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Initial Characterization of a Spontaneous Interferon Secreted During Growth and Differentiation of Friend Erythroleukemia Cells

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A gradual increase in the level of 2',5'-oligoadenylate synthetase takes place in Friend erythroleukemia cells after a shiftdown in the rate of cell growth. The increase is about 5-fold after entry of cells into the stationary phase of growth, but much higher (25-fold) when reduction in growth accompanies cell differentiation. In the latter case, the enzyme increase is similar to that which can be induced in these cells by exogenous interferon (IFN). The increase in 2',5'-oligoadenylate synthetase was shown to be due to a spontaneous secretion of IFN by the cells themselves: it is completely abolished if antiserum to murine type I IFN is added to the culture medium. In attempts to isolate some of this spontaneously secreted IFN, we show that it is stable at pH 2, not neutralized by antiserum to type II IFN, and that it also differs from the known IFN species induced by Sendai virus in Friend cells. The major component of this spontaneously secreted IFN is 20,000 M_r and differs from the corresponding virus-induced 20,000-M_r IFN by its lower affinity for antiserum to type I IFN and its antigenic characterization as β-murine IFN. The major component of the spontaneous IFN also exhibits a higher ratio of antigrowth to antiviral activity than the Sendai-induced IFNs. We suggest that Friend cells produce this specific type of IFN for the regulation of their growth and differentiation.

Interferons (IFNs) are not only antiviral factors, but are also pleiotropic modifiers of cellular functions. IFNs reduce the rate of cell proliferation (7, 21), exert antimitogenic effects during growth stimulation of G_0-arrested cells (2, 12, 13, 20), and may, in some cases, enhance differentiation and the expression of genes operating in differentiated cells (14, 15). Because of their inhibitory effect on cell growth, IFNs can be considered as naturally occurring negative growth factors. The discovery of IFN-induced translation-regulatory enzymes (for review, see references 1, 19, and 23) has provided new methods to detect whether cells have interacted with IFN (11) and to monitor the presence of even low amounts of IFN. Some cells, such as rabbit reticulocytes, contain high levels of eIF-2 kinase and 2',5'-oligoadenylate (oligo A) synthetase, two enzymes which are identical in their characteristics and functions to the IFN-induced enzymes (5, 9). Similarly, we have shown that mature T-lymphocytes contain higher levels of 2',5'-oligo A synthetase than some immature populations of thymocytes which belong to the T-cell lineage (10). We therefore wondered whether these enzymes, which appear to accumulate in the absence of externally added IFN or IFN inducers, could be induced by some IFNs produced under physiological conditions during cell differentiation. To investigate this possibility, we used Friend erythroleukemia cell lines (6, 18) as an in vitro model of cell differentiation. Our results show that cultures of Friend cells undergoing a shiftdown in the rate of cell growth start to spontaneously produce a type I mouse IFN and that this IFN production is even higher if the decrease in growth is part of the differentiation process. This spontaneous IFN activity differs from the virally induced IFNs made by the same cell line when challenged with Sendai virus.

MATERIALS AND METHODS

Cell lines. The Friend erythroleukemia cells used originate from clone 745 of the original Friend cell line (6) and were grown in Dulbecco modified Eagle medium (DMEM) (GIBCO) supplemented with 10% fetal calf serum. BALB/c 3T3 fibroblasts and L929 cells were grown in DMEM supplemented with 10% calf serum. All cultures were kept at 37°C in a humidified atmosphere of 10% CO_2 in air. Cell numbers were determined by counting either in a hemacytometer or with a Coulter Counter (model ZBI).

Induction of differentiation in Friend cells. Exponentially growing Friend cells were inoculated at a density

of 10^5 cells per ml, and 1.5% (vol/vol) dimethyl sulfoxide (Me_2SO) was added (time zero). Differentiation was monitored for 7 days. When indicated, 3 days after induction, the culture (5×10^6 cells per ml) was diluted 10-fold into fresh medium supplemented with 1.5% Me_2SO. This dilution step prevented the cultures from reaching saturation cell density and extended the period of hemoglobin accumulation of 5 to 6 days in the conventional way of induction to 7 to 8 days. For this reason, we refer to this dilution procedure as "improved conditions" of differentiation. Hemoglobin content in cell extracts was measured by the benzidine assay as previously described (15).

2',5'-Oligo A synthetase assay. Crude extracts were prepared in Nonidet P-40 (5×10^6 cells per 100 μl of lysate) as previously described (10). For determination of 2',5'-oligo A synthetase activity, 10 to 20 μg of protein was adsorbed on polyriboinosinic acid-polyribocytidylic acid-agarose beads (P. L. Biochemicals), which were then incubated with [α-^{32}P]ATP (3 Ci/mmol; 2.5 mM) as previously detailed (11). The radioactivity incorporated into 2',5'-$(Ap)_nA$ oligonucleotide was measured.

