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CHANGES IN RESISTANCE OF MICE OF VARIOUS STRAINS TO TUMORS UNDER THE INFLUENCE OF IMMUNOLOGIC FACTORS

A. I. Volegov

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In experiments on mice with administration of a carcinogen in the early period after immunization (BCG vaccine, Freund's complete adjuvant) it was shown that an increase or decrease in the resistance of the animal to tumors may be determined by hereditary features. Antitumor resistance was reduced under these circumstances only in mice of strains in which a known predisposition to the formation of a state of allergy to tuberculin has been observed. In the late stages after immunization, when the intensity of the allergic component of reactivity was reduced, antitumor resistance was substantially higher than initially.
KEY WORDS: *heredity; immunologic factors; allergy; antitumor resistance.*

The nature of changes in the functional state of an animal after exposure to various conditions is largely determined by genetic factors [6, 7, 11, 12], and this must be reflected in the character of changes in antitumor resistance. The object of the present investigation was to study this problem after immunologic procedures.

EXPERIMENTAL METHOD

Experiments were carried out on mice of various lines (C3HA, C3H/He, BALB/c, C57BL/6) and noninbred mice, 519 animals altogether. The experimental animals were immunized at the age of 3-4 months. The immunizing agent used was BCG, either alone or as a constituent of Freund's complete adjuvant (FCA). The concentration of mycobacteria in the FCA was 10 mg/ml. BCG vaccine was injected intraperitoneally in doses of 0.01 or 1 mg in 0.2 ml physiological saline. The FCA was injected into the footpad of one hind limb in a dose of 0.02 ml. The carcinogen was administered 13 days after FCA or 30 days after vaccination. These times of administration of the carcinogen were based on the fact that they correspond to the state of the strongest response of the animal to the immunologic stimulus [1, 3, 9, 10].

Changes in resistance to the development of tumors also were studied in C57BL/6 mice in experiments in which the carcinogen was administered 70 days after FCA, when the immunologic response was mainly at an end and, in particular, antibody formation was on a reduced scale [3, 10].

In all series of experiments the carcinogen (20-methylcholanthrene) was injected into the soft tissues of the thigh in a dose of 1 mg in 0.1 ml purified vegetable oil.

Tuberculin tests were used as the control of changes in reactivity. Tuberculin was injected into a footpad of a hind limb in a dose of 0.02 ml of a solution containing 50,000 tuberculin units/ml. The reactions to tuberculin were recorded in points, and two degrees of intensity were distinguished: hyperemia of the footpad and part of the medial surface of the

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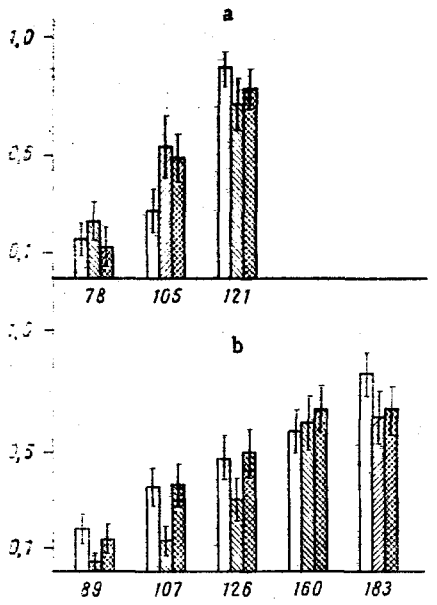

Fig. 1

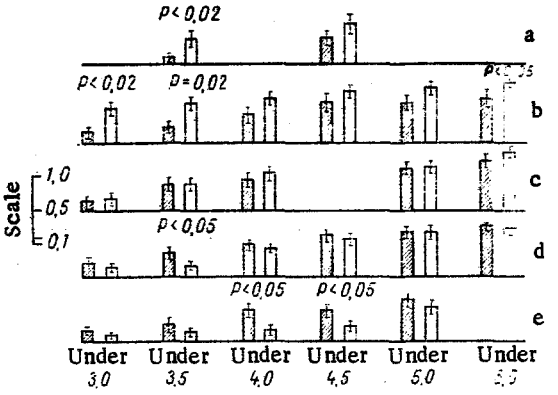

Fig. 2

Fig. 1. Tumor formation in C3H/He (a) and C57BL/6 (b) mice treated with carcinogen during response to FCA. Unshaded columns — control, shaded columns — injection of 0.01 mg BCG, cross-hatched columns — injection of 1 mg BCG. Here and in Fig. 2: abscissa, time of injection of carcinogen (in days); ordinate, yield of tumors (height of columns corresponds to ratio of number of animals with tumors to total number of mice in group).

