NORMAL ISO-AND HETEROHEMAGGLUTININS IN PATIENTS WITH CANCER

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(Received July 31, 1958. Presented by Active Member AMN SSSR S. A. Sarkisov)

The problem of the biological importance of normal antibodies cannot yet be regarded as satisfactorily solved. From the available information they may, however, be regarded as one of the factors concerned in natural immunity.

Normal antibodies, reacting with pathogenic agents which have entered the body, limit their activity to a greater or lesser degree, and facilitate their subsequent destruction. Antigenic substances of noninfectious origin (red cells, protein-polysaccharide or polysaccharide-lipid complexes) which enter the body are also neutralized by normal antibodies should they be incompatible with the antigenic structure of the body. Normal antibodies thus carry out a variety of functions directed towards maintaining the constancy of the "milieu intérieur" of the body.

Normal antibodies are also of interest as an indication of the potential ability of the body to produce immune antibodies. When normal diphtheria antitoxins are present in horses, in the process of immunization, antibodies are known to be formed in the majority of immunized animals. The production of a hemolytic serum in rabbits is most successful in cases when, before immunization, they had normal hemolysins to sheep's red cells [3]. It has also been observed [6] that antibacterial vaccination meets with greatest success in persons who, before vaccination, possessed normal antibodies in relation to the vaccine used.

Normal antibodies themselves may act as an indication of the ability of the body to produce species-specific proteins, possessing the functions of normal antibodies, and to maintain them at a level which is strictly defined for each individual.

Different pathological processes, involving the various systems and functions of the body, may cause, to a greater or lesser degree, changes in the titer of the normal antibodies [1, 7, 9]; this may, apparently, also be of prognostic importance and may sometimes be used for diagnostic purposes.

It is possible that normal antibodies are not only factors which protect the body against infectious and toxic agents, but they are also of importance to the resistance of the body against the growth of tumors.

Also of great interest are the findings relating to changes in the titer of normal antibodies arising in cancer patients. T. N. Bogdanova [1] investigated the sera of 9 patients and discovered a fall in their titer of normal agglutinins to frog's red cells. Kh. E. Mamanova [9] studied the sera of 46 cancer patients and also discovered a fall in their titer of hemagglutinins to red cells of man, sheep, guinea pigs, dogs and rabbits. However, bearing in mind the small number of cases and also the individual variations in the titer of normal antibodies in healthy persons, it is difficult to judge the significance of this phenomenon and its connection with the process of development of the tumor, especially as when studying the sera of patients known to be suffering from cancer, we are ignorant of the state of the normal antibodies of these patients before the onset of their disease. Investigation of the immunological properties of animals with experimentally transplanted tumors has obvious advantages over the study of these properties in man, since in animals it is possible to compare the antibody titer before the grafting of the tumor and in the course of its development.

The aim of our present research was to study the normal iso- and heteroantibodies in patients with cancer and also in animals with experimentally grafted tumors, and to trace the changes in the titers of the normal antibodies, in relation to the course of development of the tumor.

EXPERIMENTAL METHOD

Investigations were made of the sera of 94 patients with cancer; of these patients, 66 suffered from carcinoma of the stomach, 15 from carcinoma of the head and body of the pancreas, 2 from primary carcinoma of the liver, 2 from carcinoma of the rectum, 4 from carcinoma of the breast and 5 from carcinoma in other situations.

The diagnosis in all patients was confirmed by histological examination of biopsy specimens or by diagnostic laparotomy (in inoperable cases). Of the total number of cancer patients, we specially separated a group of inoperable patients with far advanced neoplasms (33 patients). As a control, we examined 53 sera from apparently healthy persons.

In all the sera, we determined the presence and titer of the normal heterohemagglutanins to red cells of the sheep and rabbit, and the group isohemagglutinins to human red cells. Serial dilutions were prepared from the serum with physiological saline, and 2 drops of each dilution were left behind in the agglutination tube. To each tube, including a control containing 2 drops of physiological saline, was then added 1 drop of a 5% suspension of red cells, washed three times in physiological saline. After careful agitation, the tubes were kept at room temperature for 15-20 minutes, and were then centrifuged for 1 minute at 2000 rpm, and the results of the experiment read.

The degree of the hemagglutination reaction was designated as follows: +++, when the agglutinate did not break up on gentle agitation of the tube; ++, if on agitation the agglutinate disintegrated into 2-3 large lumps; +, if a larger number of well-defined lumps was obtained; the − sign indicated absence of agglutination. The titer of the normal agglutinins of the serum was taken to be its greatest dilution at which agglutination assessed as + took place.

EXPERIMENTAL RESULTS

In Table 1 are shown the results of the study of the titers of normal antibodies in the sera of healthy human subjects and cancer patients.

