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INTERFERON GAMMA mRNA "SUPERINDUCTION" IN HUMAN LYMPHOCYTES

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SUMMARY: Human peripheral blood mononuclear lymphocytes produce interferon gamma (IFN-γ) in response to stimulation by mitogens. Previous studies on the kinetics of IFN-γ mRNA production upon mitogen induction, showed that steady-state levels of mRNA increased to a maximum at 12-24h post-induction after which they declined to levels not detectable by the assay used. We show here that in mitogen induced peripheral blood lymphocytes, inhibition of protein synthesis using three different inhibitors (cycloheximide, puromycin, pactamycin) resulted in an increase in the steady-state levels of IFN-γ mRNA. The levels of mRNA in cells treated with inhibitor 16h post-induction were up to 3-fold higher than in untreated cells. Superinduction was possible up to 40h post-induction after which the steady-state levels of mRNA had declined to limits below detection; IFN-γ mRNA was not superinduced by cycloheximide in the presence of actinomycin D.

Interferon gamma (IFN-γ) is one of the major lymphokines produced by antigenic or mitogenic stimulation of human T-lymphocytes. Previous studies have shown that following the induction of human PBML by mitogens, IFN-γ is produced transiently in these cell cultures (1,2). It has been shown that the production of IFN-γ is at least in part controlled by the levels of IFN-γ mRNA in cells; analysis of IFN-γ mRNA levels following stimulation of PBML with mitogens has shown that the mRNA is expressed transiently, with a peak level of mRNA at 12-24h post-induction (3,4,5). The levels of mRNA may be controlled in several ways by transcriptional or post-transcriptional events; one observation which may be related to the mechanism of control of mRNA levels is that of superinduction of mRNA and its protein product by inhibitors of protein synthesis. IFN-β mRNA was shown to be superinducible by a regime of inhibitors of RNA synthesis and protein synthesis (6,7). More recently it

Abbreviations used: PBML, peripheral blood mononuclear lymphocytes; IFN-γ, interferon gamma; IFN-β, interferon beta; IL-2, interleukin 2; SEA, Staphylococcal enterotoxin A.

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was demonstrated that IL-2 mRNA was superinduced in lymphocytes by use of inhibitors of protein synthesis (8,9). These types of studies have led to the possibility that the control of the expression of these mRNAs may in part be exerted by an unstable repressor protein which may act at transcriptional or post-transcriptional levels.

In the present study we have used three inhibitors of protein synthesis to examine the superinduction of IFN-γ mRNA in human PBML stimulated with mitogens and tumour promoters. All inhibitors used were capable of superinducing the IFN-γ mRNA by up to 3-fold the levels in non-treated cultures.

METHODS

Materials: cycloheximide and puromycin were obtained from Sigma, Poole, Dorset, UK; pactamycin was a kind gift from Infectious Diseases Research, The Upjohn Company, Kalamazoo, Michigan, USA.

Preparation and induction of PBML cultures: PBML were prepared from pooled human buffy coats by centrifugation on Lymphoprep (Nyegaard & Co. AS, Oslo, Norway) (3). Cells were cultured at 3 x 10^6 cells ml^{-1} in RPMI 1640 medium supplemented with 10% foetal calf serum. Cultures were induced with either a crude SEA-containing supernatant or with SEA plus the tumour promoter, mezerein, as described previously (3). At the appropriate time post-induction, cultures were treated with protein synthesis inhibitors at the concentrations indicated in the text. Cultures were incubated for a further 4h and cells were then processed for isolation of RNA by the guanidinium isothiocyanate - CsCl procedure (10).

Quantitation of IFN-γ mRNA: Samples of total RNA (20μg) were analysed by dot-blot hybridisation as described previously (11). Hybridised dot-blot filters were exposed against Fuji X-ray film (Fuji Photo Film Co. Ltd, Japan) then localised areas of the filters were cut and quantitated by liquid scintillation counting. Levels of IFN-γ mRNA in RNA samples are expressed relative to the level determined in cells induced with SEA plus mezerein but not treated for superinduction with an inhibitor of protein synthesis.

RESULTS AND DISCUSSION

PBML cultures were induced with either SEA alone or with SEA plus mezerein and 16h post-induction were either left untreated or were treated with cycloheximide, puromycin or pactamycin (Table 1). Cultures were left a further 4h then RNA was isolated from the cells. Quantitation of the levels of IFN-γ mRNA in each sample showed that the presence of an inhibitor of protein synthesis resulted in superinduction; as shown (Table 1), levels of IFN-γ mRNA were increased in cells treated with inhibitor by up to 3.7-fold relative to the levels found in non-treated cells. This effect was observed

Table 1. Superinduction of IFN-γ mRNA by protein synthesis inhibitors

Inducer	Protein synthesis inhibitor	Relative level of IFN-γ mRNA		
		Cycloheximide	Puromycin	Pactamycin
S	None	0.2	0.2	0.1
S	+	0.6	0.5	0.3
SM	None	1.0	1.0	1.0
SM	+	2.3	3.7	2.0

PBML were induced with either SEA (S) or with SEA plus mezerein (SM) as indicated. After 16h cultures were either left untreated or were treated with an inhibitor of protein synthesis as shown: cycloheximide, 200μg ml^{-1}; puromycin 50μg ml^{-1}; pactamycin 400ng ml^{-1}. Cultures were left for a further 4h and then processed for isolation of RNA. Samples of RNA (20μg) were analysed by dot-blot hybridisation assay. Following autoradiography, filters were cut and individual dots were quantitated by liquid scintillation counting. Levels of IFN-γ mRNA in each sample are expressed relative to that in SM induced cultures in the absence of inhibitor.

irrespective of whether the cultures were induced with mitogen only or with mitogen plus tumour promoter.

