INTERACTION BETWEEN RABBIT γG-ANTIBODIES AND THE HOMOLOGOUS PEPSIN Fab¹-FRAGMENT

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UDC 612.398.132.017.1

Purified rabbit antibodies against the dinitrophenyl group bind the Fab¹-fragment of γG-globulin, which is indistinguishable in its activity from normal rabbit γG-globulin. Blocking the active centers of the antibodies by the hapten has no effect on their ability to bind the fragment. It is concluded that interaction between γG-globulins and the homologous Fab¹-fragment takes place through parts of the molecule outside the region of the active center.

Proteolysis of γG-globulin (IgG) is widely used for the isolation and detailed immunochemical study of individual parts of the molecule of this protein. An unexpected finding has been that normal IgG's of the rabbit, man, and primates can interact in the passive hemagglutination test with 3.5S- and 5S-fragments of Fab¹-segments of homologous IgG molecules obtained as a result of the action of pepsin, papain, trypsin, and cyanogen bromide [6, 9-12]. It has been postulated that these properties of IgG are due to normal antibodies* against the intrinsic antigenic determinants of the IgG which they contain, and which become demasked by proteolysis of the autologous protein in vivo [11, 12]. This hypothesis is in agreement with results showing that fragments resembling Fab¹ appear in the process of catabolism of homologous and autologous IgG with the participation of phagocytes [2, 3]. However, during immunization of adult rabbits with autologous IgG and its fragments, no increase in the titer of the hypothetical antibodies was observed [13].

No attempt has yet been made to show whether purified antibodies against heterologous antigens can react with the homologous Fab¹-fragment either before or after the specific blocking of their active centers. Since it is well known that the γG-antibody molecule has only two active centers of identical structure, such an experiment could shed light on the question of whether this property of IgG to react with its fragments is determined by the presence of specific antibodies against these fragments or whether the property of forming a complex with the Fab¹-fragment or other fragments like it is characteristic of a certain group of homologous IgG molecules, regardless of their specificity as antibodies, and is determined by the structural features of parts of the molecules located outside the active centers.

The object of the present investigation was to study the reaction between purified rabbit antibodies against the dinitrophenyl (DNP) group, before and after blocking of their active centers by hapten, and the normal homologous pepsin Fab¹-fragment.

EXPERIMENTAL METHOD

Serum against DNP was obtained by immunization of nine rabbits with dinitrophenylated bovine IgG in Freund's complete adjuvant, by the method described previously [5]. The mixture of antisera was kept in a frozen state until use. The agglutinating rabbit serum against sheep's erythrocytes had a titer of

* The active factors contained in rabbit and human IgG were originally called the homoreactant and agglutinator, respectively [9, 10].

Laboratory of Immunochemistry, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 6, pp. 70-73, June, 1971. Original article submitted November 3, 1970.

TABLE 1. Agglutination of Erythrocytes Sensitized with Fab^1-Fragment* by Means of Antibodies against DNP and Normal Rabbit IgG

Preparation	Concentration of preparation (in mg)						Unsensitized erythrocytes
	2.4	1.2	0.6	0.3	0.15	0.07	
Antibodies against DNP	++++	+++	+++	++	+	–	–
Normal IgG	+++	++	++	+	+	–	–
Antibodies against DNP + normal IgG	+++	+++	++	++	+	–	–
Antibodies against DNP + Fab^1-fragment (4 mg)	–	–	–	–	–	–	–
Normal IgG + Fab^1-fragment (4 mg)	–	–	–	–	–	–	–
Physiological saline	–	–	–	–	–	–	–

*A 2.5% suspension of erythrocytes was incubated with Fab^1-fragment from IgG against sheep's erythrocytes (2.5 mg/ml) for 1 h at 37°C. Before use, the sensitized erythrocytes were carefully washed with physiological saline.

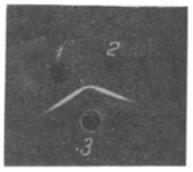

Fig. 1. Precipitation of rabbit IgG (1) and rabbit antibodies against DNP (2) in agar by antiserum against rabbit globulins (3).

1 : 10,240. Ass antiserum against rabbit serum globulins was obtained from the production department of the Gamaleya Institute of Epidemiology and Microbiology.

Rabbit IgG from normal (a mixture of sera of not less than nine rabbits) and hemagglutinating sera was obtained by ion-exchange chromatography on DEAE-Sephadex A-50 [4]. To avoid the possibility of development of any marked degree of anticomplementarity, the IgG specimens were not lyophilized and were stored at −20°C.

To obtain pepsin Fab^1-fragments from Ig of normal and hemagglutinating sera, the method of Nisonov as modified by Mandy et al. [10] was used. The Fab^1-fragments were homogenized in an ultracentrifuge, and their sedimentation constants were between 3.4 and 3.5 S.

Antibodies against DNP were isolated from the specific antiserum by the method of Frenek et al. [8]. The hapten-binding activity of the purified antibodies was determined by a modified method of equilibrium dialysis [7], using ε-2,4-dinitrophenyl-lysin (DNP lysin) as the hapten.

The passive hemagglutination test was carried out by the method of Mandy et al. [10], using a 2.5% suspension of sheep's erythrocytes sensitized with the Fab^1-fragment of γG-antibodies against sheep's erythrocytes. To 0.5 ml of antibodies against DNP and of normal IgG diluted twofold in buffered physiological saline, pH 7.2, 0.1 ml of a suspension of sensitized erythrocytes was added, and the reaction titer was estimated macroscopically after incubation for 6 h at 18°C.

The quantitative complement fixation test in the cold was carried out by Konikov's method [1] with estimation of the quantity of fixed complement in 50% hemolytic units.

