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PRODUCTION OF α-INTERFERON BY HUMAN LEUKOCYTES

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Summary. — We examined the capacity of human leukocytes to produce α-interferon subtypes following viral induction. It was shown that the β-propiolacton inactivated viral inductor stimulated the production of a complete set of native α-interferon subtypes. Acid treatment of native interferon at pH 2.0 inactivated the acid-labile portion of α-interferon. Its exposure to 37 °C for 5 days and repeated acid treatment at pH 2.0 resulted in additional inactivation of some interferon pool fractions. The acid-labile subtypes of human α-interferon were formed in a nonadherent mononuclear cell fraction.

Key words: *acid-labile interferon*; *acid-stable interferon*; *β-propiolacton*; *influenza virus*

Introduction

At present over 10 subtypes of human α-interferon (IFN) are known which are coded by appropriate genes (Khesin, 1984). These IFNs differ in a number of properties: antiviral activity in homologous and heterologous cultures, antiproliferative and immunomodulating activity (Rehberg *et al.*, 1982; Ďuk and Feldmane, 1985). It is difficult to isolate the acid labile subtypes of native IFN because treatment of virus inductor at pH 2.0 caused inactivation of these IFN subtypes. As β-propiolaction (BPL) is used for virus inactivation for vaccine production, and because BPL does not alter the antiviral activity of mouse α-, β-, γ-IFNs (Barrett *et al.*, 1984), we followed the capacity of human leukocytes to produce different subtypes of native α-IFN using BPL- and acid-inactivation of viral inductor.

Materials and Methods

Viruses. The following viruses were used: influenza virus (IV), WSN strain, Sendai virus (SV), Newcastle disease virus (NDV) strain H, vesicular stomatitis virus (VSV), Indiana strain, and mouse encephalomyocarditis virus (MEMV). All viruses except of the latter were obtained from the allantoic fluid. MEMV was passaged in ascitic carcinoma (Krebs cell) culture.

Cells. Human diploid fibroblast (HDF) cells were passaged in Eagle's medium supplemented with 10% bovine serum, 300 μg/ml glutamine and 50 μg/ml gentamycin. The continuous line of bovine kidney cells was passaged in the double concentrated Eagle's medium supplemented mented with 10% bovine serum, 600 μg/ml glutamine and 50 μg/ml gentamycin.

Obtaining of human leukocytes. Donor blood was treated with 5-fold volume of cooled 0.83% solution of ammonium chloride for 5 min; after 10 min centrifuging at 1000 rev/min the sediment was resuspended in double Eagle's medium supplemented with 5% foetal bovine serum, 600 μg/ml glutamine and 50 μg/ml gentamycin. The cell concentration was adjusted to 5×10^6 in 1 ml and used for IFN induction.

IFN production. Following the exposure of leukocyte suspension to virus inductor at 37 °C the culture fluid was treated with BPL at 4 °C for 24 hr. The rest of the culture fluid was treated with 1 N HCl, pH 2.0 for 7 days, thereafter pH was adjusted to neutral value by 1 N NaOH. BPL-treated IFN-containing material was designated as acid-labile IFN (ALI), while the acid-treated IFN-containing material — as acid-stable IFN (ASI).

IFN testing. IFN was titrated by a micromethod using 96-well plates. Cell suspension was seeded as follows: 1×10^5 cells/well for HDF and 1×10^4 cells/well for MDBK. After the monolayer was formed on day 1 and 3 (MDBK and HDF, respectively), the medium was removed and 2-fold dilutions of samples tested in double Eagle's medium supplemented with 50 μg/ml gentamycin were introduced into the wells. Following 24 hr exposure at 37 °C 100 $TCID_{50}$ of viral inductor (MEMV for HDF and VCV for MDBK) was added into the wells. As IFN titre was taken the last dilution inhibiting the CPE by 50%.

IFN typing. IFN under study (0.1 ml) was mixed with 0.1 ml of antiserum to leukocyte or immune human IFN. After 1 hr contact at 37 °C the residual antiviral activity was evaluated. Human antisera to IFN and reference preparations of human IFNs were obtained through the courtesy of Dr. V. I. Iovlev, L. Pasteur Institute of Epidemiology and Microbiology, Leningrad.

