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FACTOR DETERMINING THE ANTIGENIC TYPE OF INTERFERONS PRODUCED IN HUMAN LYMPHOBLASTOID CELL LINES

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SUMMARY The factor that determines the antigenic type of IFN produced in human lymphoblastoid cell lines was examined using live Sendai virus, ultra-violet (UV)-irradiated virus, HANA spikes exposed on L cells persistently infected with Sendai virus (L-HVJ) and poly-inosinic acid poly-cytidylic acid (poly I: C). When Sendai virus was irradiated with UV-light for 300 sec, its abilities to infect chicken eggs and induce IFN were diminished, but its HA activity was unaffected. HANA spikes exposed on L-HVJ could not induce IFN in human lymphoblastoid cell lines, although they induced IFN in mouse spleen cells. These results suggest that the induction of IFN in human lymphoblastoid cells is closely related to viral nucleic acid. Poly I: C also induced IFN in some human lymphoblastoid cell lines in which IFN production is induced by Sendai virus. The antigenic types of IFN induced by poly I: C were the same as those induced by Sendai virus. These results suggest that the antigenic type of IFN produced depends on the nature of the IFN producer cells rather than on the kind of IFN inducer.

INTRODUCTION

Virus-induced interferons are of two antigenic types, α and β (Havell and Vilček, 1972). The α type is generally produced in lymphoid cells infected by virus (Cantell et al., 1981), while the β type is mainly produced in fibroblasts treated with poly I: C (Cantell and Hirvonen, 1978). We studied the production of IFNs by various human lymphoblastoid cell lines and determined their antigenic types. Results showed that all the human T lymphoblastoid cell lines used produced only HuIFN-β, whereas human non-T and non-B lymphoblastoid cell lines produced HuIFN-α and HuIFN-β, but that of the three kinds of human B lymphoblastoid cell lines, the first produced only HuIFN-α, the second, only HuIFN-β and the third, HuIFN-α and HuIFN-β (Imanishi et al., 1982). It seemed interesting to examine the factor(s) that determined the antigenic type of IFN produced in human lymphoblastoid cell lines. Previously (Imanishi et al., 1982), we found no correlation between the antigenic type of

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IFN produced and various cell surface markers of human lymphoblastoid cells.

In this study, using several different inducers, we examined whether the antigenic types of IFNs are determined by the kind of inducer or by the nature of the producer cells.

MATERIALS AND METHODS

1. *Cells*

For studies on induction of IFN, the following cell lines were used: RPMI 8402 and CCRF-CEM cells established from patients with acute lymphoblastic leukemia as T cell lines; B35M and DG-75, Burkitt's lymphoma, BALL-1, acute lymphoblastic leukemia, ARH-77, multiple myeloma and RPMI 6410, normal B lymphoblastoid cells as B cell lines; NALM-16, acute lymphoblastic leukemia cells as a non-T, non-B cell line. The characteristics of these cell lines were described in detail elsewhere (Imanishi et al., 1982). These cells were grown and maintained in RPMI 1640 medium supplemented with 10% mixed fetal bovine serum (FBS) and calf serum (CS) and 100 U/ml penicillin. Human amnion FL cells grown and maintained in Eagle's minimum essential medium (MEM) supplemented with 5% CS were used for IFN assay and propagation of vesicular stomatitis virus (VSV).

Murine L cells grown and maintained in the same medium as FL cells were used as IFN inducer cells persistently infected with Sendai virus.

2. *Viruses*

The Nagoya and Nagata strains of Sendai virus, kindly provided by Dr. Y. Ito, National Institute of Health, Tokyo, Japan, were propagated in 10-day-old embryonated chicken eggs at 37 C and 32 C, respectively. The Nagoya strain was used for IFN induction. The Nagata strain, temperature-sensitive mutant (Nagata et al., 1972), was used for persistent infection of L cells.

The New Jersey strain of VSV was propagated in FL cells and used as challenge virus in IFN assays.

3. *Interferons*

Human leukocyte IFN (HuIFN-α(Le)) was prepared in human peripheral blood leukocytes infected with Sendai virus and partially purified by the method of Cantell and Hirvonen (1978). Its specific activity was more than 10^6 IU/mg protein.

Human fibroblast interferon (HuIFN-β), kindly provided by Dr. M. Nobuhara, Research Laboratory of Cell Science, Mochida Pharmaceutical Co., Tokyo, Japan, was produced in human fibroblasts treated with poly-inosinic acid poly-cytidylic acid (poly I: C) and partially purified by the method of Carter and Horoszewicz (1980). Its specific activity was 3×10^7 IU/mg protein.

