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PRODUCTION AND CHARACTERIZATION OF HUMAN T LEUKEMIC LYMPHOBLASTOID CELL INTERFERON

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SUMMARY Although human lymphoblastoid cells (Namalva and BALL-1 cells) which have the characteristics of B lymphocytes are known to be good producers of human lymphoblastoid interferon (Hu IFN- α (Ly)), there is only one report on IFN production by human T lymphoblastoid cells.

The present study showed that Sendai virus could induce human T lymphoblastoid cells (RPMI 8402 cells) to produce IFN. On incubation with virus, IFN was detected after 9 h, and increased until 24 h. The IFN yield depended on the dose of Sendai virus. No superinduction was observed on treatment with BUdR or antimetabolites or on irradiation with ultraviolet light. The T cell line produced IFN even in serum-free medium. The IFN produced by RPMI 8402 cells (8402-IFN) was as stable at pH 2 and on heating at 60 C for 30 min as Hu IFN- α and Hu IFN- β . 8402-IFN, Hu IFN- α and Hu IFN- β showed the same kinetics of induction of the antiviral state of FL cells. The 8402-IFN was neutralized by anti-Hu IFN- β serum, but not anti-Hu IFN- α serum, indicating that it was antigenically identical with Hu IFN- β . MDBK cells were very sensitive to Hu IFN- α , but not to this IFN or Hu IFN- β , but L₉₂₉ and RK-13 cells were equally sensitive to 8402-IFN, Hu IFN- α and Hu IFN- β . The molecular weight of this IFN was estimated as 21,000 and 16,500 daltons by SDS-PAGE and Sephadex G-100 gel chromatography, respectively.

INTRODUCTION

It is well known that human B lymphoblastoid cell lines including Namalva and BALL-1 cells, produce a high titer of interferon (IFN) (Strander et al., 1975; Vodckaert-Vervliet and Billiau., 1977; Imanishi et al., 1980). Large

scale production of human lymphoblastoid IFN (Hu IFN- α (Ly)) was achieved in a huge spinner vessel (Klein et al., 1979; Bridgen et al., 1977). The method which we described in a previous report is also useful for

large-scale production of IFN. In our method IFN is produced in BALL-1 cells grown in newborn hamsters treated with anti-thymocyte (Imanishi et al., 1980). The biological and physicochemical properties of the IFNs produced in human B lymphoblastoid cell lines have been clarified. Namalva IFN consists of two antigenic types: human leukocytic type (Hu IFN- α) and human fibroblastic type (Hu IFN- β). Havell et al. (1977) reported that 62% of the total Namalva IFN has the antigenic determinant of Hu IFN- α , and 13% that of Hu IFN- β , whereas BALL-1 IFN consists of only one antigenic type, Hu IFN- α (Imanishi et al., 1980). Thus human B lymphoblastoid IFN is well characterized.

However, there is no report on IFN production by a human T lymphoblastoid cell line or the characteristics of this IFN, except the report of Larsson et al. (1979), who demonstrated that a human T lymphoblastoid cell line produced IFN and that the IFN produced was antigenically identical to Hu IFN- β . It has been thought that normal human T lymphocytes in peripheral blood can not produce IFN. Yamaguchi et al. (1979) suggested that non-T lymphocytes, probably B lymphocytes, may produce IFN when induced with Sendai virus. Peter et al. (1980) showed that Fc-receptor-negative null lymphocytes may be involved in IFN production by the virus. It is of interest to know whether T lymphoblastoid cells other than Larsson's cell line can produce IFN and, if so, to determine the characteristics of the IFN produced by T lymphoblastoid cells.

We examined the production of IFN by a human T lymphoblastoid cell, the conditions for its production and the biological and physicochemical characters of the IFN produced, and determined the antigenic type of the IFN. We found that in many biological and physicochemical aspects the IFN produced in the human T lymphoblastoid cells that we used is the human fibroblastic type (β type) of IFN.

MATERIALS AND METHODS

1. Cells

The RPMI 8402 cell line was used for IFN production. This cell line, which was established from the blood of a patient with acute lymphoblastic leukemia and possesses the characteristics of T cells (Minowada, 1978), was grown and maintained in medium RPMI 1640 supplemented with 10% of a mixture of fetal bovine serum and newborn calf serum. For large scale growth of cells a small spinner flask (250 ml) was used first; after the cells had grown a density of about 2×10^6 cells/ml, they were transferred to a larger flask (500 ml or 1,000 ml); the cells were fed by replacing the medium by fresh growth medium every 2 or 3 days.

