THE NATURE OF INCOMPLETE ANTIBODIES

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It is shown by methods of immunochemical analysis that incomplete hemagglutinins belong to the IgG-class of serum immunoglobulins. It is also demonstrated that the indirect Coombs' test can give complete bivalent antibodies provided that they are present in low enough concentration not to cause direct hemagglutination.

The Coombs' method is widely used in modern immunology for the detection of incomplete antibodies. However, sufficient precise information regarding structure of these proteins, their molecular weight, and the class of serum immunoglobulins to which they belong is not yet available. The definition of incomplete antibodies as monovalent antibodies, i.e., capable of forming an antigen–antibody complex but not eliciting a visible phenomenon (agglutination, precipitation), likewise has not been proved [1, 2, 8, 10, 12]. The difficulties of studying the nature of antibodies are attributable to the fact that until recently it has been impossible to isolate them in a pure form and to study them in detail.

The object of this investigation was to study the nature of antibodies giving rise to the Coombs' indirect hemagglutination phenomenon (the potentiation phenomenon).

EXPERIMENTAL METHOD

Rabbits were immunized with sheep's erythrocytes by the following scheme: primary immunization – two intravenous injections of 1 ml of a 10% suspension of erythrocytes at an interval of 48 h; reimmunization 3 months later with 5 intravenous injections of a 10% suspension of sheep's erythrocytes in the same dose at intervals of 24 h. The animals were exsanguinated on the 7th day after the last injection of antigen.

To isolate highly purified preparations of IgG from antiserum, a two-stage procedure was used: salting out of the total globulin fraction with ammonium sulfate at 0.4 saturation followed by purification of the IgG by ion-exchange chromatography on DEAE-cellulose (Whatman, England) [4].

TABLE 1. Titers of Antibodies in Protein Samples at Different Stages of Isolation and Purification of IgG

Fraction	Log of titer of hemagglutinins	
	complete	incomplete
Antiserum	5.42	7.22
γ-I	4.61	9.11
γ-II	4.61	9.72
γ-III (IgG)	4.01	9.11
Supernatant	2.41	4.21

The titer of complete antibodies against sheep's erythrocytes was determined by the active hemagglutination test performed in the usual manner, and the titer of incomplete hemagglutinins was determined by the indirect Coombs' test [5] using ass serum against rabbit immunoglobulins, preliminarily inactivated on a water bath for 30 min at 56° and absorbed with sheep's erythrocytes.

To destroy the IgM-antibodies, the antiserum was incubated in a dilution of 1:10 with 2-mercaptoethanol (Loba-Chemie, Austria) in a final concentration of 0.1 M (pH 7.2-7.3) at 37° for 1 h [6]. The double diffusion

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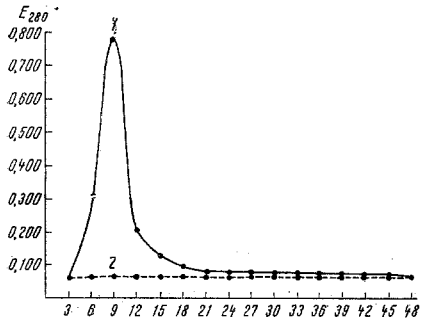

Fig. 1. Chromatograms of purified IgG (1) and supernatant fraction (2) on CM-cellulose column.

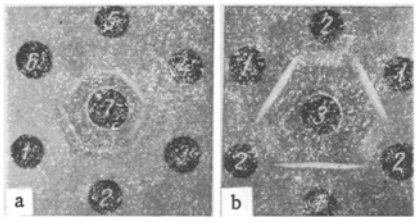

Fig. 3. Antigenic analysis of protein peak of supernatant fraction after chromatography on CM-cellulose (a) and analysis of antigenic relationship of proteins in eluate of supernatant to IgG (b). In a: 1-6) dilutions of eluate from 1:2 to 1:32; 7) anti-rabbit serum. In b: 1) IgG; 2) eluate of supernatant fractions; 3) anti-rabbit serum.

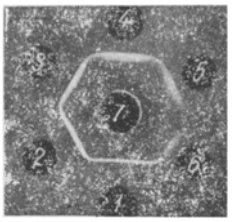

Fig. 2. Demonstration of purity of IgG preparation by precipitation in agar with ass antiserum against rabbit immunoglobulins. 1-6) Dilutions of IgG from 1:25 to 1:800; 7) anti-rabbit serum.

reaction in agar was carried out by Ouchterlony's method in the micromodification of Abelev and Tsvetkov [1], using ass antiserum against rabbit immunoglobulins for development.

EXPERIMENTAL RESULTS

The titer of complete and incomplete hemagglutinins in the rabbit antiserum (expressed in log units) was 5.42 and 7.22, respectively. After treatment of the serum with 2-mercaptoethanol, the titer of complete and incomplete antibodies was unchanged, demonstrating that they do not contain antibodies of the IgM-class.

