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INTERFERON ABROGATES THE ARREST OF DNA SYNTHESIS IN HETEROLOGOUS THYMOCYTES TREATED WITH LECTINS

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SUMMARY

Mouse or rat thymocytes are triggered to undergo blastogenesis by mitogenic doses of concanavalin A and lentil lectin, when these plant mitogens are applied to the freshly cultured thymocytes at time 0. However, these mitogens did not elicit a mitogenic response if added to mouse or rat thymocytes that were preincubated in culture medium for 24 hours. Incubation of the thymocytes with heterologous preparations of interferon during the period of 24 hours before application of the mitogens, brought about an enhanced incorporation of tritiated thymidine. The data presented suggest that heterologous interferons could significantly abrogate the block in DNA synthesis in thymocytes that were preincubated for 24 hours in culture medium prior to addition of the mitogens.

INTRODUCTION

Interferon is known basically as a potent suppressor of viral multiplication, and has also been reported to be inhibitory to a variety of immunological processes *in vivo* (1) and to DNA synthesis induced by plant mitogens or in mixed lymphocyte reactions (2,3). Interferon was shown to be suppressive to cell growth *in vitro* under normal conditions (4), or in transition phases of the cell cycle (5,6). It is accepted that interferon exerts its inhibitory effect on viral or cellular growth in homologous systems, but has no effect on heterologous cells (2). We report here on an unusual activity of human and mouse interferons that permits DNA synthesis in heterologous thymic lymphocytes treated with plant lectins, under conditions where such synthesis is arrested.

MATERIALS AND METHODS

Trypsin was purchased from Sigma Chemical Co. Ltd.; concanavalin A (Con A) and lentil lectin (LcH) came from Miles-Yeda, Israel. Mouse interferon preparations were isolated from the supernatant of monolayer

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cultures of mouse L cells induced by Newcastle disease virus, and partially purified by column chromatography using CM-Sephadex (G-25) after 10-fold concentration by vacuum dialysis. Human lymphoblastoid interferon was prepared by Sendai virus infection of Namalva cells (7) and purified by affinity chromatography using sheep anti-human-leukocyte-interferon antibody coupled to Sepharose as previously described (8). The preparations of mouse and human interferons used throughout the experiments had a specific activity of 8×10^6 units/mg and 8×10^4 units/mg, respectively.

Male Wistar rats (120-170 g) and BALB/c mice (12-15 g) were killed by ether. Thymuses and spleens were excised and minced in cold phosphate buffered saline and pressed through a large-mesh wire screen. After the cells were pelleted by centrifugation at 200 x g for 2 min, they were resuspended in Dulbecco's modified Eagle medium (9) supplemented with 5% heat-inactivated fetal calf serum, penicillin (100 U/ml) and streptomycin (100 µg/ml). Lymphocytes (4×10^6) were applied in 0.4 ml culture medium to polystyrene tubes and cultivated for 48 hours at 37°C in a 5% CO_2/air atmosphere. For the determination of DNA synthesis, 1 µCi of [^{3}H]thymidine (Nuclear Research Center, Negev, Israel, 5 Ci/mmole) was added per tube for 2 hour pulse, and acid precipitable material was collected for scintillation counting on filters (GF/C, Whatman).

RESULTS AND DISCUSSION

When freshly prepared rat or mouse thymocytes are incubated with Con A or LcH at the optimal mitogenic doses of 3 and 8 µg/ml, respectively, they respond with pronounced thymidine incorporation (Table 1). However, when these thymocytes were cultured for 24 hours prior to the application of the mitogenic lectins, they incorporated only 2-3% of the thymidine as compared with the same cells to which Con A or LcH had been added at time 0. The drastic drop in the mitogenic response of the thymocytes to the late-added mitogens was not due to excessive cell death that could have occurred in the 24 hour preincubation period, as assessed by the trypan blue exclusion test. Furthermore, the possibility of depletion of some nutrients from the culture medium during this period was ruled out, inasmuch as transfer of preincubated cells to fresh medium did not restore their response to the mitogens.

