

SOME IMMUNOBIOLOGICAL PROPERTIES OF HETEROPHILIC ANTIBODIES AND NATURAL REAGINS

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Organ specificity of heterophilic antibodies against rat erythrocytes and testicular cells contained in the sera of normal animals has been demonstrated. Normal antierythrocyte antibodies agglutinate the erythrocytes of rat embryos less strongly than erythrocytes of adult animals. In tissue culture heteroagglutinins have no inhibitory action on growth of rat kidney tissue cultures or of primary induced tumors of muscle tissue. Natural reagins have a stimulant action on growth of a rat kidney tissue culture.

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Normal antibodies are one of the factors of natural immunity which play a definite role in physiological and pathological processes [1-4]. However, the biological significance of normal antibodies from the wider point of view has been incompletely studied. This applies to their specificity, their reaction with the cells of various organs and tissues, with their action on cultures of various normal and malignant cells, and so on.

The object of the present investigation was to study some aspects of the specificity of heterophilic antibodies and natural reagins and their action on growth of cultures of normal and malignant tissues.

EXPERIMENTAL METHOD

Experiments were carried out on sera of healthy persons (14*) and of normal animals: guinea pigs (60*), cattle (29*), pigs, cocks (8*), sheep (4*), and horses (4*), and also of normal Wistar rats of both sexes aged 6-12 months. The specificity of these antibodies and natural reagins was determined by the agglutination and complement fixation reactions. The antigens used were saline extracts and whole cells obtained from tissues of the liver, kidney, spleen, and testis and erythrocytes of rats of different age groups (embryos aged 8-20 days, newborn animals, rats aged 7, 15, 30, 180, and 240 days). To determine the specificity of the normal antibodies, the test sera were absorbed once or twice for 10-15 min at 37°. The biological properties of heterophilic antibodies and natural reagins were studied in tissue culture by the method described in [5]. Kidney tissues of newborn rats and primary induced tumors of muscle tissue in rats and their metastases were cultivated. The following rat sera were used in the experiments: a) normal, native sera containing natural reagins in different titers; b) heated to 65° for 35 min; c) absorbed twice on fractions of mitochondria and nuclei isolated from rat kidney tissue for 18-24 h at 4-37°; d) sera of rats preliminarily immunized by 5 intramuscular and intraperitoneal injections of rat kidney tissue homogenate with Freund's complete adjuvant.

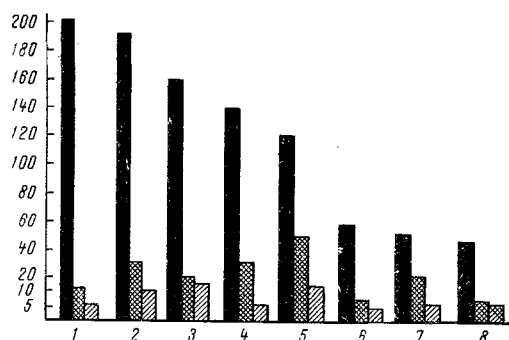


Fig. 1. Agglutination of erythrocytes of adult rats and their embryos by normal porcine sera. Ordinate: dilution of sera; abscissa; serial no. of sera. Black columns represent erythrocytes of adult rats, cross-hatched columns erythrocytes of 18- to 20-day rat embryos, obliquely shaded columns erythrocytes from 8- to 10-day rat embryos.

*Total number of specimens of sera used.

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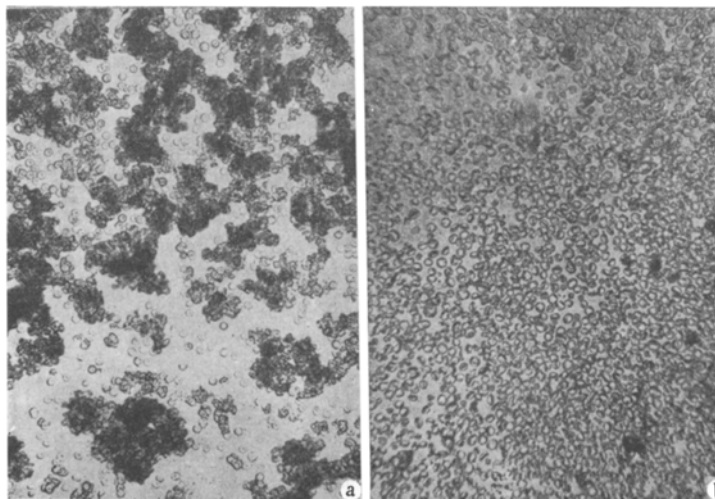


Fig. 2. Agglutination of rat erythrocytes by normal porcine serum preliminarily absorbed by erythrocytes of 10-day embryos. a) Erythrocytes of pregnant rat; b) erythrocytes of 10-day rat embryos. Dilution of serum 1:20.

