In the course of culture of the cells of a dimorphic undifferentiated adenocarcinoma and squamous-cell carcinoma of the human lung, growth of cells of the undifferentiated HLA predominated, with gradual spontaneous differentiation of the tumor into a highly differentiated HLA. Cells of the highly differentiated HLA, incidentally, exhibited considerable ability to agglutinate under the influence of lectins; they were found to have high dehydrogenase and hydrolase activity, so that it is not possible to speak definitely of the true reversion of these cells.

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INHIBITION OF TUMOR GROWTH IN SYNGENEIC MICE FOLLOWING PROCEDURES AFFECTING T LYMPHOCYTE ACTIVITY

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It is now increasingly evident that responses of the immune system of the host to tumor growth affect different levels. Processes mediated by T lymphocytes may suppress or, in general, eliminate a tumor, and they may also stimulate tumor growth and metastasization. Immunodepressive influences in some cases accelerate, in others inhibit tumor growth [4]. According to abundant evidence, the principal role in suppression of antitumor immune responses of the body is played by a subpopulation of T lymphocytes, namely T suppressors. These cells may specifically and nonspecifically suppress the response to various antigens, including tumor-specific antigens [11, 12]. It must be emphasized that these results were obtained mainly by the use of immunogenic tumors, i.e., tumors against which transplantation immunity can be induced in syngeneic animals [4]. Yet the majority of "truly spontaneous" tumors in mice and rats and also, evidently, many human tumors are weakly immunogenic in a syngeneic system [8, 9].

The aim of this investigation was to study the effect of factors modifying the immune system on growth of a spontaneous, weakly immunogenic tumor in a syngeneic mouse system.

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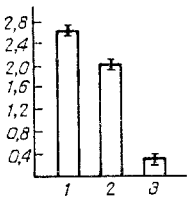

Fig. 1

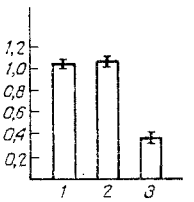

Fig. 2

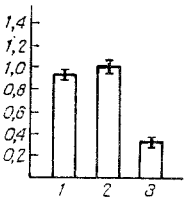

Fig. 3

Fig. 1. Inhibition of growth of Akatol tumor in B mice. 1) Control of tumor growth (2.657 ± 0.112 g); 2) tumor growth in B mice inoculated with SC from NM (2.082 ± 0.062 g); 3) tumor growth in B mice (0.326 ± 0.024 g). $P_{1-3} < 0.005$, $P_{2-3} < 0.005$, $P_{1-2} > 0.05$. Here and in Figs. 2 and 3, ordinate, weight of tumor (in g).

Fig. 2. Inhibition of growth of Akatol tumor in mice thymectomized in the adult state. 1) Control of tumor growth (1.076 ± 0.063 g); 2) tumor growth in mice after mock operation (1.098 ± 0.038 g); 3) tumor growth in thymectomized mice (0.333 ± 0.027 g). $P_{1-3} < 0.005$, $P_{2-3} < 0.005$.

Fig. 3. Effect of preliminary immunization with SC of TBM on growth of Akatol tumor. 1) Control of tumor growth (0.960 ± 0.030 g); 2) tumor growth in mice immunized with SC of NM (1.014 ± 0.051 g); 3) tumor growth in mice immunized with SC of TBM (0.334 ± 0.039 g). $P_{1-3} < 0.005$, $P_{2-3} < 0.005$, $P_{1-2} > 0.05$.

EXPERIMENTAL METHOD

Male BALB/c mice aged 2 months, obtained from "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. An Akatol tumor (a spontaneous adenocarcinoma of the large intestine obtained as a result of malignant transformation of a syngeneic ectopic graft of embryonic intestine) was obtained from the Laboratory of Tumor Strains, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, and transplanted subcutaneously in syngeneic mice. The tumor used was at 9th-15th passage. Tumor tissue was minced by pressing it through a metal sieve, filtered through three layers of gauze, and the number of living cells was determined after staining with trypan blue. The thymus was removed from the animals by the vacuum method [10] and the wound closed with a skin graft and sealed with BF-6 glue. The completeness of removal of the thymus was verified at autopsy on the mice after the end of the experiment. To obtain B mice, 20 days after the operation the thymectomized animals were irradiated in a dose of 850 rads ("Stebel'" source, mean dose rate 630 rads/min); 2 h later the mice were given an intravenous injection of 15×10^6 syngeneic embryonic liver cells. The tumor, in a dose of 5×10^6 cells, was transplanted subcutaneously on the 8th day after irradiation. Individual groups of B mice, 2 h before transplantation of the tumor, were given an intravenous injection of 5×10^7 spleen cells (SC) of intact syngeneic mice. During immunization with SC, normal mice (NM) were given two intraperitoneal injections, each of 10^8 SC, obtained from tumor-bearing mice (TBM) 15 days after inoculation of the tumor cells, at intervals of 21 days. SC from NM were injected into animals of the other group. The mice were injected subcutaneously with 3×10^6 tumor cells 7 days after the last immunization. At the same time blood was taken from the retro-orbital sinus of some of the mice and activity of the serum was tested in the two-stage complement-dependent cytotoxic test against SCof NM and TBM [2]. The cytotoxic index (CTI) was calculated by the formula $(a - b)/a \times 100\%$, where a is the percentage of living cells in the control and b the percentage of living cells in the experiment.

