

EFFECT OF SPECIFIC ANTIORGAN SERA ON METASTASIZATION
OF TUMORS INDUCED BY 9,10-DIMETHYL-1,2-BENZANTHRACENE

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UDC 616-006-092.9

Injection of heterogeneous antilung serum into rats with primary sarcoma induced by 9,10-dimethyl-1,2-benzanthracene causes increased metastasization in the lungs. The action of antiliver serum under these experimental conditions was not accompanied by metastasization in the liver.

The study of metastasization of induced tumors continues to receive increasing attention. An understanding of the nature of metastasization is impossible without knowing the causes and conditions leading both to an increase, as well as to a decrease, in the intensity of this process. Among the factors capable of influencing metastasization are organ-specific cytotoxic sera. The action of these sera on metastasization has frequently been studied by the use of models of transplanted tumors [1-6].

The objective of the present investigation was to obtain specific antiorgan sera and to study their effect on metastasization of induced tumors.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 110-120 g. The 105 rats used in the experiments were divided into 5 groups. The animals of groups 1, 2, 3, and 4 received a single injection of 4 mg 9,10-dimethyl-1,2-benzanthracene (DMBA) in 0.5 ml vegetable oil into the thigh muscles, while the animals of group 5 remained intact and acted as the control of spontaneous tumor development.

The animals of group 1 received an injection of antilung serum, the rats of group 2 received antiliver serum, the rats of group 3 received normal rabbit serum as a control, and those of group 4 acted as the control for DMBA injection.

To obtain the antiorgan sera, saline extracts of the organs (lung and liver) of rats were used as antigens. The method of immunization was that advocated by Kuo Ching-Yuan [6]. Noninbred rabbits weighing 2.5-3 kg received 9 doses of antigen by the subconjunctival route (3 series of 3 consecutive daily injections, with intervals of 2 days between the series). On the 4th day after the end of immunization, blood was taken from the rabbits to obtain the sera. Their titer and specificity were determined by the complement fixation test.

Some difficulty is encountered in the preparation of antiorgan sera of high specificity, because together with specific antibodies, antibodies with a broad spectrum of cross-reactions are usually found in the immune sera.

To overcome polyvalency, the antilung serum was absorbed with formalinized liver and spleen tissues [6a]. The absorption was carried out by the writers' modification of the method of Kosyakov and Korostel'eva [7]. In cases when the titer of specific antibodies was considerably lowered by absorption, the sera

Group of Noninfectious Immunology, Research Laboratory of Experimental Immunobiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 72, No. 7, pp. 75-77, July, 1971. Original article submitted December 25, 1970.

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TABLE 1. Effect of Antiorgan Sera on Metastasization of Tumors Induced by Administration of DMBA in the Lungs

Group of animals	Total no. of rats	No. with metastases	Percentage of metastases	Total no. of metastases	Mean no. of metastases per animal
1 (Antilung serum injected)	19	14	73.6	298	15.7
2 (Antiliver serum injected)	25	13	52.0	128	5.1
3 (Normal rabbit serum injected)	23	10	43.5	107	4.6
4 (Control, no serum injected)	20	9	45.0	96	4.8

were concentrated by McErlean's method [8], and if need be they were exhausted again. As a result of these operations, antilung sera with a titer of 1:320 and containing nonspecific antibodies in dilutions of 1:20-1:40 were obtained.

The antiliver serum was not absorbed, for although its titer of antiliver antibodies was 1:640, it contained hardly any nonspecific antibodies. Before injection, the titer of the antiliver serum was adjusted to the same value as the titer of the antilung serum (1:320).

Sera were injected into rats with developing tumors in the 4th month of carcinogenesis, as 10 injections, each of 1 ml, the intervals between injections being 2-3 days.

One month after the last injection of sera, the dead rats and those which were about to die were autopsied and their metastases counted. These autopsies were continued until the end of the 6th month of carcinogenesis, when all the remaining animals were sacrificed simultaneously in order to count their metastases.

EXPERIMENTAL RESULTS

At autopsy on the rats of the first 4 groups, metastases were found in the lungs. In their structure they corresponded to sarcomas of the mixed and spindle-cell types. Single tumors were also observed in other organs (in the abdominal and mesenteric lymph glands and also in the omentum).

In the intact animals of group 5, 2 large tumors of the cervical lymph glands were found in 1 of the 18 animals. No tumors were observed in the lungs or in the other organs of the rats of this group.

The degree of metastasization in the lungs, estimated from the mean number of metastases per animal, was 3.2 times higher in group 1 than in the control (group 4) (Table 1). The mean number of metastases in the group receiving normal rabbit serum was 3.4 times less than the number of metastases in group 1. The differences between the results for these groups are statistically significant ($P=0.01$).

In the rats of group 2 (receiving antiliver serum as additional control), the number of metastases in the lungs was about the same as in the rats receiving normal rabbit serum. No metastases were found in the liver in the rats of group 2. Metastasization in the liver perhaps must be preceded by a stronger cytotoxic action.

Analysis of these results indicates that injection of specific antilung serum can exert a definite effect on the metastasization of induced tumors.

LITERATURE CITED

1. I. N. Maiskii, Abstracts of Proceedings of a Conference on the Immunology of Tumors [in Russian], Leningrad (1961), p. 17.
2. R. B. Gragerova and Yu. A. Barshtein, Abstracts of Proceedings of a Conference on the Immunology of Tumors [in Russian], Leningrad (1961), p. 27.
3. I. N. Maiskii and L. L. Khundanov, *Neoplasma* (Bratislava), **12**, 603 (1965).
4. I. N. Maiskii and L. L. Khundanov, *Byull. Éksperim. Biol. i Med.*, No. 7, 77 (1962).
5. L. L. Khundanov, Effect of Immunization with Antigens from Autologous and Heterologous Liver Tissue on Metastasization of Walker's Carcinosarcoma in Parabiont Rats. Candidate's Dissertation, Moscow (1967).
6. Kuo Ching-Yuan, *Acta Microbiol. Sinica*, **5**, 400 (1957).

- 6a. M. I. Il'in, Lab. Delo, No. 5, 280 (1971).
- 7. P. N. Kosyakov and V. S. Koresteleva, in: Immunological Methods of Investigation of Malignant Neoplasms [in Russian], Moscow (1959), p. 59.
- 8. D. A. McErlean, Nature, 197, 507 (1963).