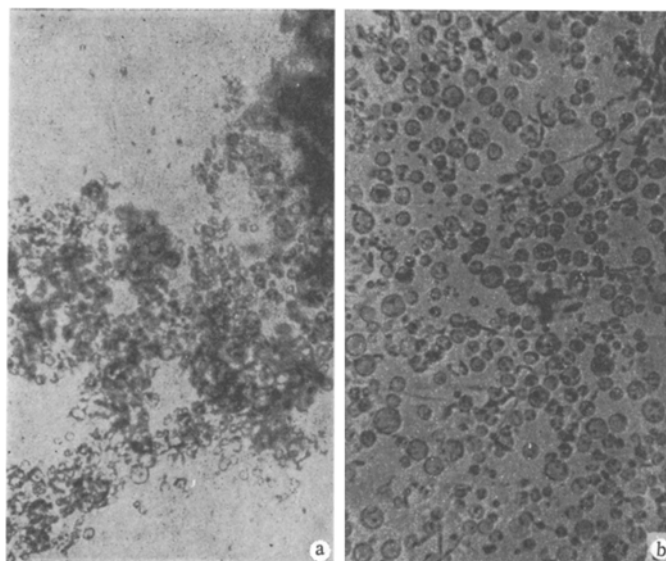


Fig. 3. Agglutination of isolated testicular cells of a rat by bovine serum. a) Testicular cells in native bovine serum; b) testicular cells in bovine serum preliminarily absorbed by testicular cells. Dilution of serum 1:8.

EXPERIMENTAL RESULTS

The results of the tests with the agglutination reaction showed that the sera of the healthy persons and normal animals which were studied agglutinated rat erythrocytes in different titers. Sera of pigs (1:25-1:230), cocks (1:15-1:210), and cattle (1:4-1:120) had the highest titer of antibodies. Human sera and the sera of guinea pigs, rabbits, sheep, and horses had comparatively low antibody titers (1:2-1:32). It is interesting to note that the sera of normal animals agglutinated erythrocytes of adult rats in higher titers and erythrocytes of rat embryos in much lower titers. Erythrocytes of 8- to 10-day embryos were agglutinated least strongly, erythrocytes of 18- to 20-day embryos and newborn rats in somewhat higher dilutions, but only erythrocytes from 7-day rats were about equal in their agglutinating properties with the erythrocytes of adult rats (Fig. 1). The sera of normal animals, absorbed by adult rat erythrocytes, lost

TABLE 1. Intensity of Growth of 2-Day Cultures of Rat Tissues in Sera of Normal Animals Containing Different Titers of Heterophilic Antibodies

Porcine sera					Bovine serum				
serum No.	titer of anti-bodies	mean coefficient of growth of tissues			serum No.	titer of anti-bodies	mean coefficient of growth of tissues		
		kidney	primary tumor	metas-tasis			kidney	primary tumor	metas-tasis
1	1:120	1,58	0,73	2,89	1	1:20	2,16	0,50	0,66
1*	1:2	0,65	0,80	2,61	2	1:20	2,43	0,56	1,10
2	1:55	1,56	0,82	2,06	3	1:10	1,68	0,92	1,22
2 ¹	1:2	1,38	—	—	4	1:18	1,71	0,69	1,12
3	1:160	2,22	0,34	1,35	4 ¹	1:2	1,38	0,36	1,22
4	1:220	2,25	0,18	2,21	5	1:16	2,87	0	1,55
5	1:120	2,18	0,80	1,51	6	1:12	2,06	0,70	0,96
6	1:110	1,18	0,99	1,45	7	1:10	0,58	0,49	2,70
7	1:90	2,43	2,02	2,09	8	1:4	1,51	0,50	1,10
8	1:50	1,25	0,89	3,54	9	1:6	1,19	—	—
9	1:40	1,87	0,53	2,92	10	1:6	1,21	—	—
10	1:35	1,17	0,11	2,49	11	1:14	1,87	0,66	1,96

Note. Mean coefficients calculated from a total number of 25-35 cultures.

* Sera absorbed by erythrocytes of adult rats.

