SUPPRESSION OF α-FETOPROTEIN PRODUCTION BY EXTRACTS OF VARIOUS TISSUES

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UDC 612.64'398.139-06:612.015.21

Injection of syngeneic serum and also of 20% extracts of the large and small intestine, liver, kidneys, and spleen of adult mice into newborn mice leads to inhibition of α-fetoprotein production. The quantity of the factor inhibiting α-fetoprotein production in muscle extracts from adult mice is very small. Mouse embryonic intestinal extract and extract of whole embryos contain hardly any of the inhibitory factor, and its content in the intestine of 21-day mice is smaller than in the intestine of adult animals. The importance of the search for hormone-like factors regulating synthesis of embryonic antigens as a means of monitoring tumor growth is discussed.

KEY WORDS: carcino-embryonic antigens (α-fetoprotein); hepatoma; hormones; liver.

One of the chief embryonic serum proteins is α-fetoprotein (α-FP). Its synthesis starts in the yolk sac, continues in the embryonic liver, and dies away in mice and rats at the beginning of the postnatal period [1, 2]. The suggestion has been made that a hormone-like factor exists, starting from the end of the intrauterine period, and inhibits α-FP production by embryonic liver cells.

The object of this investigation was to search for the factor inhibiting α-FP production.

EXPERIMENTAL METHOD

Families of newborn mice were divided half and half. One half of each family received extracts of various organs of adult mice and embryos daily, whereas the other half was not so treated (control). The α-FP titer in the serum was determined after 10 days in the mice of each group. The study began with extracts of the small intestine, on the grounds that the liver develops from the small intestine in embryogenesis and that the two organs continue to share endocrine links.

BALB/C mice were used. Extracts were prepared from the organs of mice aged 21 days and 4-5 months, and also from embryos taken during the last days of pregnancy.

The proximal part of the small intestine (8 cm) was excised from adult male and female BALB/C mice, cleansed of its contents, divided longitudinally, and washed repeatedly in Hanks' solution containing antibiotics (1000 units each of penicillin and streptomycin/ml). The piece of intestine was incubated in the same solution for 30 min at 37°C and then washed several times in Hanks' solution without antibiotics. The tissue was carefully ground in a mortar with glass and 20% extracts were prepared in Hanks' solution containing 200 units each of penicillin and streptomycin/ml. The homogenate was centrifuged at 500 rpm for 5 min. The supernatant was poured into plastic ampules and kept in liquid nitrogen (−196°C) for 18 h, and then stored at −22°C. When required the ampules with the extract were thawed and used for injections. The large intestine, liver, kidneys, spleen, and striated muscles were treated in the same way and extracts prepared from them. Extracts also were prepared from the small intestine of 21-day mice and mouse embryos taken at the end of pregnancy. In addition, extracts were prepared from whole mouse embryos (including the head), but after removal of the stomach and intestine.

Laboratory of Virology, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 2, pp. 74-76, February, 1975. Original article submitted August 9, 1974.

TABLE 1. α-FP Titer in Sera of Newborn Mice Receiving Extracts of Various Organs

No. of family of mice	Experimental half of family (receiving 20% extract of organ)	α-FP titer*	Control half of family (untreated) α-FP titer*
	Sacrifice on 10th day		
1	Small intestine of adult mice	64	512
2	" " " "	64	512
3	Large intestine of adult mice	64	256
4	" " " "	64	256
5	Muscles of adult mice	128	128†
6	" " "	256	256†
7	Kidneys of adult mice	32	256
8	" " "	64	256
9	Small intestine of adult mice	32	512
10	" " " "	32	512
11	Large intestine of adult mice	32	512
12	" " " "	32	512
13	Small intestine of embryos	256	256†
14	" " "	128	256
15	Small intestine of 21-day mice	64	128
16	" " " "	64	256
17	" " " "	64	256
18	" " " "	64	256
19	Embryos without stomach and intestine	512	512†
20	" " " " "	512	512†
21	Liver of adult mice	64	256
22	" " "	64	256
23	Spleen of adult mice	64	512
24	" " "	64	512
25	Serum of adult mice	32	512
26	" " "	32	512
27	Hanks' solution injected	256	256
28	" " "	256	256

*Reciprocal of last dilution of neonatal serum in which α-FP was detected by microprecipitation.