Assay of IFN by inhibition of VSV growth. (i) Radioimmunoassay. Twofold dilutions of the different preparations to be tested were made in 96-well microtiter plates with DMEM containing 10% calf serum. L929 cells were added (40,000 cells per well), and vesicular stomatitis virus (VSV) was added 20 h later at 5 PFU/cell in medium with 2% serum. Excess virus was removed after 2 h, and the cultures were incubated for 10 h. The level of the VSV G protein in each well (cell lysate plus medium) was determined by radioimmunoassay by using antibodies to VSV G protein and ^{125}I-labeled protein A, as previously described (22). The IFN antiviral titer in the preparation was calibrated in each assay against the mouse IFN international standard supplied by the National Institutes of Health (NIH), Bethesda, Md. In some experiments, IFN was assayed in BALB/c 3T3 fibroblasts.

(ii) CPE method. Incubation of L929 cells with IFN preparations was performed as described for the radioimmunoassay. Monolayers were then challenged with VSV at 0.1 PFU/cell in 0.1 ml of DMEM with 2% serum, and 20 h later the cytopathic effect (CPE) was scored. One unit (concentration that gives 50% reduction in CPE) usually corresponded to 1.5 to 3 NIH reference units.

Detection of IFN activity in the growth medium of Friend cells by 2',5'-oligo A synthetase induction. Twofold dilutions of the growth medium to be tested were incubated in 30-mm plates with Friend cells (2×10^6 cells per ml) 2 days after Me_2SO induction. These cells were used because they contain low basal levels of 2',5'-oligo A synthetase activity (see Fig. 1). Eighteen hours later, extracts were prepared and assayed for 2',5'-oligo A synthetase activity. It should be noted that similar results were also obtained if undifferentiated cells at 2×10^6 cells per ml were used (not shown).

Neutralization test of IFN. Neutralization of the different IFN preparations was performed by preincubating several dilutions of antiserum against type I mouse IFN (10^{-3}, 2×10^{-4}, 10^{-4}, 2×10^{-5}, and 10^{-5}) or twofold serial dilutions of antisera against α- and β-murine IFNs with 5 antiviral units per ml for 15 min at room temperature. The mixtures were then added to L929 cells and assayed for inhibition of VSV growth by radioimmunoassay. The antiserum dilution (final concentration in the culture medium) which inhibited 5 U of IFN per ml by 80% was taken as the "neutralization titer."

Preparation of the different IFN activities produced by Friend cells. (i) Sendai-induced IFN. Friend cells (2×10^6 cells per ml) at day 2 post-Me_2SO stimulation were incubated with Sendai virus (100 hemagglutination units per ml). After 24 h, the medium was collected and treated for 4 days at pH 2. The titers of IFN activity after acidification were usually around 10^5 U/ml.

(ii) "Spontaneous" IFN. Growth medium from Friend cells induced to differentiate under the improved conditions was collected at day 7 post-Me_2SO (2×10^6 cells per ml; as described in the legend to Fig. 1). The titer of antiviral activity was determined by both the CPE method and radioimmunoassay and varied between 50 and 200 NIH reference units per ml in the different experiments.

Both preparations of IFN were concentrated and fractionated by stepwise ammonium sulfate precipitation. The IFN activities in the 40 to 70% ammonium sulfate fractions were either analyzed on Sephadex G-100 columns or chromatographed on Cibacron Blue Sepharose and carboxymethyl (CM)-Sepharose columns. A total of 1.5×10^4 and 8×10^5 U for the spontaneous and Sendai-induced IFNs, respectively, were applied to Sephadex G-100 columns (5 by 90 cm). Elution was performed in 3 M acetic acid, and 10-ml fractions were collected. Parallel ammonium sulfate fractions were loaded on Cibacron Blue Sepharose-CL6B (2-ml column volume; 30 ml/h), and elution was performed in 20 mM phosphate buffer (pH 7.2) containing 1 M NaCl, followed by a 50% propylene glycol step. Under the conditions used, 50% of IFN activity was recovered from the columns, and in both preparations the bulk of IFN activity eluted at 1 M NaCl. About 10% of the IFN activity of the Sendai-induced preparation and no activity of the spontaneous preparation appeared in the flow-through fractions. For chromatography on CM-Sepharose, the ammonium sulfate precipitates were dissolved in 20 mM phosphate buffer (pH 6) containing 10 mM NaCl. Elution was performed by steps of 50, 100, 250, and 500 mM NaCl in the same buffer. In both preparations, the major peak of IFN activity was eluted at 250 mM NaCl (90% recovery).

