STATE OF CERTAIN NATURAL IMMUNITY FACTORS DURING GROWTH AND METASTASIZATION OF INDUCED TUMORS

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Investigations have shown that during development of malignant neoplasms, certain nonspecific defensive factors of the body are suppressed: the titer of natural antibodies is lowered [3, 4, 5], the concentration of properdin and C-reactive protein is reduced, and so on. It has also been shown that the process of tumor development weakens immunogenesis [1, 2].

The object of the present investigation was to study the content of natural heteroagglutinins in the blood of rats during growth and development of induced tumors.

EXPERIMENTAL

Experiments were carried out on Wistar rats of both sexes aged 5-6 months. Human erythrocytes of the four blood groups, 100 sera of normal rats, and 45 sera of rats with tumors of different sizes and different stages of development induced by 9, 10-dimethyl-1,2-benzanthracene (DMBA) were taken for investigation.

To study the agglutinating activity of the rats' sera during growth of the induced tumors, 10 rats were used. The agglutinating power of their sera was investigated before administration of DMBA and 30, 60, 90, 120, and 150 days after injection of 5 mg DMBA into the hind limb muscles. The sera of the same rats when in a terminal state were also investigated.

The agglutination reaction was carried out on slides at room temperature and by the classical method in test tubes [7]. A 3% suspension of erythrocytes washed three times in physiological saline was used. The results of the reaction on slides were read after 10 min under the microscope, and the results of the reaction in tubes after incubation for 30 min at 37°.

EXPERIMENTAL RESULTS

The study of the agglutinating power of the normal rats' sera showed that they could agglutinate human erythrocytes of all four blood groups.

The whole sera of normal rats, for instance, agglutinated human erythrocytes of group I(0) in 79.9% of cases, sera diluted 1:2 in 65.9%, diluted 1:4 in 32.6%, and diluted 1:8 in 7.6% of cases.

Human erythrocytes of groups III (B) and IV (AB) also were agglutinated by the sera of normal rats: whole sera in 94.6 and 94.6% of cases, diluted 1:2 in 82.6 and 80.9%, diluted 1:4 in 46.5 and 52.3%, and 1:8 in 7.1 and 7.2% of cases.

However, the sera of normal rats agglutinated human erythrocytes of blood group II (A) best: whole sera in 100% of cases, diluted 1:2 in 97%, 1:4 in 83.3%, and 1:8 in 72.5% of cases. The greatest difference was seen in the agglutination reaction carried out with sera in a dilution of 1:8.

It was also found that the sera of normal rats did not produce hemolysis of human erythrocytes on slides at room temperature in a period of 10 min. Hemolysis was observed in some cases only when the reaction was carried out in tubes incubated for 30 min.

Because of these observations, human erythrocytes of blood group II(A) were chosen as test object for studying the levels of natural heteroagglutinins in the sera of rats developing induced tumors.

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Reaction of Agglutination and Hemolysis of Human Erythrocytes of Blood Group II by Sera of Rats with Induced Tumors of Muscle Tissue

