



HAL
open science

Role of interferon and 2',5'-oligoadenylate synthetase in erythroid differentiation of Friend leukemia cells. Studies with interferon-sensitive and -resistant variants.

Nadir Mechti, E Affabris, G. Romeo, B. Lebleu, G. Rossi

► **To cite this version:**

Nadir Mechti, E Affabris, G. Romeo, B. Lebleu, G. Rossi. Role of interferon and 2',5'-oligoadenylate synthetase in erythroid differentiation of Friend leukemia cells. Studies with interferon-sensitive and -resistant variants.. *Journal of Biological Chemistry*, 1984, 259 (5), pp.3261-5. 10.1016/S0021-9258(17)43289-3 . hal-02361157

HAL Id: hal-02361157

<https://hal.science/hal-02361157>

Submitted on 27 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Role of Interferon and 2',5'-Oligoadenylate Synthetase in Erythroid Differentiation of Friend Leukemia Cells

STUDIES WITH INTERFERON-SENSITIVE AND -RESISTANT VARIANTS*

(Received for publication, August 4, 1983)

Nadir Mechti‡, Elisabetta Affabris§, Giovanna Romeo§, Bernard Lebleu‡¶, and Giovanni B. Rossi§||

From the ‡Laboratoire de Biochimie des Protéines, Equipe de Recherche Associée Centre National de la Recherche Scientifique 482, University of Montpellier II, Montpellier, France, and the §Departments of Virology, Istituto Superiore di Sanità, and of Cellular and Developmental Biology, University of Rome, Rome, Italy

It has been suggested that the interferon (IFN)-induced 2',5'-oligoadenylate (2-5A) synthetase, which polymerizes ATP into a series of 2',5'-linked oligomers with the general formula $\text{pppA}(2'p5'A)_n$, plays a general role in cell growth and terminal differentiation. For instance, an increase in 2-5A synthetase activity has been described during dimethyl sulfoxide (Me_2SO)-induced erythroid differentiation of Friend leukemia cells (FLC).

2-5A synthetase has been measured in two Friend leukemia cell sublines by various techniques including a radioimmunoassay of its products which would detect 10^{-16} mol of 2-5A cores. Although cells of both sublines fully differentiate (as measured by benzidine staining), only in one subline was there an increase in 2-5A synthetase activity upon treatment with Me_2SO . Hexamethylenebisacetamide, another potent agent of differentiation in this system, did not increase 2-5A synthetase activity in either of these two sublines. An IFN-resistant FLC variant differentiated normally upon treatment with Me_2SO or hexamethylenebisacetamide while it was noninducible for 2-5A synthetase activity by exogenous IFN or by the inducers themselves. A similar situation has been observed with regard to the level of phosphorylation of the IFN-induced $M_r = 67,000$ protein band. In addition, treatment of IFN-sensitive and resistant FLC sublines with mouse $\alpha\beta$ IFN antiserum did not affect differentiation. Even though we have duplicated previous findings on the increase of 2-5A synthetase activity in Me_2SO -induced FLC, the lack of any such increase with other inducers or other sublines indicates that there is no causal relationship between the enzyme activation and FLC differentiation.

Besides their antiviral activity, interferon(s) can serve as pleiotropic effector molecules in a variety of systems, including cells differentiating *in vitro* (Rossi *et al.*, 1980, 1982). Extensive studies have revealed the major role played by two

independent IFN¹-induced enzymatic systems, namely the protein kinase and 2-5A synthetase pathways, both activated by double-stranded RNAs (for a review, see Lebleu and Content, 1982). The protein kinase phosphorylates the α subunit of initiation factor eIF-2 and a $M_r = 67,000$ protein which may represent the kinase itself (for a review, see Sen, 1981). 2-5A synthetase polymerizes ATP into a series of 2',5'-linked oligomers with the general formula $\text{pppA}(2'p5'A)_n$. These in turn bind specifically and activate an endoribonuclease (RNase L) which degrades RNAs (for a review, see Lengyel, 1981).

Since 2-5A synthetase activity varies extensively in different cell lines and within a given cell line according to growth conditions, a wider role for this pathway in the regulation of cell growth and differentiation has been proposed (Stark *et al.*, 1979; Krishnan and Baglioni, 1980; Creasey *et al.*, 1980; and others). In particular, increased levels of 2-5A synthetase activity (Kimchi, 1981) and the production of β -type IFN (Friedman-Einat *et al.*, 1982) have been reported in growing and differentiating FLC exposed to Me_2SO . It has also been reported that rabbit reticulocytes contain high levels of protein kinase and 2-5A synthetase (Farrell *et al.*, 1977; Hovanessian and Kerr, 1978). Similarly, low doses of IFN enhance the expression of differentiation markers upon induction of FLC with Me_2SO (Lieberman *et al.*, 1975; Luftig *et al.*, 1977; Dolei *et al.*, 1980; Rossi *et al.*, 1980).