Materials. NIH mouse IFN standard (12,000 U/ml) was purchased from the NIH catalog no. G-002-904-511. Antiserum to type I mouse IFN was kindly provided by I. Gresser, Institut de Recherche Scientifique sur le Cancer, Villejuif, France (8), and antiserum to type II mouse IFN was obtained from E. Falcoff, Institut Curie, Paris (dilution of 1:20 of this antiserum neutralizes 10 U of type II immune IFN per ml). Antisera against α and β mouse IFNs were kindly provided by J. Trapman, Erasmus University, Rotterdam. (A 1-ml amount of anti-IFN β neutralizes 4×10^4 U of β mouse IFN and 100 U of α mouse IFN; 1 ml of anti-IFN α neutralizes 1×10^4 U of α mouse IFN and 100 U of β mouse IFN.)

RESULTS

Increase of 2',5'-oligo A synthetase during the differentiation of Friend erythroleukemia cells.

Friend erythroleukemia cells were seeded in the presence or absence of 1.5% Me_2SO and tested at different time intervals for the level of 2',5'-oligo A synthetase activity. Figure 1A shows that in both cell cultures the enzyme activity was low during the exponential growth phase (days 1 to 3) and increased by a factor of 5 to 7 after entry into the stationary phase. Under these experimental conditions, there was no significant difference in the kinetics of cell growth or in the pattern of 2',5'-oligo A synthetase between differentiating and non-differentiating cells.

To prevent the Me_2SO-induced cells from reaching the saturation cell density before completing differentiation, the cultures were diluted into fresh medium 3 days postinduction. These culture conditions, in which cells which have reached a density of 5×10^6 cells per ml (just before entry into stationary phase) are diluted 1:10 into fresh medium containing Me_2SO, are referred to as improved conditions of differentiation, since the maximal levels of hemoglobin per cell were 20 to 50% higher than those obtained in the undiluted differentiated cultures (90 μg of hemoglobin per 10^7 cells). Figure 1B shows that after the dilution step, the level of 2',5'-oligo A synthetase in the Me_2SO-induced cells increased dramatically, whereas there was only a moderate increase in the control noninduced cultures diluted in the same way. Most of the increase in synthetase activity took place between days 5 and 7 post-Me_2SO induction, reaching a 25-fold increase over the basal levels at day 7. Figure 1B also shows that after the dilution step of the Me_2SO-treated cells, there was a significant decrease in the rate of cell growth: the generation time increased from 10 to 20 h in the first 2 days postdilution (days 3 to 5), and cell growth stopped at a density of 2×10^6 cells per ml, which is below saturation cell density. Control noninduced cells continued to grow after the dilution at the same rate as before dilution (compare Fig. 1A and B).

Spontaneous elevation in the level of 2',5'-oligo A synthetase seems, therefore, to follow a shiftdown in the rate of cell growth; the elevation is moderate when this rate decreases as a result of cell entry into stationary phase, and it is maximal when the shiftdown in the growth rate is part of the differentiation process. Maximal differentiation and synthetase increase requires dilution of the Me_2SO-induced Friend cells. Under these conditions, the final levels of synthetase activity obtained at day 7 (50,000 cpm of 2',5'-oligo A per 20 μg of protein) were as high as those which can be induced in Friend cells by exogenous IFN (Table 1). This led us to examine whether IFN was produced by differentiating Friend cells.

Spontaneous secretion of IFN by Friend cells. The gradual increase in the level of 2',5'-oligo A synthetase activity occurring between days 5 and 7 under the improved conditions of differentiation was completely abolished if antiserum

