayed by these molecules or 2) their acid stability [20, 25]. Table 2 shows the results obtained in the case of human γ interferon (Expt. 1) and pH 2-inactivated murine γ interferon (Expt. 2). As reported in the first experiment, addition of human γ interferon to DBT cells resulted in a slight inhibition (1.18-fold) of virus growth, which, however, was not statistically significant. As expected, the inhibitory activity of (α, β) (10 I.U.) and γ (170 I.U.) murine interferons in combination was found to be similar to that obtained with 340 I.U. of γBCG. In contrast, the virus yield in DBT cells treated with mixed murine (α, β) (10 I.U.) and human γ (10 I.U.) interferon preparations did not differ statistically from that of untreated cells or cells treated with up to 20 I.U. of (α, β) murine interferon.

TABLE 2

Multiplication of EMC virus in control and in interferon-treated DBT cells

Experiment No.	Interferon type (I.U./ml)	Virus yield + S.E.M. ($\times 10^{-4}$)	Inhibition of virus yield (fold)
1	0	2000 ± 19.9	0
	(α, β) (10)	1763 ± 319.7	1.13
	γBCG (170)	22 ± 1.63	90.91
	γBCG (340)	8.67 ± 0.26	230.68
	(α, β) (10) + γBCG (170)	7.13 ± 0.44	280.50
	huγPHA (10)[a]	1693 ± 181	1.18
	(α, β) (10) + huγPHA (10)[a]	2366 ± 366	0.85
2	0	3830 ± 211	0
	(α, β) (20)	2933 ± 152	1.31
	(α, β) (100)	500 ± 109	7.66
	γBCG (80)	759 ± 125	5.05
	γBCG (160)	61 ± 8.6	62.79
	γBCG (320)	6.43 ± 0.29	595.65
	(α, β) (20) + γBCG (80)	18.87 ± 2.32	202.97
	γBCGpH2 (80)[b]	5400 ± 122	0.71
	(α, β) (20) + γBCGpH2 (80)[b]	3693 ± 52.08	1.04

[a] Human γPHA. All other interferon preparations were from mouse origin.

[b] pH2-Inactivated γBCG: 80 I.U./ml as determined before inactivation.

It can be seen in Table 2 (Expt. 2) that γBCG (80 I.U.) potentiated the antiviral activity of (α, β) (20 I.U.) interferon. The virus yield in cells treated with such a mixed interferon preparation was similar to that obtained in the presence of 160 I.U. γBCG and significantly lower ($P = 0.01$) than that exerted by 100 I.U. of (α, β) interferon. In contrast, combination of both pH 2-inactivated γBCG (80 I.U.) and (α, β) (20 I.U.) interferons resulted into an inhibitory activity which did not differ statistically from that obtained with 20 I.U. of (α, β) interferon alone. Therefore pH 2-inactivated murine γ interferon or human γ interferon are unable to potentiate the antiviral activity of (α, β) murine interferon. These results, as well as those obtained with more purified preparations (Table 1), suggest that interferon molecules themselves are responsible for the synergistic effect. However, the participation of other molecules (e.g. which might copurify with interferon and be species-specific and acid-inactivated) cannot be excluded.

Synergistic induction of dsRNA-dependent enzymes by mixed ((α, β) and γBCG) interferon preparations

Treatment of mouse L cells with interferon results in the induction of two dsRNA-dependent enzymes, the 2–5A synthetase and the protein kinase. It has been shown that (α, β) and γ interferons are both effective in increasing the levels of these two enzymes in L cells [13]. DBT cells did not differ from L cells in this respect. It can be seen in Fig. 3 that (α, β) and γ interferons increased the level of 2–5A synthetase in DBT cells in a dose-dependent manner. As expected from the antiviral studies, mixed interferon preparations acted synergistically with respect to the induction of the 2–5A synthetase. For example (Fig. 3, left part) the enzymatic activity (11 μM) obtained from a mixture

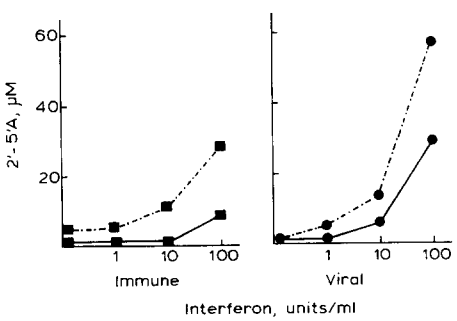

Fig. 3. Synthetase levels in DBT cells treated with (α, β) and γBCG interferon. The 2–5A synthetase was assayed as described in Materials and Methods. Left: The 2–5A synthetase levels as a function of γBCG (immune) interferon alone (■——■) or in combination with a constant amount (10 I.U./ml) of (α, β) (viral) interferon (■- - -■). Right: The 2–5A synthetase levels as a function of (α, β) (viral) interferon alone (●——●) or in combination with a constant amount (10 I.U./ml) of γBCG (immune) interferon (●- - -●). The enzyme levels in cells treated with 20 I.U./ml of γ or (α, β) interferon were 0.6 and 7.8 μM, respectively.