TABLE 2. Intensity of Growth of 2-Day Rat Kidney Tissue Cultures in Sera Containing Various Titers of Natural Reagins

Serum No.	Titer of natural reagins in complement fixation reaction	Type of rat sera	Character of growth of cultures			
			No. of cultures	mean coefficient of growth	ratio bet. epithelial and connective-tissue growth	coefficient of destruction
1	1:80++	Native	26(100)	5,42	24/2	0
2	1:5 h	Native	26(100)	3,01	23/2	0,04
2	1:5 h	Heated	20(90)	2,25	18/0	0,11
2	1:5 h	Absorbed	20(85)	2,07	17/0	0,17
3	1:80++	Native	75(88,8)	2,87	32/34	0,12
3	1:5 h	Absorbed	50(42,0)	1,34	21/0	0,58
4	1:80++	Native	30(100)	4,42	14/16	0
4	1:5 h	Absorbed	25(4,0)	0,98	1/0	0,96
5	1:40++	Native	33(100)	2,17	25/8	0
5	1:5 h	Heated	25(100)	1,41	5/20	0
6	1:40+++	Native	26(92,3)	2,23	24/0	0,03
6	1:5 h	Heated	28(81,1)	0,98	17/6	0,14
7	1:5 h	Normal, native	30(90,3)	1,61	26/2	0,07
7 ¹	1:80+++	Immune	45(100)	4,58	43/2	0
8	1:5 h	Normal, native	25(100)	1,58	23/2	0
8 ¹	1:40+++	Immune	42(100)	3,93	40/2	0

* Sera of rats immunized with homologous kidney homogenate with Freund's complete adjuvant.

Legend: h denotes hemolysis. Number of cultures in percent given in parentheses.

their agglutinating power relative to erythrocytes of rats of all age groups, whereas these same sera, if absorbed by erythrocytes of 8- to 10-day rat embryos, continued to agglutinate the erythrocytes of adult rats, although in smaller dilutions, but they did not agglutinate the erythrocytes of rat embryos (Fig. 2).

Further investigations showed that heterophilic antibodies contained in these human and animal sera did not cause agglutination of the liver, kidney, and spleen cells of adult rats. However, in some cases, guinea pig, cock, and bovine sera agglutinated not only erythrocytes, but also isolated testicular cells in dilutions of 1:4-1:170 (Fig. 3). By absorption of these sera with erythrocytes or testicular cells it was shown that antibodies causing agglutination of these cells differ in their specificity.

These results thus showed that natural antierythrocyte antibodies react specifically in the agglutination reaction only with erythrocytes, and not with the cells of organs such as the liver, kidney, and spleen. It therefore appeared interesting to study the action of heteroagglutinins on the cells of certain organs and tissues under explanation conditions. For this purpose, tissues of the kidney and primary induced tumors of rats were cultivated. As the results given in Table 1 show, the sera of normal animals, with comparatively high titers of heteroagglutinins, had no inhibitory action on growth of the experimental cultures. For instance, the mean coefficient of growth of kidney explants cultivated in No. 4 porcine serum was 2.25, but in the case of No. 10 porcine serum the titer was only 1.17. The corresponding antibody titers were 1:220 and 1:35. Furthermore, the mean coefficients of growth of the experimental cultures were rather lower in the sera of animals which did not contain normal antibodies (absorbed sera).

In the second experiment, only some of the results of which are given, rat kidney tissue was cultivated in the sera of rats containing various titers of natural reagents giving a positive complement fixation reaction with extracts from rat kidney tissue. The results showed (Table 2) that in the sera of rats containing no natural reagents the intensity of growth of rat kidney explants was much lower than in sera with a comparatively high content of natural reagents. For instance, the mean coefficient of growth of explants cultivated in sera of rats Nos. 1, 3, 4, and 6 was 5.42, 2.87, 4.42, and 2.93 respectively (titers of natural reagents 1:40-1:80), while in sera Nos. 2 and 3 absorbed, No. 4 absorbed, and No. 6 heated, which had no natural reagents, the corresponding values were 3.01, 1.34, 0.98, and 0.98. The differences are statistically significant ($P=0.01$ or $P<0.001$).

The results of these investigations thus indicate that normal antibodies, reacting specifically in immunological reactions with rat erythrocytes, or natural reagents have no inhibitory action on growth of explants of normal and tumor tissues. Moreover, the natural reagents of rat sera actually stimulate growth of kidney tissue to some extent under explanation conditions. It is interesting to note that tissues of metastases of primary induced tumors show more intensive growth in tissue culture than the primary tumors themselves.

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