TABLE 1

Titers of Normal Heterohemagglutinins in the Sera of Healthy Human Subjects and Cancer Patients to Sheep's Red Cells

Sera of	Number of sera examined	Number of sera with hemagglutinins to sheep's red cells in a titer of							Average titer
		0	1:2	1:4	1:8	1:16	1:32	1:64	
Healthy human subjects	53	−	−	2	9	23	15	4	1:22
Cancer patients	94	10	8	13	18	20	17	8	1:17
Inoperable cancer patients	33	6	6	6	2	6	6	1	1:13

It can be seen from Table 1 that, of the 53 sera from apparently healthy persons tested, not one was found in which hemagglutinins to sheep's red cells were absent. The normal hemagglutinins were usually present in a titer of 1:16 and 1:32; the average titer for this group of healthy human subjects was 1:22.

TABLE 2

Titers of the Normal Heterohemagglutinins in the Sera of Healthy Human Subjects and Cancer Patients to Rabbit's Red Cells

Sera	Number of sera examined	Number of sera with hemagglutinins to rabbit's red cells in a titer of							Average titer
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Healthy human subjects	53	–	–	–	–	14	10	29	1:89
Cancer patients	94	–	2	–	5	24	30	33	1:74
Inoperable cancer patients	33	–	2	–	3	3	14	11	1:74

Of the 94 sera obtained from cancer patients, in ten, hemagglutinins to sheep's red cells were completely absent, and in eight, they were present in a titer of 1:2; the average titer of hemagglutinins was 1:17. An even lower average titer of hemagglutinins to sheep's red cells was found in the inoperable patients – 1:13.

TABLE 3

Titers of the Normal Heterohemagglutinins in the Sera of Healthy Human Subjects and Cancer Patients

Sera	No. of sera examined	No. of sera of Group AB(IV)	Number of sera with isohemagglutinins in a titer of							Average titer
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	
Healthy human subjects	53	9	–	–	1	1	4	8	30	1:102
Cancer patients	94	13	1	1	5	6	18	29	21	1:65
Inoperable cancer patients	33	7	1	1	3	2	5	7	7	1:60

We observed changes similar to those in the sera of cancer patients compared with the sera of healthy persons in relation to agglutinins to rabbit's red cells (Table 2).

The titer of hemagglutinins to rabbit's red cells in the sera of the healthy human subjects was not below 1:32, but in cancer patients sera with a titer of 1:4 and 1:16 were found. However the fall in the average titer of agglutinins to rabbit's red cells in the cancer patients (including those with inoperable lesions) as compared with the sera of the healthy persons was insignificant.

The titers of the normal isohemagglutinins did not remain unchanged in the cancer patients. The results obtained are shown in Table 3.

As can be seen from Table 3, in the cancer patients sera with a low antibody titer were usually found: 1:2, 1:4, 1:8 and 1:16. The average isohemagglutinin titer of the sera of the cancer patients (including those with inoperable lesions) was below that in the sera of the healthy persons. This feature should be remembered when the compatibility of the serum of a cancer patient with the blood of a donor is being tested at the patient's bedside.

TABLE 4

Heterohemagglutination Reaction in the Sera of Rats before Implantation of an M-1 Sarcoma and during Its Growth, in Relation to Human Group A(II) Red Cells

Experimental animal No.	Date of examination of serum	State of animal and size of tumor	Heterohemagglutination reaction of rat sera with human Group A(II) red cells				
			1:2	1:4	1:8	1:16	1:32
	9/21/1954	Before implantation of tumor	+++	++	+	–	–
	10/2	" " " "	+++	++	++	+	–
	10/6	M-1 sarcoma implanted					
2	10/15	Tumor grown to the size of a bean	–	–	–	–	–
	10/19	Tumor the size of a pigeon's egg	–	–	–	–	–
	10/26	Tumor the size of a hen's egg	–	–	–	–	–
	10/27	Death of animal:					
	9/21	Before implantation of tumor	+++	++	+	+	–
	10/2	" " " "	+++	++	++	+	–
	10/6	M-1 sarcoma implanted					
	10/15	Tumor grown to the size of a bean	++	+	–	–	–
9	10/19	Tumor the size of a pigeon's egg	+	–	–	–	–
	10/26	Tumor the size of a hen's egg	+	–	–	–	–
	11/1	Tumor began to disintegrate	–	–	–	–	–
	11/5	Destruction of tumor	–	–	–	–	–
	11/13	Death of animal:					
	6/19	Before implantation of tumor	+	+	–	–	–
	6/23	" " " "	+	++	+	–	–
	6/24	M-1 sarcoma implanted					
	6/28	No growth of tumor observed	+++	++	+	–	–
12	7/5	Tumor grown to the size of a bean	+++	+	+	–	–
	7/13	Tumor began to disintegrate	–	–	–	–	–
	7/20	Destruction of tumor	++	+	+	–	–
	7/30	Scar formation commenced:	++	+	–	–	–
	6/19	Before implantation of tumor	+++	++	+	+	–
	6/23	" " " "	++	+	–	–	–
	6/24	M-1 sarcoma implanted					
	6/28	No growth of tumor observed	–	–	–	–	–
20	7/5	Tumor grown to the size of a bean	+	–	–	–	–
	7/13	Tumor began to disintegrate	++	+	–	–	–
	7/20	Destruction of tumor	++	+	–	–	–
	7/30	Scar formation commenced	++	+	–	–	–

A comparative study of the normal antibodies in healthy human subjects and patients with cancer thus showed that in association with carcinoma a fairly well-marked fall takes place in the titer of iso- and hetero-hemagglutinins.