† Serum titer the same in control and experiment, but intensity of precipitation band somewhat greater in control than in experiment.

The extracts (20%) were injected through a fine needle subcutaneously in the dorsal region into newborn BALB/C mice starting from the first day of life. As the animals grew, the volume of material injected increased. From the 1st to the 4th day of life the mice received 0.1 ml, on the 5th and 6th days 0.2 ml, on the 7th day 0.3 ml, and on the 8th and 9th days 0.5 ml each.

The pregnant mice were kept in pairs for 1-2 days before giving birth. Each family of newborn mice was divided half and half: one half of the newborn mice received the particular extract, the other half remained untreated (control). Extracts were injected from the 1st to the 9th day inclusive into mice killed on the 10th day. Each mouse killed on the 10th day received 2.1 ml of the corresponding 20% extract. Special experiments showed that injection of the corresponding dose of Hanks' solution into the newborn mice had no effect on the α-FP titer (Table 1).

The microprecipitation test in agar was carried out in the modification of Gusev and Tsvetkov [3]. Double dilutions of the mouse serum for testing were prepared from 1:2 to 1:4096. The test system* was diluted 1:2 and tested with the above dilutions of sera from the newborn mice. The results were read next day and each reaction was photographed.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the small and large intestine, kidneys, spleen, and serum of the adult mice contained a factor inhibiting α-FP production in young mice. Extracts of the small intestine of the 21-day mice had a weaker action than those of adult mice. Hardly any of this factor could be detected in the small intestine of mouse embryos or in whole mouse embryos. The quantity of the inhibitory factor in adult mouse muscle also was very small. Future experiments will show which other organs contain this factor, the site and conditions of its synthesis, accumulation, and excretion, and its chemical nature.

*Rabbit antiserum against purified mouse α-FP and serum of newborn mice taken in the equivalent proportions.

The function of embryonic antigens in tumor cells and the factors controlling their appearance are not yet known. Investigations in the writers' laboratory have shown that growth of transplants of the embryonic gastro-intestinal tract and of certain tumors is inhibited in newborn mice compared with their growth in adult animals [4, 5]. Hormonal factors controlling the synthesis of embryonic antigens are evidently produced in the embryo, and these hormones may control the growth of tumor cells and restore them to normal. These hormones may be absent in adult animals. By using the hormonal factors of the adult or embryo that control induction and repression of antigen in normal embryonic cells, it is possible to act on tumor cells which synthesize embryonic antigen. However, not only were these regulatory factors unknown previously, but no steps worth mentioning have been taken to find them. One such factor is described in this paper. Whether it acts on α-FP synthesis in embryonic liver cells or whether it suppresses the proliferation of these cells is unknown. In the first case it would restore hepatoma cells to normal cells, but in the second case it would inhibit their proliferation. The factor probably inhibits regeneration of the liver. The discovery of this factor evidently confirms the validity of the subsequent general approach. The search for hormone-like factors inhibiting the synthesis of this embryonic antigen must commence at that stage of ontogeny in which their synthesis is phased out, and continue after that stage.

The authors are grateful to A. K. Yazova and A. I. Gusev, working in the laboratory of immunochemistry of tumors of the N. F. Gamaleya Institute of Epidemiology and Microbiology for providing the test system for α-FP, to E. R. Karamova and M. D. Glyshkina for advice on the technique of the first microprecipitation tests, and to Professor G. I. Abelev and Professor L. N. Fontalin for discussing the results of this investigation.

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