In this report, we describe experiments carried out in this context on two FLC sublines and on an IFN-resistant variant isolated from one of them (Affabris *et al.*, 1982). This variant does not exhibit any 2-5A synthetase activity even when exposed to 10,000 units/ml of $\alpha\beta$ IFN (Affabris *et al.*, 1983).

EXPERIMENTAL PROCEDURES

Materials—[2,8-³H]Adenosine 5'-triphosphate, tetrasodium salt (25–40 Ci/mmol) was obtained from New England Nuclear and adenosine 5'-[γ -³²P]triphosphate, triethylammonium salt (3000 Ci/mmol) was from the Radiochemical Centre, Amersham, U. K. Poly(rI)·poly(rC) was from P-L Biochemicals. Benzidine dihydrochloride was from Ciba. Me_2SO and polyethylene glycol 6000 were from Merck. HMBA was a generous gift of C. Delfini, Istituto Superiore di Sanità, Rome, Italy. Bacterial alkaline phosphatase (EC 3.1.3.1) type IIIR was purchased from Sigma. All other chemicals and solvents were of reagent grade.

Cells—Two sublines, both derived from the 745A clone and ob-

* Collaborative work has been aided by the Centre National de la Recherche Scientifique. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of a Programme de Recherches Coordonnées de l'Institut National de la Santé et de la Recherche Médicale.

|| Recipient of grants from Consiglio Nazionale delle Ricerche, Progetto Finalizzato Controllo della Crescita Neoplastica (82.00406.96), and Progetto Finalizzato Controllo delle Malattie da Infezione (83.02916.52).

¹ The abbreviations used are: IFN, interferon; Me_2SO , dimethyl sulfoxide; FLC, Friend leukemia cells; HMBA, hexamethylenebisacetamide; NBS, newborn bovine serum; MEM, minimal essential medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. 2-5A designates a series of oligonucleotides of the general formula $\text{pppA}(2'p5'A)_n$ produced by the IFN-induced 2-5A synthetase.

tained from C. Friend, Mount Sinai Medical Center, New York, were independently passed for several years in the laboratories of L. Harel (Institut de Recherches Scientifiques sur le Cancer (IRSC) Villejuif) or G. B. Rossi. They will be referred hereafter as 745A_H and 745A_R, respectively. Cells of these sublines and of 745A_R 1C11, an IFN-resistant subline derived from 745A_R, (Affabris *et al.*, 1982) were grown in RPMI 1640 medium supplemented with 5% (v/v) fetal calf serum. Moloney sarcoma virus-transformed C243-3 murine fibroblasts, obtained from I. Gresser (IRSC, Villejuif), were grown in MEM supplemented with 10% (v/v) NBS. L929 cells were grown in MEM supplemented with 5% (v/v) fetal calf serum.

Viruses—Vesicular stomatitis virus (Indiana strain) stocks, obtained by infecting L929 cell monolayers with low multiplicity of infection (0.01–0.1 plaque-forming unit/cell) were titrated by plaque assay on the same cells. Titers ranged between 10^{8.5} and 10⁹ plaque-forming units/ml. Newcastle Disease virus, strain F, stocks were obtained by infecting 10–11-day-old embryonated eggs in the allantoic cavity. The virus was harvested after 3 days of incubation at 35 °C and titrated by hemagglutination assay. The titers ranged between 300 and 600 hemagglutinating units/ml.

Interferon—Mouse α IFN was prepared on C243-3 cells according to Cachard and De Maeyer-Guignard (1981). Briefly, confluent monolayers were treated for 24 h with 1 mM Na butyrate in MEM + 2% NBS (v/v) in the presence of 150 units/ml α IFN ("priming"). Cells were then infected with Newcastle disease virus (10 hemagglutinating units/10⁶ cells). The unattached virus was removed after 1 h of adsorption. The monolayers were washed with isotonic buffered saline and reincubated with MEM containing 0.5% (v/v) NBS, and 5 mM theophylline for 16–18 h. Clarified supernatant fluids were brought to pH 2.0 with 6 N HCl, kept at 4 °C for 5 days, neutralizing to pH 7.0, and stored at –80 °C. Partial purification by (NH₄)₂SO₄ precipitation between 30 and 80% saturation was followed by extensive dialysis against isotonic buffered saline. Specific activities of IFN preparations averaged about 10⁶ units/mg of protein. Amounts of IFN are given throughout this paper in laboratory units, *i.e.* the amount of IFN reducing by 50% plaque production by vesicular stomatitis virus. This unit equals 4 research reference units of the National Institutes of Health Standard Preparation, code G-002-904-511, whose titer was 12,000 IU/ml when reconstituted. Details about this standard preparation are reported in Research Reagents Note No. 15 (World Health Organization Standard, 1979).

Induction of FLC Differentiation—Friend cells were seeded at 10⁵/ml in the presence of 1.5% (v/v) Me₂SO or 5 mM HMBA. Cells were counted daily by using a Coulter Counter or a hemocytometer. Cell mortality, evaluated by the trypan blue dye exclusion method, never exceeded 2%. The degree of differentiation was determined by spectrophotometric evaluation, at 414 nm, of hemoglobin content, according to Kabat *et al.* (1975), or by the percentage of benzidine-positive cells (B⁺ cells) according to Orkin *et al.* (1975). Cell extracts were prepared from the same cell cultures and kept frozen at –70 °C.

