CHANGE IN GROWTH CHARACTERISTICS OF AISM MOUSE ASCITES TUMOR DURING CONTINUOUS PASSAGE IN VIVO

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The transplantable mouse tumor ISM exists in both solid and ascites forms. The solid ISM tumor appeared after repeated subcutaneous injection of tetrachloromethane into BALB/c mice and was characterized as a leiomyosarcoma [7]. The ascites form of this tumor (AISM) was obtained by the writer after cellular disintegration of ISM and intraperitoneal injection of a suspension of tumor cells into BALB/c mice in a dose of 10^6. Both versions of the tumor have been obtained and continuously maintained until the present time in the Laboratory of Cytology, Institute of Zoology, Bulgarian Academy of Sciences. They have been used as test objects for a number of studies of cellular proliferation and the mechanisms of its control [2, 6], and also for cardiologic [4] and immunologic research [3].

In the course of experiments conducted on different passages of AISM, certain growth characteristics were established, and these are described below.

EXPERIMENTAL METHOD

AISM was maintained in BALB/c mice by intraperitoneal inoculations of 10^6 tumor cells every 5 days. Males weighing 18-20 g were used. Mice were killed daily from the first day of inoculation until death of the animals, always at the same time of day (11 a.m.), and the volume of ascites fluid was measured by means of a syringe. The number of tumor cells per milliliter of ascites fluid was determined with a hemocytometer. The total mass of tumor cells was calculated on the basis of the number of tumor cells per milliliter of ascites fluid and its total volume. Films were prepared from the ascites fluid and stained with Schiff's reagent, and then covered with Ilford photosensitive emulsion. The films were exposed for 14 days in darkness at 4°C and then developed with amidol. The number of mitotic cells and of cells labeled with ^{3}H-thymidine was counted under the microscope and the relative percentage of multinuclear and giant cells also was counted under the microscope and the relative percentage of multinuclear and giant cells also was determined on the 5th day of tumor development. Methyl-^{3}H-thymidine (Chemapol) with specific activity of 925 GBq/mmole was injected intraperitoneally in a dose of 10 μCi/mouse 1 h before sacrifice. The mitotic index (MI) and labeling index (LI) of the cells were expressed in promille after examination of 3000 tumor cells. The investigation was conducted on the 35th and 117th passages of AISM in vivo. The numerical results were subjected to statistical analysis by the Student–Fisher test.

EXPERIMENTAL RESULTS

Growth curves of AISM in the two passages studied are shown in Fig. 1. Clearly the life span of the tumor-bearing mice differed in different passages. In the 35th passage the longest duration of survival of the mice was 8 days, whereas at the 117th passage, they survived for 12 days. It will also be clear that the total number of tumor cells in the terminal stage of life differed significantly in the two passages. In the 35th passage the total number of tumor cells reached about 10^8, whereas in the 117th passage it was more than ten times higher (1.2×10^9). According to data in [9], the final size of the cell population of ascites

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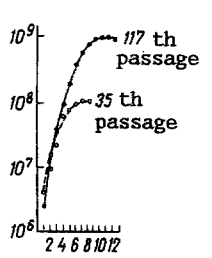

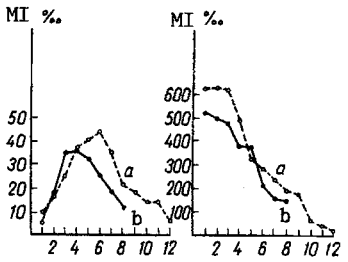

Fig. 1 Fig. 2

Fig. 1. Growth curves of 35th and 117th passages of mouse ascites tumor AISM in vivo. Abscissa, days after transplantation of tumor; ordinate, number of tumor cells in mouse peritoneal cavity.

Fig. 2. Changes in proliferative activity of mouse ascites tumor AISM during growth in vivo. Abscissa, days after transplantation of tumor; ordinate, MI and LI, in promille. a) 117th passage, b) 35th passage.

tumors grown in vivo depends on ploidy. It was shown previously [4] that the solid ISM tumor at the 46th passage was characterized by a near-tetraploid set of chromosomes. Karyologic studies by the same authors at the 36th passage of AISM revealed a decrease in the number of chromosomes in the modal class and the formation of a near-triploid set of chromosomes [4]. Our own investigations showed that the number of multinuclear and giant cells was 28% at the 35th passage, falling to 8% at the 117th passage. These findings show that during continuous reinoculation of AISM in vivo the chromosome set of the tumor population undergoes considerable changes. These changes probably are reflected in the total volume of cytoplasmic mass of the tumor and they may be the cause of differences in the extreme size of the tumor population in the two passages.

