# EFFECT OF IMMUNIZATION WITH NORMAL SYNGENEIC TISSUES ON TUMOR INDUCTION IN MICE

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Immunization of C3HA mice under controlled syngeneic conditions with homogenates of the spleen and a mixture of the viscera (liver, kidney, spleen, lungs, heart) of mice of the same line and sex inhibits to some extent the development of tumors induced by intramuscular injection of 20-methylcholanthrene. A statistically significant increase of 2-3 weeks in the latent period of tumor development and of 2.5-4 weeks in the survival period of the immunized animals compared with the control was observed.

Immunization with normal syngeneic tissues has been shown to increase the resistance of C3HA mice appreciably to subsequent inoculation with hepatoma 22a [1].

This paper describes the results of experiments to study the effect of immunization with normal syngeneic tissues on tumor induction in mice of the same inbred line following intramuscular injection of 20-methylcholanthrene.

## EXPERIMENTAL METHOD

Male C3HA mice weighing 15-18 g were used in the experiments. Homozygosity of the line was verified by the transplantation test. The animals were immunized with homogenates of normal syngeneic tissues (liver, kidneys, spleen, skeletal muscles) and also with a mixture of certain viscera (liver, kidneys, spleen, lungs, heart) in equal proportions. The homogenates were prepared in 0.14 M NaCl solution before use, and a single dose of 10-20 mg tissue per mouse was given. Immunization was by intraperitoneal injection of the material together with an adjuvant, consisting of killed BCG vaccine in a dose of 0.05 mg per mouse. Before injection of the carcinogen the mice were immunized four times (the main cycle); the intervals between injections of the antigen were 3-4 days. Reimmunization was carried out in the next 3 months: three monthly series, each of one or two injections of the antigen. The control animals received the adjuvant only.

Tumors were induced with 20-methylcholanthrene. The compound was dissolved in a 1:3 mixture of benzene and sunflower oil and injected into the thigh muscles in a dose of 2.5 mg per mouse (in 0.2 ml of solvent) on the 5th day after the end of the main cycle of immunization.

## EXPERIMENTAL RESULTS

The mean latent period of tumor development in the mice of the control group receiving adjuvant only, based on the results of three experiments, was 9.6-12.4 weeks, and their mean survival time varied from 14.9 to 17.7 weeks (Table 1). In mice immunized with homogenates of the spleen and a mixture of the viscera there was a statistically significant increase of 2-3 weeks in the latent period of tumor development and of 2.5-4 weeks in the period of survival of the animals compared with the control (see experiments nos. 1 and 2); in experiment no. 3 similar results were not obtained; it is possible that this might be due to

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TABLE 1. Effect of Immunization with Normal Syngeneic Tissues on Induction of Tumors in C3HA Mice after Intramuscular Injection of 20-methylcholanthrene

| Expt. | Material for immunization and dose | No. of mice | Assessment of induction of tumors | | | |
|---|---|---|---|---|---|---|
| | | | latent period (in weeks) | P control–experiment | length of survival of mice (in weeks) | P control–experiment |
| 1 | Skeletal muscles: | | | | | |
| | 20 mg per mouse | 21 | 13,7±0,51 | <0,05 | 18,2±1,08 | >0,05 |
| | 10 mg per mouse | 25 | 12,2±0,31 | >0,05 | 17,4±0,67 | >0,05 |
| | Mixture of viscera: | | | | | |
| | 10 mg per mouse | 20 | 15,35±0,98 | <0,01 | 21,6±1,6 | <0,05 |
| | Control | 23 | 12,4±0,33 | | 17,7±0,61 | |
| 2 | Liver: 10 mg per mouse | 22 | 11,7±0,33 | >0,05 | 16,3±0,36 | <0,01 |
| | Kidneys: 10 mg per mouse | 20 | 11,8±0,38 | >0,05 | 15,6±0,43 | >0,05 |
| | Spleen: 10 mg per mouse | 24 | 13,0±0,47 | <0,001 | 17,4±0,59 | <0,001 |
| | Control | 23 | 11,0±0,31 | | 14,9±0,35 | |
| 3 | Skeletal muscles: | | | | | |
| | 20 mg per mouse | 15 | 10,4±0,45 | >0,05 | 15,7±0,37 | >0,05 |
| | Mixture of viscera: | | | | | |
| | 10 mg per mouse | 11 | 10,2±0,45 | >0,05 | 16,2±0,43 | >0,05 |
| | Spleen: 10 mg per mouse | 14 | 10,7±0,56 | >0,05 | 15,3±0,42 | >0,05 |
| | Control | 18 | 9,6±0,29 | | 15,4±0,59 | |

