Different interferon-producing capacities of L929 cell sublines and the enhancement of interferon production by priming are controlled pretranslationally

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Received 15 September 1986

Two sublines of mouse L929 cells designated L929B and L929M were studied. The L929B cells, which displayed a 2–3-fold higher IFN production in response to Sendai virus than that of the L929M cells, had a higher sensitivity to the antiviral and priming effects of IFN and were more resistant to VSV. In good accord with the amount of IFN produced, more translatable IFN mRNA was isolated from the L929B cells. IFN production and IFN mRNA activities were proportionally increased in the IFN-primed cultures of both sublines. Results indicate that both inherent and priming-induced increased-IFN production are based on pretranslational control mechanisms.

Interferon production *Pretranslational control* *Priming effect*

1. INTRODUCTION

A wide variation of control mechanisms exists for the regulation of expressions of inducible proteins in eukaryotic cells. IFNs are proteins with antiviral, cell growth-inhibitory and immunomodulatory activities, synthesized by cells upon virus infection or treatment with double-stranded nucleic acids. The effectivity of IFN induction is dependent on the type of the producing cell and on the nature of the inducer. It has also been reported that the treatment of cells with homologous IFN before induction primes subsequent IFN production [1]. The point of attack of priming in the process of IFN synthesis has been extensively studied, but there is some disagreement as to the exact nature of the process involved [2–5]. Since we have isolated two L929 sublines that had different IFN-producing capacities and in which there were different degrees of enhancement of IFN production by priming, we considered them as advantageous tools which might provide comprehensive data on the mechanisms determining the effectivities of IFN production and of priming. The main objective of the present work was therefore to determine whether the enhanced IFN production under these conditions was also manifested in the amount of IFN mRNA or not. In order to answer this question, we compared the titres of IFN produced and the amounts of translatable IFN mRNA in these two sublines of L929 cells.

Abbreviations: FCS, fetal calf serum; HAU, hemagglutinating unit; IFN, interferon; IU, international unit; MEM, minimal Eagle's medium; PFU, plaque forming unit; $TCID_{50}$, tissue culture infectious dose 50; VSV, vesicular stomatitis virus

2. MATERIALS AND METHODS

2.1. *Cells*

The sublines L929B and L929M were isolated after serial mutagenesis from a mouse L929 cell line originally provided by G.L. Toms (Department of Microbiology, University of Birmingham,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

Birmingham, England). Both sublines were serially passaged in MEM supplemented with 5% FCS and antibiotics. Their characteristics were not reversed during more than 50 passages of the cells.

2.2. *IFN titration*

This was carried out as described [6]. IFN titres are given in reference units.

2.3. *Extraction and assay of IFN mRNA*

The poly(A)-containing RNA fraction was isolated from the IFN-producing cells with the method of Ullrich et al. [7]. The translational activity of IFN mRNA present in these preparations was quantitated by injection into *Xenopus laevis* oocytes [8]. The titre of translated IFN was determined on mouse L929 cells.

Table 1

Characteristics of L929B and L929M sublines and the IFNs they produce

Characteristics	Cells	
	L929B	L929M
Population doubling time (h)	21	21
Spontaneous IFN formation	none	none
50% virus inhibitory concentration of IFN (IU/ml)	0.15	0.8
Minimal priming dose of IFN (IU/ml)	0.1	1.0
$TCID_{50}$ of VSV (PFU/ml)[a]	6.8×10^4	7.0×10^1
Neutralization titre of MuIFN antiserum against the IFN produced[b]	2.4×10^4	2.4×10^4
Residual activity of IFN after 30 min at 56°C (%)	1.5–2	1.5–2
Molecular masses of peak IFN activities (kDa)[c]	22–24 28–32	22–24 28–32

[a] The VSV preparation used for determination of the susceptibilities of L929 cells to this virus had a titre of $10^{7.9}$ PFU/ml on primary chick embryo fibroblasts

[b] Dilution of MuIFN antiserum (G-024-501-568; provided by the Antiviral Substances Program, National Institute of Health, Bethesda, MD, USA) required for neutralization of 10 IU of IFN produced by L929B or L929M cells

[c] Gel filtration analysis of IFNs was performed on a Sephadex G-75 column (2.5 × 70 cm)

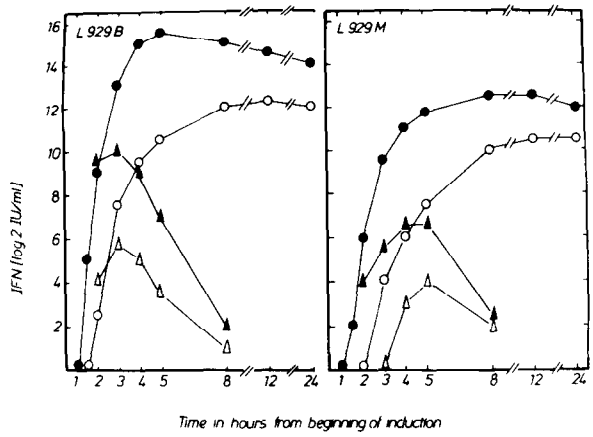

Fig.1. Effects of IFN pretreatment on IFN production and IFN mRNA content of L929B and L929M cells. Confluent cultures grown in 150 cm^2 plastic tissue culture flasks were pretreated for 10 h with 30 IU/ml of a partially purified preparation of mouse L cell IFN with a specific activity of $10^{6.5}$ IU/mg protein. For IFN induction 3 ml of 1.8×10^3 HAU/ml concentrated and purified Sendai virus (parainfluenza 1) was added to each flask, after which 1×10^8 cells were collected at different times for the determination of IFN mRNA content. The IFN production was determined on the corresponding culture media. (○) Control IFN; (●) primed IFN; (△) control IFN mRNA; (▲) primed IFN mRNA. The points represent mean values of four independent experiments.

3. RESULTS

The L929B subline was about 5–10-times more susceptible to the antiviral and priming effects of IFN, and approx. 1000-times more VSV was required to infect these cells than L929M cells. However, we found no difference between the immunological and physico-chemical characteristics of the IFNs produced by them (table 1).

Unprimed (control) L929B cells produced 2–3-times more IFN than did L929M cells. For the primed cultures the corresponding ratio was approx. 2-times higher and the kinetics of IFN production was accelerated. The amount of translatable IFN mRNA was proportional to the amount of IFN produced. The L929B cells, which produced more IFN in response to induction with Sendai virus, likewise contained more IFN mRNA than did the L929M cells. The enhancement of IFN production and the amount of IFN mRNA were also proportional in response to priming (fig.1).

4. DISCUSSION

The higher IFN mRNA levels in L929B cells indicate that the inherent differences in IFN-producing capacities are manifested pretranslationally.

Our results permit localization of the point of attack of priming at a pretranslational level. This supports the findings of Sehgal and Gupta [9] and Fujita and Kohno [10], but is at variance with the results of Abreu et al. [3] and Content et al. [5], who suggested that priming affects IFN production at the level of translation too. The proportional enhancement of the amount of IFN mRNA to that of IFN production in the primed L929B and L929M cells, together with the exclusion of a longer half-life of HuIFN-beta mRNA [11] and the enhanced rate of transcription of the HuIFN-beta gene [12] in primed human fibroblast, seem to indicate that the transcription of IFN messages is the primary target of priming.

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