COMPARATIVE STUDY OF THE IMMUNOLOGIC RESPONSE TO INJECTION OF TUMOR EXTRACT INTO NEWBORN AND ADULT ANIMALS

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Preliminary injection of tumor extract into newborn mice leads to acceleration of growth of hepatoma 22a but in adult animals it gives an immunizing effect. These differences are evidently connected with changes in the immunologic response to the tumor, for when the dynamics of cellular immunity were studied, judging from the inhibition of migration of macrophages, the lymphocytes of mice into which antigen was injected in the adult period only possessed the ability to inhibit migration of the macrophages considerably.

KEY WORDS: antitumor immunity; hepatoma 22a; immunologic tolerance.

According to data in the literature newborn animals are more sensitive to the action of carcinogenic substances [4, 5, 7] and oncogenic viruses [8]. This is probably due to certain distinguishing features of the immune system in the newborn [1]. Experiments by Hašek et al. [2] using benzpyrene-induced tumors showed that injection of tumor antigens into mice in the neonatal period weakens immunity to the same tumor in mice in the adult state. However, the authors cited did not study whether this was connected with changes in the immunologic response of the animal to the tumor.

In the investigation described below a comparative study was made of the rates of growth of the tumor and the dymanics of immunologic responses in animals receiving an injection of tumor extract in the neonatal or adult period.

EXPERIMENTAL METHOD

Experiments were carried out on newborn and adult C3HA mice receiving a preliminary injection of extract of hepatoma 22a containing 5 mg protein in 1 ml. The animals were divided into 3 groups with 30 mice in each group. The newborn mice of group 1 received an injection of 0.05 ml antigen on the 1st, 2nd, and 3rd days after birth. Adult (aged 8 weeks) mice of group 2 received 3 injections of the antigen, each of 0.5 ml, on alternate days. At the age of 10 weeks all the mice were inoculated intraperitoneally with 20,000 hepatoma cells. Mice aged 10 weeks (group 3), into which the tumor was grafted without preliminary treatment, served as the control.

Later a comparative study was made of the rates of growth of the tumor in the inoculated mice. In addition, during growth of the hepatoma the immunologic response to the tumor was studied in mice by the inhibition of macrophagal migration test as carried out by the method of Bloom et al. [6] in Poupon's modification [9], so that interaction between cellular and humoral factors of immunity could be investigated at different times after inoculation of the hepatoma. In the modification used, macrophages from normal animals, migration of which is inhibited in the presence of sensitized lymphocytes and hepatoma cells, were used.

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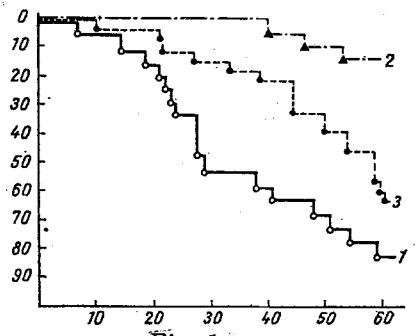

Fig. 1

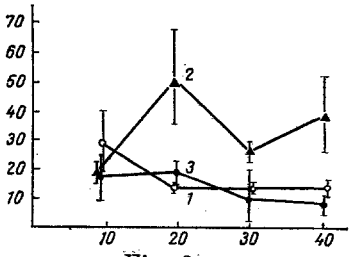

Fig. 2

Fig. 1. Comparative study of rates of growth of hepatoma 22a in mice after preliminary injection of antigen in neonatal period and in adult state: 1) antigen injected in neonatal period; 2) antigen injected into adult mice; 3) without injection of antigen. Abscissa, days after inoculation of hepatoma; ordinate, percentage death of mice.

Fig. 2. Reaction of inhibition of macrophagal migration in mice after preliminary injection of tumor extract in neonatal period and in adult state: 1) antigen injected in neonatal period; 2) antigen injected into adult mice; 3) no antigen injected. Abscissa, days after inoculation of hepatoma 22a; ordinate, percentage inhibition of macrophagal migration.

