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DIFFERENTIAL EFFECT OF INTERFERON ON THE PRODUCTION OF VIRUS- AND MITOGEN-INDUCED LEUKOCYTE INHIBITORY FACTORS IN HUMAN LEUKOCYTE CULTURES

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Summary. — Interferon treatment of human leukocytes before stimulation with Sendai virus enhanced interferon synthesis but did not affect their leukocyte migration inhibitory factor (LIF) production. The same interferon pretreatment markedly enhanced both LIF and interferon productions when leukocytes were stimulated with concanavalin A.

Key words: interferon; leukocyte migration inhibitory factor; priming; Sendai virus; concanavalin A

Introduction

It has been generally accepted that a mutual connection exists between interferon and the immune system. Interferon exerts immunoregulatory effects (Braun and Levy, 1972; Brodeur and Merigan, 1975; Heron *et al.*, 1976; Finlay *et al.*, 1977; Gresser, 1977; Stewart, 1979) and stimulation of lymphocytes by mitogens or specific antigens elicits interferon production either in vivo or in vitro (Wheelock, 1965; Green *et al.*, 1969; Youngern and Salvin, 1973).

Lymphokines such as migration inhibitory factor (MIF) and LIF are considered to be mediators of cellular immunity (Hay *et al.*, 1973; Takeshi and Cohen, 1974; Rocklin, 1974). Although these lymphokines and interferon have been found to be different entities (Neumann and Sorg, 1978; Block *et al.*, 1978; Georgiades *et al.*, 1979), they frequently appear simultaneously both in vitro (David, 1971; Wheelock, 1965; Green *et al.*, 1969) and in vivo (Zchiesche *et al.*, 1978). As interferon can regulate its own production by priming or blocking (Isaacs and Burke, 1958; Vilček and Rada, 1962; Paucker and Boxaca, 1967), in view of establishing further connections between the interferon and immune systems, it seemed of interest to study the effect of interferon pretreatment on the production of lymphokines. Experiments on the effect of interferon on LIF and interferon production by leukocytes stimulated either by Sendai virus or concanavalin A (Con A) are described below.

Table 1. Effect of partial purification on interferon and LIF activities of the Sendai virus-induced leukocyte interferon preparation

Interferon preparation	Activity	
	Interferon (U/ml)	LIF (% MI*)
Crude	4 000	42.5±9.49
Partially purified	125 000	5.8±2.08
Mock	4	8.2±2.68
Partially purified mock	4	2.4±1.85

* Mean from 5 independent experiments ± standard error.

Materials and Methods

Culture media. Eagle's minimal essential medium (MEM) supplemented with 10 % foetal cal serum was used throughout.

Viruses. Sendai virus (genus Parainfluenzavirus) was kindly supplied by Dr. K. Cantell (Helsinki) and vesicular stomatitis virus, Indiana serotype (VSV; genus *Rhabdovirus* by Dr. J. S. Porterfield (London).

Human leukocyte interferon was produced as described by Mécs and Béladi (1978). The same procedure was used for stimulation of LIF by Sendai virus.

Stimulation of LIF by Con A. Human leukocyte cultures in test-tubes were prepared exactly as for interferon induction. Each tube contained 1.5 ml of a leukocyte suspension containing 1×10 cells/ml. Con A (Calbiochem; 5 μg/ml) was added and cultures were incubated at 37 °C for 1 hr in a Girotory incubator (New Brunswick Sci. Co., U.S.A.) at 80 rev/min shaking frequency. Thereafter the cells were pellete dat $800 \times g$ for 5 min and washed twice with MEM to remove residual Con A. After resuspension in MEM and further incubation for 48 hr the cells were centrifuged off and the supernatant was stored at −70 °C until the determination of LIF activity.

Induction of type II human interferon by Con A. The method was exactly the same as described above for the stimulation of LIF by Con A.

Purification of human leukocyte interferon. The procedure used was essentially the same as described by Cantell *et al.* (1974).

Mock interferon. Culture media from 24 hr old human leukocyte cultures primed with 100 units/ /ml of interferon for 2 hr and not infected with Sendai virus served for mock interferon. It was subjected to the same purification procedure as were human leukocyte interferon preparations.

Interferon assay was done on WI-38 cells in microtiter plates as described (Mécs and Béládi, 1978). Titres were expressed as units (U) per ml in term of the reference standard of human leukocyte interferon, G-023-901-527, provided by the Reference Reagents Branch (National Institute for Allergy and Infectious Diseases, Bethesda, Maryland, U.S.A.).

