DIFFERENCES IN RESPONSE OF THE HEMATOPOIETIC SYSTEM OF NEWBORN AND ADULT MICE TO GROWTH OF SYNGENEIC HEPATOMA H-2-73

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UDC 616.36-006-092:[616.411-007.61+616.155.32]-053

KEY WORDS: newborn and adult mice; hepatoma; splenomegaly; stimulation of tumor growth.

Some syngeneic transplantable tumors grow more slowly in newborn than in adult mice [3, 4]. Tumors grow slowest of all when transplanted into recipients aged 1-6 days. The reasons for the difference in the rate of growth of tumors in animals of different ages are not yet clear. Differences in the maturity of the immune system in newborn and adult recipients of tumor cells may play a definite role.

To test this hypothesis the investigation described below was undertaken.

EXPERIMENTAL METHOD

Experiments were carried out on 136 newborn (1-4 days) and 148 adult (8-12 weeks) mice [(CBA × C57BL/6j)F_1, abbreviated subsequently to CBAB6F_1] of both sexes, obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. Hepatoma H-2-73 [2], at the 7th-10th passages, was transplanted subcutaneously or intraperitoneally as a suspension of single tumor cells, obtained by trypsinization [13], in a dose of $5 \cdot 10^5$ or $1 \cdot 10^6$ cells per mouse. The donors of the suspension of nucleated spleen cells, prepared as described previously [1], were adult female CBAB6F_1, either intact or 11 and 15 days after subcutaneous inoculation with 0.2 ml of a coarse suspension of hepatoma cells.

To perform the local neutralization test as described in [12] spleen and tumor cells were mixed immediately before injection into mice in the ratio of 50:1; newborn and adult recipients were inoculated intraperitoneally with 0.05-0.1 ml of the mixture. Mice of the control groups received tumor cells only. The mice were killed on the 10th-11th days after transplantation by cervical dislocation and the tumor was weighed.

To study the reaction of the hematopoietic tissue to growth of hepatoma H-2-73, the newborn and adult mice were given a subcutaneous injection of 0.1 ml of coarse suspension of tumor tissue obtained by means of a metal mincer. Intact mice of the same age served as the control. The spleen, thymus, and tumors were weighed and the state of hematopoiesis determined with respect to several parameters, on the 3rd-4th, 7th, 14th, and 21st days in 4-6 mice of each group. The leukocytes in 1 µl peripheral blood were counted and the leukocyte formula calculated. The absolute number of individual forms of leukocytes in 1 µl blood was calculated. The qualitative composition of the cells was determined in squash preparations from the spleen. Blood films and squash preparations from the spleen were stained with azure-eosin. The differential count was carried out on 100 cells in blood films and 1000 cells in splenic preparations. The results were subjected to statistical analysis by Student's t test at the $P \leq 0.05$ level of significance.

EXPERIMENTAL RESULTS

Hepatoma H-2-73 is one of those tumors which grow more slowly in newborn than in adult mice (Fig. 1a). The experimental results showed a number of significant differences in the response of the hematopoietic tissue of newborn and adult recipients to growth of this tumor.

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten'Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 90-92, June, 1982. Original article submitted June 22, 1981.

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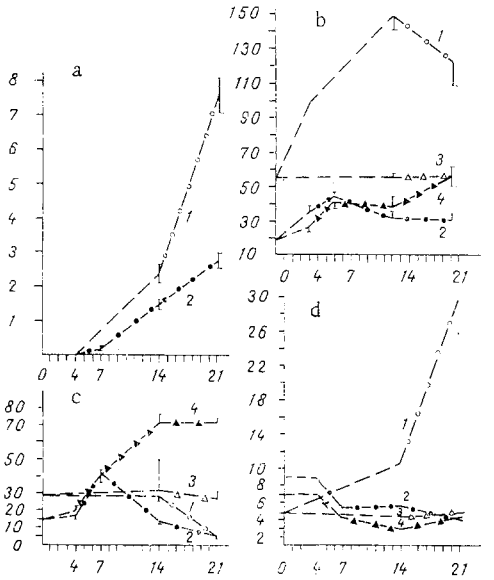

Fig. 1. Changes in hematopoietic organs accompanying growth of hepatoma H-2-73 in newborn and adult CBAB6F$_1$ mice. Abscissa, days after inoculation of tumor. 0) Time of inoculation of tumor; ordinate: a) weight of tumor (in g), b) weight of spleen (in mg), c) weight of thymus (in mg); d) number of leukocytes in 1 µl blood ($\cdot 10^3$). Each point represents mean value of parameter for group at corresponding times of tumor development. Vertical lines show standard error. 1) Adult mice inoculated with hepatoma; 2) newborn mice inoculated with hepatoma; 3) adult intact mice; 4) newborn intact mice.

In the adult animals, as the tumor grew the weight of the spleen increased by 2-2.5 times compared with its initial value. Splenomegaly did not develop in the newborn mice. Moreover, the increase in weight of the spleen connected with growth of the animal did not occur in newborn mice inoculated with tumor cells until the 7th day after transplantation, after which the weight of the spleen decreased, so that on the 21st day of observation it was about half the weight of the spleen in the control intact newborn and adult mice and about one-quarter of its weight in adult animals with hepatoma (Fig. 1b).

