The results are evidence that the mitosis-inhibiting activity of EAC extract can be manifested in the absence of adrenalin. This is in disagreement with the results of an investigation by Cooper and Smith [3], who found that the action of EAC chalone is dependent on adrenalin. In their investigation the effects of the combined action of chalone and adrenalin was determined only 4 h after their addition, and this may perhaps have led them to draw the wrong conclusion. Meanwhile, as the results of the present investigation show, the combined use of EAC extract, containing chalone, and adrenalin modifies the character of their effect on mitotic activity in a culture of EAC cells. The reasons for this effect are not clear and require further study.

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CHANGES IN AUTOIMMUNE ANTIBODY LEVELS DURING GROWTH OF TRANSPLANTABLE TUMORS IN MICE

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UDC 616-006-092.9.092-07:616-008.9-097.5-078.73

KEY WORDS: syngeneic tumors; autoantibodies.

Much evidence has been obtained in clinical oncology to show that during tumor growth autoimmune antibodies, including antibodies reacting with blood cells, can be found in the patient. Antilymphocytic and antierythrocytic antibodies are often found in patients with hematogenous tumors. According to statistics given by various workers [13] autoimmune hemolytic anemias are observed in 14.3-82.6% of patients with lymphoid tumors, and their frequency is particularly high in chronic lymphatic leukemia. Antierythrocytic antibodies [7] were found in 23 of 120 patients with lymphogranulomatosis. In some cases autoimmune hemolytic anemias have been observed in patients with Kaposi's sarcoma [6]. Autoantibodies against blood cells are also found in a wide variety of nonhematogenous tumors: carcinoma of the ovary [1], adenocarcinoma of the cecum [3], carcinoma of the cervix uteri [10, 14], epithelioma of the stomach [9], and so on. In some cases autoimmune antibodies were found initially in these patients but later the tumor itself was found [1, 6, 8, 9]. After removal of the tumor the autoimmune symptoms as a rule cleared up. Several surveys dealing with relations between autoimmune syndromes and tumor growth have been published [4, 5].

The object of this investigation was to study the dynamics of appearance of autoimmune antibodies during growth of various syngeneic transplanted tumors in mice.

EXPERIMENTAL METHOD

Experiments were carried out on mice of the CBA and 129 lines aged 4-5 months. The following syngeneic tumors were used: carcinoma of the cervix uteri RShM-5 at the 8th passage (the recipients were CBA females) and strains of teratocarcinomas maintained by a group under

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(Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 2, pp. 204-206, February, 1981. Original article submitted May 20, 1980.

0007-4888/81/9102-0195$07.50

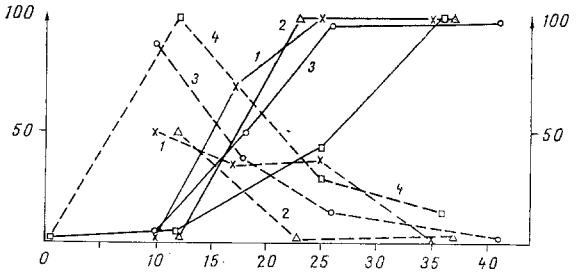

Fig. 1. Dynamics of appearance of autoantibodies in mice during growth of transplanted tumors (results of 4 experiments). Ordinate: left — percentage of mice with autoantibodies, right — percentage of mice with palpable tumors. Abscissa, time after inoculation of tumor (in days); 1) carcinoma of the cervix uteri (recipients CBA mice); 2) teratoblastoma TB-3 (recipients CBA mice); 3) teratoblastoma TB-8 (recipients mice of line 129); 4) teratoblastoma TB-24 (recipients mice of line 129). Broken lines shows percentage of animals with autoantibodies. Continuous lines show percentage of animals with palpable tumors.

V. M. Senin's direction: TB-3 8th passage (recipients CBA males), TB-24, 7th passage (recipients 129 males), and TB-8, 5th passage (recipients 129 females). Tumor tissue was shredded by extrusion through a Kapron sieve, suspended in Hanks' solution, and injected into recipients in a dose of 0.3 ml of a 5-10% suspension. The TB-3 and TB-8 tumors were injected intramuscularly and the TB-24 and RShM tumors subcutaneously. Each experimental group consisted of 7-12 mice and the control group of the same number of intact animals from the same pool. At various times after inoculation of the tumor blood was taken from the orbital sinus and the serum was frozen and kept at —20°C. Sera from each experiment were thawed and tested as far as possible at the same time. The indirect Coomb's test was used, and goat antiserum against mouse γ-globulin (from Miles-Jeda), diluted with physiological saline 16-20-fold, served as the antiglobulin reagent. The test was carried out in Takachi plates or Falcon 3034 microplates. Erythrocytes of intact syngeneic mice were washed 4 times with physiological saline and a 1% suspension was prepared. An equal volume of test serum (or of a dilution of it in physiological saline) was added to the erythrocyte suspension and the mixture incubated for 1 h at 37°C; antiglobulin serum was then added and incubation repeated, after which the reaction was read. Each test was carried out in duplicate or triplicate. The results were subjected to statistical analysis by a modification of the chi-square test for small samples.

EXPERIMENTAL RESULTS

The results of experiments with the four tumor models are illustrated in Fig. 1. In all experiments autoimmune antibodies were found in the initial phase of tumor growth in the sera of some of the mice. During tumor growth the percentage of animals with the autoimmune component gradually decreased, and by the end of the experiment, when palpable tumors were present in all animals, it was practically impossible to find autoantibodies. Sera in the corresponding control groups, taken at the same times as those of the experimental mice, contained no autoantibodies against erythrocytes. Negative correlation between the appearance of a palpable tumor and the presence of autoantibodies in the serum is clearly demonstrated in Tables 1 and 2, where data for each mouse are given separately in the course of the experiment. In both cases the negative correlation was statistically significant ($P < 0.01$).

