

MECHANISM OF THE ANTIGLOBULIN TEST

I. S. Golod

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Absorption of antigammaglobulin factors from serum leads to the appearance of a positive indirect Coombs' test which hitherto was negative. This phenomenon was observed when sera from patients with various diseases and also sera from clinically healthy donors were used.

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Human and animal blood serum contains antigammaglobulin factors possessing specificity against different parts of the γ -globulin molecule [6, 7].

The possibility of obtaining a positive Coombs' test in sera giving initially negative results of this test by means of absorption of the antigammaglobulin factors was studied in the present investigation.

EXPERIMENTAL METHOD

The sera for testing were heated in a water bath for 30 min at 56° and freed from heterophilic antibodies against rabbit erythrocytes completely by absorption in the usual manner.

Absorption of the antiglobulin factors was then carried out: rabbit erythrocytes treated with tannin and sensitized with human γ -globulin by Boyden's method [2] were added in a dose of 0.1 ml of a 2.5% suspension to 0.3 ml of the test serum taken in twofold dilutions. The mixture was incubated at 37° for 1 h, and in a refrigerator at 4° for 18-20 h, after which the erythrocytes were removed by centrifugation.

Completeness of immunoabsorption of antigammaglobulin factors from the test serum was verified by the passive hemagglutination reaction performed by Boyden's method [2]. After treatment with tanninized erythrocytes sensitized with γ -globulin, the sera under investigation were tested in the passive hemagglutination reaction using γ -globulin as antigen. If absorption of the antigammaglobulin factors was complete, the reaction was negative.

If a single immunoabsorption procedure did not produce complete removal of the antigammaglobulin factors (as was frequently observed in the serum from patients), it was repeated.

Supernatant free from erythrocytes was investigated in the indirect Coombs' antiglobulin test [3] in the slide modification. Antiglobulin serum obtained by immunization of rabbits with serum from a Rhesus-positive group 0 donor by Dausset's scheme [4] was used in the test. The titer of serum determined by titration with erythrocytes sensitized with anti-Rh antibodies was 1:2048.

TABLE 1. Indirect Antiglobulin Reaction after Removal of Antigammaglobulin Factors from Test Serum

Diagnosis	Titers of sera with positive indirect antiglobulin reaction												Negative result	Tot. no. of persons investigated
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096		
Myocardial infarction				4	13	14	19	8	17	27	5	1	3	110
Severe myasthenia					4	1	3	3		2	1	1		15
Chronic pancreatitis						4	2		2	2	3	1		14
Healthy donors	1	2	6	5	8	16	18	2	1	2			21	82

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Sera of 221 persons, healthy donors and patients with various diseases (after myocardial infarction, severe myasthenia, chronic pancreatitis), were investigated. Investigation of the serum from all subjects for incomplete antibodies, by the usual technique of the indirect Coombs' test, gave negative results.

EXPERIMENTAL RESULTS

Absorption of serum with tanninized erythrocytes loaded with human γ -globulin revealed in most cases the presence of globulins capable of fixation by human erythrocytes and giving an indirect antiglobulin reaction (Table 1). This was seen particularly demonstratively in the case of patients with the diseases listed in the table, whose sera in many cases gave a positive reaction in high titer (1:256-1:4096). It is also interesting to note that the serum of clinically healthy persons may also acquire the ability, after absorption with globulin, to give a positive indirect antiglobulin reaction.

However, when blood sera from patients with various diseases was used, the indirect antiglobulin test after absorption of the antigammaglobulin factors was positive in higher titers than in the case of healthy donors.

To assess the specificity of this phenomenon, control investigations were carried out. The scheme used was the same as that indicated above, but tanninized erythrocytes, sensitized with heterologous γ -globulin and bovine serum albumin, and tanninized erythrocytes not sensitized with antigen were used to treat the investigated sera.

The heterologous γ -globulin used for sensitization of the erythrocytes was obtained from rabbit serum by salting out with a saturated solution of ammonium sulfate [1]. The tanninized erythrocytes were treated with bovine serum albumin and rabbit γ -globulin in the same doses as those of human γ -globulin.

In the control experiments 44 sera from healthy donors with an initially negative antiglobulin reaction were studied. After treatment of the test sera with tanninized erythrocytes sensitized with bovine serum albumin, and with tanninized erythrocytes not sensitized with antigen, the indirect Coombs' test was negative. By contrast, after treatment of the test sera with tanninized erythrocytes sensitized with rabbit γ -globulin, the antiglobulin reaction became positive in titers of 1:4-1:512.

The donor's blood group did not influence the results of the investigation. The presence of a positive reaction in this case can be explained by the existence of common antigenic determinants in the molecule of human and rabbit γ -globulin. This hypothesis is confirmed by results obtained by other workers [5, 8].

It can be postulated on the basis of these results that absorption of antigammaglobulin factors leads to the liberation of chemical groups capable of reacting in the antiglobulin test.

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