In each experimental group there were at least 10 mice. The animals were killed on the 20th day of the experiment and the tumor was weighed. For statistical analysis of the results Student's t test was used.

EXPERIMENTAL RESULTS

To study the immunogenicity of the tumor, 10^6 tumor cells were injected into a hind limb muscle of the mice, and 17 days later the limb with the tumor was removed, and a further 7 days later the animal was injected with the same dose of the tumor subcutaneously. The immunized mice were indistinguishable from intact recipients as regards the time of appearance of palpable nodules (6th-7th day) and the time course of growth of the tumor. Similar results were obtained previously [1]. With this experimental scheme, the tumor thus behaved as weakly immunogenic.

The results of two analogous experiments to study tumor growth in B mice are illustrated in Fig. 1. Tumor growth in B mice was sharply inhibited compared with that in NM. Inoculation of B mice with SC of NM restored tumor growth almost to its level in NM. This stimulation of tumor growth was due, in our opinion, to the introduction of mature T lymphocytes, which are absent in B mice. Inhibition of the tumor in B mice can be explained by the action of macrophages and of natural killer cells, which play an important role in antitumor resistance [13].

Activity of T suppressors falls gradually after thymectomy without any additional procedures [3]. In the present investigation adult mice were thymectomized, and 3 months later they were injected subcutaneously with tumor cells in a dose of 3×10^6. The results of these experiments are shown in Fig. 2. At sacrifice on the 20th day the weight of the tumor was much less in thymectomized mice than in intact mice. Mock thymectomy did not affect tumor growth.

There is some evidence in the literature that injection of a sufficiently large number of antitumor T killer cells into NM causes the formation of specific T suppressors, capable of inhibiting generation of T killer cells *in vitro* on contact with cells of the same tumor. In the opinion of the authors concerned, this phenomenon can be explained by immunization of the mice against the corresponding population of T killers [6]. In the present experiments (Fig. 3), immunization of mice with SC of TBM caused significant inhibition of growth of the subsequently transplanted tumor. Growth of the tumor in mice immunized with SC of NM was indistinguishable from the control. Serum of mice immunized with SC of TBM exhibited cytotoxic activity against SC of TBM in a dilution of 1:16 (CTI = 16%), but not against SC of NM. Serum of mice immunized with SC of NM did not react in either case.

It can be concluded from these results that powerful factors of nonspecific resistance to growth of a syngeneic Akatol tumor are present in BALB/c mice, but the presence of an intact mature immune system actively interferes with the manifestation of these factors. Tumor cells, despite their weak immunogenicity, are probably sufficiently antigenic for the body and induce an active immune reaction, expressed as induction of T suppressors. Thymectomy of adult mice weakens the possibility of such induction, while leaving the functions of other subpopulations of T lymphocytes undisturbed for a long time. On injection of SC of TBM into mice, in our opinion immunization takes place against T-suppressors induced by the tumor, and for that reason inoculation of such mice with the tumor does not lead to induction of T suppressors, and tumor growth is correspondingly inhibited. Tumor growth can thus be inhibited by immunological procedures without in any way damaging the immune system as a whole.

Immunogenicity of the tumor evidently does not necessarily correlate with its antigenicity. Immunogenicity is determined by a shift of the balance toward induction of T suppressors, facilitating tumor growth by various ways, or toward T killers and other antitumor effector cells [5, 7]. A basic trend in modern immunotherapy of tumors is immunization with tumor antigen in one form or other, after removal of the main mass of tumor tissue. Meanwhile the presence of weakly immunogenic tumors presupposes the low effectiveness of such immunization. The study of the possibility of autochthonous immunization of the recipient with T suppressors may lead to a new approach in immunotherapy, unconnected with the search for specific tumor antigens. The quest for schemes of immunotherapy of weakly immunogenic tumors aimed at suppression of T suppressors induced by the tumor would seem to be a promising development.

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