The Agglutination of Antibody Coated Red Blood Cells By Antigen*

by

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"Antigen sensitized red cells" have been prepared either by incubating antigens with red cells in the presence of bis-diazotized-benzidine (BDB technique) (3, 5) or by incubating the red cells with a weak solution of tannic acid prior to their incubation with the antigen (tanned cell technique) (1, 2). This technic has permitted the demonstration of small quantities of precipitating antibodies in solution and the detection of antibodies in sera of allergic individuals (1, 3). However the reverse procedure of coupling antibodies to red cells and using these to detect antigen, to our knowledge, has not yet been reported. The present study describes a procedure for the coupling of antibodies to red cells which are subsequently capable of being agglutinated by antigen.

Methods and Materials:

Rabbits were hyperimmunized with bovine serum albumin (BSA), ovalbumin (OA), and human serum gamma globulin (HGG). The antibodies from these antisera were purified by incubation of the antiserum with antigen-polystyrene conjugates and their

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subsequent elution from these immunoadsorbents at pH 3.0 (pH 3.0 eluate) (4). The pH of antibody-containing eluate was adjusted to 7.2 and it was dialyzed, lyophilized and analyzed for antibody content by the conventional BDB technic (using antigen coated red cells). The antibody-depleted serum obtained after centrifugation of the immunoadsorbent-antiserum mixture is referred to as supernatant.

Varying concentrations of purified antibodies were coupled to constant amounts of red blood cells in the presence of constant quantities of BDB. The procedure followed is identical to that described in references 3 and 5 except that antibody was used in lieu of antigen in the coupling procedure. These antibody sensitized cells (Ab-rbc) were then incubated with varying concentrations of the respective antigens. Inhibition tests were also performed by incubating the antigen solutions with the homologous or heterologous antisera prior to incubation with the Ab-rbc.

Results and Discussion:

As can be seen from Table I, essentially all of the anti-BSA antibodies detected in the original antiserum could be recovered in the pH 3.0 eluate. Usually only a trace of antibody, 0.1% or less, remained in the supernatant. Identical results were obtained with the OA- Anti OA, and HGG-Anti-HGG systems.

As can be seen in Table II, antibody-sensitized red blood cells were agglutinated by the homologous antigen. It is interesting to note that the maximum amount of antigen capable of eliciting agglutination was exceedingly small by accepted standards (0.75 ug/ml.). Higher concentrations of antigen prevented the agglutination of the antibody-sensitized red cells, thus presenting the classical picture of a "prozone" phenomenon. This

TABLE I.

Demonstration of Antibodies by the BDB Technic

	Reagent Used	BDB Titer
1.	Whole Anti-BSA serum	200,000
2.	Purified Anti-BSA antibodies reconstituted to original volume	200,000
3.	Supernatant of antiserum after incubation with antigen-polystyrene conjugates	< 100

TABLE II.

Agglutination of Antibody Sensitized Red Blood Cells

Concentration of Antigen (BSA) (Protein)	Agglutination of Ab-rbc Incubated With:-			
	Antigen Only	Antigen Plus Original Antiserum	Antigen Plus Purified Antibody	Antigen Plus Supernatant
100 ug/ml.	-	-	-	-
12.5 ug/ml.	-	-	-	-
1.5 ug/ml.	-	-	-	-
0.75 ug/ml.	+	-	-	+
0.19 ug/ml.	+	-	-	+
0.04 ug/ml.	+	-	-	+
0.01 ug/ml.	+	-	-	+
0.0025 ug/ml.	+	-	-	+
0.0004 ug/ml.	+	-	-	+
0.0002 ug/ml.	-	-	-	-
0.00002 ug/ml.	-	-	-	-

\+ Signifies agglutination
\- Signifies no agglutination

may be the reason why previous attempts to detect antibody coupled to red cells have been unsuccessful. Furthermore, as little as 0.0004 ug protein of antigen could agglutinate the Ab-rbc. The agglutination was specific in that it could be inhibited only by prior incubation of the antigen with either the original antiserum or the eluted antibodies but not by the supernatant or other heterologous antisera. Identical results were obtained with the OA-Anti-OA, and HGG-Anti-HGG systems.

Similar experiments using red blood cells coupled to the gamma globulin fractions of the antisera rather than the purified antibodies were not agglutinated in the presence of the corresponding antigens. This finding suggests that the antibody content of the gamma globulin fractions is insufficient to produce optimally sensitized red cells due probably to competition between the antibody molecules and non-antibody molecules for the BDB during the coupling procedure.

These results demonstrate unequivocally that antibodies coupled to red blood cells are not altered immunologically and are capable of reacting with the antigen in solution to produce specific agglutination of these red cells.

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