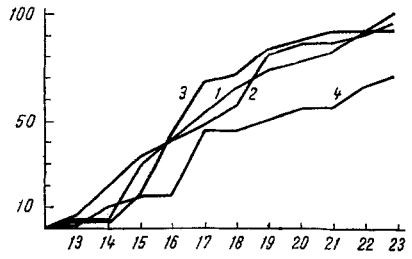


Fig. 1

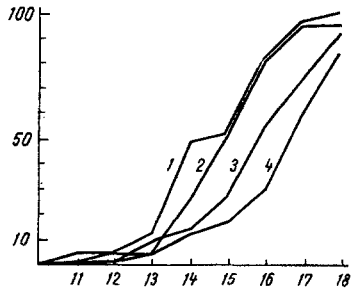


Fig. 2

Fig. 1. Curve of mortality of mice in experiment no. 1: 1) control; 2 and 3) immunization with homogenates of skeletal muscles taken in doses of 20 and 10 mg tissue per mouse respectively; 4) immunization with mixture of homogenates of viscera. Here and in Fig. 2: abscissa, time of death of mice (in weeks); ordinate, number of mice dying (in percent).

Fig. 2. Curve of mortality of mice in experiment no. 2: 1) control; 2, 3, and 4) immunization with homogenates of kidneys, liver, and spleen respectively.

changes in the scheme of immunization of the experimental animals. So far as the experiments in which homogenates of skeletal muscles, liver, and kidneys were used for immunization, in these cases there was only a tendency for the rate of tumor development to decrease and for the time of death of the mice to be delayed.

Further material for the evaluation of tumor induction in the mice immunized with normal syngeneic tissues can also be obtained by comparing the statistics for their mortality (experiments nos. 1 and 2) at different times after injection of the carcinogen (Figs. 1 and 2). The rates of development of the tumors in these experiments were not identical, as is clear from the time at which all the mice in the control groups died: in experiment no. 1 this was 10 weeks, while in experiment no. 2 it was 6 weeks. This factor also influenced differences in the times of death of the immunized and control mice, for these differences were more marked in animals in which the tumors developed more slowly.

These results show that immunization with normal syngeneic tissues inhibits tumor development induced by intramuscular injection of 20-methylcholanthrene to a certain extent. Preparations from a mixture of viscera and from the spleen were most effective in this respect, evidently because they contained a wider spectrum of normal cell antigens. These may also have included antigens whose synthesis is intensified in maligant cells. In other words, the effectiveness of immunization with normal tissues is linked with the problem of intensification of the synthesis of hetero-organic antigens in tumor cells [2].

According to Reiner [3], treatment of animals with normal tissues induces mechanisms which, under ordinary conditions of tissue repair, promote removal of the necrobiotic cells. The writers consider that during immunization with normal syngeneic tissues there is an increase in the intensity of the homostatic response of the recipient, immunological in nature, aimed at eliminating cells in which, under the influence of various factors, there is an ectopic intensification of the synthesis of certain normal antigens. The possibility cannot be ruled out that these mechanisms may contribute to the elimination of neoplastically transformed cells.

## LITERATURE CITED


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