Assay of 2-5A Synthetase Activity—Cells were homogenized in 2 volumes of lysis buffer (20 mM HEPES buffer, pH 7.5, 15 mM KCl, 1.5 mM Mg(OAc)₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and 0.5% (v/v) Nonidet P-40). The homogenate was centrifuged for 3 min at 10,000 × g and the supernatant fluid (S10) was stored in liquid nitrogen. Protein concentration was determined by the method of Whitaker and Granum (1980). 5 μ l of cell extracts were incubated for 1 h at 37 °C in 20 μ l of the incubation mixture described by Minks *et al.* (1979), *i.e.* 20 mM HEPES-KOH, pH 7.4, 15 mM KCl, 25 mM Mg(OAc)₂, 1 mM dithiothreitol, 5 mM ATP, 4 mM fructose 1,6-diphosphate, and 20 μ l of poly(rI)·poly(rC). The radioactive 2-5A oligomers synthesized from [³H]ATP in the S10 extract were isolated by chromatography on DEAE-cellulose, according to Minks *et al.* (1979), and counted by liquid scintillation. The results are expressed in nanomoles of ATP transformed/h/mg of protein.

Radioimmunological Assay of 2-5A Activity—Cell extracts and incubations were performed as described above, except for the absence of [³H]ATP. Radioimmunoassay of 2-5A products was performed as described by Cailla *et al.* (1982a). Briefly, dilutions of the samples containing 2-5A oligomers were incubated with alkaline phosphatase for 30 min at 37 °C in 100 mM Tris-HCl, pH 9.3, 10 mM MgCl₂ buffer. Samples were boiled 3 min at 100 °C to inactivate the phosphatase and were diluted 3-fold with a citrate buffer (100 mM, pH 6.3). 50 μ l of samples were mixed with 50 μ l of the [¹²⁵I]-labeled iododisuccinyl-A2'p5'A tyrosine methyl ester probe, and 100 μ l of monoclonal antibody, both prepared according to Cailla *et al.* (1982b). After 18 h of incubation at 4 °C, the antigen-antibody complexes were precipi-

tated with 300 μ l of polyethylene glycol 6000 in the presence of human plasma as carrier. Samples were centrifuged 10 min at 3000 × g and the radioactivity of the precipitate was measured in a γ -counter. The 2-5A concentration of each sample was obtained by comparison with a reference curve. The results were expressed as nanomoles of 2-5A/10⁶ cells for the indicated period of time. None of the samples that were not treated with phosphatase displaced the radioactive probe, indicating that only phosphorylated 2-5A was synthesized in the cell-free extracts. Likewise, phosphatase-treated samples did not displace the radioactive probe if they were digested with snake venom phosphodiesterase.

Assay of Protein Kinase Activity—Extracts (postmitochondrial supernatant fractions) were prepared at 4 °C from packed cells lysed into 1.5 volumes of homogenization buffer (10 mM Tris-HCl, pH 7.5, 7 mM β -mercaptoethanol, 10 mM KCl, 1.5 mM Mg(OAc)₂, 0.5% (v/v) Nonidet P-40). The homogenate was brought to 3 mM Tris-HCl, pH 7.5, 90 mM KCl, 3.5 mM Mg(OAc)₂, and 7 mM β -mercaptoethanol, and centrifuged at 10,000 × g for 10 min. The supernatant fluid was either assayed immediately or stored in aliquots in liquid nitrogen. 5 μ l of cell extract were incubated for 15 min at 37 °C in 10 mM HEPES buffer, pH 7.6, 90 mM KCl, 10 mM Mg(OAc)₂, 1 mM dithiothreitol, 100 μ M ATP, 20% (v/v) glycerol, 2 μ Ci of [³²P]ATP, with or without poly(rI)·poly(rC) (1 μ g/ml), in a final volume of 25 μ l. After incubation, 2 volumes of electrophoresis sample buffer (125 mM Tris-HCl, pH 6.8, 100 mM β -mercaptoethanol, 10% (v/v) glycerol, 2% (w/v) Na dodecyl sulfate, 0.005% (w/v) bromophenol blue) were added to the kinase assay mix which was then heated to 95 °C for 15 min and analyzed by electrophoresis on 9% polyacrylamide slab gels containing 0.5% (w/v) Na dodecyl sulfate (Laemmli, 1970). The phosphorylated proteins were detected by autoradiography.

