INOSINE DECREASES GLOBIN-RNA CONTENT IN FRIEND ERYTHROLEUKEMIA CELLS INDUCED TO DIFFERENTIATE BY AMINONUCLEOSIDE OF PUROMYCIN

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1. Introduction

Since the discovery that dimethylsulfoxide (DMSO) induces differentiation of murine Friend erythroleukemia cells [1] a wide variety of differentiation-promoting agents have been reported [2–5].

The aminonucleoside of puromycin, AMS, (6-dimethyl amino 9 3′-amino 3-deoxyribosyl purine) studied as a possible antitumor drug [6] has been re-investigated [7]. Low concentrations of AMS (10^{-5} M) promote erythroïd differentiation of Friend cells without blocking cell multiplication and simultaneous administration of inosine markedly decreases the percentage of hemoglobin-producing cells, although inosine does not affect the cellular uptake of AMS [7]. These data, based on quantitative estimations of globin messenger RNA, confirm that AMS is a very efficient inducer of Friend cell differentiation and demonstrate that the inhibitory effect of inosine, involves quantitative regulation of specific mRNA.

2. Materials and methods

2.1. *Cell culture*

Friend erythroleukemia cells (clone 745-A) were cultivated in the absence or presence of AMS alone (5 μg/ml) or AMS plus inosine as in [7]. In some experiments, higher concentrations of inosine (⩽100 μg/ml) were utilized because of variations in the efficiency of different inosine batches. Cells were collected on day 4 of treatment when the total number of cells and the percentage of differentiated cells reach a maximum.

2.2. *Preparation of cellular RNA*

Total cytoplasmic RNA was extracted from the postmitochondrial supernatant of cells lysed with Nonidet P40 and cleared from DNA and tRNA by precipitating in 2 M LiCl.

2.3. *Isolation of globin mRNA and synthesis of globin cDNA*

Total RNA was prepared from reticulocytes of phenylhydrazine-treated Swiss mice using methods in [8] and polyadenylated 9 S globin mRNA was purified by two successive passages through oligo(dT) cellulose columns followed by sucrose gradient centrifugation. The highly radioactive globin cDNA probe was cell-free synthetised in optimal conditions [9] using 2 μg 9 S globin mRNA and dc[^{3}H]Tp (23 Ci/mmol) at 8.10^{-5} M. It was further characterized by alkaline sucrose gradient centrifugation which showed peak fractions sedimenting at 5–7 S and hybridization to a mixture of DNA from PCR1α H_4 and PCR_1 βH_9 plasmids carrying the mouse DNA sequences that encode the α and β globin chains, respectively [10]. The plasmid DNA preparations were a gift from F. Rougeon.

2.4. *Hybridization assays*

All solutions and containers were pretreated with 0.2% diethylpyrocarbonate. Annealing assays were performed at 68°C in 0.4 M NaCl, 0.01 M Tris buffer (pH 7.4), 0.01 M EDTA, 0.2% SDS and the percentages of cDNA hybridized were determined by the use of S_1 nuclease as in [11].

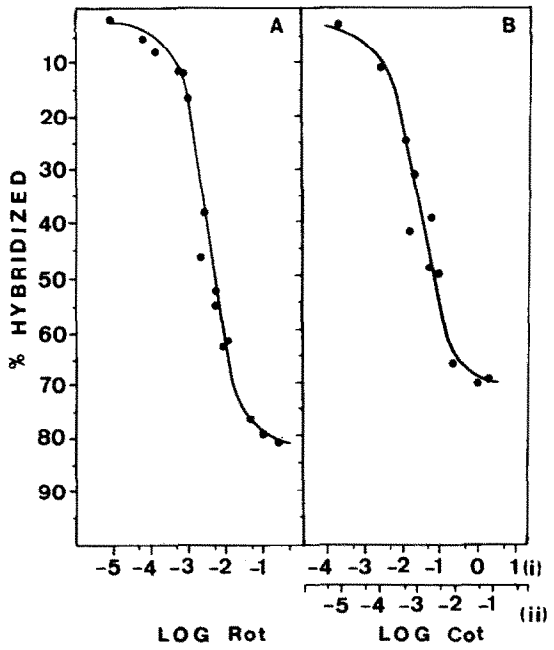

Fig.1. (A) Hybridization of c[^{3}H]DNA probe with 9 S globin RNA ($R_0t_{1/2}$ = 3.5 . 10^{-3}). (B) Hybridization of c[^{3}H]DNA probe with plasmid DNA carrying the globin DNA sequences. (I) Log C_0t. C_0, initial concentration of DNA plasmid ($C_0t_{1/2}$ 3.6 . 10^{-2}). (II) Corrected Log C_0t. C_0, initial concentration of globin sequences contained in plasmid DNA (~5%) ($C_0t_{1/2}$ 1.8 . 10^{-3}).

