# CHANGES IN RESISTANCE TO TUMOR DEVELOPMENT IN MICE OF DIFFERENT LINES IMMUNIZED IN THE NEONATAL PERIOD

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Depending on the genotype of an organism, changes in its functional systems as a result of exposure to various influences may reflect significant differences [4]. This is also reflected in the dynamics of antitumor resistance [3]. Since the organism is exposed to immunologic influences from birth, the aim of the investigation described below was to determine how this may affect antitumor resistance in animals of different genotypes.

## EXPERIMENTAL METHOD

The effect of immunologic influences in the neonatal period on resistance of the organism to the development of tumors induced by a chemical carcinogen was studied in mice of the C3H/He, C57BL/6, and BALB/c lines and noninbred mice, and also in C3H/He mice predisposed to develop mammary gland carcinoma, and also spontaneous tumors. Altogether 547 mice were used. The immunologic factors studied were BCG and Freund's complete adjuvant (FCA). The adjuvant used contained *Mycobacterium tuberculosis* (BCG strain) cells in a concentration of 10 mg/ml.

Half the mice from each litter, not later than 24 h after birth, were injected subcutaneously in the dorsal region with a BCG culture in a dose of 0.1 mg in 0.02 ml of physiological saline (experiments of series I) or with 0.02 ml of FCA (series II); the other mice received physiological saline only. Experimental and control mice were exposed to the carcinogen at the age of 2.5-3 months. The carcinogen (20-methylcholanthrene) was injected into the soft tissues of the thigh in a dose of 1 mg in 0.1 ml of vegetable oil. Mice in which dependence of the onset of spontaneous tumors on immunologic factors was studied received FCA only. The effectiveness of the factors used was judged from the time of appearance of tumors: induced — from the day of administration of the carcinogen; spontaneous — from the day of birth. Some animals in the series of experiments with FCA were killed so that the immunocompetent organs could be studied morphologically. Each group contained 10-14 mice.*

## EXPERIMENTAL RESULTS

Tumors induced by 20-methylcholanthrene in vaccinated C3H/He mice were more numerous at all times of observation than in control mice of the same line (Fig. 1). Differences in the yield of tumors between the experimental and control animals after 3.5, 4.5, and 6 months were statistically significant ($P < 0.01$, $< 0.02$, and $< 0.001$ respectively). The picture was different in the C57BL/6 mice. In the experimental mice of this line the yield of tumors was smaller than in the control animals: $P < 0.02$, time of observation 3.5 months.

Differences between C3H/He and C57BL/6 mice as regards changes in resistance to the action of the chemical carcinogen under the influence of BCG vaccination were significant. For times of observation of 3, 3.5, and 6 months $P < 0.05$, $< 0.05$, and $< 0.02$ respectively. Similar results were obtained in mice of this line receiving FCA (Fig. 2).

Fewer tumors appeared in the experimental group of C57BL/6 mice than in the control in the early stages of observation (3 months). Later the differences in the yield of tumors

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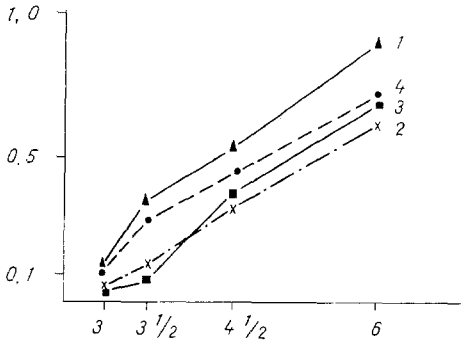


Fig. 1

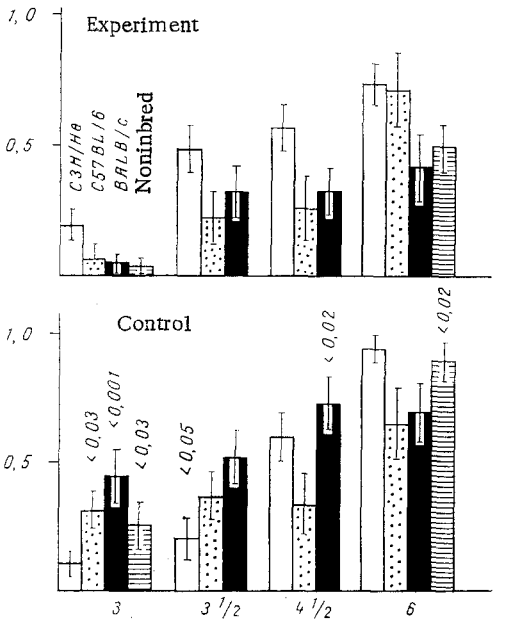


Fig. 2

Fig. 1. Yield of tumors in C57BL/6 and C3H/He mice vaccinated with BCG in the neonatal period. Here and in Fig. 2: abscissa, time after injection of carcinogen (in months); ordinate, ratio of number of animals with tumors to total number of mice in corresponding group. 1) C3H/He (experimental), 2) C3H/He (control), 3) C57BL/6 (experimental), 4) C57BL/6 (control).

