INCOMPLETE ANTIBODIES AGAINST SHEEP'S RED CELLS IN HUMAN SERUM

N. I. Kuznetsova

Research Institute of Rheumatism (Director, Active Member AMN SSSR A. I. Nesterov) and Professor V. P. Serbskii Central Research Institute of Forensic Psychiatry (Director, Docent G. V. Morozov), Moscow)
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 3, pp. 73-76, March 1963
Original article submitted January 2, 1962

The heterogenic sheep antigen discovered by Forssman [8] occurs widely among different species of animals [1, 2, 9, 13, 14]. Antibodies against this antigen are found in normal human serum in a titer of 1:4 to 1:32. In many diseases an increase in the titer of heterophilic antibodies against Forssman antigen is observed [6, 10, 11]. In some diseases, at a certain stage the titer of these antibodies falls [3]. Besides ordinary agglutinating antibodies, incomplete antibodies against Forssman antigen have been detected. Antibodies of this type can be found in the serum of neonates by means of the Coombs test [15].

In the present paper we describe the results of an investigation of the titer of incomplete antibodies to sheep's red cells in the sera of healthy adults and of adult patients. This investigation was carried out because of our discovery that the sera of certain patients with infectious nonspecific polyarthritis, adsorbed with sheep's red cells (the normal preliminary treatment of test sera before performance of the Waaler-Rose diagnostic reaction), contains antibodies detectable by means of antiglobulin serum.

EXPERIMENTAL METHOD

The test serum was inactivated by heating to 56° for 30 min, and adsorbed twice with a precipitate of thrice-washed sheep's red cells. To 1 ml of the undiluted serum 0.2 ml of the red cell suspension was added each time, and the mixture was allowed to stand at room temperature for 30 min. The adsorbed serum was tested for completeness of adsorption. Two drops of serum (whole and diluted 1:2) was added to a test tube 0.5 cm in diameter and 1.2 cm high, after which one drop of a 1.5% suspension of sheep's red cells in phosphate buffer solution (pH 7.6) was added. The mixture of serum and red cells was allowed to stand overnight at room temperature (18°). The reaction was read by means of a hand lens, taking note of the configuration of the precipitate. Serum freed from antisheep hemagglutinins was mixed with an equal volume of a 3% suspension of sheep's red cells in buffer solution and incubated for 1 h at 37°, after which the red cells were washed three times with buffer solution and tested with immune rabbit antiserum against γ-globulin or with whole human serum. These sera (later, for convenience, they will be called antiglobulin) were chosen for their activity in agglutinating red cells treated with sera containing incomplete antibodies against sheep's red cells. If the antiglobulin serum itself contained antisheep hemagglutinins, it was adsorbed before the experiment.

Two drops of antiglobulin serum was poured into test tube as described above, and to it was added one drop of a suspension of red cells treated with the test serum. The reaction was read after standing overnight at room temperature. Two variants of the test with antiglobulin serum were used. In the first variant the red cells, treated with undiluted test serum, were added to different dilutions of antiglobulin serum; the result was taken as positive if agglutination took place with dilutions of antiglobulin serum of 1:10 and higher. In the second variant the red cells were treated with the test serum, whole and in various dilutions, and added to antiglobulin serum diluted 1:10 (or, more rarely, 1:20). If agglutination took place in these conditions, the result was taken as positive. In controls to both variants the agglutinating activity of the antiglobulin serum in relation to untreated sheep's red cells and the agglutinability of the treated red cells in buffer solution were tested.

Tests were carried out on the sera from 386 persons: the sera of 65 persons were tested by the second variant of the reaction; 155 subjects were patients at the clinic of the Institute of Rheumatism and 148 were patients at mental hospitals; 83 sera were obtained from clinically healthy persons.

TABLE 1. Results of Determination of Incomplete Antibodies to Sheep's Red Cells in Sera of Patients and Healthy Subjects

Group of subjects	Total No. tested	Diagnosis	No. of positive reactions		No. of negative reactions		Percentage of positive reactions	Mean error
Patients at clinic of Institute of Rheumatism	155	Rheumatism	15		26			
		Infectious nonspecific polyarthritis (rheumatoid)	37		36			
		Systemic lupus erythematosus and scleroderma	9	66	8	89	42.5	±3.9
		Other collagen diseases	1		3			
		Diagnosis of rheumatism and infectious arthritis not confirmed	4		16			
Mental patients	148	Schizophrenia	6		73			
		Organic diseases of the brain of varied etiology, epilepsy, syphilis	11	24	31	124	16.2	±3
		Reactive state, chronic alcoholism, simple intoxication, no mental disease found	7		20			
Clinically healthy	83		6		77		7.2	±2.8

EXPERIMENTAL RESULTS

It will be clear from Table 1 that sera containing incomplete antibodies, adsorbed on to sheep's red cells, were found in persons of all groups, although more frequently in patients from the clinic of the Institute of Rheumatism. The difference in frequency was statistically significant. It will be seen in Table 2 that incomplete antibodies were found in a titer of 1:1 to 1:32.