When rat and mouse thymocytes were preincubated for 24 hours with purified preparations of heterologous interferons, and subsequently treated with the lectins, the level of thymidine incorporation was significantly elevated. As can be seen in Table 1, human and mouse interferon preparations, induced in mitogen-treated rat thymocytes over 20 and 15 fold

TABLE 1 Mitogenic Response of Rat and Mouse Thymocytes to Con A and LcH and the Effect of Human and Mouse Interferons[a]

Lectin	*Time of lectin addition[b] (hours)*	*Origin of interferon[c]*	*[^{3}H]Thymidine incorporated by thymocytes of Wistar rat cpm*	*BALB/c mouse cpm*
Con A	0	-	350,000	120,000
"	24	-	8,000	2,800
"	0	Human	330,000	115,000
"	24	"	220,000	66,000
"	0	Mouse	290,000	40,000
"	24	"	190,000	2,200
LcH	0	-	280,000	51,000
"	24	-	7,000	4,000
"	0	Human	260,000	48,000
"	24	"	190,000	34,000
"	0	Mouse	250,000	11,000
"	24	"	160,000	3,000
-	-	Human	2,000	1,000
-	-	Mouse	1,700	1,200

[a] The data represent the average of triplicate determinations.

[b] Lectin was added either together with the interferon (time 0) or after 24 hours.

[c] Human interferon was used at a concentration of 500 units/ml, and mouse interferon at 1,000 units/ml.

increases in thymidine uptake, respectively. BALB/c thymocytes preincubated with human interferon and subsequently treated with LcH and Con A, incorporated more than 8 and 20 fold thymidine, respectively, over control cultures that were not treated with interferon. The enhancing effect of human interferon was dose-dependent, increasing with concentrations up to 500 U/ml, and falling gradually at higher interferon concentrations. Mouse interferon was most effective at 1,000 U/ml, with no significant change at 2,000 and 4,000 U/ml. It is apparent from Table 1 that mouse interferon inhibited mitogenic stimulation when applied to homologous BALB/c lymphocytes, simultaneously with the lectins, as was reported elsewhere (2), thus emphasising the distinct effect of heterologous inter-

ferons in our system. Since our preparation of human interferon had a specific activity of 8×10^4 units/mg only, the possibility was raised of a non-specific contaminant that could bring about the observed effects on DNA synthesis. We have therefore prepared "mock" human interferon by adding acid-treated Sendai virus to Namalva cells, and proceeded with the various steps described for the purification of human interferon, including affinity chromatography. Fractions from the affinity column were collected, serially diluted and assayed for their effect on thymidine incorporation in lectin treated rat and mouse thymocytes. It was apparent that under experimental conditions identical to those in assays with human interferon, no effect whatsoever was found with preparations of "mock" interferon. This lends support to the suggestion that the release from the block in DNA synthesis observed upon late addition of lectins to thymocytes can be ascribed to human interferon.

The effects of the heterologous preparations of interferon on mitogenic stimulation by Con A and LcH were completely abolished by trypsin (5 µg/ml, 10 min), sodium periodate (0.05 M, pH 4.4, 30 min), or by 2-mercaptoethanol treatment, yet these preparations lost over 80% of their activity after being heated for 30 min at 80°C. All of these treatments were shown to affect significantly the biological activities of interferon (10).

The interferon preparations tested in our system did not affect thymidine incorporation in cells to which they were added simultaneously with the plant lectins at time 0 (Table 1), neither did they induce thymidine incorporation to any measurable extent when added alone to cells. The effect of interferon on DNA synthesis in thymic lymphocytes was not due to any measurable promoting activity on the viability of the cells, as was assessed by trypan blue exclusion test. Thymocytes that were incubated during the mitogenic assay for 72 hours had shown 35 to 45% viability at the end of the period, whether or not interferon was present in the culture medium.