Two conclusions can be drawn from these results: 1) In the initial (without clinical manifestation) phase of tumor growth antibodies capable of reacting with intact syngeneic erythrocytes appear in the blood of mice receiving the tumors; during tumor growth, with the appearance of a palpable tumor, these antibodies are found in a much smaller percentage of cases or not at all; 2) the phenomenon is not unique for any one tumor and it is manifested in two lines of mice, differing in their H-2 haplotype, and in all four tumor models.

TABLE 1. Dynamics of Autoimmune Antibodies in Mice of Line 129 after Inoculation of Teratoblastoma TB-8 (5th passage)

No. of days after inoculation of tumor (time of taking blood)	Nos. of mice							
	1	2	3	4	5	6	7	8
10	+/—	+/—	+/—	+/—	+/—	+/—	—/—	+/—
18	+/—	—/+	—/+	+/+	+/—	—/—	—/+	—/—
26	+/+	—/+	—/+	—/+	—/—	—/+	—/+	—/+
41				—/+	—/+	—/+	—/+	—/+

Legend. Here and in Table 2: ±/± — presence (absence) of autoimmune antibodies in animal's serum/presence (absence) of palpable tumor.

TABLE 2. Dynamics of Autoimmune Antibodies in Mice of Line 129 after Inoculation of Teratoblastoma TB-24 (7th passage)

No. of days after inoculation of tumor (time of taking blood)	Nos. of mice						
	1	2	3	4	5	6	7
0	—/—	—/—	—/—	—/—	—/—	—/—	—/—
12	+/—	+/—	+/—	+/—	+/—	+/—	+/—
25	—/+	+/—	—/+	—/+	—/—	+/—	—/—
36	—/+	+/+	—/+	—/+	—/+	—/+	—/+

According to preliminary data, the autoantibodies discovered are not simply antitissue (i.e., reacting with any erythrocytes), for in some cases the sera were line-specific and reacted only with erythrocytes of syngeneic mice or of mice of not all the tested lines. This is a problem for further investigation.

Despite the fact that a connection between autoimmune syndromes and tumor growth is assuming ever-increasing importance in the eyes of oncologists, no single theory has yet been put forward to explain the many phenomena of this type. The principal existing explanations can be reduced to three groups: I) During tumor growth there is a general disturbance of immunologic homeostasis which can lead to autoimmune reactions [12]; II) the organism reacts to a combination of its own antigens and of tumor-specific transplantation antigens, as a result of which the autoimmune components of the reaction arises [14, 15]; III) tumor-specific antigens are the normal antigens of the given organism, but during tumor growth they are present ectopically, and consequently they induce an immune response to themselves. The appearance of a tumor may be preceded by a prolonged autoimmune process, and the tumor develops only after this protective reaction of the host has been overcome [5].

Without entering into discussion with the supporters of these theories, the following hypothesis can be proposed to explain the results of the present experiments. It is now known that antigens foreign for H-2 haplotypes of a given line are expressed on cells of many mouse tumors [2, 11]. Probably in the "preclinical" phase of tumor development the mouse responds to it by producing antibodies that cross-react with these "foreign" antigens and with antigens of its own H-2 complex. In the presence of a large tumor nodule the antibodies may be completely adsorbed by the tumor tissue. Such antibodies may both interfere with elimination of tumor cells and the surrounding normal tissue, thereby strengthening the autonomy of the tumor. According to our hypothesis, after removal of the tumor the antibodies gradually disappear but they may reappear in the preclinical phase of recurrence or during metastasis. Verification of this last hypothesis may make possible the early diagnosis of recurrence of tumors by a method that is technically simple and which is the same for tumors of different origin. Investigations in this direction are proceeding.

The authors are grateful to V. M. Senin for constant interest in and help with the work.

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EFFECT OF SOME CHEMOTHERAPEUTIC AGENTS ON DNA SYNTHESIS AND DISTRIBUTION IN TRANSPLANTABLE HUMAN GASTROINTESTINAL TUMORS

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UDC 615.277.3.015.42:616.3-006-008.939.633.2

KEY WORDS: DNA; chemotherapeutic agents; gastrointestinal tract.

To determine the mechanism of action of chemotherapeutic agents the study of cell kinetics by quantitative methods is widely used at the present time. With the appearance of continuous flow cytophotometry and B-spectrometry it is now possible to undertake mass cytological studies of tumors (A. S. Petrova). However, investigations of human tumor cell populations are conducted on biopsy material, which cannot give a complete answer to the question of the character and distribution of cells in a tumor nodule. An interesting model from this point of view is that of a human tumor transplanted into an athymic mouse. Although during the last ten years many investigators have studied transplantation of human tumors into athymic mice, so far only paper [6] on cell kinetics has been published. The reason may be difficulties in obtaining tumor strains with standard growth parameters.

The writers previously studied the duration of phases of the cell cycle, the size of the proliferative pool, and the character of ploidy in twelve original human tumor strains with stable growth characteristics. On the basis of the patterns of cell kinetics of human transplanted tumors thus revealed it was decided to study the effect of some widely used clinical chemotherapeutic agents on the cell kinetics.

The aim of the present investigation was to use cytophotometry and B-spectrometry to study the synthesis and distribution of DNA in cells of strains of human gastrointestinal tumors transplanted into athymic mice.

EXPERIMENTAL METHOD

Human strains of carcinoma of the large intestine (RTK-1 and RTK-2), carcinoma of the stomach (RZh), and carcinoma of the liver (RPech) were transplanted subcutaneously into

Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 2, pp. 206-208, February, 1981. Original article submitted July 23, 1980.

 0007-4888/81/9102-0198$07.50