

THE IMMUNOLOGICAL PROPERTIES OF NORMAL TISSUE ANTIBODIES

(UDC 612.017.1-084)

M. S. Lomakin, E. V. Sokolova and S. S. Feigel'man

Growth and Development Immunology Laboratory, Institute of Experimental Biology,
Academy of Medical Sciences, USSR

(Presented by Active Member, Academy of Medical Sciences, USSR,
N. N. Zhukov-Verezhnikov)

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 59, No. 4,
pp. 84-89, April, 1965

Original article submitted October 8, 1963

The study of the normal tissue antibodies which were found by a number of investigators[2-7] in the sera of experimental animals, and also their role in the organism is of great interest, although in this respect certain theoretical considerations have been expressed by some scientists [1].

The purpose of the present work was a study of some immunological properties of normal tissue antibodies.

METHODS

Vistar rats and rats without pedigree of different ages, and also chinchilla rabbits weighing 3 kg were used in the study. The sera of the experimental animals was examined using the complement fixation test for the presence of normal tissue antibodies. Water-salt extracts of auto- and homologous rat tissues served as antigens: kidney, liver, lung, spleen, heart, marrow, testicle, uterus, uterus with carcinoma, normal mammary gland, mammary gland with fibroadenoma and (metastasizing) spreading cancer of the mammary gland. Rabbit antigens were water-salt extracts of kidney, liver and Brown-Pearce carcinoma tissue.

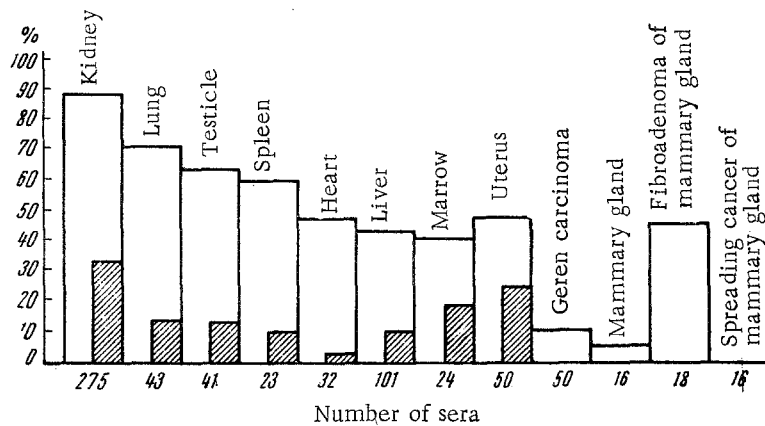
The complement fixation test was done by the classical method at 37°. To determine the character of the specificity of normal tissue antibodies normal sera was absorbed with formalinized rat tissues: kidney, liver, spleen and uterus. Three experiments were set up with sera of newborn rats, and in each of these a mixture of sera from 20-25 individuals was used.

In view of the importance of a study of factors changing the antigenic properties of tissue, some antigens (kidney) were heated at 56° for an hour, after which the antigens were used in the complement fixation test.

RESULTS

In all, 275 sera of normal rats and 26 sera of normal rabbits were used in the complement fixation test. A summary of the data from the complement fixation test from the interaction of normal rat sera with antigens from rat tissue is presented in the figure. Those reactions were considered positive in which the diluted sera reacted ++, +++, and++++ with the corresponding antigens. Two-hundred forty-two sera reacted with kidney tissue antigens in a dilution of 1:10 and 99 sera in a dilution of 1:80. With liver tissue antigens 43 sera reacted at a dilution of 1:10; in 72% of the cases these sera reacted with lung tissue antigens. Positive reactions were noted also with other antigens. However, only one serum out of 16, and only in a weak dilution, reacted with mammary gland tissue antigens. An interesting fact was noted in the complement fixation reaction of sera of normal rats with antigens from normal and homologous tumor tissue. For example, rat sera reacted with uterus tissue antigens in a dilution of 1:10 in 23 out of 50 cases, while with antigens of uterus carcinoma tissue at the same dilution, only in 5 out of 50 cases.