of 10 I.U. of γ interferon and 10 I.U. of (α, β) interferon was greater than that obtained with 100 I.U. of γ interferon (7.5 μM). In Fig. 3 (right part) it can be seen that the level of 2–5A synthetase activity (4.5 μM) achieved in the presence of both 1 I.U. of (α, β) and 10 I.U. of γ interferon, which are practically inactive by themselves, is similar to that obtained in the presence of 10 I.U. of (α, β) interferon (5.5 μM).

In contrast, no synergistic induction of 2–5A synthetase activity was observed upon mixing (α, β) interferon with control mouse serum. In fact, the simultaneous addition of (α, β) interferon (10 or 100 I.U./ml) and 1.66% of control mouse serum (a concentration which is equivalent to 100 I.U./ml of γBCG in the case of immune mouse serum) did not result into an increase of enzyme activity over the values obtained with (α, β) interferon alone (5.5 μM and 28 μM respectively).

The ability of γ interferon to potentiate the (α, β) type was not restricted to crude γBCG preparations but also observed with semipurified interferon (γPHA). In Table 3 it can be seen that addition of both (α, β) (100 I.U.) and γPHA (100 I.U.) interferons resulted into a 2–5A synthetase activity which was greater than that obtained with either 100 I.U. of γPHA or 200 I.U. of (α, β) interferon.

Fig. 4. shows that both interferon types increased the level of the protein (67,000 MW) kinase, as it has been shown for L cells. Although Fig. 4 does not allow a correct quantification of the results, it appears nevertheless that the two interferon types have at least an additive effect on the induction of the kinase. There is actually some suggestion that the two interferons act synergistically. The intensity of the 67,000 MW phosphorylated band (10.91 cm^2) obtained from DBT cells treated with 10 I.U. of both interferon types (gel No. 6), appears greater than the sum of those obtained in the case of 10 I.U. (α, β) interferon (gel No. 3 : 4.54 cm^2) and of 10 I.U. of γ interferon (gel No. 8 : 2.03 cm^2).

DISCUSSION

The type I (α, β) and type II (γBCG; γPHA) interferon preparations which we have used in our experiments did not differ significantly in their inhibitory activity against

TABLE 3

2–5A synthetase levels in DBT cells treated with (α, β) and γPHA interferons

Interferon type (I.U./ml)	Enzyme activity (μM)
0	< 1
(α, β) (100)	21.5
(α, β) (200)	36.6
γPHA (10)	< 1
γPHA (100)	7.8
(α, β) (100) + γPHA (10)	73.3

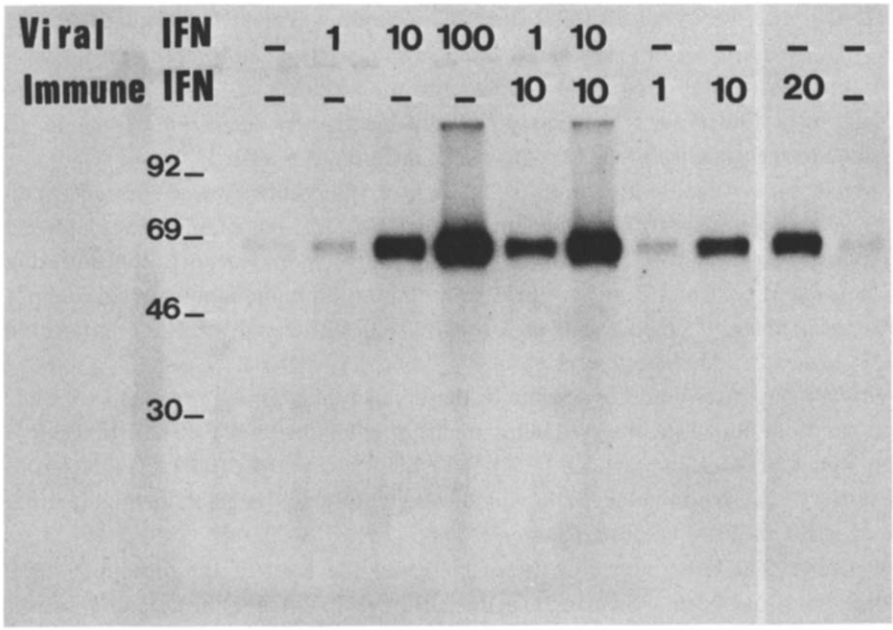

Fig. 4. Phosphorylation of the 67,000 MW protein in control and interferon-treated DBT cells. Treatment with different concentrations of viral (α, β) and immune (γBCG) interferon (0–1–10–100 I.U./ml) was as indicated. The effect of mixed, (α, β) and γBCG, interferon preparations is represented in the fifth and sixth gel from the left. The numbers on the left give the molecular weights of protein markers in thousands: 92, phosphorylase B; 69, bovine plasma albumin; 46, ovalbumin; 30, chymotrypsin. Each sample represents kinase activity from 0.5 mg cell extract.