Human amnion FL cells, which were grown in Eagle's minimum essential medium (MEM) supplemented with 5% calf serum, were used for IFN assay and propagation of vesicular stomatitis virus (VSV).

Bovine MDBK cells, murine L₉₂₉ cells and rabbit RK-13 cells were used for examination of the species specificity of IFN.

2. Viruses

Sendai virus, which was propagated in fertilized chicken eggs and concentrated by centrifugation at 8,000 rpm for 1 h, was used for induction of IFN. Sendai virus solution was stocked at -80°C until used for induction of IFN.

The New Jersey strain of vesicular stomatitis virus (VSV), which was propagated in FL cells, was used as a challenge virus in IFN assay.

3. IFNs

Human leukocyte IFN (Hu IFN- α (Ly)) was prepared in human peripheral leukocytes infected with Sendai virus. The supernatant was concentrated by ammonium sulphate precipitation and, purified first by CM- and DEAE-cellulose column chromatographies, and then by Sephadex G-100 gel filtration. Its specific activity was about 1×10^7 IU/mg protein (Matsuo et al. 1974).

Human lymphoblastoid IFN (Hu IFN- β (Ly)) was prepared in BALL-1 cells grown in newborn hamsters treated with antithymocyte serum and was infected with Sendai virus. This preparation was concentrated and purified by SP-Sephadex C-25 chromatography and Sephadex G-100 gel filtration. Its specific activity was about 1×10^7 IU/mg protein

(Imanishi et al., 1980).

Human fibroblast IFN (Hu IFN- β) was kindly provided by Dr. Nobuhara of the Research Laboratory of Cell Science, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan. It was produced in human fibroblasts treated with polyriboinosinic acid-polyribocytidylic acid (poly I: C) and was purified by a modification of the method by Carter and Horoszewicz (1980). The purified Hu IFN- β had a specific activity of 3×10^7 IU/mg protein.

4. Anti-IFN serum

Anti-Hu IFN- α serum was obtained from rabbits immunized with Hu IFN- α produced by BALL-1 cells. At 1,000-fold dilution, this antiserum neutralized 2,000 IU of Hu IFN- α completely.

Anti-Hu IFN- β serum was kindly supplied by Dr. S. Kobayashi, Basic Research Laboratories, Toray Industries Inc., Kamakura, Japan. At 3,000-fold dilution, this antiserum neutralized 10 IU of Hu IFN- β completely.

5. IFN induction

RPMI 8402 cells grown in medium RPMI 1640 containing 10% of a mixture of sera was used for IFN induction. When the cell density became 1 to 2×10^6 cells/ml, the culture was centrifuged at 1,000 rpm for 5 min and the cell density was adjusted to 5 to 10×10^6 cells/ml. A sample of 500 hemagglutination (HA) units/ml of Sendai virus was inoculated onto RPMI 8402 cells and incubated at 37 C for 24 h in a humidified atmosphere of 5% CO₂ in air. Then the culture fluid was collected and centrifuged at 1,000 rpm for 5 min. For inactivation of residual inducing virus, the supernatant was irradiated with ultraviolet light from a sterilizing lamp (Toshiba: 10 W) at a distance of 10 cm from the IFN preparation for 2 h or was acidified to pH 2.0 with 1 N HCl and stood for 3 days. The supernatant was stocked at -80 C until IFN assay.

6. Method for IFN assay

Details of the method are described elsewhere (Imanishi et al., 1977). Samples of 2×10^5 cells/ml of FL cells were dispersed into the wells of a microplate (Nunc, Denmark) and incubated at 37 C for 2 days under an atmosphere of 5% CO₂ in air. After incubation, confluent monolayer cells was treated with 0.025 ml of serially 2-fold diluted IFN, and further incubated overnight. Then the medium was removed and the cells were challenged with about 10 to 50 TCID₅₀ of VSV. Cytopathogenic

effects (CPE) were examined after 48 h. The reciprocal of the IFN concentration which reduced the CPE by 50% was expressed as laboratory IFN units, and laboratory IFN units were calibrated against an international reference preparation (code 16/69) and expressed in terms of International Units (IU).

