CHEMICAL MODIFICATION OF HUMAN IgG AS A METHOD OF DEMONSTRATING DIFFERENCES BETWEEN PROPERTIES OF ANTIBODIES AND NATURAL ANTIGLOBULINS (HOMOREACTANTS)

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Naturally arising antiglobulin factors, or homoreactants (HR) contained in preparations of human IgG are inactivated by incubation in a solution of sodium thiocyanate (3-5 M) and sodium deoxycholate (0.005 M). Staphylococcal antitoxin contained in the same human IgG preparations is resistant to the action of these reagents. The results point to differences in the structure of HR and antibodies.

KEY WORDS: homoreactants; $F(ab')_2$-fragment; staphylococcal antitoxin.

Proteins with the property of interacting with Fab-fragments of the IgG molecule have been found in healthy human and animal blood serum. These naturally arising antiglobulin factors have been called homoreactants (HR) or agglutinators [8, 10-13]. HR are present in the serum IgG fraction; they have antigenic kinship with this protein, but they differ in certain physicochemical and immunological properties from antibodies: They have a relative molecular weight of 240,000 daltons, relatively low affinity for specific ligands (Fab-fragments), and they evidently cannot pass through the human placental barrier [7, 9].

Attempts to isolate HR in a purified form by an immunosorption method, such as is widely used to purify antibodies, have proved unsuccessful [7]. One reason for this is the lack of systematic information about the chemical properties of these proteins and, in particular, about their sensitivity to the action of reagents used to produce dissociation of the antigen—antibody complex.

The present investigation was carried out to compare antibodies and HR by the use of a chemical modification method [3]. Human γ-globulin, used as the source of HR and antibodies, was treated by the chaotropic reagent sodium thiocyanate (NaSCN) and the ionic detergent sodium deoxycholate (Na-DCh). Unlike antibodies, HR are highly sensitive to the action of these reagents, indicating differences in the structure of the proteins compared.

METHODS

Preparations of human γ-globulin obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology and a preparation of human γ-globulin obtained from the firm of "Serva" were used. Each preparation was freed from contamination by aggregated protein by gel-chromatography on Sephadex G-200. Antigenic analysis showed that the purified preparations consisted of IgG only. Both preparations contained HR reacting with human $F(ab')_2$ fragments obtained with the aid of pepsin (pepsin HR), and also antibodies against staphyloccal toxoid.

$F(ab')_2$ from antirhesus human IgG was obtained as described previously [5, 7]. Pepsin HR was determined in the passive hemagglutination test, using group I Rh-positive erythrocytes sensitized by $F(ab')_2$ fragments of antirhesus antibodies by the method in [13].

The content of staphylococcal antitoxin was determined in the passive hemagglutination test [4] using purified staphylococcal toxoid as the antigen. The erythrocyte diagnostic serum was generously provided by G. L. Ratgauz.

Gel-chromatography was carried out on a column with Sephadex G-200 (2.5×140 cm), equili-

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TABLE 1. Activity of Pepsin HR and of Staphylococcal Antitoxin after Treatment of Human IgG with NaSCN and Na-DCh

IgG	HR (minimal agglutinating dose, mg)	Antitoxin (minimal agglutinating dose, mg)
Treated with NaSCN		
1M	0,75	0,025
3M	>3	0,025
5M	>3	0,025
Treated with Na-DCh* (0,005 M)	>3	0,025
IgG (control)	0,375	0,025

*These results were obtained after treatment with Na-DCh at 37°C.

brated with physiological saline, pH 7.2. The dead volume (V_0) of the column was determined with the aid of dextran blue 2000 ("Serva").

The agar precipitation test with antisera against human IgG and its Fab and Fc fragments ("Hyland") was carried out in a micromodification [1].

RESULTS

To study the effect of NaSCN on activity of HR and staphylococcal antitoxin, IgG in a concentration of 10 mg/ml was incubated for 30 min at 20°C in solutions of this reagent of different concentration (from 1 to 5 M). Dialysis in the cold was then carried out against physiological saline. Changes in the activity of the antitoxin did not take place under the influence of NaSCN in any of the concentrations used. Activity of HR was considerably reduced after incubation of IgG in NaSCN solutions with concentrations of 3 M or over (Table 1).

In the presence of high concentrations of NaSCN a precipitate was thrown down from the protein solution. To rule out the possibility of adsorption of HR by this precipitate, it was carefully washed and then incubated for 1 h with the original IgG. The precipitate was then separated by centrifugation and the HR titer determined in the supernatant. No decrease in activity of HR was observed.

