CHARACTER OF GROWTH AND DIFFERENTIATION OF OC15S1 IN SYNGENEIC AND ALLOGENEIC MICE

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A unique feature of embryocarcinoma cells (ECC) — the stem cells of teratocarcinomas, which preserve the properties of malignancy in the course of many subcultures, and cause death of the animals — is their ability, under certain conditions, to be transformed into phenotypically normal cells, when they form definitive tissues of various kinds, i.e., they exhibit a similarity (in their potential powers) to polypotent embryonic cells [1-3].

One aim of this investigation was to study the dynamics of proliferative processes at the initial stages of differentiation, i.e., in the stage of phenotypic normalization of ECC. Elucidation of the hitherto unstudied features of growth and differentiation of teratocarcinomas in allogeneic animals was particularly interesting.

EXPERIMENTAL METHOD

The test object was teratocarcinoma OC15S1, a tumor obtained from mouse embryos of line 129 [6, 9]. Syngeneic line 129 mice, with embryoid bodies (ascites form of teratocarcinoma) in their peritoneal cavity, were given an injection of ^{3}H-thymidine (specific activity 17 Ci/mmole) in a dose of 40 μCi per animal. The mice were killed 30 and 40 min, 1, $1^1/_2$, 2, 3, $3^1/_2$, 4, $4^1/_2$, 5, 7, $7^1/_2$, 9, 10, 13, $14^1/_2$, $16^1/_2$, 19, 21, 24, and 26 h after injection of the isotope. Type M emulsion was applied to dewaxed sections of embryoid bodies and exposed for 30 days. The duration of the mitotic cycle T [7] and the number of DNA-synthesizing ECC — the index of labeled nuclei (ILN, in %) were determined on sections stained with hematoxylin and eosin.

In the next series of experiments syngeneic (line 129) and allogeneic (line C57BL) male mice weighing 18-20 g were given an intraperitoneal injection of 5500 embryoid bodies. The animals were killed 7, 9, 13, 19, 27, and 35 days after transplantation (C57BL mice again 72 days after transplantation). An intraperitoneal injection of 5 ml of medium 199 was given to the killed mice and the number of embryoid bodies in 1 ml fluid was determined. To determine the percentage of trophic embryoid bodies the test material was fixed with Bouin's fluid and serial sections were stained with hematoxylin and eosin. To test whether embryonic bodies present in the peritoneal cavity of the allogeneic mice for 19 and 72 days still preserved their oncogenic properties, the test objects were retransplanted into syngeneic animals.

EXPERIMENTAL RESULTS

The kinetics of cell proliferation of ECC, located in embryoid bodies in the form of diffusely scattered elements (T 11 h, ILN 32.7%) and of ECC forming cavities (T 14 h, ILN 23.9%) was demonstrated. A similar lengthening of the mitotic cycle and reduction of the number of DNA-synthesizing cells also were observed during aggregation (differentiation) of ECC [8].

There are two possible pathways of ECC development: 1) reproduction of malignant ECC similar to themselves; 2) in the course of differentiation the ECC form phenotypically normal tissues. The data showing lengthening of the mitotic cycle confirmed observations of electron microscopy [5], indicating that cytodifferentiation of ECC begins and can be identified actually in embryoid bodies. ECC forming cavities, which are similar in structure to poly-

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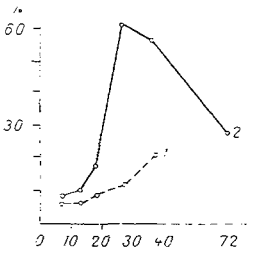

Fig. 1

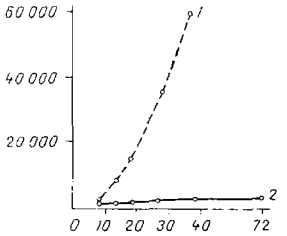

Fig. 2

Fig. 1. Number of necrotic embryoid bodies in syngeneic (1) and allogeneic (2) mice. Abscissa, time after transplantation (in days); ordinate, percentage of necrotic embryoid bodies.

Fig. 2. Number of embryoid bodies in syngeneic (1) and allogeneic (2) mice. Abscissa, time after transplantation (in days); ordinate, number of embryoid bodies in 1 ml.

potent presumptive ectoderm of the early embryo [5], are probably committed, i.e., they differentiate along the path of further formation of normal tissues. The structural features of ECC diffusely distributed among the embryoid body [5] are evidence in support of the view that these cells are probably undifferentiated.