7. Induction by polyinosinic acid polycytidylic acid (poly I: C)

Poly I: C was purchased from Yamasa Shoyu Co., Ltd., Choshi and dissolved in RPMI 1640 medium. It was added to cultures of RPMI 8402 cells at 1000, 100 or 10 μ g/ml. After incubation for 6 h at 37 C, the RPMI 8402 cells were rinsed twice with fresh medium and suspended in medium RPMI 1640 containing 10% mixed serum. After incubation in a humidified atmosphere of 5% CO₂ in air at 37 C for 18 h, the supernatant was harvested and stocked at -80 C until assay of IFN activity.

8. Neutralization of IFN by anti-IFN serum

Anti-Hu IFN serum was serially diluted 2-fold on a transfer plate and serially 2-fold diluted IFN was added to each concentration of antiserum. The mixtures of IFN and antiserum were incubated at 37 C for 60 min in a humidified atmosphere of 5% CO₂ in air, and then transferred to monolayers of FL cells in the wells of a microplate. After incubation at 37 C for 24 h, the culture fluid was removed and 10 to 50 TCID₅₀ of VSV was inoculated onto the FL cells. After incubation for 48 h, CPE was examined and neutralizing activity was determined.

9. Determination of molecular weight of IFN

The molecular weight of IFN was determined by two methods: gel filtration on Sephadex G-100 and SDS-polyacrylamide gel electrophoresis (PAGE). For gel filtration, the concentrated IFN preparation was charged onto Sephadex G-100 and eluted with phosphate buffer containing 1 M NaCl. The following molecular weight markers were used: blue dextran (2,000,000 daltons), bovine serum albumin (67,000 daltons) chymotrypsinogen A (25,000 daltons) and cytochrome C (12,384 daltons).

For SDS-PAGE, polyacrylamide was dissolved in Tris-HCl buffer containing 0.1% SDS, and the gel concentration was adjusted to 15% for the separating gel and 3.0% for the stacking gel. The concentrated IFN preparation and the marker proteins (albumin, ovalalbumin, carbonic anhydrase, trypsin inhibitor and γ -lactalbumin) were applied to the

stacking gel with bromophenol blue and subjected to electrophoresis at 20 mA for 1 h and then at 40 mA for 4 to 5 h. The polyacrylamide gel was stained with Coomassie brilliant blue and the molecular weight was determined from the mobilities of IFN and marker proteins.

10. Kinetics of development of the antiviral state by IFN

FL cells were treated with 300 IU/ml of IFN, and 0.5, 1.0, 4.0, 6.0, 10.0 and 24.0 h later the medium was removed and 10 TCID₅₀ of VSV was added. After incubation for 24 h, the supernatant was collected and the yield of VSV in the supernatant was measured by the microplaque method.

RESULTS

1. Kinetics of IFN production in RPMI 8402 cells

Cultures of RPMI 8402 cells in plastic petri dishes (3 cm) were inoculated with 500 HA units/ml of Sendai virus, and 3, 6, 9, 12, 24 and 48 h later the culture fluid was recovered by centrifugation and irradiated with ultraviolet light to inactivate residual Sendai virus. IFN activity was detected in the culture fluid after 9 h, increased gradually until 24 h, and then decreased (Fig. 1).

2. Relation between dose of Sendai virus and IFN production

Sendai virus was added to cultures of RPMI

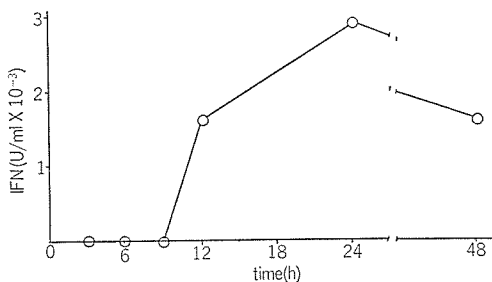


FIGURE 1. Kinetics of 8402-IFN production.

RPMI 8402 cells were infected with 500 HA units/ml of Sendai virus. After the incubation at 37 C for 3, 6, 9, 12, 24 and 48 h the culture fluid was harvested, and assayed for IFN activity.

8402 cells at doses of 20, 100, 500 and 2,000 HA units/ml. The culture fluid was harvested 24 h later and irradiated with ultraviolet light. The highest IFN activity was detected after inoculation of 2,000 HA units/ml of Sendai virus. Similar results were obtained in 4 independent experiments.