Rat No.	Sera of rats taken before injection of DMBA				Sera of rats taken after injection of DMBA												Sera taken from rats in a terminal state			
	dilution of sera				90 days				120 days				150 days							
	whole	1:2	1:4	1:8	Size of tumor (in cm)	dilution of sera			Size of tumor (in cm)	dilution of sera			Size of tumor (in cm)	dilution of sera			Size of tumor (in cm)	dilution of sera		
						whole	1:2	1:4	1:8		whole	1:2	1:4	1:8		whole	1:2	1:4	1:8		whole	1:2	1:4	1:8
1	+++	+++	+++	+++	1×1×1	+++	—	—	—	2×2×2	±	—	—	—	4×4×5	H	—	—	—	5×6×6	H	—	—	—
2	+++	+++	+++	—	No tumor	+++	+++	+	—	0,5×1×1	+++	+++	—	—	3×3×4	H	—	—	—	4×5×5	H	—	—	—
3	+++	+++	+++	—	No tumor	+++	+++	+	—	No tumor	+++	+++	+	—	No tumor	+++	—	—	—	—	—	—	—	—
4	+++	+++	—	—	1×1×2	+++	+++	—	—	2×3×3	+++	+++	—	—	4×4×5	H	—	—	—	5×5×7	H	—	—	—
5	+++	+++	—	—	0,5×1×1	+++	+++	—	—	0,5×1×1	+++	+++	—	—	3×4×6	H	—	—	—	4×5×7	H	—	—	—
6	+++	+++	—	—	0,5×1×1	+++	+++	—	—	2×2×3	+++	+++	—	—	5×6×6	H	—	—	—	6×7×8*	H	—	—	—
7	+++	+++	+	—	No tumor	+++	+	—	—	No tumor	+++	+++	—	—	No tumor	+++	—	—	—	—	—	—	—	—
8	+++	+++	—	—	1×1×2	+++	—	—	—	2×2×3	±	—	—	—	4×5×6	H	—	—	—	5×7×7	H	—	—	—
9	+++	++	—	—	2×2×2	+++	—	—	—	2×3×4	++	—	—	—	4×4×4	—	—	—	—	5×6×7	—	—	—	—
10	+++	++	—	—	0,5×1×1	++	+	—	—	1×2×2	++	—	—	—	5×4×5	—	—	—	—	5×5×6	—	—	—	—

Legend: +++ well marked agglutination; ++ and + moderate and weak agglutination of erythrocytes; — no agglutination; H—hemolysis of erythrocytes.

*Serum of a rat with multiple metastases.

The sera of rats with tumors of various sizes (from 2×3×4 to 6×7×8 cm) caused agglutination of the test erythrocytes in 18.5% of cases. The erythrocytes were also agglutinated by most of the whole sera of the rats with small tumors. Of the 45 sera investigated, 5 (11.1%) from rats with tumors caused hemolysis of the human erythrocytes of blood group II(A) in the course of 10 min at room temperature. The rats whose sera caused hemolysis of the human erythrocytes had larger tumors and metastases in the internal organs.

The results of titration of the natural heteroagglutinins in the rats' sera during development of induced tumors are given in the table.

As the table shows, the sera of the rats before receiving the carcinogen caused agglutination of the test erythrocytes in dilutions up to 1:8.

On the 30th and 60th days after injection of the carcinogen the agglutinating power of the sera of the experimental animals remained essentially unchanged from that of the controls.

From the 90th day after injection of the carciongen, a decrease in the titer of heteroagglutins was observed. For instance, the serum of rat No. 6 before receiving DMBA caused agglutination of the erythrocytes in a dilution of 1:4 to the extent of ++. On the 90th day, when the size of the tumor had reached 0.5×1×1 cm, agglutination of the erythrocytes was observed only by whole serum and by serum diluted 1:2. On the 120th day after injection of DMBA, when the tumor had reached a comparatively large size, only the whole serum caused agglutination of the erythrocytes. On the 150th day after injection of the carcinogen, when the tumor in the rat was large (5×6×6 cm), not even the undiluted serum agglutinated the erythrocytes. At the same time, hemolysis of the erthrocytes was observed in the course of 10 min at room temperature.

The serum of a rat in a terminal state, with a tumor measuring 7×7×8 cm caused hemolysis of the erythrocytes in a dilution of 1:4. At necropsy, multiple metastases were found in the peritoneal cavity and lungs of the rat.

Heating the sera causing hemolysis of erythrocytes to 56° for 30 min destroyed their hemolyzing properties.

In rats resistant to the carcinogen, no decrease in the titer of natural heteroagglutinins to human erythrocytes of blood group II(A) and no appearance of hemolysins were observed.

The results obtained thus show that the content of natural heteroagglutinins in rat's sera against human erythrocytes of blood group II(A) falls as the induced tumors grow in size. In rats with large induced tumors and in rats with metastases no natural heteroagglutinins as a rule can be found. The sera of such rats in some cases are capable of producing hemolysis of human erythrocytes of blood group II(A).

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