RESULTS

2-5A Synthetase Activity in Several Growing and Differentiating FLC Sublines Treated with Me₂SO—Cells of two FLC sublines (745A_R and 745A_H) and of one IFN-resistant variant (745A_R 1C11) were seeded at 10⁵ cells/ml in medium supplemented with Me₂SO and were grown for 6 days under stationary conditions. As shown in Fig. 1A, the three growth curves were similar. Under these conditions, erythroid differentiation, as measured by hemoglobin-containing (benzidine-positive) cells, attained nearly 100% in all three lines (Fig. 1B). In accordance with published data (Kimchi, 1981), 2-5A synthetase activity increased in parallel with the accumulation of benzidine-positive cells in the 745A_H subline. On the contrary, neither the wild type 745A_R subline nor the IFN-resistant variant derived therefrom, exhibited any detectable enzyme activity (Fig. 1C). It was verified that 745A_R cells responded to exogenous mouse α IFN with the expected elevation in 2-5A synthetase activity (as shown by Affabris *et al.*, 1983) while 745A_R 1C11 cells did not respond to such treatment (data not shown), as previously described (Affabris *et al.*, 1983).

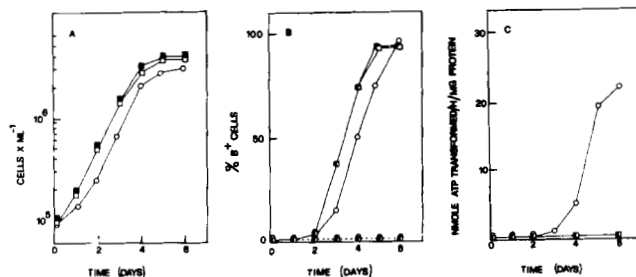


FIG. 1. Cell growth, erythroid differentiation, and 2-5A synthetase activity of IFN-sensitive and -resistant FLC sublines. Cells were seeded at 10⁵/ml in the presence of 1.5% (v/v) Me₂SO. Cell growth (A), percentage of benzidine-positive cells (B), and 2-5A synthetase activity (C) were evaluated daily as described under "Experimental Procedures" for 745A_H (○), 745A_R (□), and 745A_R 1C11 (■) sublines. The dotted lines in B refer to the differentiation of cell cultures in the absence of the inducer.

As 2-5A synthetase levels have been reported to be related to growth conditions (Krishnan and Baglioni, 1980; Creasey *et al.*, 1980), two additional protocols were also followed. 745A_R cells seeded at 5×10^5 /ml in Me₂SO-rich medium were counted daily and diluted in Me₂SO-containing medium to the initial concentration for 8 days. Alternatively, cells of the same subline were seeded at 10^5 /ml in the same medium, counted 3 days later (when cell saturation density had not yet been attained), diluted to 2×10^5 /ml in Me₂SO-rich medium, and grown for 8 days, essentially as described by Friedman-Einat *et al.* (1982). Although the percentage of benzidine-positive cells attained nearly 100% in all these conditions, no increase in 2-5A synthetase activity could be detected (data not shown). Likewise, no increase in 2-5A synthetase was observed when cells were diluted in Me₂SO-free medium (data not shown).

Since even very low levels of 2-5A synthetase activity could suffice to activate RNase L and therefore be biologically meaningful, these negative data have been re-assessed with a radioimmunological assay of 2-5A cores (Cailla *et al.*, 1982a). The cell-free products of 2-5A synthetase were dephosphorylated with alkaline phosphatase and quantified using monoclonal antibodies specifically detecting as low as 10^{-16} mol of 2-5A cores (Cailla *et al.*, 1982b). Fig. 2 shows the linear conversion of ATP into 2-5A expected for an enzymatic assay. The use of appropriate dilutions allowed detection of both the induction of 2-5A synthetase activity upon exogenous IFN treatment of 745A_R cells (Fig. 2A) and a low but measurable basal 2-5A synthetase activity in cell-free extracts prepared from untreated cells (Fig. 2B). Similar low levels of 2-5A synthetase activity were detected in extracts of IFN-resistant variants, regardless of stimulation by IFN (data not shown). As described in Table I, however, no modulation of 2-5A synthetase activity was detectable by this sensitive assay procedure in extracts prepared from Me₂SO-treated cells, even in the conditions described by Friedman-Einat *et al.* (1982).

2-5A Synthetase in FLC Sublines Treated with Hexameth-

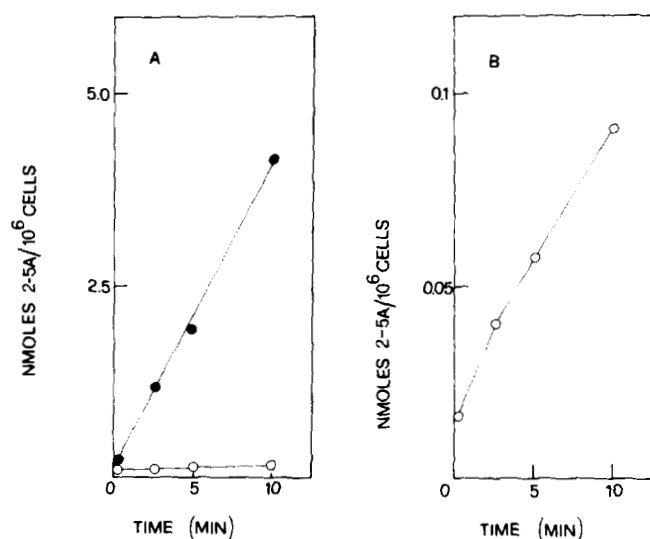


FIG. 2. Radioimmunological determination of 2-5A synthetase activity in cell extracts of FLC. Extracts were prepared from untreated (○) or IFN-treated (●) (200 units/ml) 745A_R cells and assayed for 2-5A synthetase activity as described under "Experimental Procedures." 2-5A oligomers were dephosphorylated with bacterial alkaline phosphatase and processed for radioimmunoassay as described in the text. 2-5A concentrations are expressed in nanomoles of 2-5A/10⁶ cells. B shows the data obtained for untreated 745A_R cells on an expanded scale.