Besides the human sera, we also examined the sera of 20 rats with subcutaneously transplanted M-1 sarcomas. Blood was taken from the caudal vein of each animal on 7 occasions and the presence of heterohemagglutinins to human Group A(II) red cells was determined in the sera before implantation of the tumor and in the course of its growth. According to data in the literature, the normal antibodies to human red cells in rats are a reasonably sensitive index of the immunological reactivity of these animals [12]. In Table 4 are shown the results of the experiments on animals with implanted tumors.

It can be seen from Table 4 that with increasing development of the tumor in the rats (experimental animals Nos. 2 and 9), a fall in the titer of antibodies to human red cells was observed, and later their complete disappearance, after which death of the animal ensued. In those cases when the tumors ulcerated and regressed (experimental animals Nos. 12 and 20), during development of the tumor, the antibodies also disappeared, but during the period of cicatrization of the lesion, they reappeared.

These investigations thus showed that in rats the process of progressive development of tumors is accompanied by a fall in the titer of normal antibodies. Our observations on experimental animals suggest that the fall in the titers of normal antibodies in cancer patients also depend on the development of the malignant lesion.

The factors responsible for causing the fall in the titers of normal antibodies in cancer patients are still unknown. It has been shown in many investigations devoted to the study of the plasma proteins in cancer that depletion of the body protein (which could account for the fall in the titers of normal antibodies) occurs mainly at the expense of the albumin fraction of the plasma protein; the globulin fraction, which is known to carry the antibodies, remains unchanged until the terminal period of the animal's life [2, 4, 5, 8, 10, 11]. It may be assumed that, during the development of the tumor, there is a fall in the antigenic function of the body which shows itself, in particular, by a fall in the titer of normal iso- and heterohemagglutinins. In this connection, the experimental findings showing that the appearance of the tumor is most often observed in animals characterized by a low titer of normal antibodies, are themselves of undoubted interest [13-15].

SUMMARY

The titer of normal hemagglutinins relative to sheep, rabbit and human erythrocytes was studied in the blood serum of healthy persons and cancer patients. Besides, the changes in the titre of normal antibodies were studied in the blood serum of rats during the growth of inculcated M-1 sarcoma. It was established that development of the cancer process in man is associated with reduction of the titer of normal iso- and heterohemagglutinins, observed also in experimental animals, parallels the progressive development of the tumor. Spontaneous resolution of the tumor is associated with the restoration of the titer of the normal antibodies.

LITERATURE CITED

[1] T.N. Bogdanova, The Study of the Heteroagglutinating Properties of Human Blood. Author's abstract of Candidate's Dissertation. Makhachkala, 1954 [In Russian].

[2] R. Vintsler, "The plasma proteins in cancer". Progress in the Study of Cancer, vol. 1, pp. 388-436, Moscow, 1955 [In Russian].

[3] S.I. Ginzburg, V.S. Kalinina and R.E. Zelikova, Zhur. Mikrobiol, Epidemiol. i Immunol., 17, No. 3, 425-431 (1936).

[4] D. Grinshtein, The Biochemistry of Cancer, Moscow, 1951 [In Russian].

[5] S. Debov, Sovremennye Problemy Onkologii, No. 5, 3-8 (1954).

[6] S.M. Enaleeva, Kazan. Med. Zhur., No. 7, 661-668 (1930).

[7] N.N. Zhukov-Verezhnikov and T. Guseva, Zhur. Mikrobiol., Epidemiol.i Immunol., No. 5, 33-36 (1950).

[8] G.G. Ivanov, Abstracts of Research during 1947 at the AMN SSSR, No. 6, pp.81-82, Moscow, 1949 [In Russian].

[9] Kh. E. Mamanova, Heteroagglutination in Normal Conditions and in Certain Diseases in Man. Author's abstract of Candidate's Dissertation. Frunze, 1954 [In Russian].

[10] N.B. Medvedeva, General and Special Oncology, vol. 1, pp. 369-401, Moscow-Leningrad, 1940 [In Russian].

[11] P. Bernfeld and F. Homburger, Proc. Amer. Ass. Canc. Res., 1954, v.1, N 2, p.5.

[12] A.E. Bogden and P.M. Aptekman, Canc. Res., 1953, v.13, p. 890-894.

[13] I. Davidsohn and K. Stern., Proc. Soc. Exper. Biol. a. Med., 1949, v.70, p. 142-146.

[14] I. Davidsohn and K. Stern., Canc. Res., 1949, v.9, p. 426-435.

[15] R. Johnson, S. Albert, H. Pinkus, and R. Wagshal, Proc., Amer. Ass. Canc. Res., 1954, v.1, N.2, p.23.