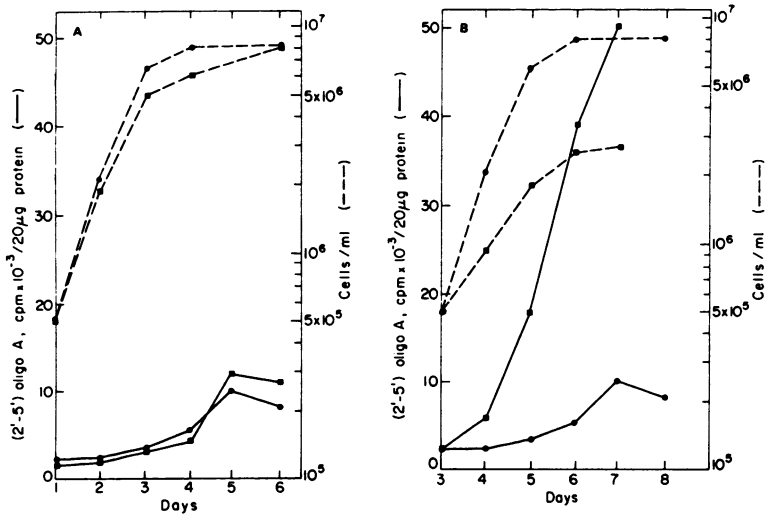

FIG. 1. 2',5'-Oligo A synthetase activity and growth of Friend cells with or without Me_2SO. (A) Friend erythroleukemia cells were seeded at 10^5 cells per ml with (■) and without (●) 1.5% Me_2SO (day 0). At the indicated times, cells were counted and 2',5'-oligo A synthetase activity was measured in the cell extracts, as described in the text. Dotted lines, cell number; solid lines, 2',5'-oligo A synthetase activity measured per 20 μg of protein. (B) Cells were seeded at 10^5 cells per ml with or without Me_2SO, as described in (A) (day 0), and 72 h later (day 3) cells were diluted 1:10 into fresh medium (improved conditions of differentiation). cpm, Counts per minute.

TABLE 1. Properties of the 2',5'-oligo A synthetase-inducing activity from the culture medium of differentiating Friend cells

Additions[a]	2',5'-Oligo A synthetase (cpm/20 μg of protein)	Antiviral activity (U/ml)
None	4,400	<1
Culture medium supernatant[b]	48,900	10^2
After pH 2 treatment	46,300	10^2
With anti-type I IFN	6,100	
With anti-α-IFN	51,000	
With anti-β-IFN	9,500	
With anti-type II IFN	49,100	
Virus pellet from culture medium[c]	5,000	<1
After sucrose gradient purification	5,600	<1
Mouse L-cell IFN (200 U/ml)	49,500	
Sendai virus (100 HAU/ml)	51,300	10^5

[a] Friend cells received the indicated additions and were assayed 24 h later for 2',5'-oligo A synthetase activity as described in the text. HAU, hemagglutination units.

[b] Medium from a culture of differentiating Friend cells at day 7 of improved conditions was centrifuged at 100,000 × *g* for 1 h, and the supernatant was added to cells at a dilution of 1:8 or tested directly for antiviral activity by radioimmunoassay. Antisera to type I IFN (10^{-3} dilution), to α- and β-IFNs (1:100 dilution), and to type II IFN (1:20 dilution) were added together with culture medium supernatant.

[c] The 100,000 × *g* pellet from culture medium was assayed for Friend leukemia virus by reverse transcriptase activity, either directly or after sucrose gradient purification (3). An amount of virus at least 10 times higher than that present in 2-day Friend cells was added. The activity of 2',5'-oligo A synthetase in the treated cells and the antiviral activity in the growth medium were determined 24 h later.

against type I mouse IFN was added to the culture medium. Figure 2 shows that a 10^{-3} dilution of this antiserum completely prevented any increase in the synthetase activity. This result was the first indication that the enzyme induction is related to a spontaneous secretion of IFN by these cells. With lower amounts of the same antiserum (10^{-4} dilution), the synthetase did not increase up to day 6 of the improved conditions, but increased strongly on day 7 (Fig. 2). This suggests that IFN accumulates continuously in the growth medium between days 5 and 7, in line with the gradual increase in enzyme activity seen in the cells.

To demonstrate the presence of IFN, we tested the culture medium of differentiating Friend cells by two main methods: inhibition of VSV growth and induction of 2',5'-oligo A synthetase. Two different assays of antiviral activity with VSV were used in this work: the CPE reduction assay and the radioimmunoassay described in Materials and Methods. By using the radioimmunoassay, we found that at day 7, under the improved conditions of differentiation, there was, on the average, 100 U of mouse IFN per ml in the medium, as expressed in NIH reference units (Table 1). No IFN activity could be detected until day 5 of differentiation; IFN started to accumulate on day 6 and reached maximal levels on day 7 (not shown). The assay of 2',5'-oligo A synthetase induction in cells exposed to the culture medium proved to be the most sensitive assay for detecting the spontaneous IFN activity: a 1:256 dilution of the growth medium could induce detectable levels of 2',5'-oligo A synthetase in Friend cells (Fig. 3). By using this assay, we could establish (Fig. 3) that the culture medium of cells at day 7 of the improved conditions of differentiation contained 12 times more IFN activity than the corresponding medium from Me_2SO-treated cells which