the multiplication of EMC virus or HSV-1 in DBT cells. Furthermore, data which have been presented elsewhere [29] have indicated that replication of mouse hepatitis virus in thioglycollate-induced macrophages is equally affected by (α, β) and γ interferons. In contrast to the lack of a differential antiviral activity which we report here for the various interferon types, other workers have claimed that human γ interferon exerts a much higher inhibition on reovirus and vaccinia virus multiplication than human leucocyte (α) interferon [21]. For EMC virus, however, both interferon types possessed a similar antiviral activity, which is in agreement with our results obtained in a murine system. Therefore it appears that, if any differences exist in the antiviral activities of type I and type II murine interferon, these are restricted to only a few viruses.

It has been found [8] that (α, β) and γ interferons potentiate each other with respect to the inhibition of Mengo virus multiplication in L cells. Our results show that in another cell system (DBT cell line), both EMC virus and HSV-1 are sensitive to such a synergistic effect. In fact, we have found that the degree of inhibitory activity of mixed interferon preparations is approximately twice that expected on the basis of the sum of their separate activities. In our study, however, the magnitude of the synergistic effect was lower, one-half to one-tenth, than that reported previously by Fleischmann et al. [8].

Since the γ interferon preparations used in our study possessed a low degree of purity, one might argue that a contaminant rather than interferon itself was responsible for the synergistic phenomenon. We have found, however, that γ interferon preparations of low (10^2 I.U./mg) and high (10^5 I.U./mg) specific activity were all capable to potentiate (α, β) interferon. These results indicate that the 'potentiating substance' is γ interferon itself or copurifies with γ interferon. One possibility to distinguish between γ interferon and contaminants, particularly lymphokines, is to examine the species specificity of the potentiating substance and its sensitivity to acid treatment. It is well known that γ interferon is unstable at acid pH and it is species specific [23]. In contrast, it has been shown that monokines and lymphokines, such as human interleukins I and II, are acid-stable and display their activity on murine cells [20, 25]. Furthermore, preliminary results obtained by one of us (E.F.) indicate that at least three lymphokines produced by PHA-stimulated human lymphocytes (T-cell growth factor, immunoglobulin binding factor, T-cell replacing factor) are not species-specific, being able to exert their biological effects on heterologous (murine) cells.

Since no synergistic antiviral activity has been observed in DBT cells treated with either human γ interferon or pH 2-inactivated mouse γ interferon, it appears likely that γ interferon itself is involved in the potentiation of (α, β) interferon. However, the participation of other substances, with interferon-like characteristics, cannot be excluded at the present time.

Although the precise role of the 2–5A synthetase and the protein kinase in the overall action of interferon remains to be elucidated, it is assumed that, at least in some cells, the antiviral and anticellular activities of interferon are mediated by the inhibitory reactions of these enzymes on protein synthesis.

We have shown that (α, β) and γ interferons potentiate each other with respect to the induction of at least one of these enzymes, namely the 2–5A synthetase. Therefore, dsRNA-dependent enzymes, such as the 2–5A synthetase, appear to be suitable biochemical markers for studying the interaction of different types of interferons in some cellular systems.

The synergistic activities of mixed interferon preparations implies that the two interferon types exert their biological effects on cells through metabolic pathways which, up to a certain point, have to be different. Our results concerning the induction of the 2–5A synthetase in DBT cells, suggest that such pathways already differ at a step which precedes the induction of this enzyme. This is supported by the demonstration of Branca and Baglioni [3] that the cell surface receptors for type I and type II interferons are different.

At the present time, any hypothesis concerning the mechanism(s) involved in the synergism between type I and type II interferons would be speculative. It might be that binding of one type of interferon to its own receptor enhances the expression of the receptor for the other type (and vice versa), thereby leading to the potentiation of the biological effects seen after simultaneous treatment of cells with both interferon types. This hypothesis is consistent with i) the fact that the two interferon types interact at

the cell surface with different receptors [3] and ii) the observation that interferons exert pronounced effects on cellular membranes, being able to increase the expression of membrane components such as Fc receptors [10], β_2-microglobulin [6, 7] and histocompatibility antigens [7, 17, 18].

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