TABLE I

Radioimmunological analysis of 2-5A synthetase activity in FLC extracts

Cells seeded at 10^6 /ml with or without 1.5% (v/v) Me₂SO, were diluted to 2×10^5 cells/ml 3 days later in Me₂SO-supplemented medium and grown in the same medium for 6 days. Radioimmunological assay of 2-5A synthetase activity was performed as described under "Experimental Procedures."

Subline	Inducer	2-5A synthetase activity	
		B ⁺ cells %	nmol 2-5A/10 ⁶ cells in 10 min
745A _R	Me ₂ SO, 4 days	<1	0.128
	Me ₂ SO, 6 days	78	0.118
745A _R 1C11	Me ₂ SO, 4 days	96	0.132
	Me ₂ SO, 6 days	<1	0.112
	Me ₂ SO, 6 days	64	0.106
		82	0.108

TABLE II

2-5A synthetase activity in several FLC sublines treated with hexamethylenebisacetamide

Cells were seeded at 10^6 /ml with or without 5 mM HMBA and grown in stationary conditions. B⁺ values were determined as described under "Experimental Procedures." 2-5A synthetase activities, measured with the conventional assay of Minks *et al.* (1979), were expressed in nanomoles of ATP transformed/h/mg of protein and those measured with the radioimmunological assay were expressed in nanomoles of ATP transformed/10⁶ cells (values in parentheses), as described in the text.

FLC subline	Inducer	B ⁺ cells %	2-5A synthetase activity
745A _R		<1	<1 (0.134)
	HMBA 4 days	76	<1 ND ^a
745A _R 1C11	HMBA 6 days	98	<1 (0.054)
		<1	<1 (0.112)
745A _H	HMBA 4 days	74	<1 ND
	HMBA 6 days	99	<1 (0.058)
745A _H		<1	<1
	HMBA 4 days	49	<1
745A _H	HMBA 6 days	100	<1
		ND	<1
	IFN	ND	215
	IFN-HMBA	ND	221

^aND, not done.

ylenebisacetamide—Since Me₂SO has been shown to increase 2-5A synthetase activity in several cell lines (Besançon *et al.*, 1981),² the effects of HMBA, another potent inducer of differentiation in the FLC system (Reuben *et al.*, 1980), have been investigated. As shown in Table II, nearly 100% of either 745A_H or 745A_R cells became benzidine-positive after 6 days of exposure to 5 mM HMBA. 2-5A synthetase activity remained undetectable in fully differentiated 745A_R and 745A_R 1C11 cells after HMBA treatment, thus confirming the data obtained with Me₂SO for these two sublines. Surprisingly, HMBA did not stimulate 2-5A synthetase activity in 745A_H cells (Table II), at variance with the data obtained with Me₂SO (see above). HMBA even diminished the basal level of 2-5A synthetase activity which could be measured with the more sensitive radioimmunoassay of 2-5A products (Table II). The latter observations could not be accounted for by a trivial inhibitory effect, since HMBA did not affect 2-5A synthetase activity whether it was added to intact IFN-treated cells (Table II) or to cell-free extracts (data not shown).

Protein Kinase Activity in Growing and Differentiating

² S. Scoarnc, P. Milhaud, and B. Lebleu, unpublished observations.

