EFFECT OF HETEROGENIC ORGAN ANTISERA ON METASTASIZATION OF PRIMARY MAMMARY GLAND CARCINOMA OF RATS (RMC-1)

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The role of immunological factors and, in particular, of antibodies in the etiology and pathogenesis of malignant disease has been inadequately studied.

Meanwhile, during the last two decades reports have been published indicating that a leading role in the origin and development of tumors must be ascribed to autoantibodies [1-8]. Antibodies may also be concerned in the etiology and pathogenesis of tumor metastasization.

The author's earlier investigations [1-4] showed that administration of heterogenic immune sera against organs influences the metastasization of the Brown-Pearce primary carcinoma of rabbits.

The object of the present investigation was to study the effect of heterogenic organ antisera on metastasization of a primary mammary gland carcinoma of rats (RMC-1).

EXPERIMENTAL METHOD

The organ antisera (antispleen, antiliver, antikidney, and antilung) were obtained by immunizing rabbits with tissue antigens of the corresponding organs of the rat.

The titers of the sera were determined in the complement fixation reaction. The immune sera and normal rabbit serum were injected into noninbred male rats weighing approximately 90-100 g with an RMC-1 tumor implanted intratesticularly. The sera were used to depress the functions and lower the resistance of the tissues of the

TABLE 1. Mean Number of Metastases in Organs of Rats after Administration of Corresponding Anti-Organ and Normal Sera

Serum	Organ tested	Dose of serum		Control group (no serum injected)
		0.5 ml, 10 times	0.1 ml, 3 times	
Antispleen	Spleen	8.3 P < 0.01	1.3 P = 0.7	0.1
Antilung	Lungs	3.5 P < 0.01	0.6 P = 0.5	0.3
Antikidney	Kidneys	1.0 P < 0.01	0	0
Antiliver	Liver	5.3 P = 0.3	0.6 P < 0.01	0.5
Normal	Spleen	2.6	1.5	0.1
	Lungs	1.0	1.1	0.3
	Kidneys	0.1	0	0
	Liver	2.6	1.8	0.5

Note. Here and in Table 2 the value of P was calculated by the Fisher–Student formula. The significance or otherwise was determined by comparison with the number of metastases in the same organs of animals receiving the corresponding dose of normal serum. The difference was considered significant when P < 0.01.

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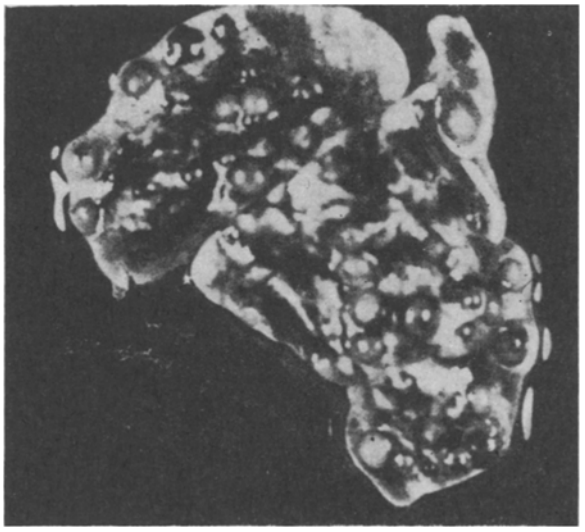
TABLE 2. Mean Number of Metastases on Organs of Rats after Injection of Corresponding Organ Antisera

Serum	Tested organ	Large dose of immune serum (4.5 ml)	Normal serum (4.5 ml)	Small dose of immune serum (0.3 ml)	Normal serum (0.3 ml)	Control group (no serum injected)
Antikidney	Kidneys	0.5	0	0	0	0
Antilung	Lungs	10.5 $P < 0.01$	0	0.6	0.7	0.6
Antiliver	Liver	3.5 $P < 0.01$	1.1	0.5	0.4	0.9



Multiple metastases of tumor in lungs of a rat after injection of a large dose of antilung serum.

organs to the development of metastases of the tumor; the small doses were given to stimulate the functions and increase the resistance of the tissues of the organs. Two series of experiments were performed.