Fractionation of donor leukocytes. Donor mononuclear cells were isolated by the method of Boyum (1968) and adsorbed on plastic Petri dishes 15 cm in diameter for 2 hr as follows: 90×10^6 cells in 10 ml of Eagle's double medium supplemented with 10% of foetal bovine serum, 600 μg/ml glutamine and 50 μg/ml gentamycin. Non-adherent cells were centrifuged at 1000 rev/min for 10 min while adherent cells were mechanically removed, placed into warm medium 199 and centrifuged using the same regime. The original cell population and both fractions were adjusted to the concentration of 3×10^6 per ml; IFN production was induced and culture fluid was treated as described above.

Results

In the course of the first experiments the dilution of BPL was determined which would not inactivate the antiviral activity of human α- and γ-IFNs (Table 1). α-IFN proved to be more resistant to BPL treatment than γ-IFN. At the dilution of 1 : 4000 BPL did not inactivate both types of IFN, and in further experiments only that dilution of BPL was used. Further on, we followed the effect of BPL treatment on the infectivity of IV, SV, and NDV and their capacity to cause interference in cell culture used for IFN testing (Table 2). BPL was shown to inhibit completely the infectivity of

Table 1. The effect of β-propiolacton on interferon activity

Interferon	β-propiolacton dilution				Control
	1 : 100	1 : 1000	1 : 4000	1 : 10000	
α	<2*	128	128	128	128
γ	<2	8	128	128	128

* IFN titre (units/ml).

Table 2. The effect of β-propiolacton on viral infectivity and the capacity to cause interference

Virus	Treatment	Infectivity log EID_{50}	Interference with the virus indicator in cultures	
			HDF	MDBK
Influenza	−	9	±*	−
	+	0	−	−
Sendai	−	9.5	+	−
	+	0	±	−
Newcastle disease	−	9.5	+	+
	+	0	+	+

* Footnote. + marked interference; ± insignificant; − no interference.

the viruses used. However, only BPL-treated IV did not cause interference in HDF and MDBK cultures in relation to reference virus. Inactivated SV caused weak interference in HDF but not MDBK cultures while NDV free of infectivity caused interference in HDF and MDBK cultures comparable to that by the initial infectious virus. IV and in some experiments SV only were used in further experiments.

IFN production in human leukocytes was induced by exhibiting the cell suspension to 10^7-10^9 EID_{50} of IV or SV; the samples of culture fluid were taken in the course of leukocyte cultivation (Table 3). When a higher inducing dose of IV was used, IFN was detected in the culture media 3 hr following leukocyte induction and its activity in BPL-treated material was increased 4-fold as compared to that in acid-treated material (64 and 16 units/ml, respectively). By 6 hr the activity of both IFN subtypes increased reaching its maximum by 18 hr while their correlation remained stable (512 and 128 units/ml). Following IFN induction in leukocytes with 10^7 EID_{50}

Table 3. Interferon production in human leukocytes following their induction by influenza and Sendai viruses

Virus	Virus dose, log EID_{50}	Time (hr)							
		1		3		6		18	
		BPL	pH 2.0	BPL	pH 2.0	BPL	pH 2.0	BPL	pH 2.0
Influenza	9	<2	<2	64	16	128	32	512	128
	7	<2	<2	<2	<2	16	8	64	64
Sendai	9	128	<2	128	<2	256	64	1064	128
	7	2	<2	16	<2	16	4	32	16

Footnote. BPL — BPL treatment of the material; pH 2.0 — treatment with 1 mol/ HCl at pH 2.0.

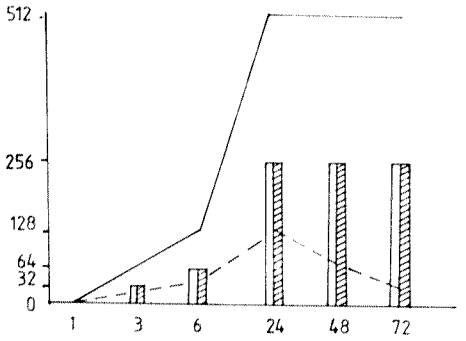

Fig. 1

Interferon production in human leukocytes following influenza virus induction
Abscissa: time after induction; Ordinate: interferon titres, units/ml.
Uninterrupted line = ALI tested in HDF; interrupted = ASI tested in HDF; white columns = ALI tested in MDBK; hatched = ASI tested in MDBK.

of IV, the antiviral activity in the culture fluid was first detected 6 hr later and was rising by 18 hr. The titre in BPL-treated material was increased two-fold as compared to that in acid-treated material only at the first testing (16 and 8 units/ml, respectively). At the second testing irrespective of the kind of culture fluid treatment the IFN titre was 64 units/ml.