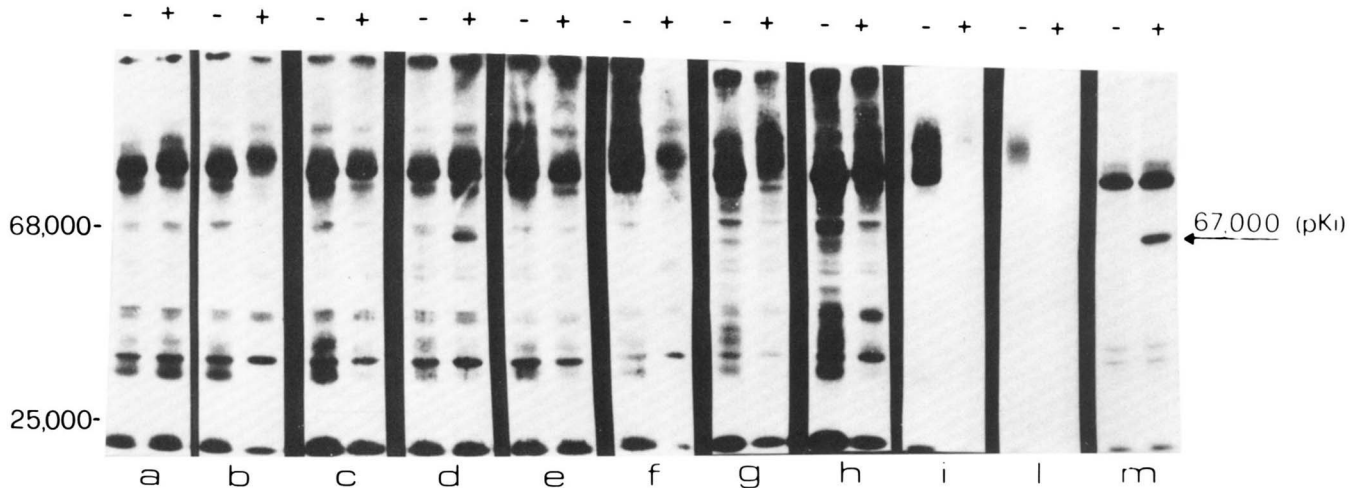


FIG. 3. **Autoradiography of SDS-polyacrylamide gel electrophoresis of protein kinase assay mixtures.** 745A_R cells were seeded at 10^5 /ml and grown in the presence of 1.5% (v/v) Me₂SO, 5 mM HMBA, or 200 units/ml of IFN. After 3 days, cells were diluted to 2×10^5 /ml with medium containing the same inducer and grown for an additional 4 days. Cell extracts were prepared, as described under "Experimental Procedures," 3, 5, and 7 days after cell seeding. Percentages of B⁺ cells were 49, 85, and 89 for Me₂SO-treated cells and 80, 97, and 99 for HMBA-treated cells, when measured 3, 5, and 7 days after seeding, respectively. The patterns of phosphorylation of the cell extracts, in the absence (-) or presence (+) of poly(rI)·poly(rC), were analyzed by polyacrylamide gel electrophoresis as described in the text. Assay mixture applied to each lane were as follows: a, 745A_R (3 days); b, 745A_R + Me₂SO (3 days); c, 745A_R + HMBA (3 days); d, 745A_R + $\alpha\beta$ IFN (3 days); e, 745A_R (5 days); f, 745A_R + Me₂SO (7 days); g, 745A_R + HMBA (5 days); h, 745_R (7 days); i, 745A_R + Me₂SO (7 days); l, 745A_R + HMBA (7 days); m, L929 + $\alpha\beta$ IFN (1 day). The indicated molecular weights correspond to those of bovine serum albumin (68,000) and chymotrypsinogen (25,000).

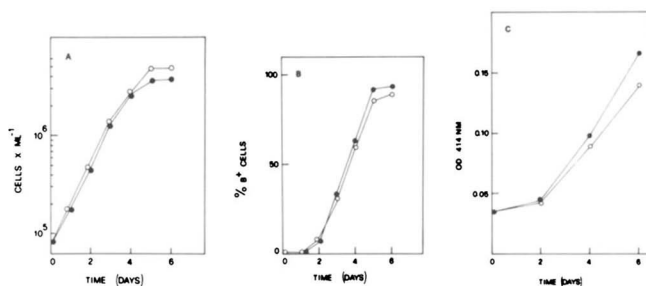


FIG. 4. **Effects of $\alpha\beta$ IFN antiserum on growth and differentiation of FLC.** 745A_R cells were seeded at 10^5 /ml in the presence of 1.5% (v/v) Me₂SO with (○) or without (●) sheep antiserum against murine $\alpha\beta$ IFN. Cell growth (A), cell differentiation determined by measuring the percentage of B⁺ cells (B), or by spectrophotometric determination of hemoglobin content (C). Differentiation of untreated cells with (△) or without (▲) antiserum was taken as a control. 2-5A synthetase activity was measured as an internal control, in extracts prepared, as described under "Experimental Procedures," from cells treated with Me₂SO, Me₂SO + IFN (320 units/ml) and Me₂SO + IFN (320 units/ml) + antiserum. The obtained values were <1, 40, and <1 nmol of ATP transformed/h/mg of protein, respectively.

FLC—If IFN were released during FLC differentiation, as recently proposed (Friedman-Einat *et al.*, 1982), it should increase the double-stranded RNA-activated protein kinase activity. Extracts were thus prepared from FLC induced to differentiate with either HMBA or Me₂SO, for increasing periods of time and incubate with γ -³²P-labeled ATP with or without double-stranded RNA. None of these conditions led to an increased level of phosphorylation of the $M_r = 67,000$ protein band in 745A_R and 745A_R 1C11 sublines (Fig. 3) and in 745A_H up to day 6 (data not shown). On the contrary, extracts prepared from exogenous IFN-treated FLC did exhibit the characteristic double-stranded RNA-dependent increase of the $M_r = 67,000$ band phosphorylation, although

less pronounced than for IFN-treated L929 cells taken as a positive reference (Fig. 3).