Fig. 2. Yield of tumors in mice of different lines receiving carcinogen during response to FCA. a) Noninbred mice; b) BALB/c; c) C3HA; d) C57BL/6; e) C3H/He. Unshaded columns — control, shaded columns — experiment.

calf — 1 point; hyperemia of the footpad and of the whole calf with well marked signs of edema — 2 points. The results of the tuberculin tests were read 24 and 48 h after their performance.

EXPERIMENTAL RESULTS

In the C3H/He mice vaccinated with BCG in a dose of 0.01 mg tumors appeared significantly later than in the control animals of that group (Fig. 1). For times of observation of 89 and 107 days after injection of the carcinogen, $P < 0.04$. In C57BL/6 mice vaccinated with the same dose of BCG, on the other hand, compared with the control animals there was only a tendency (not significant) for the tumors to appear earlier (Fig. 1). Comparison of the differences in the direction of the changes in antitumor resistance in C3H/He and C57BL/6 mice for early periods of tumor formation (105 and 107 days) show that they were significant ($P < 0.01$).

With an increase in the dose of BCG to 1 mg in the experimental C3H/He mice, by contrast with the results described above, no slowing of tumor formation was observed (Fig. 1). Under analogous conditions, in the C57BL/6 mice, just as after administration of BCG in a dose of 0.01 mg, the tumors appeared earlier in the experiment than in the control. For times of observation of 107 days, $P < 0.05$.

The results obtained when the carcinogen was injected soon after FCA (in the acute period of the response) are shown in Fig. 2. In these experiments tumor formation was accelerated somewhat in the experimental C3H/He and C57BL/6 mice (3.5 and 4 months after injection of the carcinogen). During later observation the differences between the experimen-

tal and control groups disappeared. In the experimental C3HA mice no changes in antitumor resistance were observed. In BALB/c and noninbred mice preliminary injection of FCA delayed tumor formation (results statistically significant; see Fig. 2).

If the carcinogen was injected into C57BL/6 mice (18 experimental and 20 control mice) in the late period after injection of FCA, by contrast with the results described above obtained in mice of this strain, tumor formation was reduced in intensity, significant for all times of observation (109-166 days). For the later period (166 days) after injection of the carcinogen the ratio between the number of animals developing tumors and the total number of mice in the group was 0.30, compared with 0.92 in the control ($P < 0.01$).

The results of the tuberculin tests on the experimental mice 10 days after injection of FCA were as follows: The mean intensity of the reactions in the C57BL/6 mice after 24 h was 1.2 ± 0.16 point, in C3H/He mice 0.70 ± 0.12 point, BALB/c mice 0.45 ± 0.07 point, and in the noninbred mice 0.20 ± 0.12 point. After 48 h the intensity of the tuberculin test also was greatest in the C57BL/6 mice (0.44 ± 0.12 point) and least in the noninbred mice (0.05 ± 0.11 point). Differences in the intensity of the reactions after 24 h were significant between C57BL/6 and BALB/c mice ($P < 0.01$) and between C57BL/6 and noninbred mice ($P < 0.01$).

When tuberculin tests were carried out on C57BL/6 mice in the late period (60 days) after injection of FCA the mean intensity of the reactions after 24 and 48 h was 0.33 and 0.15 points respectively.

The results are evidence that the same immunologic procedure carried out on animals with different hereditary characteristics, all other conditions being the same, may lead to different, or even opposite changes in the resistance of the animal to the development of tumors. Meanwhile the direction of changes in antitumor resistance may be determined by the strength and timing of the immunologic procedure.