3. IFN induction by polyribinosinic acid-polyribocytidylic acid (poly I: C)

Poly I: C is a potent IFN inducer in human fibroblast cells, but not in human leukocytes or in human lymphoblastoid cells. When 1,000, 100 or 10 µg/ml of poly I: C was added to cultures of RPMI 8402 cells, no IFN activity was detected in the culture fluid.

4. Large-scale production of IFN in RPMI 8402 cells

RPMI 8402 cells grown in a spinner vessel (1,000 ml) were washed by centrifugation at 1,000 rpm for 5 min and adjusted to a cell density of 10×10^6 cells/ml in serum-free RPMI 1640 medium. The cell suspension was put into a spinner vessel (500 ml), and inoculated with Sendai virus at a dose of 1,000 HA units/ml. The culture fluid was harvested and centrifuged at 9,000 rpm for 30 min after incubation for 24 h at 37 C in a humidified atmosphere of 5% CO₂ in air. The supernatant was collected, adjusted to pH 2.0 with 1 N HCl and stood for 3 days. Under these conditions about 2,300 IU/ml of IFN with a specific activity of 3.25×10^4 IU/mg of protein was produced.

The biological and physicochemical properties and antigenic types of the IFN produced by the RPMI 8402 (8402-IFN) were examined.

5. Kinetics of development of the antiviral state by IFNs

The antiviral state was induced in FL cells 1 h after the treatment with 8402-IFN, and 6 h later a completely antiviral state developed. This state continued for more than 24 h. There was no difference in the kinetics of de-

velopment of the antiviral state with Hu IFN- α , Hu IFN- β and 8402-IFN.

6. *Species specificity of the antiviral activity of IFN*

Hu IFN- α (Ly), Hu IFN- β and 8402-IFN were assayed on FL cells, L₉₂₉ cells, MDBK cells and RK-13 cells. As shown in Table 2, Hu IFN- α , Hu IFN- β and 8402-IFN caused low antiviral activity in L₉₂₉ cells and RK-13 cells. There was no significant difference in the sensitivities of the cells to Hu IFN- α , Hu IFN- β and 8402-IFN. On the other hand, MDBK cells were sensitive to Hu IFN- α , but

TABLE 1. *Species specificity in the antiviral activity of IFN^a*

Expt.	cell	8402-IFN	Hu IFN- α (Le)	Hu IFN- β
1	FL	1,427	1,200	1,200
	MDBK	106	1,200	45
2	FL	570	900	900
	RK-13	29	57	36
	L ₉₂₉	28	180	113

^a FL cells, MDBK cells, L₉₂₉ cells and RK-13 cells were treated with 8402-IFN, Hu IFN- α (Le) and Hu IFN- β for 24 h, and infected with VSV.

After 48 h, CPE was judged and IFN activity was determined.

not to Hu IFN- β or 8402-IFN, suggesting that 8402-IFN may be similar to Hu IFN- α , but not to Hu IFN- β (Table 1).

7. *Neutralization of IFNs by anti-IFN sera*

Serially 2-fold diluted anti-Hu IFN- β serum was mixed with serially 2-fold diluted IFNs and incubated at 37 C for 1 h. Then the mixtures were transferred to cultures of FL cells in microplate wells. After 24 h the cells were inoculated with VSV. CPE was observed after 48 h incubation at 37 C. Results showed that Hu IFN- β and 8402-IFN were neutralized by anti-Hu IFN- β serum, but Hu IFN- α was not (Fig. 2a). In similar experiments with anti-Hu IFN- α serum, the activity of Hu IFN- α was neutralized, but those of Hu IFN- β and 8402-IFN were not (Fig. 2b). These results indicate that the IFN produced in RPMI 8402 cells is antigenically identical to Hu IFN- β .