Effects of IFN Antiserum on FLC Erythroid Differentiation—Neutralization of IFN effects with a specific antiserum should provide an alternative approach to the elucidation of the role of IFN-induced enzymes in erythroid cell differentiation, if indeed any increase in their activity resulted from IFN release. FLC were treated with an antiserum raised against an unfractionated preparation of mouse $\alpha\beta$ IFN at doses shown in preliminary experiments to completely inhibit exogenous IFN-induced 2-5A synthetase activity. As described in Fig. 4, such a treatment did not significantly affect FLC growth (A) or differentiation induced by Me₂SO as measured by the increase in benzidine-positive cells (B) or by spectrophotometric determination of hemoglobin content (C). IFN was included in parallel experiments as a control of antiserum efficacy, as described in Fig. 4.

DISCUSSION

The terminal differentiation of one FLC subline is accompanied by an increase of the IFN-induced 2-5A synthetase, as originally described by Kimchi (1981). However, the data presented in this paper do not support a general role of 2-5A synthetase induction in the differentiation of erythroid cells, as: 1) an increase in 2-5A synthetase activity following Me₂SO treatment is not observed in a closely related but independently passaged FLC subline; and 2) HMBA does not modulate 2-5A synthetase activity even in the FLC subline (745A_H) which shows an increase of 2-5A synthetase activity following Me₂SO treatment.

These data are in keeping with the absence of any detectable increase in 2-5A synthetase activity in five of five of the IFN-resistant variants isolated in this laboratory (Affabris *et al.*, 1983)³ while all of them respond to inducers of differentiation

³ Unpublished observations.

indistinguishably from their wild type parent.

The observed differences in the ability of Me₂SO and HMBA to increase 2-5A synthetase activity in at least one FLC subline is not surprising. It has been shown that differentiation inducers such as Me₂SO or Na butyrate increase 2-5A synthetase activity in several unrelated lines such as C243 murine fibroblasts (Besançon *et al.*, 1981) or HeLa cells,² which are not known to express markers specific to a given differentiation lineage.

Since subnanomolar amounts of 2-5A suffice to activate RNase L (Lengyel, 1981), conventional assays for measuring 2-5A synthetase activity might not have been sensitive enough to detect low amounts of 2-5A products still of possible biological significance. Indeed, a recently developed sensitive radioimmunoassay of 2-5A (Cailla *et al.*, 1982a) did allow us to measure previously undetectable basal levels of 2-5A synthetase activity in all three sublines. Yet, Me₂SO was unable to modulate these levels in 745A_R and the IFN-resistant variant cells. We cannot exclude, however, a role of the 2-5A synthetase recently detected in the nuclei (Nilsen *et al.*, 1982; St. Laurent *et al.*, 1983) in the differentiation process of FLC, as experimental procedures conventionally used to assay 2-5A synthetase do not lead to a major disruption of the nuclei. Likewise, a localized activation of the 2-5A system (as hypothesized by Nilsen and Baglioni, 1979) in the intact differentiating FLC could have escaped our detection and yet play a significant biological role.

2-5A synthetase induction could merely reflect the excretion of IFN, as reported by Friedman-Einat *et al.* (1982). There are examples of excretion of minute amounts of IFN undetectable by its biological assay. As we could not detect any IFN level in the supernatants of differentiating FLC,⁴ cells were exposed to a potent mouse αβIFN antiserum which would neutralize any IFN produced under these circumstances. As the differentiation process was not affected by this treatment, we can exclude a role of "spontaneously" produced αβIFN in the course of Me₂SO-induced hemoglobinization. In this respect, it is not surprising that protein kinase is not induced by Me₂SO or HMBA under these experimental conditions in 745A_R sublines. Likewise, IFN antiserum suppresses the increased level of 2-5A synthetase activity accompanying mouse myeloid leukemia cell differentiation without preventing differentiation itself (Sokawa *et al.*, 1981). This does not exclude, however, a role of IFN and of the 2-5A system in the regulation of growth and differentiation in other systems such as rat liver regenerating after partial hepatectomy (Etienne-Smekens *et al.*, 1981) or lymphocyte activation (Kimchi, 1981).

Considering the presence of an elevated 2-5A synthetase activity reported in mammalian reticulocytes (Hovanessian and Kerr, 1978) and the data reported in this paper, we might hypothesize that any such increase takes place very late in the process of erythroid cell differentiation, *e.g.* at a stage preceding closely the extrusion of the nucleus. Differences in FLC sublines would then easily explain why only some of them (see the data reported here for 745A_H and those reported by Kimchi (1981)) do reach this stage while others do not (see data reported here for 745A_R and for HMBA-induced 745A_H). Whatever the case, the 2-5A system *per se* (*i.e.* independently from exogenous IFN addition) would not play any role either in the commitment of FLC to terminal differentiation or in the hemoglobinization process which is fully expressed in this *in vitro* system (Cioé *et al.*, 1978).

Acknowledgments—We wish to thank Dr. H. Cailla (Centre d'Immunologie de Marseille-Luminy) for her help with the radioimmunological quantification of 2-5A products. We are indebted to Drs. L. Harel and I. Gresser (Institut de Recherches Scientifiques sur le Cancer, Villejuif) for providing us with an FLC subline and mouse anti-IFN antiserum, respectively. Skillful secretarial assistance has been provided by C. D'Addazio and C. Zambelli.