4. *Anti-interferon sera*

Anti-HuIFN-α serum was obtained from rabbits immunized with HuIFN-α produced in BALL-1 cells. At 320-fold dilution, this antiserum neutralized 500 IU of HuIFN-α completely.

Anti-HuIFN-β serum was kindly supplied by Dr. S. Kobayashi, Basic Research Laboratories, Toray Industries, Inc., Kamakura, Japan. At 1280-fold dilution, this antiserum neutralized 500 IU of HuIFN-β completely. These antisera were stored at -80 C.

5. *IFN induction by Sendai virus and ultraviolet-irradiated Sendai virus*

Human lymphoblastoid cells were suspended in RPMI 1640 supplemented with 5% FBS at a density of 1×10^6 cells/ml and infected with 500 HA/ml of original activity. The culture fluid was harvested after incubation for 24 h at 37 C in a humidified atmosphere of 5% CO_2 in air and irradiated with UV-light for 2 h to inactivate the residual inducing virus.

6. *IFN induction by poly I: C*

Poly I: C was purchased from Yamasa Shoyu Co., Choshi, Japan. Human lymphoblastoid cells were adjusted to a density of 1 or 5×10^6 cells/ml and treated with 100 μg/ml of poly I: C in the presence of 100 μg/ml of DEAE-dextran at 37 C for 4 h. The cells were washed with serum-free RPMI 1640 and incubated in RPMI 1640 supplemented with 5% FBS at 37 C. The supernatant was harvested 24 h later.

7. *Establishment of murine L cells persistently infected with Sendai virus*

L cells were infected with the Nagata strain of Sendai virus at 100 multiplicity of infection (moi) at 37 C for 2 h by the method of Nagata et al. (1972). The Sendai virus-adsorbed L cells were thoroughly washed with physiological saline, and

Eagle's MEM containing 1% CS was added to the L cell culture. The L cells persistently infected with Sendai virus (L-HVJ) were subcultured in Eagle's MEM containing 5% CS 24 h after incubation at 37 C.

8. *IFN assay*

IFN activity was assayed by a modification of the dye-uptake method described by Imanishi et al. using FL cells and VSV (1981). FL cells (6×10^4 cells) were added to wells of a microplate containing serial two-fold dilutions of IFN and incubated at 37 C in a humidified atmosphere of 5% CO_2 in air for 18 h. Then 10 to 50 $TCID_{50}$ of VSV was added to each well. After incubation at 37 C for 18 h, the cells were stained with 0.05% neutral red solution at 37 C for 2 h, and then washed with saline. The dye taken up by living cells was eluted with 50% ethanol in 0.05 *M* sodium phosphate solution, and the optical density of each well was measured in a Titertek Multiskan (Flow Laboratories, USA) at 546 nm. The reciprocal of the IFN concentration that reduced the optical density by 50% was expressed as laboratory IFN units, and values were calibrated against those with an international reference preparation (code 16/69) and expressed in terms of international units (IU).

9. *Determination of antigenic types of IFN*

Antigenic types of IFNs were determined by neutralization of IFN with anti-HuIFN-α and HuIFN-β. The neutralization method was based on direct antiviral assay (Havell and Vilček. 1972) using FL cells and VSV as challenge virus. Namely, 500 IU/ml of IFN was mixed with anti-HuIFN-α or -β serum, which could completely neutralize HuIFN-α or -β at a ratio of 1: 1. The mixtures were incubated at 37 C for 1 h, and then residual IFN activity was assayed as described above.

10. *UV-irradiation*

The Nagoya strain of Sendai virus was partially purified by centrifugation at 8,000 rpm for 2 h and the pellet was resuspended in PBS(-). A sample of 2 ml of virus solution (5,000 HA/ml) was placed in a 60-mm plastic dish and irradiated from a 15 W germicidal lamp (Toshiba Electric Co., Tokyo) at a distance of 13.5 cm from the UV-light source.

RESULTS

1. *IFN induction by UV-irradiated Sendai virus*

UV-irradiated Sendai virus was reported to have decreased infectivity, but to maintain HA activity and induce IFN in mouse spleen cells (Ito et al., 1974; Ito et al., 1978). These results suggest that not only viral nucleic acid but also HA spikes on the Sendai virus have ability to induce IFN. To examine the possibility that IFN induced by viral nucleic acid is different from that induced by HA

TABLE 1. *IFN induction in human lymphoblastoid cells by UV-irradiated Sendai virus*

	IFN activity (IU/ml)					
	Time of UV-irradiation (sec)					
	0	15	60	300	1,800	3,600
BALL-1	1,408[b]	1,791	482	ND[c]	ND	ND
RPMI 6410	887	591	132	ND	ND	ND
CCRF-CEM	745	430	51	ND	ND	ND
RPMI 8402	2,018	1,727	1,402	ND	ND	ND
HA	320	320	320	320	ND	ND
Infectivity[a] to eggs	+	+	+	−	−	−

[a] UV-irradiated virus was inoculated into 10-day-old chicken embryonated eggs and incubated at 37 C for 3 days. The chorioallantoic fluid was collected and its HA activity was determined. + and −, presence and absence of HA activity.