Changes in MI and LI in AISM during its development at the 35th and 117th passages are illustrated in Fig. 2. MI rose steadily from the 1st through the 4th day (35th passage) and from the 1st through the 6th day (117th passage), after which it gradually fell. The increase in MI corresponded in time to the exponential stage of tumor growth. LI was high already on the first days of tumor development, and it decreased in the course of growth. This difference in the time course of the two parameters of proliferation may probably reflect differences in the over-all temporal organization of proliferation, for DNA synthesis precedes mitosis chronologically. At the plateau stage the number of mitotic and DNA-synthesizing cells steadily decreased, indicating a general decrease in the number of proliferating cells in the tumor. We know that a decrease in the total number of proliferating cells in ascites tumors in the course of their development is due to emergence of cells from the G_1- and G_2-phases of the mitotic cycle and lengthening of the mitotic cycle as a whole [8]. A definite role in these processes is played by tissue-specific regulators of cell multiplication (chalones), which can arrest cells in the G_1- and G_2-phases of the cell cycle temporarily and reversibly. It was shown previously [1, 5] that chalones are produced in ascites tumors and that their content increases during growth of the tumor. The present writer recently showed that a 5-day AISM has a chalone system for controlling cellular multiplication, and that it obeys a circadian rhythm [6].

On the basis of the results, and also taking into account changes in the modal class of chromosomes of ISM during in vivo passage [4], it can be tentatively suggested that changes in ploidy of tumor cells during continuous retransplantation and chalone control of proliferation are factors limiting both the rates of growth and the size of the tumor cell population.

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EFFECT OF ORGANOFLUOROSILICON COMPOUNDS ON DEVELOPMENT OF CERTAIN TUMORS IN MICE

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Every year sees an increase in the number of publications on the synthesis of organosilicon compounds possessing various antitumor properties. The most interesting of them have been examined in a review [4]. Of all the different derivatives of organosilicon compounds preference is awarded today to silsesquioxanes of the $[O_{1;5}\ Si(CH)_{2n}CH(COOM)_2]$ type, in which n is a number from 0 to 4, and M = H denotes an alkali metal [3]. Injection of these compounds into mice with an Ehrlich's ascites tumor prolongs their survival by 1.5-2 times compared with the control [8]. Japanese workers have shown that silsesquioxanes containing a perfluorinated radical of the $[CF_3CF_2CF_220CF(CF_3)\ COO(CH_2)_3Si]_2\ O_3$ type are the most active and prolong the survival of mice by 2.5-3 times [9].

We have studied for the first time the action of organosilicon compounds containing a fluorinated radical of the $(CF_3CHX_1CHX_2)n - SiR^1R^2R^3$ type, where n = 3 $X_1 = X_2 = H$, $R^1 = OH$ [3], $R^1 = OH$ [4], when $n = 1$, $X_1 = X_2 = H$, $R^1 = R^2 = R^3 = OC_2H_5$ [1], $R^1 = R^2 = OC_2H_5$, $R^3 = CH_3$ [2], when $n = 3$, $X_1 = X_2 = H$, $R^1 = O\ (CH_2CH_2CF_3)_3$ [5], when $n = 1$ $X_1 = X_2 = H$, $R^1 = R^2 = R^3 = (OCH_2CH_2)_3N$ [6], and when $n = 1$, $X_1 = H$, $X_2 = Cl$, $R^1 = R^2 = R^3 = (OCH_2CH_2)3N$ [7], on the cytotoxic activity and on the development of a virus-induced Rauscher leukemia and tumors induced by MCh-11 cells, in mice.

EXPERIMENTAL METHOD

Cytotoxic activity was studied in experiments with human ovarian carcinoma cells (line CaOv) in culture. The indicator of activity was inhibition of incorporation of ^3H-thymidine into the cell DNA. The cells were cultured in a monolayer on medium 199 with 10% bovine serum. The substances were tested in concentrations of 5×10^{-4}, 1×10^{-4}, and 1×10^{-5} M. Exposure of the cells to the substances lasted 24 h, after which the cells were incubated for 1 h in medium with ^3H-thymidine (37 mBq/ml). After washing to remove radioactivity and after removal of the acid-soluble fraction from the cells by hydrolysis in 10% $HClO_4$ at 80°C

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