Rabbit sera also gave a significantly weaker complement fixation reaction with antigens from Brown-Pearce carcinoma tissue than with antigens from liver and kidney tissue. Thus, of 26 sera from normal rabbits only 14 gave a positive reaction with antigens from Brown-Pearce carcinoma tissue. Conversely, all the sera reacted with kidney



Results of the complement fixation reaction during interaction of normal rat sera with water-salt extracts of rat tissues. White columns—complement fixation reaction with minimal titer 1:10; crosshatched columns—complement fixation reaction with maximal titer 1:80.

TABLE 1. Complement Fixation Reaction During Interaction of Normal Rat Sera with Antigens from Tissues of Auto-(A) and Homologus (H) Kidney

Dilution of serum	Rat 1		Rat 2		Rat 3		Rat 4	
	A	H	A	H	A	H	A	H
1:20	++++	+++	++++	++	++++	+++	++++	+++
1:40	+++	++	+++	+	+++		+++	
1:80	++	+	±				±	
1:160	++							

Table 1 (cont.)

Dilution of serum	Rat 5		Rat 6		Rat 7		Rat 8	
	A	H	A	H	A	H	A	H
1:20	++++	+++	++++	+++	++++	+++	++	+
1:40	+++	+++	++++	++	++++	+++	+	
1:80	++	+	++		++++	+		
1:160					±			

Notation: +, ++, +++, ++++ degrees of positive complement fixation reaction;—negative complement fixation reaction; ± unclear reaction.

antigens in the complement fixation test, and 21 out of 26 sera with liver tissue antigens, which shows the greater immunological affinity of normal animal sera for antigens from normal tissues as compared with tumor tissue.

It is interesting to note that rabbit sera gave a positive complement fixation reaction with antigens from rat tissue: kidney, liver, lung, marrow, spleen, heart, uterus and normal mammary gland. However, in distinction from the preceding experiments, rabbit sera gave a positive reaction with almost the same value with antigens from tumor tissues (carcinoma of the uterus and cancer of the mammary gland) and normal tissues (uterus, normal mammary gland).

The data obtained indicates that there are substrates in the sera of normal rats and rabbits which react positively with antigens both from homologous and heterologous tissues.

TABLE 2. Results of the Complement Fixation Reaction of Normal and Immune Rabbits and Rats with the Corresponding Antigens

Animals from which serum was taken	Serum dilution						Serum dilution					
	1:10	1:20	1:40	1:80	1:160	1:320	1:40	1:80	1:160	1:320	1:640	1:1280
	antigens from rat kidney tissue						antigens from rat kidney tissue					
	adults						embryos or new born			adults (heated at 56° for 60 min.)		
Adult rats												
1	++	++	++	++	++	++	—	—	—	—	—	—
2	++	++	++	++	++	++	—	—	—	—	—	—
3	++	++	++	++	++	++	—	—	—	—	—	—
4	++	++	++	++	++	++	—	—	—	—	—	—
5	++	++	++	++	++	++	—	—	—	—	—	—
6	++	++	++	++	++	++	—	—	—	—	—	—
7	++	++	++	++	++	++	—	—	—	—	—	—
8	++	++	++	++	++	++	—	—	—	—	—	—
9	++	++	++	++	++	++	—	—	—	—	—	—
10	++	++	++	++	++	++	—	—	—	—	—	—
Newborn rats	—	—	—	—	—	—	Reaction not done	Reaction not done	Reaction not done	Reaction not done	Reaction not done	Reaction not done
Normal rabbits												
1	++	++	++	++	++	++	++	++	++	++	++	++
2	++	++	++	++	++	++	++	++	++	++	++	++
3	++	++	++	++	++	++	++	++	++	++	++	++
4	++	++	++	++	++	++	++	++	++	++	++	++
Rabbits immunized with rat kidney (No. 213, 256, 1 and 205)												
1	++	++	++	++	++	++	++	++	++	++	++	++
2	++	++	++	++	++	++	++	++	++	++	++	++
3	++	++	++	++	++	++	++	++	++	++	++	++
4	++	++	++	++	++	++	++	++	++	++	++	++

Notation of degree of complement fixation reaction same as in Table 1.

Rat sera reacts more intensively in the complement fixation reaction with antigens from tissue of autologous kidney (Table 1).