It is a noteworthy fact that in C57BL/6 and C3H/He mice in which the appearance of tumors was accelerated when the carcinogen was injected in the early period after injection of FCA, the response to tuberculin at these times was relatively high. In BALB/c and noninbred mice in which, under similar conditions, tumor development was retarded, allergic reactions to tuberculin were either absent or doubtful. The absence of papules in the region of development of the reactions and their rapid disappearance (within 48 h) are confirmation that they are mainly caused by the rapid component of allergic reactivity (i.e., by humoral antibodies).

After injection of the carcinogen into C57BL/6 mice at relatively long intervals of time after injection of FCA, the low level of allergic reactivity was accompanied, not by a decrease but, on the contrary, by a marked increase in the resistance of the animal to tumors. These results can be explained on the grounds that the allergic state of the animal may have an adverse effect on the mechanisms of its immunologic defense [2, 5, 6, 9]. In this connection it is an interesting fact that allergic antibodies, like antibodies stimulating tumor growth, are fixed to the cell surface, have a sedimentation constant of 7S, and do not fix complement.

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LOWER CARCINOGENICITY OF URETHANE FOR THE LUNGS OF ATHYMIC NUDE MICE THAN FOR IMMUNOLOGICALLY NORMAL MICE OF THE SAME LITTERS

V. I. Kaledin, E. V. Gruntenko, and T. S. Morozkova

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After two injections of urethane in a dose of 1 mg/g adenomas of the lungs developed in 2 of 10 nude mice and in 7 of 10 normal animals from the same litter. The mean number of adenomas per mouse was 0.2 ± 0.13 and 1.2 ± 0.36 respecitvely ($P < 0.05$).
KEY WORDS: *nude mice; urethane; immunologic surveyance.*

Mutant nude mice (nu/nu), characterized by congenital absence of the thymus, with consequent inability to form immunologic reactions of cellular type [4, 7, 10], provide a promising model for the study of the role of immunity in carcinogenesis. However, the short life span of these animals when kept under ordinary conditions (mean 3-4 months) makes it difficult to use them for comparison with normal mice with respect to the frequency of development of spontaneous tumors. They are more suitable for experiments with induced carcinogenesis. Several reports of the induction of tumors in nude mice by means of viruses and methylcholanthrene have already been published [10]. However, urethane was used for this purpose in only one investigation, despite its many advantages: solubility in water, comparatively low toxicity, highest activity after early postnatal administration, and so on. Tumors of the lungs induced by urethane can be detected macroscopically 1.5-3 months after injection. The multiple character of these tumors increases the reliability of the results, even when the number of animals used in the experiments is small. All this suggests that urethane carcinogenesis in athymic nude mice is a suitable model for testing Burnet's hypothesis [3] of the role of immunologic surveyance in carcinogenesis.

In the investigation described below an attempt was made to use this model for the above purpose.

EXPERIMENTAL METHOD

Experiments were carried out on nude (nu/nu) and immunologically normal (+/+ and +/nu) mice belonging to the same litters. The mice were obtained in 1972 from the Institute of Animal Genetics (Edinburgh, Scotland) and kept since that time in the animal house of the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, by mating heterozygous +/nu individuals. One quarter of the animals born under these circumstances have the nu gene in a homozygous state and are clearly distinguished from phenotypically normal +/+ and +/nu mice by the absence of hair. On April 15, 1975, urethane was injected intraperitoneally in a dose of 1 mg/kg into all the nude and an equal number of normal mice of the same sex in each of the 14 available litters. After 7 days a further injection of the same dose of carcinogen was given. The animals remained under observation until death of one of the partners (this was always a nude mouse), after which the other partner was immediately killed. The lungs were removed and fixed in 10% formalin. The adenomas were counted by examining the lungs under the MBF-1 loupe with a magnification of 16×. Altogether 10 pairs of mice were studied; 6 pairs in which the nude mice died earlier than 2 weeks after injection of the carcinogen were excluded.

Laboratory of Genetics of Cancer, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 574-576, November, 1978. Original article submitted March 20, 1978.

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