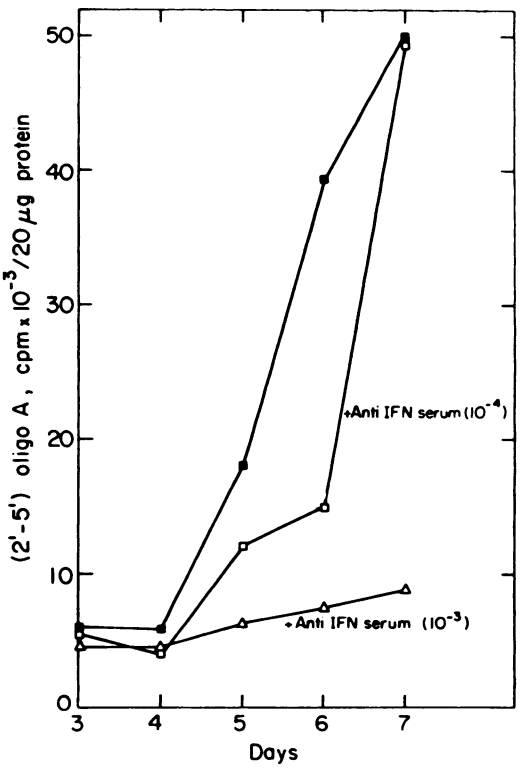

FIG. 2. Effect of antiserum to type I IFN on the increase in 2',5'-oligo A synthetase occurring during differentiation. Exponential cultures were induced to differentiate under the improved conditions. Antiserum to type I IFN was added immediately after the dilution step (day 3). Symbols: ■, no antiserum; □ and △, antiserum at 10^{-4} and 10^{-3} dilutions, respectively. Extracts were prepared at the indicated times and tested for 2',5'-oligo A synthetase activity.

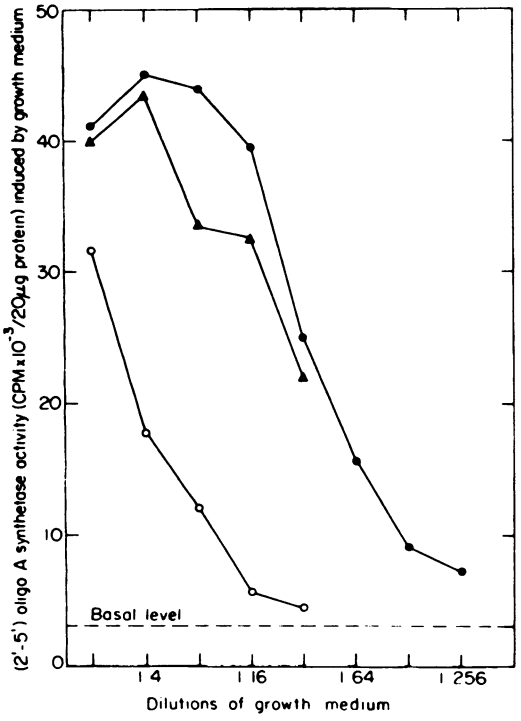

FIG. 3. Titration of IFN activity in the growth medium of Friend cells by the assay of 2',5'-oligo A synthetase induction. Friend cells were induced by 1.5% Me_2SO. On day 3, a portion of the culture was diluted 1:10 into fresh medium with (●) or without (▲) Me_2SO, and the rest of the cultures remained undiluted (○). Culture medium was collected on day 7 of differentiation and was tested for its ability to induce 2',5'-oligo A synthetase activity in 2-day cells, which contain low basal levels of the enzyme (dotted line). Details of the assay for synthetase induction are given in the text.

were kept undiluted. It is likely, therefore, that the larger increase in synthetase activity which takes place under the improved conditions (Fig. 1B) results from higher IFN production. High levels of IFN activity could also be detected if dilution of the differentiating cells was performed at day 3 into medium lacking Me_2SO (Fig. 3), indicating that the Me_2SO does not have to be present during the IFN secretion period (days 5 to 7). Me_2SO is therefore not a direct inducer of IFN production in this system.

The activity in Friend cells growth medium which induces 2',5'-oligo A synthetase in the cells was shown to be soluble, stable at pH 2, not neutralized by antiserum to mouse IFN type II (Table 1), and not neutralized by antiserum to α-IFN but neutralized by antiserum to β-IFN. Friend erythroleukemic cells are chronically infected with the Friend murine leukemia viruses, and it is known that differentiation is accompanied by an enhanced release of these viruses into the medium (15). We therefore tested the possibility that the Friend viruses could mediate activation of IFN gene expression. For this purpose, the virus pellet was spun down from the culture medium at day 6 of the improved conditions of differentiation, further purified by fractionation on sucrose gradients, and tested for its ability to induce the synthetase activity in Friend cells. For comparison, a virus commonly used to induce IFN synthesis (Sendai virus) was added to Friend cells, and the response to the different viruses was tested 24 h after the challenge. Table 1 shows that Friend virus could not induce the synthetase in the cells, whereas maximal response was induced by the Sendai virus. In the growth medium, 10^5 U of IFN activity per ml was detected after the challenge with Sendai virus, whereas in the case of the challenge with Friend virus, the levels were below the limit of detection (Table 1). It seems, therefore, that the secretion of IFN during differentation is not triggered by Friend virus particles accumulating in the medium.

