INHIBITION OF STEROID INDUCIBLE TYROSINE AMINOTRANSFERASE BY MOUSE AND RAT INTERFERON IN HEPATOMA TISSUE CULTURE CELLS

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1. Introduction

Interferons are cell-derived glycoproteins induced by viral infection and by a variety of non-viral agents. They are characterized principally by their capacity to establish an antiviral resistance in sensitive cells [1].

Recent reports from several laboratories [2–4] have shown that the translation of messenger RNAs, from viral and cellular origin, was impaired in cell-free systems derived from interferon treated cells. The synthesis of total cellular proteins, measured either in whole cells or in cell-free systems, was inhibited by the interferon treatment, but to a lesser extent [5].

In the present work, the effect of interferon on the synthesis of a specific enzymic protein has been studied in vivo. We utilised the particularity of the outstanding property of hepatoma tissue culture (HTC) cells which respond to adrenal steroids by a 5–15-fold increase of the synthesis of tyrosine aminotransferase (TAT) [6]. Our results show that partially purified mouse interferon, which has antiviral activity in rat cells, and crude rat interferon act on HTC cells by an inhibition of steroid-induced TAT.

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2. Materials and methods

2.1. *Interferon preparations and assays*

Rat fibroblast interferon (RF-I) was prepared on 2–3 days old confluent monolayers of primary rat embryo cells (PREC) infected with Newcastle disease virus (NDV, Hertz strain). After 24 hr incubation at 37°C the culture fluids were harvested, the remaining virus inactivated by pH 2 treatment for 5 days, and ultracentrifuged. The preparations were then concentrated 5 to 10 times by pressure dialysis. Rat serum interferon (RS-I) was produced in rats by intravenous injection of 1 ml NDV suspension (about 5×10^9 EID 50). Interferon appeared in the sera with a peak at 6 to 8 hr after inoculation. The viral inducer was inactivated as described above. Both interferons were titrated by the plaque reduction test on PREC cells using Vesicular stomatitis virus (VSV) as challenge virus. RF-I had a titer of 250–500 units/ml. The titer of RS-I was 500 units/ml.

Mouse L cell interferon (ML-I) was prepared and purified as previously described [2]. Its final specific activity ranged from 5×10^6 to 10^7 international units/mg protein when the assay was performed on L cells. This interferon also showed an antiviral effect when tested on cells of related species [7]. In our hands, the ML-I exerted about 2% of its homologous activity. In the experiments detailed here, all the interferon titers are expressed in rat units.

2.2. *Cell cultures, protein and TAT determinations*

HTC cells were grown at 37°C in suspension cultures in Swim's 77 medium supplemented with 10% calf serum [8]. Cell counting was performed in the

presence of Trypan Blue for evaluation of viability. TAT enzymic activity was assayed by the method of Diamondstone [9]. Protein concentration was determined by the method of Lowry et al. [10].

3. Results

All experiments were performed on HTC cells in suspension cultures. The various preparations of interferon were added 15 hr prior to the addition of 10^{-7} M dexamethasone as inducer of TAT synthesis. Samples were taken 4, 10, 16 and 24 hr after addition of the hormonal inducer in order to estimate the cell concentration, the total protein concentration and the enzymic activity of TAT. Fig. 1 shows that the treatment with interferon produced a decrease in the specific activity of TAT, which was dose dependent. Two consecutive aspects of interferon action can be pointed out: (i) during the first 10 hr following the