LIF assay. The conventional capillary tube method (David and David, 1971; Rocklin, 1974) was used. Briefly human polymorphonuclear (PMN) cells were prepared from peripheral blood of healthy donors. Blood samples were mixed in test-tubes with ACD solution at a ratio of 4:1. They were placed in an approximately 45° angle position to the plane of the holder and left stationary for 30 min. The leukocyte-rich plasma was collected and the cells were centrifuged off at

Table 2. Effect of trypsin, pH 2 and heat treatment on interferon and LIF activities of crude human leukocyte interferon preparations

Treatment	Activity in per cent of control	
	Interferon	LIF
None	100.0	100.0
Trypsin	1.7	53.5
pH 2	66.7	19.0
56 °C, 30 min	80.0	103.8

200 × g for 5 min. Resuspended cells were separated in a Ficoll-Hypaque gradient according to Boyum (1968). Red blood cells in the pellet were lysed by suspending the cells in ice cold 0.83 % ammonium chloride for 10 min. The pooled PMN cells were washed twice and resuspended in MEM and filled into capillary tubes which were sealed with paraffin at one end. According to microscopic determinations, the cell suspensions in capillary tubes consisted of 90—98 % PMN and 2—10 % mononuclear cells. The filled and sealed capillary tubes were centrifuged on the sealed end at 200 × g for 5 min and then cut at the cell-fluid interphase. The cut cell-containing portions were placed into migration chambers (Sterilin) filled with MEM or with experimental supernatants and sealed with a cover glass. The chambers were incubated at 37 °C for 18 hr.

The area of cell migration was magnified by a microfilm reading apparatus (Carl Zeiss, Jena, GDR), drawn on transparent paper and quantified by planimetric measurement. Migration inhibition (MI) was expressed as follows:

$$\text{per cent MI} = 100 - \frac{100 \times \text{migration area in the presence of experimental supernatant}}{\text{migration area in the presence of supernatant from control cultures}}$$

Interferon pretreatment. Partially purified human leukocyte interferon at a concentration of 100 U/ml was added to leukocyte cultures and incubated for 4 hr at 37 °C. It was removed by centrifugation before stimulation with Sendai virus or Con A.

Heat inactivation. Samples were heated in a water bath at 56 °C for 30 min and then tested for residual activity.

pH 2 inactivation. Preparations were brought to pH 2 with 1 N HCl for 1 hr then neutralized with 1 N NaOH and assayed for residual activity.

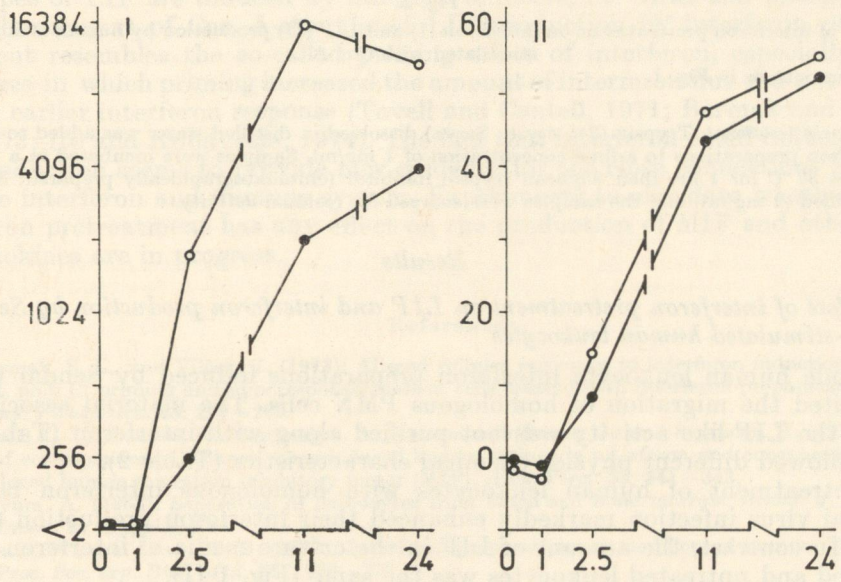

Fig. 1.