At different stages of isolation and purification of IgG from the antiserum, samples of protein were taken and the concentration of complete and incomplete antibodies in them was investigated. The first sample was taken after precipitation of the globulins with ammonium sulfate and dialysis against buffered 0.15 M sodium chloride. This fraction was described as γ-I. The second sample (γ-II) was taken after dialysis of the protein against 0.01 M phosphate buffer (pH 7.55). It did not contain euglobulins, which are precipitated during dialysis against buffer of low ionic strength. The third sample (γ-III) was taken after chromatography of the proteins on DEAE-cellulose, and corresponded to highly purified IgG. In addition, the content of complete and incomplete hemagglutinins was determined in the fraction of serum proteins (supernatant fraction) not precipitated at 0.4 saturation with ammonium sulfate (after careful dialysis against 0.15 M sodium chloride), and also in the original antiserum (Table 1).

As Table 1 shows, when purified preparations of IgG (γ-III) were used, a marked increase in strength of the hemagglutination reaction was observed in the presence of antiglobulin serum compared with that for native serum.

The results in Table 1 do not, however, show conclusively what is responsible for the phenomenon of potentiation of hemagglutination in the Coombs' test when fraction γ-III is used: the bivalent IgG antibodies themselves or the monovalent antibodies contained in this fraction. For this reason, the presence of monovalent fragments of IgG antibodies, such as the Fab-fragments whose formation in vivo by catabolite splitting of IgG has been demonstrated experimentally [3], in the IgG preparation could be postulated. To verify this hypothesis, fraction γ-III was passed through a column with CM-cellulose in 0.01 M acetate buffer, pH 5.76. As was demonstrated previously [9], Fab-fragments of IgG are eluted under these conditions from the column, whereas IgG is adsorbed on the ion-exchanger. To a column measuring 1×12 cm, filled with CM-cellulose in 0.01 M acetate buffer, pH 5.76, 1.64 mg of fraction γ-III, previously dialyzed against the same buffer, was added. The whole of the protein added to the column was adsorbed on the ion-exchanger (Fig. 1). When three fresh portions of fraction γ-III (a total of 5 mg protein) were subsequently passed through the same column, in no case was protein found in the eluate.

Consequently, fragments resembling Fab were not present in the IgG preparation.

The homogeneity ot the γ-III fraction was tested by double diffusion in agar using ass antiserum against rabbit immunoglobulins (Fig. 2). In all concentrations used, IgG formed one distinct precipitation line, indicating that it was free from contamination with other serum globulins.

Activity of complete and incomplete hemagglutinins was also found in the supernatant fraction (Table 1). To establish the nature of this activity, the supernatant (5.5 mg protein), after dialysis against 0.01 M acetate buffer (pH 5.76), was passed through CM-cellulose (Fig. 1). A single protein peak was obtained, and after concentration it was found to contain 4 antigenic components (Fig. 3a), unrelated to IgG (Fig. 3b). The agglutination test and the indirect Coombs' tests revealed only a trace of activity in the composition of the protein peak (titer of incomplete antibodies 0, of incomplete 1 : 8). Hence, the serologic activity of the supernatant was due principally to IgG-antibodies not precipitated completely by ammonium sulfate at 0.4 saturation.

The facts described above suggest that the phenomenon of potentiation of hemagglutination in the Coombs' test is given by bivalent antibodies belonging to the IgG-class of serum immunoglobulins, capable of participating in direct serological reactions. This phenomenon is manifested when the concentration of bivalent antibodies is insufficient for direct hemagglutination. This hypothesis was put forward initially by McDuffie and Kabat [11], and the results of the present investigation confirm it experimentally.

The significantly greater potentiation of the hemagglutination reaction when purified IgG preparations were used compared with native serum can be explained on the basis of observations [7, 13], showing that the blood serum of man and animals contains factors inhibiting the hemagglutination reaction.

Hence, if an investigator is dealing with an antiserum which gives a direct hemagglutination reaction, and on addition of antiglobulin serum to the system the phenomenon of potentiation of hemagglutination is exhibited, this potentiation cannot be completely attributed to incomplete antibodies, but their presence must be excluded.

LITERATURE CITED

1. G. I. Abelev and V. S. Tsvetkov, Vopr. Onkol., No. 6, 62 (1960).
2. P. N. Kosyakov, Antigenic Substances of the Body and Their Importance in Biology and Medicine [in Russian], Moscow (1954).
3. A. Ya. Kul'berg, L. M. Bartova, I. A. Tarkhanova, et al., Biokhimiya, No. 1, 105 (1968).
4. A. Ya. Kul'berg, Yu. V. Docheva, I. A. Tarkhanova, et al., Biokhimiya, No. 6, 1178 (1969).
5. R. R. A. Coombs, A. E. Mourant, and R. R. Race, Brit. J. Exp. Path., 26, 255 (1945).
6. N. Costea, V. Yakulis, and P. Heller, Blood, 26, 323 (1965).
7. F. J. Dixon, R. T. Schultz, and F. Milgrom, Lab. Invest., 121, 503 (1956).
8. M. Heidelberger and F. E. Kendall, J. Exp. Med., 62, 697 (1935).
9. W. J. Mandy, M. K. Stambaugh, and A. Nisonoff, Science, 140, 901 (1963).
10. J. R. Marrack, H. Hock, and R. G. S. Johns, Brit. J. Exp. Path., 32, 212 (1951).
11. F. C. McDuffie and E. A. Kabat, J. Immunol., 77, 61 (1956).
12. A. S. Wiener, Proc. Soc. Exp. Biol. (New York), 56, 173 (1944).
13. R. C. Williams and H. G. Kunkel, Ann. New York Acad. Sci., 124, 860 (1965).