The agar diffusion test was carried out in the micromodification of Ouchterlony's method.

EXPERIMENTAL RESULTS

Rabbit antibodies against DNP, used in the experiments described below, bound 1.94 moles of hapten (DNP-lysin), indicating that they contained not more than 3% of inactive protein. They sedimented in the ultracentrifuge as a single peak with $S_{20} = 6.6$. Antibodies against DNP formed one precipitation line with antiserum against rabbit globulins, and this merged with the precipitation line formed by normal rabbit IgG (Fig. 1).

Erythrocytes sensitized with Fab^1-fragment agglutinated in the presence both of normal IgG and of antibodies against DNP (Table 1). The minimal concentration of both proteins which possessed agglutinating activity was 150 μg/ml. Evidence was obtained that the hemagglutinating activity of antibodies against DNP and normal IgG was due to their reaction with the Fab^1-fragments fixed on the erythrocytes. Neither protein agglutinated erythrocytes not sensitized by the Fab^1-fragment. If the Fab^1-fragment of normal IgG (4 mg) was added to the original solutions of antibodies against DNP and normal IgG 60 min before their use

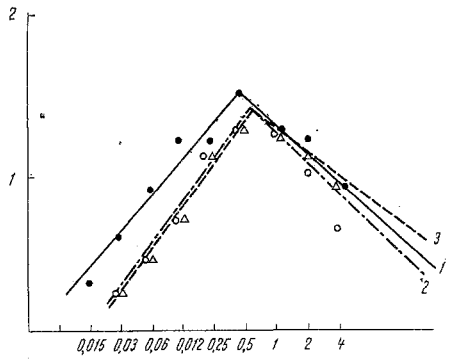

Fig. 2. Fixation of complement by normal IgG, by antigens against DNP, and by their complex with hapten in the presence of different concentrations of Fab[1]-fragment of normal IgG: 1) normal IgG; 2) antibodies against DNP; 3) antibodies against DNP + DNP-lysin. Concentration of IgG and antibodies 217 μg/ml. Ordinate, fixed compleplement (in 50% hemolytic units); abscissa, concentration of Fab[1]-fragment (in mg/ml).

in the hemagglutination test, they did not agglutinate the sensitized erythrocytes. Antibodies against DNP can react only with the isolated Fab[1]-region of the IgG molecule and not with the original IgG molecule. If the antibodies were mixed in equal volumes with normal IgG, their titer in the passive hemagglutination test remained unchanged. It was impossible to test the effect of DNP-lysin on the activity of antibodies against DNP in the passive hemagglutination test because the hapten, in a concentration of 7.5-15 mg (2-4 moles hapten per mole antibodies, used in a concentration of 2.4 mg/ml), caused spontaneous agglutination of the sensitized erythrocytes.

The ability of normal IgG and of antibodies against DNP to react with the Fab[1]-fragment was studied in the CFT.

It was found to begin with that on addition of increasing quantities of Fab[1]-fragment to a constant amount of IgG in the presence of heterologous complement (guinea pig serum), with certain ratios between the reagents fixation of the complement was observed and was zonal in character (Fig. 2). IgG was used in concentrations possessing either no anticomplementarity or only traces of it. The Fab[1]-fragment was not anticomplementary throughout the range of concentrations used. Altogether 8 batches of normal IgG from mixed sera of not less than nine rabbits were studied. With the same preparation of Fab[1]-fragment a zone of fixation was observed with identical ratios between IgG and Fab[1]-fragment. Meanwhile, during titration of IgG with Fab[1]-fragments obtained from individual IgGs or small pools of IgGs (from 2-3 rabbits), the position of the zone of fixation varied substantially. The observations showed that Fab[1]-fragments of individual rabbits differ in their content of the carbohydrate component: the higher this content, the greater the activity of the fragment with respect to complement fixation in the presence of homologous IgG.

It follows from Fig. 2 that antibodies against DNP fix complement in the presence of Fab[1]-fragment. If the same specimen of Fab[1]-fragment and equal concentrations of normal IgG and antibodies were used, the position of the zones of fixation coincided, although the quantity of complement fixed was slightly smaller in the system with antibodies than in the system with normal IgG.

Fixation of complement by antibodies against DNP and Fab[1]-fragment was not significantly changed after blocking of the active centers of the antibodies by hapten (DNP-lysin) added previously to the antibodies in a 4-molar excess.

The ability of the antibodies to react with Fab[1]-fragment of normal IgG, both before and after blocking of their active centers by hapten, means, first, that this reaction takes place through structures located outside the active centers of the antibody. Since the antibodies used in the investigation belonged to the IgG class and were indistinguishable in their ability to bind the fragment from a normal rabbit, it can be concluded that the property of IgG to react with the homologous fragment cannot be regarded as the result of the possession of normal antibodies against this fragment in its composition, as was postulated previously [6, 9-12]. There are therefore no grounds for considering that this phenomenon is connected with an autoimmune process developing as the result of demasking of concealed antigenic determinants of the autologous IgG during its catabolism [13]. So far as the definite selectivity of reaction of individual groups of the IgG molecules with pepsin, papain, or trypsin fragments is concerned [11, 12], the degree of steric correspondence between the partners in the reaction may depend, on the one hand, on the formation of the corresponding fragment and, on the other hand, on differences in the structure of the IgG molecules due to their heterogeneity.

The ability of immunoglobulins, irrespective of their specificity as antibodies, to react with the products of their proteolytic degradation may be of great importance in the regulation of immunobiological processes if it is considered that fragments of the Fab[1] type are intermediate products of the catabolism of γG-globulin [2, 3].

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