Effect of interferon pretreatment on interferon (I) and LIF (II) production by human leukocytes stimulated with Sendai virus

○ — Pretreated with interferon
● — Control
Abscissa: time in hr; ordinate: interferon titre (units/ml I) or % MI (II)

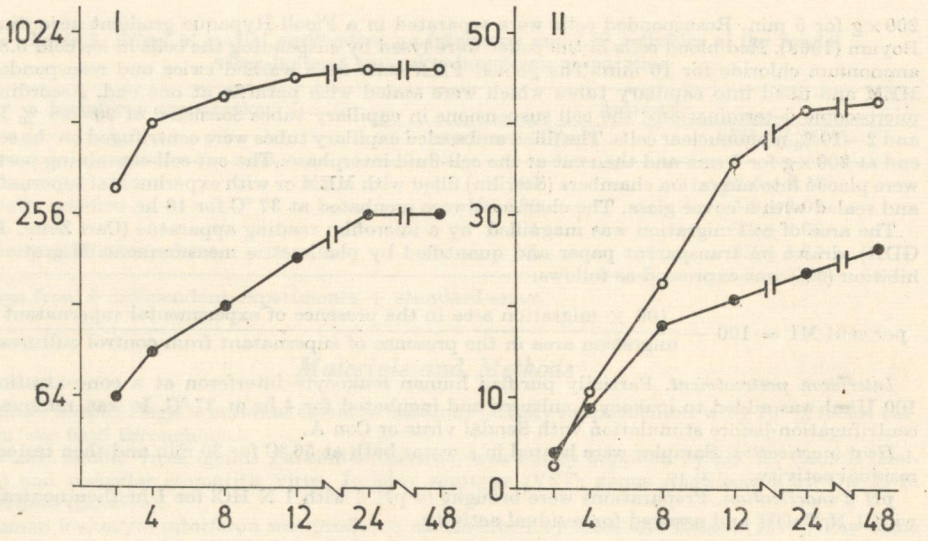

Fig. 2.
Effect of interferon pretreatment on interferon (I) and LIF (II) production by human leukocytes stimulated with Con A
Explanations as in Fig. 1.

Trypsin treatment. Trypsin (2× cryst., Serva) dissolved in distilled water was added to crude interferon preparations to a final concentration of 1 mg/ml. Samples were incubated in a water bath at 37 °C for 1 hr; then soybean trypsin inhibitor (chromatographically prepared; Serva) was added (2 mg/ml) and the samples were assayed for residual activity.

Results

Effect of interferon pretreatment on LIF and interferon production by Sendai virus-stimulated human leukocytes

Crude human leukocyte interferon preparations induced by Sendai virus inhibited the migration of homologous PMN cells. The material associated with the LIF-like activity was not purified along with interferon (Table 1) and showed different physico-chemical characteristics (Table 2).

Pretreatment of human leukocytes with homologous interferon before Sendai virus infection markedly enhanced their interferon production (Fig. 1-I). In contrast, the amount of LIF in the culture media of interferon pretreated and untreated leukocytes was the same (Fig. 1-II).

Effect of interferon pretreatment on LIF and interferon production by Con A-stimulated human leukocytes

Priming the leukocytes with Sendai virus-induced leukocyte interferon enhanced the Con A-stimulated type II interferon production (Fig. 2-I). LIF activity in culture media from leukocytes not primed with interferon

reached significant levels at 8—12 hr and maximal levels at 24—48 hr after stimulation. Release of LIF from interferon-pretreated cells followed the same pattern at the beginning of incubation. The duration of the intensive LIF production period in these cultures was, however, longer and LIF activity in samples taken at 12 hr and later after stimulation was approximately 2-fold that of leukocytes without interferon-pretreatment (Fig. 2-II).

Discussion

Interferon pretreatment of human leukocytes did not influence LIF production, but markedly enhanced their interferon production after stimulation with Sendai virus. This could be considered as another characteristic property unshared by these two biological mediators produced simultaneously by Sendai virus-stimulated human leukocytes. On the contrary, interferon pretreatment resulted in enhancement of both interferon and LIF production by Con A-stimulated human leukocytes. The difference observed between the effects of interferon pretreatment on LIF production stimulated by Sendai virus and Con A seems to indicate that LIF production might take place by different mechanisms depending upon the stimulator used or that different types of LIF are induced by different inducers, i.e. virus and mitogen. The enhancement of Con A-stimulated LIF production by interferon pretreatment resembles the so-called priming effect of interferon, especially those cases in which priming increased the amount of interferon but did not trigger an earlier interferon response (Tovell and Cantell, 1971; Barmak and Vilček, 1973; Ito and Kobayashi, 1974). The fact that interferon could influence LIF production seems to provide new evidence for the close connections between the interferon and immune system. Experiments to elucidate whether interferon pretreatment has any effect on the production of MIF and other lymphokines are in progress.

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