The following results were obtained when the sera were absorbed with formalinized tissues. If the rat serum before absorption gave a positive reaction with antigens from kidney, liver, uterus and spleen tissue, then after absorption with kidney tissue all the normal antibodies were removed from the sera which led to the absence of a reaction with the given antigens even at a dilution of 1:10. After absorption of the sera with liver tissue positive complement fixation reactions were noted only with antigens from kidney tissue, but not with antigens from liver, spleen and uterus tissue. A similar picture was observed upon absorption of the sera with spleen tissue. Serum absorbed with uterus tissue did not react with any of the specified antigens.

These experiments show that normal tissue antibodies have a relative specificity, which develops to the greatest degree with respect to normal antibodies reacting positively with antigens from kidney tissue.

In further work, it was found that the serum of normal rats gave positive results in the complement fixation test with antigens from human, mouse, guinea pig and rooster kidney tissues. The serum of healthy humans also gave a positive reaction with antigens of rat kidney tissue.

In order to ascertain whether there are differences in the capacity of normal rat sera to give a complement fixation reaction with kidney antigens, 20 sera of rats of different ages and weights were examined. Water-salt extracts of kidney from different rats were used as antigens. It was established as a result of this study that the serum of normal rats give a different degree of complement fixation reaction with antigens from different kidneys. Thus, some sera reacted with antigens from all the kidneys of adult rats used in the experiment, but did not react with antigens from kidneys of newborn rats. Other sera had an affinity for antigens from kidneys of adult males, but did not react with antigens from kidneys of adult females and vice versa. However, we did not succeed in establishing the effect of sex differences on the results of the complement fixation reaction in this experiment.

In Table 2 is presented one of the records of the study of the immunological properties of normal rat kidney antibodies. For comparison, the sera of normal rabbits and rabbits immunized with antigens from rat kidney tissue were also taken.

The sera of adult rats reacted intensively with antigens from adult rat tissue and less intensively or not at all with antigens from kidney tissue of newborn rats. Thus, of 60 sera used in the experiment, 55 reacted with antigens from adult rat kidney tissue (91.6%) and only 14 sera with antigens from newborn rat kidney tissue. The sera of normal rabbits reacted in approximately the same titer with antigens from kidney tissue both of adult rats and newborn rats. It was also established that sera of normal rats reacted in considerably fewer number and less intensively with antigens from kidney which were heated at 56° for an hour than with native kidney antigens. The sera of normal and immunized rabbits gave a positive complement fixation reaction with heated antigens of rat kidney approximately as often as with native antigens.

Thus, the data obtained shows that in the sera of normal rats and rabbits are found normal tissue antibodies which react positively with antigens from auto-, homo- and heterologous tissues, and the sera of normal animals possess the most activity in reacting with antigens from kidney tissue. It was established that formalinized rat kidney and uterus tissue is able to absorb normal tissue antibodies, while liver and spleen tissue is less active in this respect. The sera of newborn rats and embryos does not give a positive complement fixation reaction with antigens from rat kidney tissue, while the sera of adult rats reacts weakly or not at all with antigens from kidney tissue of newborn rats and with kidney antigens heated at 56° for an hour. The sera of normal rats reacts more intensively with antigens from normal tissues and much more weakly or not at all with antigens from homologous tumor tissues, while sera from normal rabbits react with the specified antigens to approximately the same degree. Finally, the sera of rabbits immunized with rat kidney shows the same degree of affinity for antigens from kidney tissues of adult rats, newborn rats and kidney antigens heated at 56° for an hour.

LITERATURE CITED

1. N. N. Zhukov-Verezhnikov, Summary reports of the 5th Session of the Academy of Medical Sciences, USSR [in Russian], Moscow, (1948), p. 16.
2. M. S. Lomakin, Byull. éksper. biol., No. 9, (1956), p. 40.
3. M. S. Lomakin, Summary Reports of the 2nd All-Union Conference on the Problem of Tissue Incompatibility, Conservation and Transplantation of Tissues and Organs [in Russian], Odessa, (1961), p. 135.

4. M. S. Lomakin, in the book: Data of the 3rd Conference on the Problem of Regeneration and Cellular Multiplication [in Russian], Moscow (1962), p. 100.
5. M. N. Nilovskii, Byull. éksper. biol. No. 11, (1958), p. 94.
6. J. Kidd and W. Friedewald, J. exp. Med., 76, (1942), p. 543.
7. L. Muschel, L. Simonton, P. Wells, et al., J. clin. Invest., 40, (1961), p. 517.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.