The study of the cell composition of the spleen showed that the absolute number of lymphocytes was greatest in adult mice with tumors. Meanwhile as the tumor grew the weight of the thymus fell considerably in both newborn and adult animals (Fig. 1c). Growth of the tumor in adult mice was accompanied by the development of leukocytosis, whereas in the newborn mice the number of leukocytes in the blood was virtually unchanged (Fig. 1d). The number of monocytes and polymorphs in newborn mice with tumors was significantly greater than in intact mice of the same age (Fig. 2a, c). The number of lymphocytes, which increased with age in the blood of the intact animals, decreased in newborn mice inoculated with hepatoma (Fig. 2b). Growth of the tumor in the adult mice led to an increase in the number of all the above-mentioned types of cells in the blood (Fig. 2). The most significant differences were thus found in the reaction of the spleen to growth of the hepatoma in newborn and adult animals.

In recent times interest in the role of the spleen in the response of the body to tumors has increased considerably [2, 5, 6, 9-11]. It has been shown [9, 11] that splenomegaly in animals with progressively developing tumors coincided in time with increased ability of the spleen cells to stimulate tumor growth, whereas removal of the primary tumor led to restoration of the normal weight of the spleen [11]. Splenectomy often increases resistance to

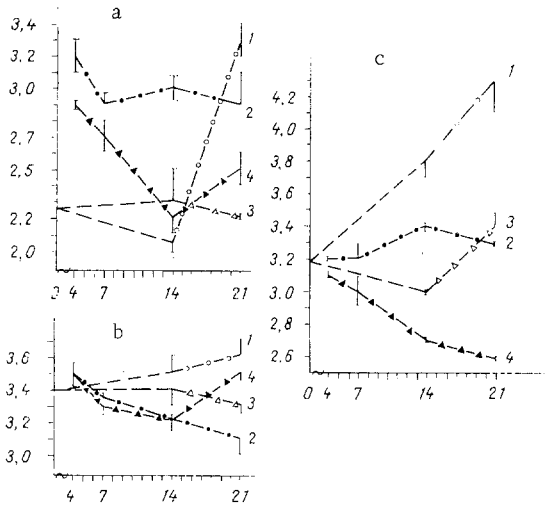

Fig. 2. Changes in cell composition of blood accompanying growth of hepatoma H-2-73 in newborn and adult CBAB6F$_1$ mice. Abscissa, days after inoculation of tumor. Ordinate: a) number of monocytes in 1 µl blood; b) number of lymphocytes in 1 µl blood; c) number of polymorphs in 1 µl blood. Each point represents log of mean value of parameter for group at corresponding times of tumor development. Remainder of legend as to Fig. 1.

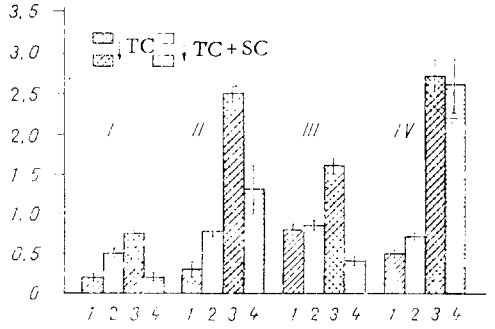

Fig. 3. Effect of spleen cells from adult mice with tumors on growth of hepatoma H-2-73 in newborn (1, 2) and adult (3, 4) mice in a syngeneic system. Ordinate, weight of tumor (in g). Height of each column represents mean weight of tumor in group ± standard error. TC) Tumor cells, SC) spleen cells of adult mice with tumors. I, II, III, IV) Nos. of experiments.

transplanted syngeneic tumors [2, 6, 11]. Accordingly it was interesting to discover how transfer of the spleen cells from adult animals with tumors would affect growth of the hepatoma in newborn mice. The results of these experiments are shown in Fig. 3.

Tumors in newborn mice receiving only tumor cells in a dose of $1 \cdot 10^6$ were significantly smaller in all cases than in the corresponding group of adult mice. Intraperitoneal injection of a mixture of $5 \cdot 10^7$ sensitized spleen cells and $1 \cdot 10^6$ tumor cells into newborn mice stimulated tumor growth in three of four cases compared with values observed in the control group of newborn mice (Fig. 3, experiments I, II, IV). Injection of this same cell pool into adult animals caused significant retardation of growth of the hepatoma in three of four cases (Fig. 3, experiments I, II, III). In the same test spleen cells from intact adult syn-

geneic mice did not affect the rate of growth of the hepatoma in the peritoneal cavity of the newborn recipients, but inhibited it in adult mice (the results are not shown).

These experiments thus showed that during development of hepatoma H-2-73, cells capable of causing acceleration of tumor growth appear in the spleen of the adult animal. Activity of this sort was manifested only when the spleen cells were transferred into newborn recipients. When a similar cell mixture was transferred to adult mice, it exhibited antitumor activity. Consequently, the end result in this system depends on interaction between the donor's cells and humoral or cellular factors of the recipient organism.

The necessity for interaction between the recipient's cells and donor's cells was demonstrated both by inhibition of tumor growth in Winn's test and also by its acceleration in the same test [9, 10]. The formation of cytotoxic cells directed against tumors is known to be accompanied by generation of suppressor cells. Relations between these two types of cells, on which the fate of the tumor depends, probably take place differently in immunologically immature newborn mice and immunocompetent adult mice. The next step must be to study the nature and mechanism of action of cells from adult tumor-bearing mice which cause acceleration of growth of the hepatoma in newborn mice.

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