PRODUCTION OF HYPOREACTIVITY FACTOR BY MOUSE CELLS

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KEY WORDS: interferon; repressor.

The state of hyporeactivity to repeated induction of interferon prevents the clinical use of interferon inducers and requires the preliminary drawing up of schemes for their application. Mechanisms of development of the state of hyporeactivity during repeated injection of interferon inducers into animals or addition to a cell culture are unknown. It has been suggested that a protein repressor of interferon production participates in this process [1-4]. It was reported previously that one such repressor is contained in the cell sap of chick fibroblasts in a state of hyporeactivity [2, 3]. On addition to homologous cells it inhibited interferon production by 100-1000 times. The repressor had no antiviral action, but by the conditions for manifestation of its activity and some of its physicochemical properties, it closely resembled interferon.

In the investigation described below the possibility of releasing interferon production repressor into the incubation medium of cells after creation of a state of hyporeactivity in them was investigated on a model of mouse L929 cells.

EXPERIMENTAL METHOD

To create a state of hyporeactivity a monolayer of mouse L929 cells was treated twice with the interferon inducer poly(I) · poly(C) in the presence of DEAE-dextran. The poly(I) · poly(C) was added to the cells in a concentration of 50 μg/ml for 1 h at 37°C. After removal of the inducer the cells were incubated in nutrient medium 199. The second treatment with inducer was given at various times after the first and under the same conditions. Repressor and interferon were determined in samples of culture fluid after the first and second inductions of interferon. To detect repressor in the culture fluid, the method of its determination in cell sap suggested previously [2, 3] was used. The monolayer of L929 cells was treated with samples of culture fluid for 2 h at 37°C, after which interferon formation was induced in them by treatment with poly(I) · poly(C) in the presence of DEAE-dextran. The culture fluid was collected after 24 h for interferon determination. Interferon was determined by inhibition of the cytopathic action of VEE virus in homologous cells [5].

EXPERIMENTAL RESULTS

The time course of interferon production by L929 cells in the medium is illustrated in Fig. 1. Interferon began to be detected in the medium after 14 h and it accumulated until 24 h. After the second induction with poly(I) · poly(C) the quantity of interferon secreted into the medium decreased, evidently as a result of inhibition of its formation in the cells (Table 1). The phenomenon of hyporeactivity was seen most clearly when the second induction took place up to 6 h after the first.

The next step was to study the possibility of liberating the inhibitor of interferon production into the incubation medium of cells in a state of hyporeactivity. For this purpose, after the second induction of interferon samples of culture fluid were collected at different intervals, and used to treat homologous cells before induction of interferon in them (see the section: "Experimental Method"). Half of the samples of culture fluid were incubated with 1000 units of purified antiserum against mouse interferon for 30 min at 37°C, and they were then also investigated for repressor activity. It will be clear from Table 2 that treatment of the cells with samples

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TABLE 1. Development of State of Hyporeactivity in L929 Cells

Time of second induction, h	Interferon production, IU/ml	Multiplicity of inhibition relative to control
3	160	8
4	160	8
6	160	8
8	320	4
14	320	4

Legend. Interferon production in control after first induction with poly(I) · poly(C) was 1280 IU/ml. Interferon was determined in samples of culture fluid collected 24 h after first and second inductions.

TABLE 2. Time Course of Production of Hyporeactivity Factor in Incubation Medium

Time of collecting samples of culture fluid after second induction, h	Incubation with antiserum against mouse interferon	Multiplicity of inhibition of interferon production
4	−	64
	+	64
6	−	64
	+	32
8	−	64
	+	32
14	−	64
	+	2
16	−	64
	+	2
18	−	4
	+	1
20	−	1
	+	2

Legend. Interferon production in absence of hyporeactivity factor after induction of poly(I) · poly(C) was 1280 units/ml. Treatment of the cells with antiserum against mouse interferon (2 h at 37°C) did not affect the interferon yield.

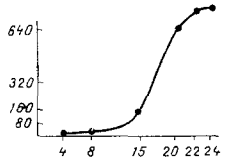

Fig. 1. Time course of interferon production by mouse L929 cells. Abscissa, time (in h); ordinate, interferon titer (in IU/ml). Interferon was determined in culture medium 24 h after treatment of cell monolayer with poly(I) · poly(C).

of culture fluid collected up to 16 h after the second induction caused considerable depression of interferon production (by 32-64 times). Incubation with antiserum against mouse interferon neutralized the activity of the repressor in samples of culture fluid collected only after 8 h.

It can be concluded from these results that hyporeactive cells secrete a factor inhibiting interferon production into the incubation medium. This factor differs from interferon in its antigenic composition. Further investigations will establish its nature and its connection with the intracellular repressor. Besides hyporeactivity factor, interferon itself can evidently control its production by the feedback principle. Two opposite phenomena, namely "priming" and "hyporeactivity," due to interferon, have been described in the literature. The mechanisms whereby interferon regulates its own production are unknown.

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TIME COURSE OF ACTIVITY OF NATURAL MOUSE SPLEEN KILLER CELLS AFTER PARTIAL SPLENECTOMY

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The mouse spleen is capable of reparative regeneration after removal of a large part of the organ. The operation and repair processes in the spleen are accompanied by a drastic fall in the ability of splenocytes to form antibodies, and this is accompanied by changes in the content and functional activity of the T and B lymphocyte populations [3].

In recent years many investigators have directed their attention toward normal natural killer cells (NKC), cells mediating natural cell cytotoxicity. The reasons are not only that NKC are possible effectors against malignantly transformed cells, but also the suggestion that they may play an important role in the maintenance of homeostasis, with their participation in the regulation of normal proliferation and differentiation in renewing systems [4]. The writers showed previously that repair processes in the liver are accompanied by marked changes in NKC activity in the spleen, a reduction of natural cytotoxic activity during the 2 days after the operation, followed by an increase in this activity on the 5th-9th day [1].

The aim of this investigation was to study the dynamics of NKC activity during reparative regeneration of the spleen.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 16-20 g. Under ether anesthesia two-thirds of the spleen was removed from the animals. As a special control, in a separate series of experiments the right submandibular salivary gland, which is incapable of regeneration under these experimental conditions, was completely removed [2]. The animals were decapitated at various times after the operation and cell suspensions prepared from the spleens.

To assess the level of DNA synthesis $2 \cdot 10^6$ spleen cells were incubated in medium RPMI-1640 with 10% fetal calf serum and 1% glutamine, in the presence of [^{3}H]thymidine (5 μCi/ml, specific radioactivity 23 Ci/mmole) for 1 h at 37°C. Radioactivity incorporated into the acid-insoluble fraction was estimated with a Tricarb Packard scintillation counter.

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