Since the red cells of animals of different species contain identical antigenic substances [2], we were interested to discover to what extent the antibodies adsorbed on to sheep's red cells were active in relation to the red cells of other species of animals. Serum containing incomplete antibodies was accordingly adsorbed twice with sheep's red cells, and then adsorbed a third time with sheep's, human, rat's, and guinea pig's red cells. To 0.5 ml of serum was added 0.1-0.25 ml of a red cell precipitate, after which the titer of incomplete antibodies was determined by the technique described above.

The results of investigation of the serum of four subjects are given in Table 3. In the first two subjects, incomplete antibodies were detected by the first variant of the reaction, and in the remaining two subjects by the second variant. It is clear from Table 3 that the sheep's red cells possessed the greatest power of adsorbing incomplete antibodies. This demonstrates that the incomplete antibodies which were found were mainly against antigen present in sheep's red cells.

In addition to determining the incomplete antibodies against sheep's red cells, the titer of ordinary antisheep hemagglutinins was also determined in the sera from certain subjects. Of the 30 persons whose serum was found to contain incomplete antibodies, in 16 their titer was between 1:4 and 1:32, and in 14 between 1:64 and 1:128. Hence, incomplete antibodies against antigen of sheep's red cells may be present in the serum in association with either a normal or an increased titer of agglutinating antibodies.

It is interesting to compare the results of the determination of incomplete antibodies with the titer of rheumatoid factor in patients with infectious nonspecific (rheumatoid) arthritis. The titer of rheumatoid factor was determined by the method of Waaler and Rose, modified to include adsorption [4]. Incomplete antibodies against sheep's red cells were found in 21 of 28 patients with a positive Waaler-Rose reaction, and in 9 of 31 patients with a negative reaction. The parallel trend of the titer of incomplete antibodies to sheep's red cells and the concentration of rheumatoid factor in the serum of the patients with rheumatoid arthritis demonstrates indirectly that incomplete antibodies appear when the disease is relatively long in duration, since rheumatoid factor is known not to appear in the blood in the earliest stages of the disease [5].

TABLE 2. Titers of Incomplete Antibodies to Sheep's Red Cells

Serial No.	Dilution of test serum					
	whole	1:2	1:4	1:8	1:16	1:32
1			+++	++	++	+
2	++	+++	++	++	++	
3			++	++	+	—
4	+++	+++	+++	+++	+	—
5	+++	++	++	++	++	
6			++	+	—	—
7		++	+	—	—	—
8	+++	++	+	—	—	
9	+++	++	++	—	—	
10	++	+	+	—	—	
11	++	+	+	—	—	
12	++	++	+	—	—	
13	++	++	++	±	—	
14	++	++	+	—	—	
15	+++	+++	+++	±	—	
16	+++	+++	++	±	—	
17	±	++	++	—	—	
18	+	±	—	—	—	
19	+	±	—	—	—	
20	++	—	—	—	—	
21	++	—	—	—	—	
22	+	—	—	—	—	
23			++	—	—	
24	++		—	—	—	

TABLE 3. Absorption Activity of Red Cells of Different Animals in Relation to Incomplete Antisheep Antibodies

Serial No.	Dilution of test serum	Dilution of antiglobulin serum	Absorption by red cells				Serum before absorption
			sheep's	guinea pig's	rat's	human	
1	Whole	1:32	—	+		++	++
		1:64	—	+		++	++
		1:128	—	+		+	++
		1:256	—	±		+	+
2	»	1:4	—			++	++
		1:8	—			++	++
		1:16	—			++	++
		1:32	—			++	+
		1:64				++	+
3	1:2	1:10	++		+++	+++	+++
	1:4		+		+++	+++	+++
	1:8		—		+++	+++	++
	1:16		—		±	±	—
4	Whole	1:20	+		++	++	++
	1:2		—		+	++	+
	1:4		—		±	±	+

This investigation thus showed that incomplete antibodies to Forssman antigen can be found in the serum of certain individuals. These antibodies were detected more frequently in the serum of patients, and are therefore probably normal antibodies of the type of the iso- and heteroagglutinins of the blood. Incomplete antisheep antibodies were found more frequently in the serum of patients with collagen diseases than in the serum of other patients and healthy subjects. This stresses the need for further research in this direction. The extent to which the appearance of these antibodies is specific for those diseases with a similar pathogenesis must be determined. Results of great interest in this direction have been obtained, showing that heterophilic antibodies against Forssman antigen possess a particular tropism for blood vessels which contain this antigen [7, 12, 14]. These results demonstrate the importance of the study of the biological significance of Forssman's antigen in diseases of an allergic character, in the pathogenesis of which vascular disturbances play a leading part.

SUMMARY

Incomplete antibodies to sheep erythrocytes were revealed in the serum of the following adults: in 42.5% out of the 155 patients in the Institute of Rheumatism, in 16.2% out of the 148 patients in the psychiatric clinic, and in 7.2% out of the 83 apparently healthy persons. These antibodies were specific to sheep erythrocytes in comparison with the guinea pig, rat, and human erythrocytes; this was confirmed in absorption experiments. Among patients with infectious nonspecific polyarthritis incomplete antibodies were revealed mostly in individuals with the rheumatoid factor in the blood (in 21 out of 28) as compared to those without the rheumatoid factor (in 9 out of 31). It is assumed that incomplete antibodies to Forssman antigen are not normal antibodies, but appear in pathological conditions.

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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.