Following IFN induction in human leukocytes with SV (10^9 EID_{50}) the antiviral activity was detected in BPL-treated culture fluid one hour later. Apparently it was associated with an interference caused by SV (present in the culture fluid of HDF). The antiviral activity increased 6 hr later reaching its maximum by 18 hr (256 and 1024 units/ml, respect-ively). Following acid treatment, the antiviral activity was first detected by 6 hr later increasing by 18 hr (64 and 128 units/ml, respectively). IFN level in BLP-treated material was 4—8 times higher than following acid treatment. In human leukocytes treated with 10^7 EID_{50} of SV the antiviral activity in BPL-treated culture fluid was detected within 3 hr, later on it increased up to 18 hr (16 and 32 units/ml, respectively). Following acid treatment of the culture fluid the antiviral activity was detected from 6 to 18 hr after leukocyte induction (4 and 16 units/ml, respectively). ALI was completely neutralized by human α-IFN but not γ-IFN antisera. Consequently, it was α-ALI.

Table 4. Interferon activity following 5-day incubation at 37 °C

Interferon	Initial activity	Activity after the incubation	
		without treatment	pH 2.0
ASI	512	256	16
α-reference	256	256	16
α_2-recombinant	32 000	16 000	16 000

Table 5. The capacity of human leukocyte fractions to produce interferon under the effect of influenza virus

Expt	Leukocytes		Cells			
	BPL	pH 2.0	adherent		non-adherent	
			BPL	pH 2.0	BPL	pH 2.0
1st	128	128	256	256	128	32
2nd	128	64	128	64	256	32

In experiments described below IV was used as a viral inductor. Fig. 1 depicts the data of one of the experiments in which ALI and ASI production was followed from 3 to 72 hr post induction of human leukocytes. ALI production reached its maximum within 24 hr. During subsequent leukocyte cultivation its activity remained unchanged (512 units/ml). ASI production also reached its maximum within 24 hr, however, by 72 hr its activity decreased (128 and 32 units/ml, respectively). While testing ALI and ASI in MDBK culture the antiviral activity was first detected 3 hr later, the titres being the highest in the culture fluid following 24 hr after leukocyte induction showing no alteration during subsequent leukocyte cultivation (256 units/ml). ALI and ASI titres proved equal in the samples tested at the same term: lower than ALI titres in HDF culture but higher than ASI titres in the same culture.

ALI and ASI were produced after leukocyte induction for the first 24 hr since following the removal of IFN-containing culture fluid at his term and after washing of leukocytes they did not produce IFN during further cultivation. It seems likely that the activity of IFNs produced as a result of leukocyte induction during cultivation for over 24 hr depends on their sensitivity to incubation temperature. This assumption was confirmed by the following experiments. ASI as well as reference α-IFN and recombinant α_2-IFN were incubated at 37 °C for 5 days with subsequent acid treatment, pH 2.0. The ASI titre did not alter after 5-day exposure at 37 °C, however, following acid treatment at pH 2.0 it fell from 256 to 16 units/ml (Table 4). The similar exposure of reference α-IFN to 37 °C did not alter its activity, its titre decreased considerably (from 256 to 16 units/ml) following acid treatment. The exposure of the recombinant α-$_2$-IFN at 37 °C for 5 days and subsequent acid treatment did not cause any changes in its initial activity levels.

To detect ALI-producing cells following viral induction, human leukocytes were divided into adherent and non-adherent populations and IFN production was induced (Table 5). ALI titre produced by non-adherent blood cells was 4—8 times higher than ASI titre (the first experiment, 128 and 32 units/ml; the second experiment, 256 and 32 units/ml). The adherent cells produced equal titres of ASI and ALI in the first experiment (25

units/ml) whereas in the second, ALI titre was 128 units/ml and that of ASI, 64 units/ml. We conclude that ALI is produced by non-adherent blood cells.