Initial purification and characterization of the different IFN activities produced by Friend cells. The following experiments were done to compare the spontaneous IFN to the conventional IFNs induced in Friend cells after challenge with Sendai virus. The starting crude IFN preparations were culture medium from Sendai-induced Friend cells (10^5 U/ml) and culture medium from day 7 of the improved conditions of differentiation (80 U/ml), as described in Materials and Methods. The 40 to 70% ammonium sulfate fractions of each preparation were applied to Sephadex G-100 columns (1.5×10^4 and 8×10^5 U for the spontaneous and Sendai-induced IFN, respectively). Figure 4 shows that the major peak of the Sendai-induced IFN antiviral activity eluted from G-100 as a 40,000-M_r protein, whereas the major peak of the spontaneous IFN antiviral activity (over 70% of total) eluted as a 20,000-M_r protein. In both preparations, minor peaks (5 to 10% of total activity) were also detected at 20,000 or 40,000 M_r. It is clear, therefore, that the relative proportion of the large and small IFN peaks differs in the spontaneous preparation from that detected in the Sendai-induced IFN preparation.

The different IFN fractions from Sephadex G-100 were then tested for neutralization of the antiviral activity by various antisera against type I murine IFNs α and β. The first indication that the spontaneous 20,000-M_r IFN differed immunologically from virus-induced IFNs was obtained with an antiserum against unfractionated type I IFN: over 10 times more antiserum was needed to neutralize the spontaneous IFN than for either of the two Sendai virus-induced frac-

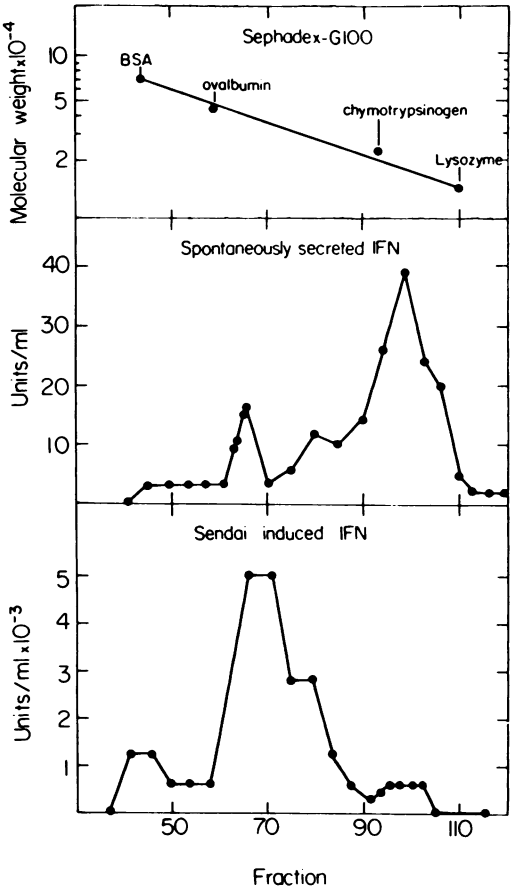

FIG. 4. Fractionation of the antiviral activities in spontaneously secreted and Sendai-induced IFN from Friend cells by filtration on Sephadex G-100. IFNs from the culture medium of differentiating Friend cells (1.5×10^4 U) and from Sendai-infected Friend cells (8×10^5 U) were ammonium sulfate precipitated, made 3 M in acetic acid, and applied to Sephadex G-100 as described in the text. Samples from eluting fractions were assayed for inhibition of VSV growth by radioimmunoassay, as described in the text.

tions (Table 2), suggesting that the spontaneous IFN has a lower affinity for these anti-IFN antibodies. We then used antisera specific for the IFN α and β species of virus-induced murine IFN. As expected (24), in the Sendai-induced IFN from Friend cells, the 20,000-M_r IFN peak had the immunological characteristics of the IFN α, whereas the 40,000-M_r peak corresponded to IFN β (Table 3). In contrast, the 20,000-M_r major component of the spontaneous Friend IFN was neutralized efficiently by anti-IFN β and not by anti-IFN α (Table 3). The spontaneous IFN seems, therefore, to consist mainly of a β-type species with an apparent molecular size one-half that of virus-induced IFN β. Further evidence that the major component of the spontaneous Friend IFN is β type was obtained by chromatography on Cibacron Blue Sepharose and on CM-Sepharose. The spontaneous IFN was adsorbed and salt eluted from both columns together with the virus-induced IFN β species (Table 3). It should be noted that all the data in Table 3 were obtained after treatment at pH 2.