Peritoneal macrophages were isolated from the peritoneal cavity of intact mice 72 h after intraperitoneal injection of 2 ml 5% Difco peptone in Hanks' solution. Spleens of experimental and control mice were used as the source of sensitized lymphocytes. A mixture consisting of equal volumes (0.2 ml) of suspensions of the following cells was prepared for the test: macrophages $40 \cdot 10^6$/ml, spleen cells $20 \cdot 10^6$/ml, and tumor cells $10 \cdot 10^6$/ml. Capillary tubes (0.6 mm in diameter, 70 mm long) were filled with this mixture and then incubated at 37°C in chambers with medium No. 199 containing 15% normal mouse serum or serum of the experimental mice. The reaction was read after 24 h; the zones of migration of the cells were drawn on paper by means of a drawing apparatus, cut out, and weighed. The percentage inhibition of macrophagal migration was calculated by the equation:

$$\text{Percentage inhibition of migration} = 100\,\% - \frac{\text{Mean weight of zones of migration in experiment}}{\text{Mean weight of zones of migration in control}} \times 100\%.$$

Inhibition was regarded as significant if the degree of inhibition of migration exceeded 20% [6].

In order to study changes in the immune status of the animals on the 10th, 20th, 30th, and 40th days after inoculation of the tumor, groups of 3 mice were sacrificed and no fewer than 15 capillary tubes were tested on each mouse.

EXPERIMENTAL RESULTS

To detect differences in the rates of growth of the tumors in the experimental and control mice a comparative study was made of the times of their death, using the test of 50% mortality of the animals as criterion of the differences. As is clear from Fig. 1, the greatest rate of growth of the hepatoma was observed in mice receiving an injection of tumor extract in the neonatal period (group 1): 50% of the mice in this group died by the 27th day. In the control group (3) only 14% of the animals had died by this time, whereas the mice of group 2, receiving antigen in the adult state, were all alive. At the time of 50% death of animals in the control group (56th day) 80% of the mice in group 1 were already dead, but the number of dying mice in group 2 at this time did not exceed 13%.

Preliminary injection of tumor extract into newborn mice thus led to acceleration of the rate of growth of the inoculated hepatoma. Meanwhile, injection of the tumor extract into adult mice led to an immunizing effect.

Definite differences were observed in the immunologic reaction of mice to the tumor as reflected in the inhibition of macrophagal migration tests. As Fig. 2 shows, in mice receiving a preliminary injection of tumor extract in the adult state definite inhibition of macrophagal migration was observed (51.2%). The degree of inhibition of migration depended on the rate of growth of the tumor: inhibition was greatest on the

20th day after inoculation, after which the level of immunologic responses of the animals fell somewhat. Spleen cells from mice into which the antigen was injected in the neonatal period not only had less ability to inhibit macrophagal migration (28.9%), but this inhibition was observed only on the 10th day after inoculation of the tumor. The level of inhibition of macrophagal migration in the control, as Fig. 2 shows, did not exceed 20%. Addition of the serum of mice receiving the antigen in the neonatal period to the incubation medium abolished the effect of macrophagal inhibition, but in the later periods of growth of the tumor the serum actually stimulated migration. By contrast to this, the serum of mice receiving antigen in the adult state had no blocking action on the lymphocytes. Hence, in mice receiving tumor extract in the neonatal period the immunologic response to the tumor was reduced, as shown by an increase in the rate of growth of hepatoma 22a, by the reduced ability of the lymphocytes to inhibit macrophagal migration, and by the appearance of factors blocking the action of immune lymphocytes in the blood stream.

These results are not only important for the study of differences in the immunologic response to the tumor in mice into which the tumors were injected in the neonatal period and in the adult state, but they are also of definite interest in connection with the study of the role of immunologic tolerance in malignant growth [3].

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