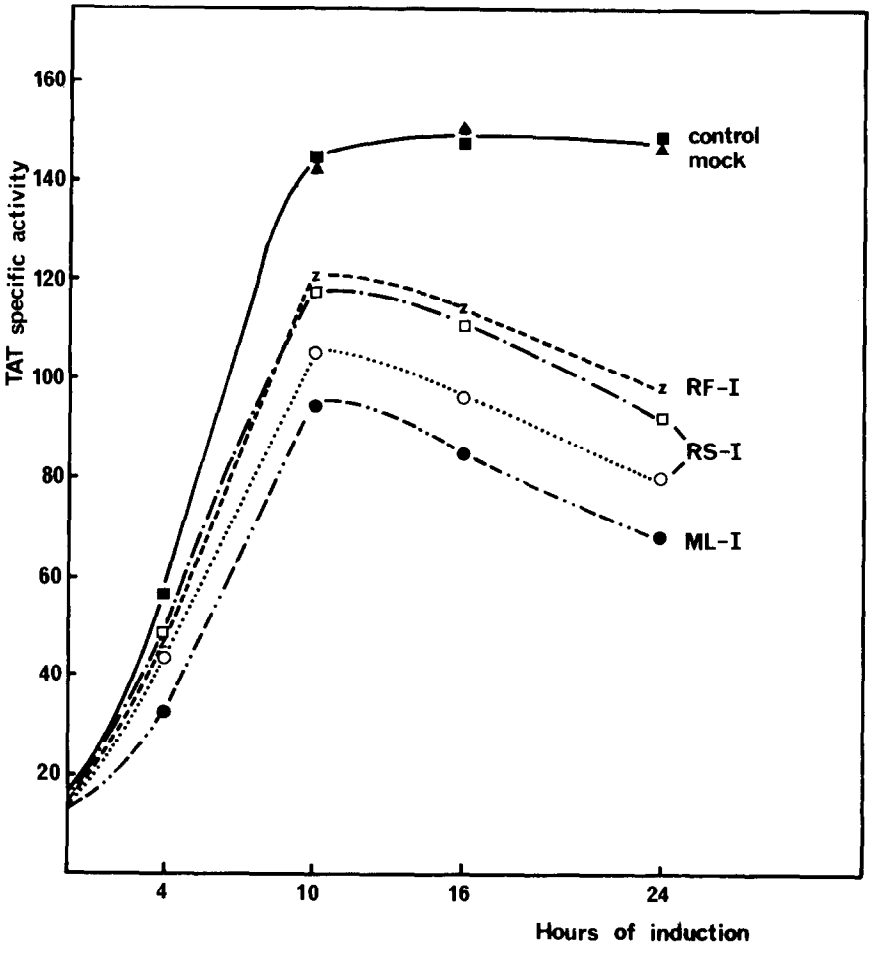

Fig. 1. Time course of the inhibition of steroid induced TAT in HTC cells by interferon of different origins. HTC cells were grown in batches of 30 ml suspension cultures at a concentration of 3×10^{-5} cells/ml. 3 ml of PBS (control), mock interferon diluted in PBS or interferon diluted in PBS in the concentration indicated below was added. 15 hr later, HTC cells were induced to synthesize tyrosine aminotransferase by adding 10^{-7} M dexamethasone. TAT specific activity (units/mg protein) was determined for various induction times: control (■–■–■), cells pretreated with mock interferon (▲–▲–▲), mouse interferon, ML-I, 300 units/ml (●– – –●), rat fibroblast interferon, RF-I, 3 units/ml (z– –z), rat serum interferon, RS-I, 3 units/ml (□–·–□) and 15 units/ml (○.....○).

addition of the hormone, TAT induction was partially inhibited; after this period, the maximum level of enzymic activity was reached in the control as well as in the interferon treated cells; the decrease of specific activity relative to control cells ranged from 14 to 34%, depending on the dose of interferon; (ii) after reaching the maximum level of induction, the specific activity of the enzyme decreased in the interferon treated cultures although it remained unchanged in the control cultures for the next 14 hr. At that time the decrease of specific activity reached 33 to 54%, and was also dose dependent.

In addition to this specific effect, we confirmed (table 1) the previously reported action of interferon on cell division associated with a decrease in the total protein synthesis [11]. For instance, with the highest dose of interferon tested (300 units/ml), no cell division could be observed. Nevertheless the interferon added did not display any toxic effect, as no dead cells could be observed in the medium.

4. Discussion

Our results confirm the inhibitory effect of interferon on the translation of cellular messenger RNAs [5]; the present demonstration concerns the in vivo synthesis of a hormone-inducible specific protein.

The experiments reported here were performed with crude preparations of two different kinds of rat interferon and with a partially purified mouse interferon. Even if we cannot exclude the possibility that the inhibition we describe is related to the presence of impurities, it is nevertheless unlikely that all the preparations, which differ in origin, should all contain the same active impurity. Moreover the inhibition is dose dependent and the doses have been measured as a function of the antiviral action.

Recently, Vassef et al. [12] reported, with techniques somewhat different from ours, a 20% inhibition of TAT induction in layer cultures of HTC cells pretreated with an interferon prepared on the same cells with poly I:poly C as inducer. Our results include the kinetic study of the induction of TAT synthesis, in the presence or absence of interferon.

In fact, interferon not only inhibits the synthesis of this specific enzymic protein, but also acts on the total cellular proteins. However TAT is more affected than the overall protein synthesis. Thus, it seems that the proteins which are synthesized at a faster rate are more inhibited by the interferon action.

The steady state reached in control cultures is the

Table 1
The effects of interferon pretreatment on cell growth and protein content during TAT induction

	Hormone induction time			
	0		24 hr	
Interferon treatment	Cell number $\times 10^5$	Protein μg/ml	Cell number $\times 10^5$	Protein μg/ml
Control (no addition)	4.22	246	6.56	360
Mock interferon	4.32	243	6.70	365
Rat serum interferon				
3 units/ml	3.99	236	4.56	279
5 units/ml	3.28	226	3.98	242
Rat fibroblast interferon 3 units/ml	3.96	220	4.38	281
Mouse interferon				
300 units/ml	3.04	158	2.98	168

HTC cells were grown and treated as described in fig. 1.

consequence of an equilibrium between the synthesis and the 'normal' degradation of the enzyme [13]. In the interferon-treated cultures the decrease of the specific activity of the enzyme presents some similarities with the decrease observed upon removal of the inducer, or with the 'enhanced' degradation of TAT in HTC cells grown in 'step-down' conditions [8]. The molecular basis of the decrease of the specific activity of the enzyme in interferon treated cells remains to be established.

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