

CHANGES IN THE PROPERTIES OF THE SERUM
DURING GROWTH OF A BROWN-PEARCE CARCINOMA IN RABBITS

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In recent years the problem of the immunology of cancer has received the most extensive study. The body responds to the development of a tumor by definite immunological reactions [1, 2, 4, 7, 9]. Besides the natural immunity to the development of malignant tumors [8], some research workers [5] distinguish 2 further forms of immunity: immunity to an already developed tumor and antimetastatic immunity.

Certain authors [3, 8] have shown that in persons with malignant tumors, diagnosed at early stages, in some cases it is possible to discover specific anticarcinoma antibodies. Moreover at later stages of the malignant disease these antibodies can no longer be found [8].

It has been shown [11] that feeble metastasis of a tumor is associated with early appearance of specific anticarcinoma antibodies. In animals with well marked metastases, specific anticarcinoma antibodies appear very late or may not be found at all.

It may be thought from these and other findings that a tumor, developing in the body, may exert a specific effect on the body by suppressing its power of antineoplastic immunological reaction. The present paper is devoted to the study of this problem.

EXPERIMENTAL METHOD

As experimental material we selected the strongly metastasizing Brown-Pearce rabbit adenocarcinoma. All the experiments were carried out with tumors of the same strain. Experiments were performed on 2 groups of sexually mature male rabbits of the chinchilla breed, weighing 2.5-3 kg; there were 11 animals in each group. The tumor was inoculated intratesticularly in the rabbits of both groups. Before inoculation of the tumor and then every 3rd day afterwards, 10 cc of blood was taken from each rabbit, and serum was prepared from this

TABLE 1

Results of Morbid Anatomical Examination of Rabbits After Inoculation with Brown-Pearce Adenocarcinoma

Time of examination of rabbits after inoculation, in days	Total no. of rabbits dying and killed	Tumor without metastases	Tumor with metastases
From the 6th to the 10th	5	2	3
" " 10 " " 16	13	1	12
" " 16 " " 25	6	—	6

TABLE 2

The Course of the Complement Fixation Reaction of Serum No. 740 (Antigens: from a Brown-Pearce Tumor, from Rabbit's Testicle, from Rabbit's Liver)

Time of taking blood	Antigens	Dilution of sera		
		1:10	1:20	1:40
Before inoculation of the tumor	Brown-Pearce tumor	+++	++	+
	Testicle	+++	++	+
	Liver	+++	++	+
On the 3rd day after inoculation of the tumor	Brown-Pearce tumor	+++	++	+
	Testicle	+++	++	+
	Liver	++++	++(+)	++
On the 6th day after inoculation of the tumor	Brown-Pearce tumor	++++	++(+)	+
	Testicle	++++	++(+)	+
	Liver	++++	+++(+)	++
On the 9th day after inoculation of the tumor	Brown-Pearce tumor	+++	++	+
	Testicle	+++	++	+
	Liver	++++	+++	+
On the 12th day after inoculation of the tumor	Brown-Pearce tumor	++	+(+)	±
	Testicle	++	+(+)	±
	Liver	+++	++	+
On the 15th day after inoculation of the tumor	Brown-Pearce tumor	+	+	±
	Testicle	+	+	±
	Liver	++	++	+
On the 18th day after inoculation of the tumor	Brown-Pearce tumor	+	±	h
	Testicle	+	±	h
	Liver	++	+	+
On the 21st day after inoculation of the tumor	Brown-Pearce tumor	+	±	h
	Testicle	+	±	h
	Liver	++	+	±
On the 24th day after inoculation of the tumor	Brown-Pearce tumor	+	±	h
	Testicle	+	±	h
	Liver	++	+	+
On the 29th day after inoculation of the tumor	Brown-Pearce tumor	±	h	h
	Testicle	±	h	h
	Liver	+	±	h

Note: The letter h signifies complete hemolysis.

blood. After each removal of blood, the animals were injected subcutaneously with physiological saline, also in a volume of 10 cc. All the sera obtained were inactivated at 56°C and tested for anticomplement.

In order to determine the presence of antibodies in the sera obtained, and to assess their specificity, we utilized the complement fixation reaction. Fixation of complement took place in the cold (at a temperature of 3-5°C). As antigens in the reaction we used saline extracts of a Brown-Pearce tumor, and also of tissue from the liver and testicles of both healthy rabbits and of rabbits affected by tumors.