Discussion

The present work was aimed at studying production of various subtypes of native IFN following viral induction of human leukocytes. This became possible using BPL for inactivation of viral inductor, a compound which does not affect the activity of α-, β-, and γ-mouse IFN (Barrett *et al.*, 1984). In our experiments the final dilution of BPL 1 : 4000 did not decrease the antiviral activity of leukocytic and immune human IFNs. When used in the same dilution, BPL completely inactivated the infectivity of IV, SV, and NDV. However, only inactivated IV did not lead to interference with indicator viruses in HDF and MDBK cultures whereas inactivated SV induced weak interference in HDF culture and inactivated NDV induced marked interference in HDF and MDBK cultures just as the infectious virus did.

When IV and SV were used for human leukocyte induction the antiviral activity was detected in the BPL-treated culture fluid 24 hr later. Its treatment with 1 N HCl at pH 2.0 led to lower antiviral activity. ALI proved to be α-IFN as shown by typing because it was completely neutralized by human α-IFN antisera. As far as we know there are single reports in the literature on the capacity of viruses to induce α-ALI production in human leukocytes. Thus, Balkwill *et al.* (1983) detected α-ALI in culture fluid of leukocytes obtained from IV-vaccinated volunteers following additional stimulation *in vitro* by IV. Matsuoka *et al.* (1985) obtained ALI after induction of human leukocytes with SV and inactivation by UV-irradiation of interferon-containing fluid.

In our experiments the level of ALI and ASI production following IV and SV induction was dose-dependent: the higher viral dose the quicker induction and more active production of both IFN subtypes. ALI and ASI were revealed in the culture fluid following 3 hr post induction with IV, thereafter their activity reached its maximum by 24 hr. Further cultivation of induced leukocytes did not affect ALI activity whereas that of ASI decreased significantly. This difference in the dynamics of activity was not related to that in production of both IFN subtypes as both ALI and ASI were produced within the first 24 hr post induction. We assumed that this phenomenon may be caused by conformation changes in some part of ASI molecules during long-term exposure at 37 °C, and this leads to their inactivation during acid treatment at pH 2.0. Following 5-day exposure at 37 °C ASI did not lose its antiviral activity, however, additional acid treatment at pH 2.0 decreased its activity more than by 90%. The activity of human α-reference IFN did not alter following the same procedure but markedly decreased by more than 90% after the treatment at pH 2.0. The activity of recombinant α-$_2$-IFN did not change either following the exposure at 37 °C or following acid treatment.

Consequently, the culture fluid obtained 24 hr post induction of human leukocytes by IV and subsequent BPL treatment contained the most complete set of α-IFN subtypes. Acid treatment at pH 2.0 inactivated the acid-labile portion of the IFN pool while its acid-stable portion remained. More than 90% of antiviral activity was inactivated following additional exposure to 37 °C and acid treatment at pH 2.0. The remaining subtypes (or a subtype) are the true ASI and possibly, the production of one of them was regulated by the gene which expression resulted in α_2-IFN production.

The antiviral activity of ALI and ASI was similar in MDBK culture. Against the background of decreased ASI activity in HDF its activity did not alter in MDBK. Apparently, acid treatment leads to destruction of the IFN pool portion which is less active in MDBK culture while acid-stable fraction of the pool determines the activity of native IFN in heterologous culture. This assumption is based on the data reported by Lin *et al.* (1978) who showed that following SDS PAGE and isoelectric focusing the most acid peak of human leukocytic IFN was 100 times more active in bovine cell culture compared with the homologous culture. The antiviral activity in a heterologous culture was likely to be related to the IFN subtype with a low mol. mass. According to the data reported by Braude *et al.* (1979) and Desmyter and Stewart II (1976) human leukocytic IFN treatment with α-chemotrypsin or its mercaptoethanol and urea denaturation led to the elimination of IFN pool fraction with high and preservation of that with low (15000—16000) mol. mass, while in the homologous culture the activity was not preserved persisting at the same level without changes in the heterologous culture.

The biological meaning of α-ALI production is obscure. Its sensitivity to acid makes it similar to γ-IFN. That is why α-ALI as well as γ-IFN is likely to have an important role in intercellular interactions but only under the effect of viruses. According to our data, the producers of α-ALI are non-adherent cells obtained from human blood leukocytes. Further studies should furnish results showing what particular cell population produces α-ALI following viral induction and shedding more light on the role of α-ALI in immunity.

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