8. *Stability to pH 2 and heat-treatment*

Hu IFN- α and Hu IFN- β are stable at pH 2. The activity of 8402-IFN was also stable at pH 2 (Table 2). When 8402-IFN was heated at 60 C for 30 min, it was partially inactivated, like the activities of Hu IFN- α and Hu IFN- β . All these IFNs were inactivated by heat treatment at 80 C for 30 min. The stabilities of IFN on heat-treatment at 100 C for 2 min in the presence of 1% SDS with or

2a

	8402-IFN						Hu IFN- β						Hu IFN- α Le					
	$\times 1$	$\times 2$	$\times 4$	$\times 8$	$\times 16$	IFN (-)	$\times 1$	$\times 2$	$\times 4$	$\times 8$	$\times 16$		$\times 1$	$\times 2$	$\times 4$	$\times 8$	$\times 16$	
$\times 2$	+	+	+	+	+	$\times 2$	+	+	+	+	$\times 2$	-	-	-	\pm	-		
$\times 4$	+	+	+	+	+	$\times 2$	+	+	+	+	$\times 4$	-	-	-	\pm	-		
$\times 8$	-	\pm	+	+	+	$\times 8$	+	+	+	+	$\times 8$	-	-	-	-	-		
$\times 16$	-	-	+	+	+	$\times 16$	-	\pm	+	+	$\times 16$	-	-	-	\pm	-		
$\times 32$	-	-	\pm	+	+	$\times 32$	-	-	+	+	$\times 32$	-	-	-	-	\pm		
$\times 64$	-	-	\pm	+	+	$\times 64$	-	-	-	+	$\times 64$	-	-	-	\pm	\pm		
$\times 128$	-	-	-	+	+	$\times 128$	-	-	-	+	$\times 128$	-	-	-	-	-		
anti IFN (-)	-	-	-	+	+	anti IFN (-)	-	-	-	-	anti IFN (-)	-	-	-	-	\pm		

	8402-IFN							Hu IFN- β					Hu IFN- α Le					
	$\times 1$	$\times 2$	$\times 4$	$\times 8$	$\times 16$	IFN (-)		$\times 1$	$\times 2$	$\times 4$	$\times 8$	$\times 16$	$\times 1$	$\times 2$	$\times 4$	$\times 8$	$\times 16$	
X2	-	-	+	+	+	+	$\times 2$	-	-	-	-	-	$\times 2$	+	+	+	+	+
X4	-	-	+	+	+	+	$\times 4$	-	-	-	\pm	\pm	$\times 4$	+	+	+	+	+
X8	-	-	+	+	+	+	$\times 8$	-	-	-	\pm	+	$\times 8$	+	+	+	+	+
X16	-	-	+	+	+	+	$\times 16$	-	-	-	+	+	$\times 16$	+	+	+	+	+
X32	-	\pm	+	+	+	+	$\times 32$	-	-	-	\pm	\pm	$\times 32$	-	\pm	\pm	+	+
X64	-	-	+	+	+	+	$\times 64$	-	-	-	+	-	$\times 64$	-	-	+	+	+
X128	-	-	+	+	+	+	$\times 128$	-	-	-	+	-	$\times 128$	-	-	-	+	+
anti IFN (-)	-	-	+	+	+	+	anti IFN (-)	-	-	-	+	+	anti IFN (-)	-	-	-	+	+

FIGURE 2. Neutralization of IFNs by anti-IFN sera.

Serially 2-fold diluted anti-Hu IFN- β serum (Fig. 2a) or anti-Hu IFN- α serum (Fig. 2b) was mixed with serially 2-fold diluted Hu IFN- α or Hu IFN- β and incubated at 37 C for 1 h, and then the mixture was transferred to wells containing FL cells. Twenty-four hours later the cells were inoculated with VSV. CPE was examined after incubation at 37 C for 48 h.

+: CPE in two wells of the microplate, -: No CPE in two wells of the microplate, \pm : CPE in one well of the microplate.