[b] Values are average IFN titers in 3 separate experiments.

[c] Not detectable.

spikes, we examined the induction of IFN by UV-irradiated Sendai virus in human lymphoblastoid cell lines.

Partially purified Sendai virus was UV-irradiated for 15 to 300 sec and then its induction of IFN in the human lymphoblastoid cell lines BALL-1 cell, RPMI 6410 cell, CCRF-CEM cell and RPMI 8402 cell were examined. Sendai virus irradiated with UV-light for 15 and 60 sec induced IFN in all these cell lines, although its inductive effect decreased with increase in the dose of UV-irradiation. After irradiation for more than 300 sec, Sendai virus induced no IFN. Furthermore, decrease in IFN induction was parallel with decrease in infectivity by Sendai virus (Table 1). However, the HA activity of the Sendai virus was maintained for the first 300 sec of irradiation. These results suggest that the nucleic acid was closely related to the induction of IFN in human lymphoblastoid cell lines, whereas the surface glycoprotein of Sendai virus had no ability to induce IFN. Therefore, the antigenic type of IFN produced in human lymphoblastoid cell lines did not seem to be determined by the kind of inducer.

2. *IFN induction in L cells persistently infected with Sendai virus*

L-HVJ was dispersed into the wells of a multiwell plate and incubated at 37 C for 2 days, and then L-HVJ was added and incubation was continued at 32 C for 24 h. The existence of HANA spikes on the surface of L-HVJ was confirmed by a hemadsorption test. Inocula of 10^6 human lymphoblastoid cells were added to the L-HVJ monolayer cultures and incubation was continued at 37 C for 18 h, and then the culture fluid was harvested for IFN assay. As positive controls, mouse spleen cells were cocultured with L cells or L-HVJ cells, instead of human lymphoblastoid cells, because Ito et al. (1978) reported that L-HVJ induced IFN in mouse spleen cells. The results showed that L-HVJ could not induce IFN in any of the human lymphoblastoid cell lines, but it induced murine IFN in mouse spleen cells (Table 2). Only low IFN activity was detected when L cells were cocultured with spleen cells. These results suggested that HANA spikes did not induce IFN in human lymphoblastoid cell lines, but that they could induce IFN in mouse spleen cells, because higher IFN was produced by cocultivation of spleen cells with L-HVJ cells than with L cells. In this experiment, we could not find a factor that determines the antigenic type of IFN produced in human lymphoblastoid cell lines.

TABLE 2. *IFN induction in human lymphoblastoid cells cocultured with L cells persistently infected with Sendai virus*[a]

Cell line	IFN activity (IU/ml)
RPMI 8402	ND[c]
BALL-1	ND
DG-75	ND
ARH-77	ND
RPMI 6410	ND
NALM-16	ND
C3H mouse spleen cells[b]	460
C3H mouse spleen cells + L cells[b]	14

[a] Human lymphoblastoid cells (1×10^6 cells/ml) were cocultured with L cells persistently infected with Sendai virus at 37 C overnight. Culture fluid was assayed for IFN activity using FL cells and VSV.

[b] C3H mouse spleen cells (1×10^6 cells/ml) were cocultured with L cells persistently infected with Sendai virus or L cell monolayers, at 37 C overnight, as a positive control. Culture fluid was assayed for IFN activity using L cells and VSV.

[c] Not detectable.

3. *IFN induction of poly I: C*

The above results suggested that the viral component causing IFN induction in human lymphoblastoid cells may be viral RNA, but nor viral glycoproteins or HANA spikes, and that the same viral components may be essential for induction of HuIFN-α and HuIFN-

TABLE 3. *IFN induction in human lymphoblastoid cell lines by poly I: C with DEAE-dextran*[a]

Cell line	IFN activity (IU/ml)	
	1×10^6 cells/ml	5×10^6 cells/ml
NALM-16	319	252
ARH-77	112	35
BALL-1	68	NT[c]
B35M	ND[b]	ND
DG-75	ND	ND
RPMI 6410	111	511
CCRF-CEM	ND	NT
RPMI 8402	ND	NT

[a] Human lymphoblastoid cells were adjusted to a density of 1 or 5×10^6 cells/ml, and treated with 100 μg/ml of poly I: C and DEAE-dextran for 4 h. The cells were then washed and incubated in RPMI 1640 containing 5% FBS. The culture fluid was harvested 24 h later.

