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POTENTIATION OF THERMAL INJURY IN MOUSE CELLS BY INTERFERON

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Mouse cells, when exposed to high temperature (43°), shut off overall protein synthesis and continue to synthesize "heat shock proteins". Such heat shocked cells, upon reincubation at 37°C, recover and proliferate. However, when mouse cells are pretreated with mouse interferon (IFN) and then exposed to 43°, more than 99% of the cell population fail to recover. Synthesis of the major heat shock protein is unaffected in cells treated with IFN. Experiments designed to assess the role of intracellular glutathione (GSH) during cells' recovery from hyperthermia indicated that there is an irreversible depletion of glutathione when IFN treated cells are heat shocked. Neither depletion of GSH, nor potentiation of thermal injury was observed in a IFN-resistant line of mouse cells.

Interferons (IFN), a group of low molecular weight proteins, induce pleiotropic effects on animal cells. Apart from its well-known antiviral effects IFN can also enhance, modify or inhibit the expression of cellular genes (for a review see 1). At present, the exact basis of IFN's multiple effects on gene expression remains obscure.

Several laboratories are using the heat shock response of eukaryotic cells as a model system for studying regulation of transcription (for a review see 2). A variety of cells, when exposed to temperature 5-15°C above their optimum for growth, respond by inducing the synthesis of a few polypeptides called heat shock proteins (HSPs) with concomitant reduction in the synthesis of other cellular proteins. Both de novo transcription and translation are needed for the expression of the HSPs. In light of the varied effects of IFN on gene expression it was of interest to determine whether the induction of HSPs is affected by IFN. Here we report that IFN did not influence the induction of HSPs in mouse cells. Nevertheless, IFN treatment dramatically reduced the survival rate of mouse cells following heat shock.

MATERIALS AND METHODS

Cell culture: Swiss 3T3 cells were grown at 37°C in Dulbecco's modified Eagle's medium, 10% calf serum, 100 units/ml penicillin and 100 µg/ml streptomycin in a

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humidified atmosphere of 10% CO_2. Cell survival analysis was made by removing them from the petri dishes using 0.05% trypsin and ethyleneadiamine tetracetic acid (0.1g/liter). The cell suspension was stained with trypan blue and the viable cell number was determined by counting the unstained cells using a hemocytometer.

Interferon: Preparations of mouse L cell interferon (α and β) with specific activity of 4.4×10^7 units/mg protein were purchased from Enzo Biochem. Inc., New York. Human lymphoblastoid interferon (specific activity 3×10^7 units/mg protein) was a gift from Dr. Joyce Taylor-Papadimitriou, Imperial Cancer Research Fund, London, U.K. Human IFN α A/D (bgl) was a gift from Genentech Inc., San Francisco, Calif. and had a specific activity of 1×10^8 units/mg protein.

Heat shock: Cultures to be heat shocked were prepared using exponentially growing cells and seeded at a density 1 to 2×10^5 cells per 33 mm Nunc dish and allowed to divide at 37°C for 48 h. Such subconfluent monolayers of cells were floated in a precision temperature-controlled circulating water bath (Beckman Instruments) at 43° $\pm$ 0.03°C with 10 mM Hepes (pH 7.4) added to the medium. After hyperthermia treatment growth medium in the cultures was replaced with fresh pre-warmed medium and incubation continued at 37°C for 24 hours.

Glutathione assay: The method used was identical to that reported by Mitchell *et al.* (3). The cultures were washed and the cells removed from the plates using trypsin. The cells were washed twice with cold phosphate buffered saline and resuspended in cold 0.6% sulfosalicylic acid (10^6 cells/2 ml), lysed by sonication and then centrifuged at 4°C. The supernatant was assayed for total GSH by the GSH reductase procedure (4).

Other Materials and Methods:

Protein was determined by the method of Lowry *et al.* (5). All chemicals used were purchased from Sigma Chemical Co., St. Louis.