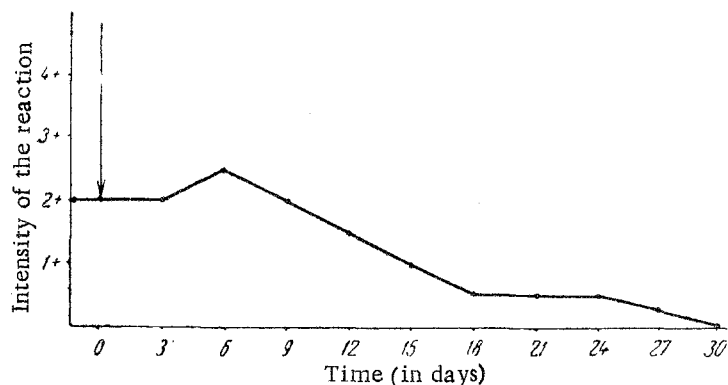
In each experiment parallel tests were made of sera taken from the same animal before inoculation with the tumor and at different periods after inoculation. Simultaneously, on the basis of pathological anatomical investigation, in a third group of rabbits the time of appearance of metastases was determined in animals which had been inoculated with a tumor. In this group observations were made altogether on 24 rabbits.

EXPERIMENTAL RESULTS

The results of the examinations are shown in Table 1. As seen from the findings given in this table, on the 10th-16th day after inoculation metastases, visible to the naked eye, were found in 18 rabbits of the third experimental group; on the 26th day after inoculation metastases were found in all the animals without exception. The results in Table 1 thus show that metastases in animals affected by tumors are first found starting on the 10th day. Sixteen days after inoculation metastases were present in all the rabbits examined.

The results of the experiments to study the sera of rabbits already affected by a Brown-Pearce tumor are given in Table 2. As may be seen from Table 2, which is a typical record of an experiment, the sera of the rabbits taken before inoculation of the tumor reacted positively at the same titer and with the same intensity both with antigen from the Brown-Pearce tumor and with antigens from normal tissue of the testicle and liver of a rabbit. The same may be said of the sera obtained from the rabbits on the 3rd-9th day after inoculation.

All these sera reacted with the above named antigens +++ or ++++ in a dilution of 1:10, ++ or +++ in a dilution of 1:20, and + in a dilution of 1:40. The sera obtained from rabbits on the 12th and 15th day after inoculation of the tumor gave a positive reaction of ++ or +++ in a dilution of 1:10, + or ++ in a dilution of 1:20, and in a dilution of 1:40 a positive reaction of + was observed only with antigen from normal liver tissue. The sera obtained on the 18th-29th day after inoculation gave a feebly positive reaction in a dilution of 1:10 only. In higher dilutions the reaction was to all intents and purposes negative.



Changes in the titer of normal antibodies (serum No. 740 in a dilution of 1:20) in the course of the experiment. Inoculation of the Brown-Pearce tumor is indicated by the arrow.

Analysis of these findings showed that specific antibodies cannot be found in the sera of rabbits already affected by Brown-Pearce tumors. It further follows from these results that the sera of these rabbits evidently contain normal antibodies to tissues from rabbit's organs. The titer of these antibodies begins to fall on the 12th day after inoculation, and on the 29th day they are practically absent. The changes in the titer of the normal antibodies during the course of the experiment are shown graphically on the figure.

It must also be pointed out that all the sera reacted with slightly greater intensity with antigen from normal liver tissue of the rabbit (see Table 2) but so far we are not able to account for this fact.

The results of the experiments thus show that from the 12th day after inoculation a fall takes place in the immunological reactivity of the animal affected by a Brown-Pearce tumor; this is manifested by a fall in the titer of normal antibodies. At the same time, as was pointed out above, this is the period at which the tumor begins to give rise to metastases. It can be concluded from these findings that the onset of the process of metastasization of the tumor is connected with the fall in the immunological reactivity of the animal affected by the tumor.

The question whether in cancer patients there is immunity to the developing tumor is so far unanswered; different investigators have obtained contradictory results on this subject.

It has been established in the present investigation that production of anticarcinoma antibodies to a developing and metastasizing Brown-Pearce carcinoma does not take place in the rabbit. The results which we have obtained show also that the serum of these rabbits evidently contains normal antibodies, whose titer is not raised in the process of development of the tumor, but, on the contrary, begins to fall on the 10th-12th day. At the moment of death of the animals the normal antibodies disappear completely. The fall in the titer of normal antibodies coincides with the moment of appearance of metastases and the onset of violent growth of the tumor at the place of inoculation.

From the results obtained it can be postulated that the normal antibodies of a diseased rabbit play a definite part in the defense of the body against a developing tumor only in the first stages of its development, and for verification of this hypothesis special experiments are required.

SUMMARY

It was established in experiments conducted on 22 chinchilla male rabbits that normal antibodies may be revealed in the blood serum of healthy animals by the cold complement fixation test. In transplantation of Brown-Pearce's tumor the titer of these antibodies begins to decrease from the 10th-12th day, which coincides with the time of tumor metastasis. There is a complete disappearance of normal antibodies at the moment of the animal's death.

The author assumes that a definite role is played by the normal antibodies in combatting the developing tumor.

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