[b] Not detectable.

[c] Not tested.

β in these cells. To determine whether both HuIFN-α and HuIFN-β can be produced in response to an inducing agent other than the viral component, we examined IFN production by poly I: C in these cells. Induction of IFN in human lymphoblastoid cells by poly I: C was considerably less than that by Sendai virus. Human lymphoblastoid cells, which produced IFN on induction with Sendai virus, did not always produce IFN on induction with poly I: C; e.g., human T lymphoblastoid cells, RPMI 8402 and CCRF-CEM cells, did not produce IFN on induction with poly I: C at all, although they produced IFN on induction with Sendai virus. However, some human B lymphoblastoid cells and one non-T, non-B lymphoblastoid cell line produced low activity of IFN with poly I: C, as shown in Table 3. These results indicate that not only the nature of the producer cells, but also the kind of IFN inducer determines whether IFN is produced in human lymphoblastoid cell lines.

4. *Antigenic types of IFN induced by poly I: C*

The antigenic types of IFN induced by poly I: C in various lymphoblastoid cell lines, determined by the neutralization test as de-

TABLE 4. *Antigenic types of IFN induced by poly I: C*[a]

Cell line	Original IFN activity (IU/ml)	Activity of poly I: C-induced IFN after neutralization with antisera (IU/ml)			Antigenic type	Antigenic type of IFN induced by Sendai virus
		anti-IFN-α	anti-IFN-β	anti-IFN-α +anti-IFN-β		
NALM-16	306	46	229	ND	α, β	α, β
ARH-77 (conc.)[c]	284	105	336	ND	α, β	α, β
RPMI 6410	383	ND[b]	180	ND	α	α
BALL-1 (conc.)	292	ND	171	ND	α	α
Human leukocytes	320	ND	258	ND	α	α
Human fibroblasts	548	720	ND	ND	β	β

[a] Human lymphoblastoid IFN was mixed with anti-HuIFN-α or -β serum and incubated at 37 C for 1 h. The residual activity of IFN was assayed by microassay as described in the text.

[b] Not detectable.

[c] Human lymphoblastoid IFN was concentrated in a collodion bag when its activity was low.

scribed in Materials and Methods were compared with those induced with Sendai virus. The results are summarized in Table 4. ARH-77 and NALM-16 cells, which produced HuIFN-α and HuIFN-β on induction with Sendai virus, also produced these types of IFN on induction with poly I: C. BALL-1 and RPMI 6410 cells, which produced only HuIFN-α on induction with Sendai virus, also produced only HuIFN-α with poly I: C. These data indicate that both HuIFN-α and HuIFN-β can be induced with poly I: C, and that the antigenic types of IFN induced by poly I: C are the same as those induced by Sendai virus. Thus the nature of the producer cells, rather than the kind of IFN inducer, seems to determine the antigenic type of IFN produced by human lymphoblastoid cells.

DISCUSSION

There have been no studies on factors that determine the antigenic type of IFN produced, but Havell et al. reported that the antigenic type of IFN produced by human fibroblasts seems to depend on the nature of the IFN inducer (Havell et al., 1978). Thus it is possible that a certain component of virus induced one antigenic type of IFN and another component induced another antigenic type. On the basis of this hypothesis we examined whether HANA spikes or nucleic acids of Sendai virus determine the antigenic type of IFN produced in human lymphoblastoid cell lines. Results showed that after UV-irradiation, Sendai virus showed decreased ability to induce IFN when its infectivity of chicken eggs was lost while its HA activity was still maintained, and that HANA spikes exposed on mouse L cells persistently infected with Sendai virus could not induce IFN in human lymphoblastoid cell lines. Thus only viral nucleic acid is related to IFN induction in human lymphoblastoid cells, and other viral components are not. These data do not show what determines the antigenic type of IFN produced in human lymphoblastoid cell lines. Therefore, we used poly I: C for IFN induction in human lymphoblastoid cells instead of Sendai virus, because the IFN induced in human fibroblasts by poly I: C is different from that induced by paramyxoviruses including Sendai virus and Newcastle disease virus (Mozes and Vilček, 1978). The human lymphoblastoid cells that produced IFN on induction with poly I: C were not the same as those that produced IFN on induction with Sendai virus. However, the antigenic types of IFN induced by poly I: C were the same as those induced by Sendai virus in human lymphoblastoid cell lines. This means that the nature of the IFN producer cells was more closely related to determination of the antigenic types of IFN produced in human lymphoblastoid cell lines than the kind of IFN inducer.

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