RESULTS AND DISCUSSION

The majority of polypeptides cease to be synthesized when mouse cells are incubated at 43°C for 60 min. However, the heat shock messages are preferentially transcribed and translated during this period (6-8). The heat shocked cells, upon reincubation at 37°C, recover within 24 h and begin to proliferate. Our preliminary experiments indicated that this recovery did not occur when cells were treated with IFN. Therefore, we determined whether the observed effects depended upon the dose and the type of IFN used. The results (Table 1) indicated that even at a concentration of 10 IU/ml of IFN, the survival rate of the heat shocked cells was considerably reduced and at 250 IU/ml 99.99% of the cells failed to survive. Human lymphoblastoid IFN, known to be biologically inactive in mouse cells, failed to potentiate cell killing following heat shock. However, human α IFN A/D (bgl), a recombinant IFN capable of inducing the antiviral and anti growth effect in murine cells, behaved like mouse IFN in potentiating thermal injury. These results suggest that the enhanced cell killing is an IFN mediated effect. Treatment of control cells

Table 1. Effect of IFN on the ability of mouse 3T3 cells to recover from heat shock

Treatment	% Survival after heat shock
None	52.0
Cycloheximide	99.0
Mouse IFN, 250 IU/ml	< 0.01
Mouse IFN, 100 IU/ml	5.2
Mouse IFN, 10 IU/ml	24.0
Mouse IFN, 250 IU/ml + Cycloheximide	< 0.01
Human lymphoblastoid IFN 1000 IU/ml	45.2
Human leukocyte IFN α-A/D (bgl) 1000 IU/ml	< 0.01

Swiss Mouse 3T3 cell cultures were prepared in 33 mm dishes by seeding $2x10^5$ cell/dish. The cultures were used forty eight hours later at which time there were $5x10^5$ cells/culture. HEPES buffer (100 mM) was added to achieve a final concentration of 10 mM. The cultures were floated in a circulating water bath held at 43°C + 0.01. At the end of 60 min of incubation the medium was removed, cultures rinsed three times with growth medium and fresh growth medium added. The cultures were incubated for 24 h, at the end of which the number of viable cells per culture was determined. Duplicate cultures were used for each determination. Wherever IFN was used the cultures were incubated with growth medium containing IFN for 16 h before the heat shock. Cycloheximide at 10 µg/ ml, was added to the cultures 15 min prior to heat shock. The results presented are an average of three independent determinations.

with cycloheximide during incubation at 43°C protected such cells from thermal stress; a finding consistent with the observation of McCormick and Penman (9) who reported that the thermal stress induced inhibition of protein synthesis can be abrogated by treatment of cells with cycloheximide during heat shock. Yet the IFN induced potentiation of thermal injury was evident even in cells heat shocked in the presence of cycloheximide.

A variety of other mouse cell lines were tested to determine whether IFN could enhance cell death following heat shock. These included both normal and primary mouse-embryo cells as well as murine cells transformed by DNA or RNA tumor viruses or chemical carcinogens. In all instances mouse IFN markedly potentiated cell death during recovery from heat shock (data not presented). However, human IFN (α or β) could not potentiate thermal injury in a variety of normal or transformed human cells.

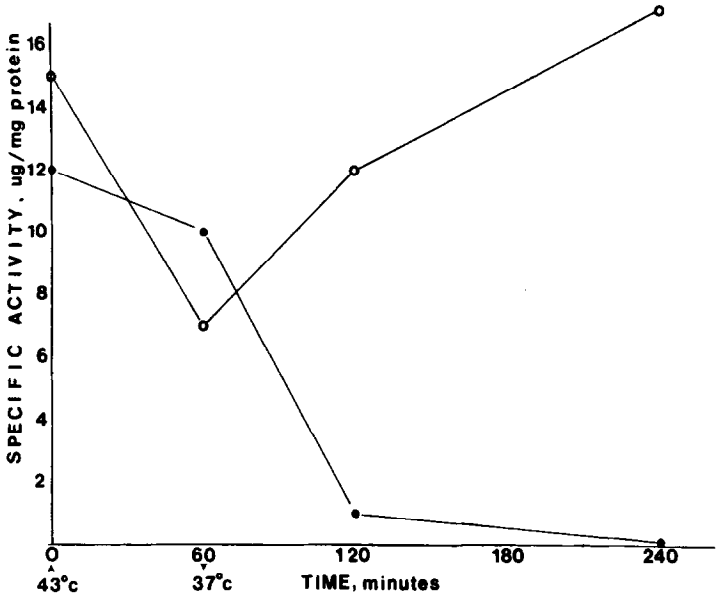

Fig. 1. Effect of IFN on intracellular glutathione level in Swiss 3T3 cells. The experimental details were similar to those described in Table 1. The total GSH levels (oxidized and reduced forms) were determined and the specific activities are shown per mg protein. ○— untreated cells ●— IFN treated cells.