REFERENCES

- Affabris, E., Jemma, C., and Rossi, G. B. (1982) *Virology* **120**, 441-452
- Affabris, E., Romeo, G., Belardelli, F., Jemma, C., Mechti, N., Gresser, I., and Rossi, G. B. (1983) *Virology* **125**, 508-512
- Besançon, F., Bourgeade, M. F., Justensen, J., Ferbus, D., and Thang, M. N. (1981) *Biochem. Biophys. Res. Commun.* **103**, 16-24
- Cachard, A., and De Maeyer-Guignard, J. (1981) *Ann. Virol.* **132**, 307-312
- Cailla, H., Laurence, L., De Borgne de Kaouel, C., Roux, D., and Marti, J. (1982a) in *Radioimmunoassay and Related Procedures in Medicine* (International Atomic Energy Agency, ed) pp. 33-44, IAEA, Vienna
- Cailla, H., Le Borgne de Kaouel, C., Roux, D., Delaage, M., and Marti, J. (1982b) *Proc. Natl. Acad. Sci. U. S. A.* **79**, 4742-4746
- Cioé, L., Dolei, A., Rossi, G. B., Belardelli, F., Affabris, E., Gambari, R., and Fantoni, A. (1978) in *In Vitro Aspects of Erythropoiesis* (Murphy, M. J., ed) pp. 159-171, Springer-Verlag, New York
- Creasey, A., Bartholomew, J. C., and Merigan, T. C. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 1471-1475
- Dolei, A., Colletta, G., Capobianchi, M. R., Rossi, G. B., and Vecchio, G. (1980) *J. Gen. Virol.* **46**, 227-236
- Etienne-Smekens, M., Vassart, G., Content, J., and Dumont, J. E. (1981) *FEBS Lett.* **125**, 146-150
- Farrell, P. J., Balkow, K., Hunt, T., Jackson, R. J., and Trachsel, H. (1977) *Cell* **11**, 187-200
- Friedman-Einat, M., Revel, M., and Kimchi, A. (1982) *Mol. Cell. Biol.* **2**, 1472-1480
- Hovanessian, A. G., and Kerr, I. M. (1978) *Eur. J. Biochem.* **84**, 149-159
- Kabat, D., Sherton, C. C., Evans, L. H., Bigley, R., and Koler, R. D. (1975) *Cell* **5**, 331-338
- Kimchi, A. (1981) *J. Interferon Res.* **1**, 559-569
- Krishnan, I., and Baglioni, C. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 6506-6510
- Laemmli, U. K. (1970) *Nature (Lond.)* **227**, 680-685
- Lebleu, B., and Content, J. (1982) in *Interferon* (Gresser, I., ed) Vol. 4, pp. 47-94, Academic Press, New York
- Lengyel, P. (1981) in *Interferon* (Gresser, I., ed) Vol. 3, pp. 78-99, Academic Press, New York
- Lieberman, D., Volloch, Z., Aviv, H., Nudel, V., and Revel, M. (1975) *Mol. Biol. Rep.* **1**, 447-451
- Luftig, R. B., Conscience, J.-F., Skoultchi, A., McMillan, P., Revel, M., and Ruddle, F. H. (1977) *J. Virol.* **23**, 799-810
- Minks, M. A., Benvin, S., Maroney, P. A., and Baglioni, C. (1979) *J. Biol. Chem.* **254**, 5058-5064
- Nilsen, T. W., and Baglioni, C. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 2600-2604
- Nilsen, T. W., Wood, D. L., and Baglioni, C. (1982) *J. Biol. Chem.* **257**, 1602-1605
- Orkin, S. H., Harosi, F. I., and Leder, P. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72**, 98-102
- Reuben, R. C., Rifkind, R. A., and Marks, P. A. (1980) *BBA Rev. Cancer* **605**, 325-346
- Rossi, G. B., Dolei, A., Capobianchi, M. R., Peschle, C., and Affabris, E. (1980) *Ann. N. Y. Acad. Sci.* **350**, 279-293
- Rossi, G. B., Dolei, A., Cioé, L., and Peschle, C. (1982) *Tex. Rep. Biol. Med.* **41**, 381-387
- Sen, G. C. (1981) *Prog. Nucleic Acid Res.* **27**, 105-156
- Sokawa, Y., Nagata, K., and Ichikawa, Y. (1981) *Exp. Cell Res.* **135**, 191-197
- Stark, G. R., Dower, W. J., Schimke, R. T., Brown, R. E., and Kerr, I. M. (1979) *Nature (Lond.)* **278**, 471-473
- St. Laurent, G., Yashie, O., Floyd-Smith, G., Samanta, H., Sehgal, P. B., and Lengyel, P. (1983) *Cell* **33**, 95-102
- Whitaker, R., and Granum, P. (1980) *Anal. Biochem.* **109**, 156-159
- World Health Organization Standard (1979) *J. Biol. Stand.* **7**, 383-395

⁴ G. B. Rossi *et al.*, unpublished data.