In addition to the differences in size and immunological properties, the spontaneous IFN preparations also exhibited a different ratio of antigrowth over antiviral activities. The antigrowth activity was measured in quiescent BALB/c 3T3 fibroblasts synchronized in G_1/G_0 and stimulated by serum to synthesize DNA. We have previously described in detail the inhibitory effect of L-cell IFN and of 2',5'-$(Ap)_nA$ on entry of BALB/c 3T3 cells into S phase in this system (12). In the present work, DNA synthesis was assayed by two methods: rate of [*methyl*-^{3}H]thymidine incorporation into acid-precipitable material and determination of the labeling index (percentage of labeled nuclei). From 0.1 to 100 antiviral units of the different fractions eluting from Sephadex G-100 per ml was added immediately after serum stimulation, and the effect on DNA synthesis was determined 20 h

TABLE 2. Comparison between different IFN activities eluting from Sephadex G-100

Producer cell	Inducer	Relative proportion of the different molecular size IFNs[a]		Neutralization titer[b]		Antigrowth effect[c]	
		20K	40K	20K	40K	20K	40K
Friend cells at day 2 post-Me_2SO induction	Sendai	+	+++	2×10^5	2×10^5	34	36
Friend cells at day 7 post-Me_2SO induction	None	+++	+	10^4	Not done	0.75	50

[a] +++, Major IFN activity in the preparation (as in Fig. 4); +, minor IFN activity in the preparation.
[b] Antiserum dilution required to inhibit 5 U/ml. The peak fractions containing IFN activity eluting from Sephadex G-100 (as in Fig. 4), were tested for their neutralization by anti-type I IFN antiserum.
[c] IFN concentration (units per milliliter) required to inhibit cell growth. The peak fractions containing IFN activity eluting from Sephadex G-100 were tested for antigrowth activity as described in the text.

TABLE 3. Antigenic characterization and antigrowth properties of the different IFN fractions

IFN fraction[a]	Source of IFN	Neutralization titer[b]		Antigrowth activity[c]
		Antiserum to α-IFN	Antiserum to β-IFN	
20K component from Sephadex G-100	Spontaneous	50	1,600	1
	Sendai induced (minor peak)	3,200	10	40
40K component from Sephadex G-100	Sendai induced (major peak)	10	6,400	50
Blue Sepharose (1 M NaCl)	Spontaneous	50	1,600	3
	Sendai induced	10	6,400	60
CM-Sepharose (0.25 M NaCl)	Spontaneous	50	3,200	1.5
	Sendai induced	10	6,400	50

[a] The 40 to 70% ammonium sulfate preparations of Sendai-induced and spontaneous IFNs were fractionated by Sephadex G-100, Blue Sepharose, or CM-Sepharose columns, as described in the text. In each case, fractions containing the majority of spontaneous IFN activity are compared to the corresponding fractions eluting from Sendai-induced IFNs. 20K, 20,000 M_r; 40K, 40,000 M_r.

[b] Antiserum dilution required to inhibit 5 U/ml. Fractions were tested after treatment at pH 2 for 24 h at 4°C. The antiviral activity was determined by radioimmunoassay.

[c] IFN concentration (units per milliliter) required to inhibit cell growth. Fractions were tested after treatment at pH 2 for 24 h at 4°C. Antigrowth activity was determined in BALB/c 3T3 cells as described in the text.

later, at the time when DNA synthesis in control stimulated cells is maximal (Fig. 1 of reference 12). For these experiments, the antiviral titer of each fraction from Sephadex G-100 was determined by using the VSV radioimmunoassay in the same BALB/c 3T3 cells and at the same time as the antigrowth properties of these fractions were assayed. We then calculated the number of antiviral units which inhibited DNA synthesis by 50%. Table 2 shows that the major peak of the spontaneous IFN preparation eluting at 20,000 M_r differed from the other IFN fractions by its relatively high ratio of antigrowth over antiviral activity. Less than 1 antiviral unit per ml reduced DNA synthesis by 50%, as compared with 30 to 50 antiviral units per ml for the other IFN fractions, including the minor 40,000-M_r fraction of spontaneous IFN. When the major component of spontaneous IFN was purified on Cibacron Blue Sepharose or CM-Sepharose, the same higher ratio of antigrowth over antiviral activity was observed (Table 3) compared with viral-induced IFN. These results suggest that the higher antigrowth effect may be an intrinsic property of the spontaneous IFN, although further purification is clearly needed.