The polypeptides synthesized in 3T3 cells during thermal treatment and 37°C were compared by SDS-PAGE analysis of ^{35}S methionine labeled proteins. The results (not presented here) indicated the following: preferential synthesis of a protein with Mr of 70,000 was observed only at 43°C. The synthesis of two polypeptides (Mr 92,000 and 72,000) was enhanced in the heat-shocked cells. These results are identical to those reported by Khandjian and Turler in mouse kidney cells (10). The pattern and the extent of synthesis of these heat-shock proteins was unaffected by treating 3T3 cells with IFN. Thus, although the heat shock proteins were synthesized in IFN treated cells the cells failed to survive. These results suggest that cells' recovery from thermal stress should include events in addition to that of HSP synthesis.

At present, data exist in the literature supporting the notion that thermal resistance of animal cells is related to the intracellular glutathione (GSH) levels. Glutathione, a tripeptide, has been implicated in a variety of cellular functions including protection of cells against free radicals (11). Mitchell et al. (3) reported a rapid elevation in GSH levels in Chinese hamster V79 cells during heat shock. The cellular GSH level increased within the first hour of incubation at

Table 2. Glutathione level and cell survival after heat shock

Treatment	3T3		IFN^r	
	# of viable cells	GSH μg/mg protein	# of viable cells	GSH μg/mg protein
None	$2.2x10^5$	4.6	$3.0x10^5$	7.9
Mouse IFN 1000 IU/ml	$<10^4$	<0.5	$1.1x10^5$	7.4

The details of the experiment are identical to those described in Table 1. The level of GSH shown above represents the sum of the oxidized and reduced forms.

43°C and remained elevated for a considerable period of time during the cells' recovery period at 37°C. We determined the levels of GSH in control and IFN treated cells during heat shock and subsequent recovery at 37°C. The results (Fig. 1) indicated that cells incubated with or without IFN at 37°C had similar levels of GSH. Incubation of cells at 43°C for 60 min was accompanied by a drop in the level of GSH in both control and IFN treated cells: a finding in contrast to that reported by Mitchell et al. (3). The GSH level in control cells began to rise within an hour into the recovery period at 37°C, and by three hours the cells had regained the original (37°C) level of GSH. However, the GSH level in IFN treated cells fell precipitously. Is the observed depletion of GSH level in IFN treated cells related to the inability of such cells to recover from thermal injury? To answer this we examined the effect of IFN on potentiating thermal stress in IFN^r cells, a mutant 3T3 cell line. Unlike the parent 3T3 cells in which IFN can induce both anti viral and anti cell growth effects only anti viral effects are induced in IFN^r cells. Thirty percent of IFN^r cells treated with IFN and heat shocked survived (Table 2). The potentiating effect of IFN to thermal injury was considerably less than that observed in the parent cell line. The levels of GSH in control and IFN treated cells were also similar. Thus, it appears that depletion of GSH level may be related to cell death induced by heat shock in IFN treated cells. How the depletion of GSH occurs in IFN treated cells remains to be established. IFN has been reported to increase the rigidity of plasma membranes in several cell types (13). Whether such changes have any relevance in IFN's ability to potentiate thermal injury remains to be determined.

The data presented here have established that IFN can potentiate injury induced by heat shock in mouse cells. The mouse IFN preparation used here consisted of a mixture of IFN-α and β; whether both types possess similar activity in this respect cannot be ascertained from the present results. We have not tested the effect of mouse IFN-γ for its ability to potentiate thermal injury since purified preparations of the same are not available at present. Surprisingly, human IFN did not potentiate thermal injury in human cells. In this respect it is interesting to note that, as compared to mouse or hamster cells, human cells are more resistant to hyperthermia. At present several types of pharmacokinetic studies with mouse IFN in mice are being done with the hope that the results from such studies could be extrapolated to the human system. One should be cautious of extrapolating results from the mouse model to the human system in light of the present observations.

Finally, the ability of mouse IFN to potentiate thermal injury in mouse cells can be exploited for the isolation of IFN-resistant cells. IFN-sensitive cells can be killed readily by thermal stress. In this manner we have already isolated several IFN-resistant cell lines from Balb C/3T3 mouse cells.

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