Embryoid bodies adherent to internal organs are transformed into solid tumors, in which subsequent stages of cytodifferentiation also take place. Besides collections of undifferentiated ECC, occupying the main part of the tumor, differentiated structures also are present, including some of enterodermal (simple isoprismatic and high prismatic epithelium) and epidermal (ciliated epithelium) origin, with numerous neutral derivatives [6].

The characteristics of growth of teratocarcinomas transplanted intraperitoneally into syngeneic and allogeneic animals also were studied. In syngeneic mice of line 129, approximately 17 days after transplantation, both solid tumors and ascites fluid could be identified visually. The number of embryoid bodies (ascites form of the tumor), the dimensions of the solid tumors (to 2-4 cm^3), and the volume of ascites fluid (to 6 ml) increased progressively, and these animals died after about 40 days (Fig. 1). In allogeneic C57BL mice, however, limitation of growth of the teratocarcinoma was observed. Solid tumors and ascites fluid were not formed and the number of embryoid bodies (ascites form of the tumor) 35 days after transplantation was 40 times less than in syngeneic mice: 1500 and 60,000 bodies in 1 ml (Fig. 1). The percentage of necrotic embryoid bodies rose sharply in allogeneic mice (Fig. 2), and as a result mainly structures with diffusely distributed ECC, i.e., probably with undifferentiated cells, rather than bodies containing cavities (i.e., with committed cells, starting out along the path of normal cell differentiation), predominantly remained viable. Since solid tumors do not form in allogeneic mice, definitive differentiated tissues naturally also are absent.

In allogeneic C57BL mice after transplantation of teratocarcinoma OC15S1, only very few embryoid bodies with undifferentiated ECC remain. The probable reason for this is that stem (undifferentiated) ECC do not possess H-2 histocompatibility antigens. ECC starting out on the path of cytodifferentiation, on the other hand, produce H-2 antigens [4], as a result of which an immunologic attack takes place, with death of the corresponding differentiated population.

In conclusion it must be pointed out that embryoid bodies which have remained 19 and 72 days in allogeneic mice, on subsequent retransplantation into syngeneic animals, undergo a typical type of development (the ascites form of tumor — solid form, ascites fluid, and death of the animal), i.e., the ECC stem cells preserve their oncogenic properties. The composition of these solid tumors formed as a result of retransplantation is the same as that of tumors developing from the beginning in syngeneic mice, and this is evidence that the ECC preserves its own mode of differentiation.

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GLUCONEOGENESIS IN ANIMALS WITH EXPERIMENTAL TUMORS TREATED BY HYDRAZINE SULFATE

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Hydrazine sulfate (HS) has antitumor activity under experimental conditions [6, 11] and is an effective agent in clinical oncology [10]. It has no marked side effects, such as are characteristic of most known antitumor agents [3, 9]. It has been suggested [12] that HS goes some way toward normalizing carbohydrate metabolism, when disturbed during tumor growth, by sharply inhibiting gluconeogenesis. This effect is achieved through inhibition of phosphoenolpyruvate carboxykinase, the key enzyme of gluconeogenesis. As a result the vicious circle of glycolysis—gluconeogenesis—glycolysis, arising in malignant disease, which demands high energy expenditure and leads to the well-known cancer cachexia, is broken. The author cited gives indirect evidence in support of this hypothesis, but no direct experimental data on inhibition of gluconeogenesis during effective antitumor treatment with HS has hitherto been obtained.

The aim of this investigation was to study gluconeogenesis *in vivo* in the liver and kidneys (the main organs in which it takes place) of experimental animals with transplanted tumors after treatment with HS. The blood sugar also was determined to reflect changes in gluconeogenesis.

EXPERIMENTAL METHOD

Noninbred rats weighing 130-150 g with transplanted Zajdela's ascites hepatoma and noninbred albino mice with transplanted NK/LI ascites lymphoma were used. Daily intraperitoneal injections of HS (80 mg/kg) were given to the animals starting 24 h after transplantation; the control animals received 0.9% NaCl solution. This course of treatment leads after 7 ± 2 days to significant inhibition of tumor growth [5]. The rats were decapitated on the 7th-9th day after transplantation of the tumor, the mice on the 5th-7th day, and in all cases one day after the last injection of HS.

To assess gluconeogenesis *in vivo*, labeled glucose newly formed from [^{14}C]-2-alanine was determined. The labeled alanine was injected intraperitoneally (30 μCi into mice, 300 μCi into rats) and the animals were decapitated 1 h after injection of the isotope. Glucose was isolated by column ion-exchange chromatography [1]. Glucose was estimated quantitatively by the enzymic method with glucose oxidase [2]. Radioactivity in the samples was measured on a Mark 2 counter, using dioxin scintillator.

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