Since the inhibitors cycloheximide and puromycin both inhibit protein synthesis by blocking the elongation step of translation (12,13), it seems possible that the superinduction may result from ribosomes being locked onto the actively translated mRNA molecules and thereby protecting them during the critical stages of mRNA isolation. However, pactamycin inhibits translation via a different mechanism by blocking the initiation stage of protein synthesis (13), therefore, superinduction cannot be simply considered to be an artefact of the mechanism by which a particular inhibitor acts but must be a consequence of inhibiting translation *per se*. We also examined how late after induction it was possible to superinduce IFN-γ mRNA with cycloheximide. As in our previous study (11), addition of cycloheximide prior to induction of the cultures showed either no effect on the mRNA with SEA induction or abolished the synergistic induction of mRNA with SEA plus mezerein (fig. 1). Addition of cycloheximide at 8h, 16h and 40h post-induction resulted in superinduction with both induction protocols. Beyond approximately 48h, levels of mRNA present in cells were at or below the limits of detection using this

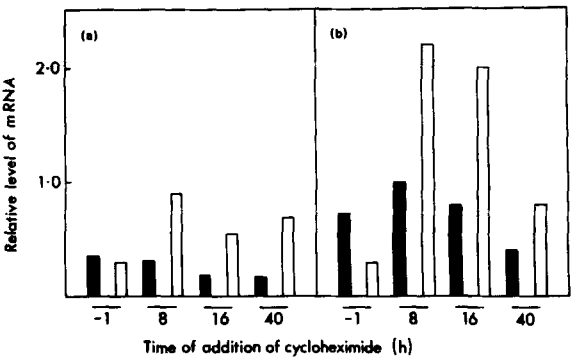

Fig. 1 Time course of superinduction of IFN-γ mRNA by cycloheximide. PBML were induced with (a) SEA, (b) SEA plus mezerein. Cycloheximide was added either 1h before induction (-1) or at the indicated time after induction. RNA was isolated 4h after addition of the cycloheximide (4h after induction for culture pretreated for 1h prior to induction). Levels of IFN-γ mRNA are expressed relative to the level in samples induced with SEA plus mezerein (not treated with cycloheximide) and harvested at 12h post induction. Closed bars, no cycloheximide added; open bars, cycloheximide added.

hybridisation analysis system. The IFN-γ mRNA is, therefore, superinducible at any stage after the initial induction of the IFN-γ gene expression.

In order to determine whether in the presence of cycloheximide there were significant changes in the degradation rate of the IFN-γ mRNA, actinomycin D was added 8h post-induction with or without cycloheximide and the mRNA steady-state levels were determined at 12h. The data in table 2 showed that cycloheximide did not significantly stabilise the mRNA, since levels in the presence of actinomycin D had both declined by about the same extent irrespective of the presence of cycloheximide.

The mechanism by which inhibition of protein synthesis causes superinduction of IFN-γ mRNA may be explained in several ways. Firstly, the steady-state level of mRNA in the cells is controlled by the balance between synthesis of the mRNA and its degradation. Therefore, if the control mechanism involved a labile protein repressor factor acting at the level of transcription, then inhibition of protein synthesis could lead to superinduction by loss of the repressor factor. If such a factor were, however, associated with the degradation of the mRNA then once again removal of the factor by inhibition could give rise to superinduction. Finally, it is possible that since these inhibitors perturb the natural sequence of events

Table 2. Effect of actinomycin D on superinduction of IFN-γ mRNA by cycloheximide

Sample	Treatment after induction with SEA plus mezerein	Relative level of IFN-γ mRNA
(a)	RNA isolated 8h p.i.	1.2
(b)	RNA isolated 12h p.i.	1.0
(c)	CHX added 8h p.i. RNA isolated 12h p.i.	2.5
(d)	Actinomycin D added 8h p.i. RNA isolated 12h p.i.	0.5
(e)	CHX plus actinomycin D added 8h p.i. RNA isolated 12h p.i.	0.7

PBML were induced with SEA plus mezerein and were treated as indicated with cycloheximide (CHX), 200μg ml^{-1} and actinomycin D, 3μg ml^{-1}. Relative levels of IFN-γ mRNA in each sample were quantitated as in table 1.

associated with translation of the mRNA then the fate of the mRNA in terms of its normal degradation pathway may also be influenced. This may give rise to an extended half-life for the mRNA due to a disturbance of its normal chain of events. In this case it is not necessary to hypothesise on the existence of labile repressor factors since superinduction would result simply from an increased half-life of the mRNA, associated with the continued transcription of the IFN-γ gene.

The data presented here together with other published data raises the possibility that IFN-γ and IL-2 may share common mechanisms of induction and control of expression. Several groups have shown that IFN-γ and IL-2 mRNA kinetics of induction are similar in terms of rate of accumulation of mRNA and time of appearance of peak levels (5,14,15). Here, we demonstrate that as with IL-2 mRNA (8,9), the IFN-γ mRNA in induced PBML is superinduced by inhibition of protein synthesis. Furthermore, comparison of the 5' sequences upstream of the IL-2 gene and of the IFN-γ gene has shown that there are regions of high homology between these promoter regions of the two genes (16). Whether these regions serve a functional role in the expression of these genes is yet to be shown, and also whether the IFN-γ and IL-2 genes are controlled by the same or different mechanisms.

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