3. Results and discussion

Following alkaline gradient centrifugation, most of the cell-free synthesised c[^{3}H]DNA molecules sedimented at 5–7 S (not shown), which indicated they represented copies of the largest portion or all of the globin RNA template. Also the greatest part of c[^{3}H]DNA could be reassociated to 9 S RNA (fig.1A) or plasmid DNA carrying the globin DNA sequences (fig.1B). Furthermore when the C_0t values calculated for the total amounts of recombinant plasmid DNA were corrected, according to their globin DNA content, the hybridization kinetics of c[^{3}H]DNA appeared faster with plasmid DNA than with 9 S RNA ($C_0t_{1/2}$ = 1.8 . 10^{-3} and $R_0t_{1/2}$ = 3.5 . 10^{-3} respectively). Since the recombinant DNA contained no other mouse DNA sequences than those of the globin genes, this result is good indication that both the 9 S RNA and the cDNA probe used here were essentially globin specific.

Typical hybridization curves between globin c[^{3}H]DNA and total cytoplasmic RNA from induced or non-induced Friend cells are shown in fig.2. Only a small part of the radioactive probe could be reassociated to RNA from non-induced cells. This is consistent with the low proportion of benzidine positive cells (1–3%) which is found in control cultures. In contrast the greatest portion of globin cDNA hybridized very much faster to RNA from all AMS-induced cells, but at significantly slower rates to RNA from cells treated with inosine plus AMS, than to RNA from cells treated with AMS alone. The table gives the $R_0t_{1/2}$ values derived from these curves and the corresponding globin RNA contents. As seen, AMS-treated cells appeared to contain an average of 17 000–18 000 globin RNA molecules/cell. In comparison DMSO treated cells contained 10 000–14 000 globin mRNAs according to [12–14] and

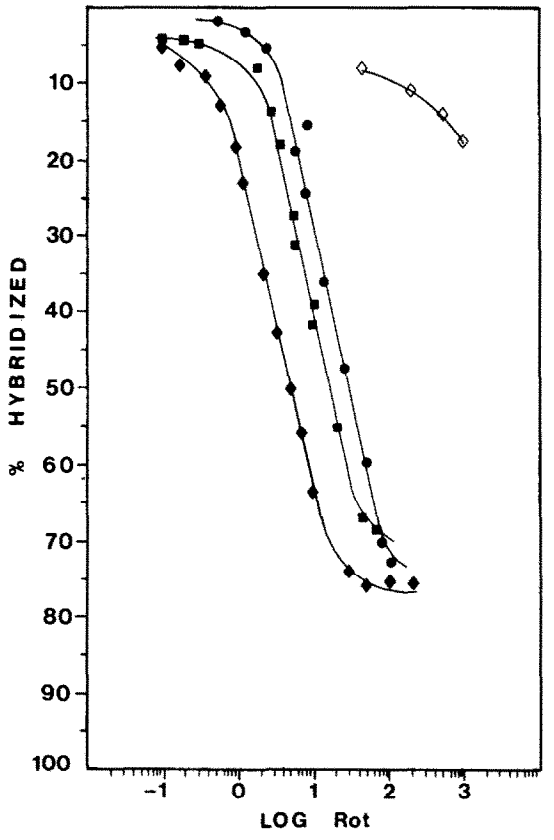

Fig.2. Hybridization of globin c[^{3}H]DNA with cytoplasmic RNA from Friend cells treated with: AMS (♦——♦); AMS + inosine (10 μg/ml) (■——■); AMS + inosine (100 μg/ml) (●——●); non-induced cells (◇——◇).

Table 1
Inhibitory effects of inosine on erythroïd differentiation induced by AMS

Treatment of cells		% Differentiated cells (Benzidine positive)	Globin cDNA hybridized to cellular RNA $R_0t_{1/2}$	% Globin mRNA in cellular RNA	Mean number of globin mRNA sequences/cell	Inhibition index	
						I	II
AMS 5 μg/ml		80	2.6	0.135	17 400	–	–
id + inosine	10 μg/ml	25	7.0	0.05	6200	3.2	2.8
	100 μg/ml	15	17.0	0.02	2600	5.3	6.6

The percentages of globin mRNA were calculated from the ratios of the indicated $R_0t_{1/2}$ values to the $R_0t_{1/2}$ obtained with globin cDNA hybridized to purified globin mRNA and the mean numbers of globin mRNA sequences were derived from these percentages. Inhibition index: I, percentage benzidine positive cells with AMS alone/percentage benzidine positive cells with AMS + inosine; II, mean number of globin one RNA sequences with AMS alone/mean number of globin mRNA sequences with AMS + inosine. The average cell RNA content was ~4.2 μg in all experiments and was the same for cells treated with AMS alone or AMS plus inosine

9000–10 000 according to us (not shown). This degree of abundance confirms that AMS is a potent inducer of erythroïd differentiation in this cell system. Finally, it is interesting to note that despite some variations in the efficiency of different inosine batches, the globin mRNA content always decreased in about the same proportion as the percentage of hemoglobin producing cells, in induced cell cultures subjected to inosine. This allows the conclusion that inhibition of AMS-induced differentiation by inosine, appears to involve quantitative regulation of specific mRNAs at either the transcriptional or post-transcriptional level or both.

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