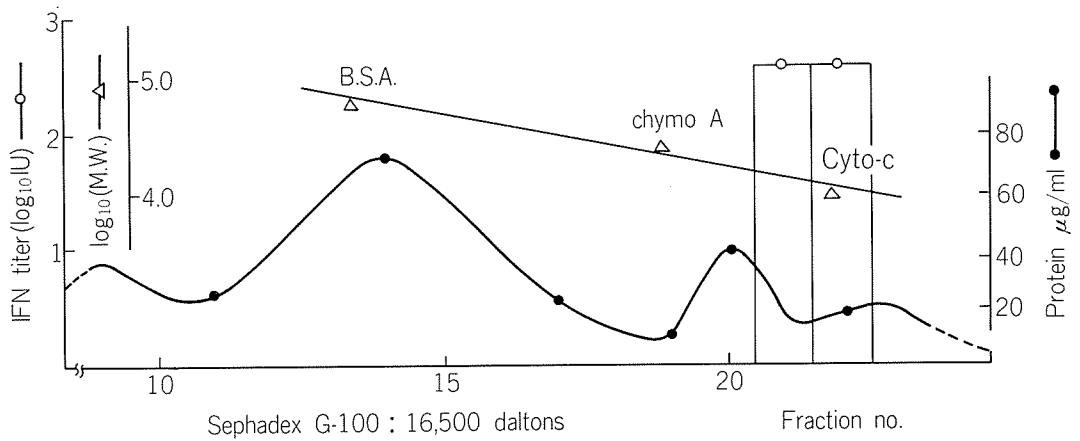


FIGURE 3. Determination of molecular weight of 8402-IFN by Sephadex G-100 gel filtration.

The 8402-IFN preparation was concentrated by precipitation with 80% saturated ammonium sulphate, loaded onto a Sephadex G-100 column and eluted with phosphate buffered saline containing 1 M NaCl. Marker proteins (blue dextran, bovine serum albumin, chymotrypsinogen A and cytochrome c) were loaded onto the same column.

- B.S.A. : bovine serum albumin M.W. 67,000
- chymo A: chymotrypsinogen A M.W. 25,000
- cyto-c : cytochrome-c M.W. 12,384

TABLE 2. *Stability of IFN to heat and acid*^a

	8402-IFN		Hu IFN- α (Le)		Hu IFN- β	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
untreated control	600	1200	1200	1200	— ^b	570
60 C 30 min	300	180	510	210	—	210
80 C 30 min	n.d. ^c	n.d.	n.d.	n.d.	—	n.d.
pH 2	1400	860	1000	390	—	420

^a Hu IFN- α , Hu IFN- β and 8402-IFN were heated at 60 C or at 80 C for 30 min, or acidified to pH 2 with 1N HCl. These heated or acidified IFNs were assayed for antiviral activity.

^b —: not done.

^c n.d.: not detected.

TABLE 3. *Stability of IFN activity to heat in the presence of SDS with or without reducing agents*^a

Treatment			Residual IFN activity (%)		
1% SDS	2ME-urea	heat-ing	8402-IFN	Hu IFN- α	Hu IFN- β
—	—	—	100	100	100
—	—	+	<1	17	<1
+	—	+	18	92	74
+	+	+	8	44	19

^a 8402-IFN, Hu IFN- α and Hu IFN- β were heated at 100 C for 2 min in the absence or presence of SDS with or without 2-mercaptoethanol and urea. Then residual IFN activity was determined.

without 1% 2-mercaptoethanol and 5 M urea were examined. All these IFNs were inactivated by heating at 100 C for 2 min in the absence of SDS. When dissolved in 1% SDS solution, the activities of Hu IFN- α and Hu IFN- β were maintained during heat-treatment at 100 C for 2 min, but that of 8402-IFN was not. About 50% of the activity of Hu IFN- α was maintained during heat-treatment at 100 C for 2 min in the presence of SDS with 2 ME and urea, but most of the activities of Hu IFN- β and 8402-IFN were lost (Table 3).

9. *Molecular weight of 8402-IFN*

8402-IFN was concentrated by precipitation

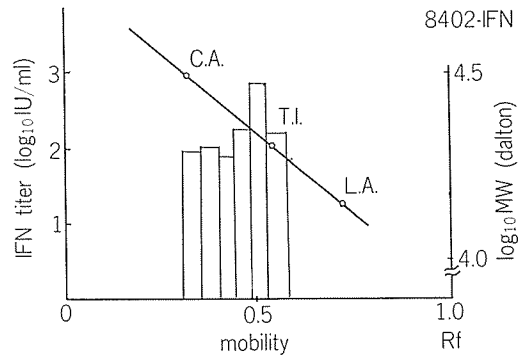


FIGURE 4. Determination of molecular weight of 8402-IFN by SDS-PAGE.

Concentrated 8402-IFN and marker proteins were charged onto polyacrylamide gel containing 0.1% SDS with bromophenol blue and subjected to electrophoresis at 20 mA for 1 h and then at 40 mA for 4 to 5 h.