It is noteworthy that rat and mouse spleen lymphocytes from the same strains used for thymocyte preparations, or human peripheral blood lymphocytes, responded strongly to Con A or LcH when incubated with these mitogens 24 hours after they were cultured. Thus, the extent of thymidine incorporation by BALB/c splenocytes, Wistar rat splenocytes and human peripheral lymphocytes treated with lectins after 24 hours in culture were 65-75% of that measured for these cells triggered with Con A or LcH at time 0. The reason for the complete arrest in thymidine incorporation in thymocytes, not found with rat and mouse splenocytes or with human peripheral lymphocytes, is not clear at present but may represent a nuclear arrest that is peculiar to preincubated thymocytes. In this regard it should be noted that in the absence of mitogens the rate of DNA synthesis in thymocytes freshly removed from rats and mice is much higher than that of splenocytes of the same animals. As shown in Figure 1, BALB/c mouse thymocytes pulsed at time 0 incorporated 8 times more [^{3}H]thymidine than BALB/c splenocytes, and Wistar rat thymocytes were 4 times as active as Wistar splenocytes in their rate of DNA synthesis when pulsed immediately after they were cultured. It is possible that the accelerated thymidine incorporation in thymocytes is responsible for a degradation of precursors that are crucial for the mitogenic stimulus by Con A and LcH to occur. If such hydrolytic processes indeed take place in the thymic lymphocytes, the data presented here suggest that the heterologous interferon preparations may inhibit these processes during the preincubation period. In homologous systems interferon exhibits its dominant inhibitory effects on DNA synthesis, yet in heterologous cells other activities of interferon could be manifested, some of which could give rise to promoting effect on DNA synthesis that is found in our system.

Miörner *et al.* (11) have reported recently that human fibroblast and leukocyte interferons at very low doses (<1 unit/ml) enhanced mitogenic effects induced by phytohemagglutinin and Con A in human lymphocytes. They

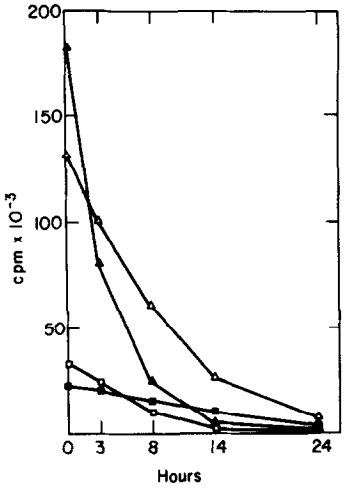

Figure 1 Spontaneous [^{3}H]thymidine incorporation by rat and mouse thymic and splenic lymphocytes. Lymphocytes prepared from thymuses or spleens were cultured at 3 x 10^6 cells/tube in Dulbecco's modified Eagle medium supplemented with 5% fetal calf serum. At different times after cell culturing, tubes were pulsed for 2 hours with 1 µCi [^{3}H]thymidine/tube, and trichoroacetic acid precipitable material was collected on filters and counted. Each point is the average of triplicate determinations. ▲—▲, BALB/c mouse thymocytes; ■—■, BALB/c mouse splenocytes; Δ—Δ, Wistar rat thymocytes; □—□, Wistar rat splenocytes.

also found that the most marked effects were obtained when interferon was added 24 hours before the lectins, and that addition of interferon at later times diminished the effect on lymphocyte DNA synthesis. We were not able to demonstrate any enhancing or inhibitory activity of mouse interferon tested in the homologous system of BALB/c thymocytes at the very low concentrations of 0.01 - 1 U/ml. It will be interesting to investigate whether the promoting effect of interferon on DNA synthesis reported for homologous or heterologous cell systems is due to a similar mechanism. Experiments are in progress to test this possibility that could shed light on the cellular components that are required for a mitogenic stimulus to occur.

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