EXPERIMENTAL RESULTS

In the experiments of series I, conducted on 110 rats, four organ antisera were used: antilung and antispleen in a titer of 1:640, and antikidney and antiliver in a titer of 1:1280. All the rats were inoculated with the tumor as a 20 % suspension of fragments of tumor tissue in physiological saline, in a dose of 0.6 ml per animal. The animals were divided into 6 groups. The rats of 5 groups were injected with one of the sera, and the animals of the sixth (control) group were inoculated with the tumor but did not receive the injection of serum. Some of the animals in the group were injected with large doses of serum (0.5 ml) ten times, subcutaneously, while the other rats of the group received small doses (0.1 ml) three times, also subcutaneously. On the 23rd day after inoculation of the tumor the animals were sacrificed and the metastases in their organs were counted (Table 1).

It may be seen in Table 1 that normal serum in large doses stimulated the development of metastases (compared with the number of metastases in the animals receiving small doses of normal serum and in the controls). However, statistical analysis showed that these differences are not significant, so that in fact the process of metastasization was not stimulated by the use of large doses of normal serum. The differences in the intensity of the process of metastasis formation after injection of small doses of normal serum and in the control group were not significant. Consequently, the administration of normal serum in either large or small doses had no significant effect on the mestasization of the tumor.

The mean number of metastases developing in the organs after injection of small doses of the corresponding immune sera was only a little different from the number of metastases obtained when small doses of normal serum were given or in the animals of the control group. Large doses of organ antisera caused many more metastases to develop in the homologous organs than the same doses of normal serum. This difference is statistically significant. The only exception was the difference between the number of metastases in the liver following injection of antiliver and normal serum; this difference is not statistically significant.

Hence large doses of tumor antisera led to the development of a larger number of metastases in the tissues of the homologous organs than large doses of normal serum.

Small doses of the organ antisera caused slight stimulation of metastasization of the tumor in the homologous organs. It may be that the doses used were relatively large for rats, and instead of the expected effect – depression of metastasization – they slightly stimulated the development of metastases.

In the experiments of series II, undertaken on 106 male rabbits, three antisera were used (antiliver, antilung, and antikidney) in titers of 1:1280. In this series of experiments the dosage of the sera and their mode of administration were changed, in order to produce greater stimulation of metastasization of the tumors in the corresponding organs than in the animals of series I.

As the large dose 0.9 ml of serum was injected intraperitoneally five times (if injected more than five times the serum proved toxic for the animals); the small dose was 0.06 ml of serum, diluted in 0.9 ml of physiological saline; five intraperitoneal injections were given also. The animals were inoculated intratesticularly with the tumor in the form of a 20% suspension of minced tumor tissue in physiological saline, each rat receiving 1 ml.

The results of the counts of the metastases in the organs of the animals are given in Table 2.

It is clear from Table 2 that small doses of all the immune and normal sera had hardly any effect on the development of metastases in the organs. Possibly the small doses of sera used were not optimal. When large doses of normal serum were injected into the lungs and kidneys, no metastases were found, whereas when the homologous immune sera were injected into these organs metastasization was intensified (see figure). The difference between the number of metastases in the lungs of the control animals and the number in the lungs of the rats receiving large doses of antilung serum are statistically significant. Stimulation of the process of metastasization in the liver after injection of antiliver serum also was statistically significant (compared with the number of metastases in the animals of the control group and in the rats receiving large doses of normal serum).

Hence, in this series of experiments also, large doses of organ antisera stimulated development of metastases of the tumor in the homologous organs. Evidently the antiorgan antibodies contained in the immune sera, by their influence on the tissues of the homologous organs, led to changes in these organs facilitating the development of tumor metastases.

The results of these experiments demonstrate that the antiorgan heterogenic antibodies of immune sera may influence the metastasization of a primary carcinoma of the mammary gland of the rat (RMC-1).

SUMMARY

Heterogenous antiorganic sera in large doses stimulate metastasization of transplantable mammary carcinoma in rats (RMC-1) in homologous organs. This is evidence of a certain role played by the immunological factors, in particular by the antiorganic antibodies, in the process of tumor metastasization.

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