The polyacrylamide gel was stained with Coomassie brilliant blue and the molecular weight was determined by comparison of the relative mobilities (Rf) of IFN and marker proteins. Rf is defined as:

$$Rf = \frac{\text{distance migrated from origin}}{\text{distance from origin to reference point}}$$

C.A.; Carbonic Anhydrase (M.W. 30,000)

T.I.; Trypsin Inhibitor (M.W. 20,000)

L.A.; d-Lactalbumin (M.W. 14,400)

SDS-PAGE analysis of concentrated IFN:

21,000 daltons

with 80% saturation of ammonium sulphate, loaded onto a Sephadex G-100 column and eluted with phosphate buffered saline contain-

ing 1 M NaCl. As shown in Fig. 3, the activity was eluted in a position corresponding to a molecular weight of about 16,500 daltons (Fig. 3).

The molecular weight of the IFN was also determined by SDS-PAGE. Concentrated 8402-IFN was charged onto polyacrylamide gel containing 0.1% SDS and subjected to electrophoresis with marker proteins. From its mobility, its molecular weight was estimated to be 21,000 daltons (Fig. 4).

DISCUSSION

Human peripheral leukocytes (probably B lymphocytes) infected with Sendai virus produce mainly IFN- α , less than 1% of the total IFN molecule being Hu IFN- β (Havell, 1980). Namalva cells (a B cell line) produce about 62% Hu IFN- α and 13% Hu IFN- β (Havell et al., 1977), but another B cell line (BALL-1 cells) produces only one antigenic type (α) of IFN (Imanishi et al., 1980). Thus, normal lymphocytes or B cell lines produce chiefly IFN- α .

The present study showed that a human T lymphoblastoid cell line also produced IFN when induced with Sendai virus, and that this IFN (8402-IFN) was antigenically identical with Hu IFN- β . This IFN was produced from 9 h after inoculation of virus, increased until 24 h after virus inoculation and then decreased, just as IFN production by Namalva cells and BALL-1 cells. Moreover, the highest titer of IFN was produced by inoculating 2,000 HA units/ml of virus onto RPMI 8402 cells, as in the cases with Namalva and BALL-1 cells.

The production of human fibroblast IFN (Hu IFN- β) is enhanced by treating the fibroblasts with antimetabolites (actinomycin D and cycloheximide) (Havell and Vilcek., 1972) or by irradiating them with ultraviolet light (Maehara et al., 1980). However, we could not detect superinduction of IFN on treatment of the RPMI 8402 cells with antimetabolites or ultraviolet light (data not shown). Tovey et al.

(1977) reported that treatment of Namalva cells with BUdR before IFN induction enhanced IFN production. Pretreatment of RPMI 8402 cells with BUdR did not enhance IFN production (data not shown). Thus, except for the absence of superinduction, the conditions of IFN production by RPMI 8402 cells were similar to those in B cell lines and fibroblasts.

The biological properties of 8402-IFN were examined. 8402-IFN, like Hu IFN- α and Hu IFN- β , induced a completely antiviral state in FL cells within 6 h. These IFNs showed species specific effects. RK-13 cell and L₉₂₉ cells were not very sensitive to any of the three IFNs. MDBK cells were reported to be very sensitive to Hu IFN- α , but not to Hu IFN- β (Gresser et al., 1974). Our results showed that MDBK cells were very sensitive to Hu IFN- α , but not to 8402-IFN or Hu IFN- β . This means that 8402-IFN resembles Hu IFN- β in chemical structure.

IFNs have many common physicochemical properties. The stability of 8402-IFN to pH 2 and to heat were the same as those of Hu IFN- α and Hu IFN- β , although in SDS solution 8402-IFN was inactivated by heating at 100 C for 2 min, while Hu IFN- α and Hu IFN- β were not. The molecular weight of 8402-IFN was found to be 16,500 to 21,000 daltons. The molecular weights of Hu IFN- α and Hu IFN- β also range from 15,000 to 21,000 daltons. Thus, the IFN produced in human T lymphoblastoid cells was similar to human fibroblast IFN in many biological and physicochemical aspects. Our results are consistent with the findings of Larsson et al. (1979) that the CCRF-CEM cell line of human T lymphoblastoid cells produced IFN that was antigenically identical to Hu IFN- β .

Recently, the base sequences of the Hu IFN- α and Hu IFN- β genes were determined by gene recombination (Taniguchi et al., 1980). It will be possible to determine the difference between human T lymphoblastoid IFN and human fibroblast IFN in detail by techniques of genetic engineering.

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