DISCUSSION

In the present work, Friend erythroleukemic cells were shown to produce IFN without being exogenously treated by any conventional inducer of IFN synthesis (e.g., virus or double-stranded RNA). This IFN production followed a decrease in the growth rate of cells occurring in two instances: when cells entered the stationary phase of growth and when the shiftdown in the growth rate was part of the differentiation process. In the latter case, the extent of IFN production (100 U/ml, on average) was 10- to 12-fold higher than in the former case and was highest when improved conditions of differentiation were used, conditions under which the typical differentiation-related limitation in the proliferative capacity of cells (16) could be clearly expressed. IFN was produced at a relatively late time after exposure of cells to Me_2SO and was accompanied by a gradual induction of 2',5'-oligo A synthetase in the cells. The murine leukemia viruses which are released from the cells during differentiation (4) did not induce either IFN secretion into the medium or 2',5'-oligo A synthetase accumulation in the cells when added exogenously to the cells. In addition, Me_2SO was not the direct inducer of the IFN synthesis during differentiation, since the cells could be committed to secrete IFN even if Me_2SO was removed from the culture medium at day 3, before the onset of IFN secretion (Fig. 3). Since no other conventional inducer of IFN synthesis was identified in this system, we defined this process as spontaneous production taking place under certain growth conditions.

Spontaneous production of some species of human leukocyte IFN was previously reported in several lymphoblastoid cell lines (17), but it should be emphasized that in these systems production took place during the entire logarithmic growth phase, suggesting a constitutive expression of some of the IFN genes in these cell lines. Unlike this situation, in the case of the Friend cell system, we suggest that the spontaneous production is an inducible event which occurs only after shiftdown in cell growth, although the nature of the induction is not yet clear at this stage.

We have characterized the spontaneous IFN and shown that it is stable at pH 2 and neutralized by antiserum to type I mouse IFN and not

by antiserum to type II immune mouse IFN. A comparison with the conventional viral-induced IFNs produced by the same cell line challenged with Sendai virus in the exponential growth phase revealed a difference in the relative proportion of the two main molecular size IFNs within each preparation. It was previously reported in other murine cell systems that the relative proportion of the two main IFN species, the α-IFN (20,000 to 24,000 M_r) and the β-IFN (36,000 to 40,000 M_r), which differ in their structures and antigenic properties, varies depending on the cell type and the nature of the inducer (24). However, in the case of the spontaneous IFN preparation, we find that the 20,000-M_r species not only represents 70% of the total activity, but also seems to be antigenically related to the 36,000- to 40,000-M_r species of virus-induced IFN and exhibits chromatographic properties similar to those of the β-murine IFN on Blue Sepharose and CM-Sepharose. In the Sendai-induced IFN preparation produced by the same cell line, we found no evidence for this component. Taking into account also its lower affinity toward antiserum to type I IFN, we are led to the conclusion that the spontaneous 20,000-M_r IFN appears to differ from the major α and β species of virus-induced murine IFNs.

The antigrowth properties of the spontaneously secreted Friend cell IFN were analyzed in quiescent BALB/c 3T3 fibroblasts stimulated to grow by serum, a system which was shown to be very sensitive to the inhibitory effect of IFN on cell entry into S phase (21). In previous work, we determined that the titer of Newcastle disease virus-induced L-cell IFN, which reduces DNA synthesis in the 3T3 system by 50% (11), is equivalent to 30 to 50 antiviral units per ml. The same range of growth-inhibitory activity was found in this work for the Sendai-induced 20,000- and 40,000-M_r species and for the minor 40,000-M_r fraction of spontaneous IFN. The high efficiency of the major component of the spontaneous IFN to inhibit growth is therefore exceptional (between 0.75 and 3 antiviral units per ml inhibited 50% of DNA synthesis). Although the antigrowth activity of the spontaneous IFN was neutralized by anti-β-murine IFN (data not shown) and the higher antigrowth properties were observed with different purification methods, it is clear that further purification is required to determine whether this is an intrinsic property of the major IFN species produced during differentiation.

A most interesting question in this system concerns the biological role of this IFN during differentiation and its possible stimulatory effect in the expression of the globin gene (15). These studies are in progress. Another question is whether spontaneous production of IFN during differentiation takes place in vivo and includes other cell lineages of the hemopoietic system. A strong indication which supports this suggestion is the observation that mature reticulocytes (9) and peripheral lymphocytes (10) contain high levels of 2',5'-oligo A synthetase activity, which may indicate the involvement of IFN during their maturation.

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