Fig. 2. Yield of tumors in mice of different lines treated with FCA in the neonatal period. Unshaded columns — C3H/He, dotted columns — C57BL/6, black columns — BALB/c, horizontally shaded columns — noninbred mice. Value of P shown when differences between experimental and control mice of the same line are significant.

between the experimental and control mice of this line diminished and ceased to be statistically significant. At observation 6 months after administration of the carcinogen there was actually a tendency for more tumors to be formed in the animals of the experimental group. A significantly lower yield of tumors continued in the experimental BALB/c and noninbred mice at this time compared with the control animals.

In some C57BL/6 mice treated with FCA or BCG in the neonatal period, nutritional disorders developed (a wasting syndrome). These animals were excluded from the experiments. No nutritional disorders were found in mice of other lines.

Administration of FCA in the neonatal period accelerated spontaneous tumor formation in C3H/He mice. After 17 months tumors appeared in 12 of 23 mice in the experiment and in six of 22 mice in the control.

It will be noted that resistance to the development of tumors as a result of immunization was reduced in a line of mice which are carriers of oncogenic virus (NJV virus), which is transmitted vertically with the mother's milk. It was shown previously that if Rauscher leukemia virus is administered artificially together with FCA the resistance of the animal to this virus is reduced [5]. These data are important for the problem of so-called cancer families. The possibility cannot be ruled out that under certain conditions immunological procedures may facilitate the expression of malignant neoplasms in members of such families.

The thymus, spleen, and retroperitoneal lymph nodes of 6-week-old C57BL/6, C3H/He, and BALB/c mice treated with FCA in the neonatal period were examined morphologically. The organs were first weighed. The mean weight of the thymus in the experimental BALB/c mice was 73 ± 1.7 mg and in the controls 62 ± 2.8 mg; the corresponding figures for C57BL/6 mice were 63 ± 7 and 64 ± 8.3 mg and for C3H/He mice 53 ± 9.4 and 56 ± 8.2 mg. The mean weight of the spleen in the experimental BALB/c mice was 154 ± 13.5 mg and in the controls 147 ± 17.3 mg; the corresponding figures for C57BL/6 mice were 80.1 ± 6.5 and 75 ± 11.2 mg, and for C3H/He mice 89 ± 4.9 and 101 ± 3.5 mg.

In BALB/c mice treated with FCA an increase was observed in the relative proportion of medulla in the thymus, and in the lymph nodes there was very slight hyperplasia of the endothelium. No differences from the control were found in the spleen. In the experimental C57BL/6 mice the cortex of the thymus showed hyperplasia compared with the control and moderate plasmatization of the medulla. The splenic follicles were hyperplastic and large in size and they contained large pale cells. In certain areas the boundary of the follicles was indistinct and focal hyperplasia gave way to diffuse. No changes were found in the lymph nodes. There were no differences in the morphology of the thymus and lymph nodes in the experimental and control C3H/He mice. In the spleen of the experimental animals moderate hyperplasia of the follicles was observed, megakaryocytes were numerous, and germinative centers appeared.

The results of the morphological investigations are evidence that cellular immunologic reactions (especially thymus-dependent) dominate in the experimental mice, whose resistance to the development of tumors was increased, over components of reactivity which adversely affect antitumor defense. Morphologically this corresponds to hyperplasia of the cortex of the thymus (in C57BL/6 mice) and to an increase in the total weight of the thymus with hyperplasia of the endothelium of the lymph nodes (BALB/c mice), in the absence of changes characteristic of increased immunoglobulin production. By contrast with this, the decrease in resistance to tumor development in C3H/He mice was combined with the appearance of germinative centers in the spleen, which is characteristic of the production of antibodies (which may be of a type stimulating tumor growth).

These findings are in agreement with facts established previously: the ability of C57BL/6 mice to give marked local reactions of lymphoid type [4]; the earlier appearance of the second phase of the reaction to FCA in ontogeny (under thymus control) in these mice compared with C3H/He mice [1], and also the fact that the relative number of lymphocytes in the blood of C57BL/6 mice treated with FCA in the neonatal period was increased, whereas in C3H/He mice under the same conditions their number was reduced [2].

The data described above thus show that the hereditary features of an organism must be taken into account when the influence of immunologic procedures in the neonatal period, and also in the period of puberty [3], on the immunologic system and antitumor resistance is assessed. This is a matter of both theoretical and practical importance.

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