Besides insoluble aggregates, treatment of IgG with 5M NaSCN also led to the formation of a large quantity of aggregated protein which remained soluble in physiological saline. This was shown by gel-chromatography of the treated IgG on a Sephadex G-200 column. Soluble aggregates eluted from V_0 of the column accounted for about 60% of the total IgG applied to the column (Fig. 1). Each of the peaks thus obtained contained an antitoxin which was indistinguishable in specific activity from the original IgG. The fractions of IgG treated with NaSCN compared did not possess homoreactant activity.

The action of Na-DCh on HR and staphylococcal antitoxin was studied in other experiments. For this purpose IgG in a concentration of 10-15 mg/ml was incubated with 0.005 M Na-DCh (from "Koch-Light") at pH 7.2 for 1 h at 0 or 37°C. The treated protein was dialyzed against physiological saline and subjected to chromatography on a Sephadex G-200 column. The IgG incubated with Na-DCh in the cold remained homogeneous and was not contaminated by aggregated protein (Fig. 1). In its antitoxic activity it was indistinguishable from the original IgG. The HR titer in the preparation was considerably lower than in the initial IgG. As a result of treatment of IgG with Na-DCh at 37°C the appearance of an aggregated protein, accounting for about 30% of the total IgG applied to the column, was observed (Fig. 1). Staphylococcal antitoxin was present in the composition of both peaks. Their specific activity was indistinguishable from the activity of the original IgG. HR was not found in any of the fractions of IgG tested after treatment with Na-DCh at 37°C.

The results are evidence that HR and antibodies contained in the same IgG preparation and detectable by similar serological methods (passive hemagglutination test) differ in their sensitivity to the action of NaSCN and Na-DCh. Neither NaSCN, in the highest of its concen-

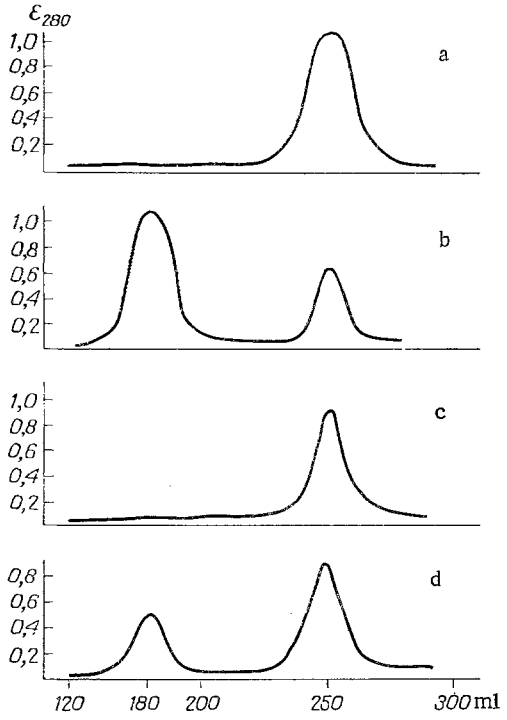

Fig. 1. Chromatography of human IgG preparations on Sephadex G-200: a) untreated human IgG; b) IgG treated with 5M NaSCN; c) IgG treated with 0.005M Na-DCh at 0°C; d) IgG treated with 0.005M Na-DCh at 37°C. Abscissa, volume of eluting solution (in ml); ordinate, optical density (E 280).

trations used, nor Na-DCh inactivates antibodies. This could mean that neither reagent changes to any significant degree the structure of the Fab region of the molecule, within which lies the combining site of the antibody. The wide use of NaSCN in order to obtain antibodies in purified form from the antigen—antibody complex is in agreement with this conclusion [6]. Meanwhile the same reagents destroy or inactivate HR. This may mean that the region in its molecule responsible for binding the ligand (Fab fragment) has a different structure and is evidently located in another part of the IgG molecule with the properties of HR. A similar conclusion was drawn previously from other experiments [2, 7]. It can be tentatively suggested that the combining site of HR lies on the Fc region of the molecule. This region of the IgG molecule is known [5] to have a less compact structure and to be more sensitive to the action of denaturing agents than the Fab region.

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INCREASED RESISTANCE OF (CBA × C57BL/6)F_1 HYBRIDS TO THE SYSTEMIC GRAFT versus HOST REACTION DURING REGENERATION OF THE SPLEEN

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Partial splenectomy was performed on (CBA × C57BL/6)F_1 hybrid mice 2, 12, 15, and 20 days before induction of the graft versus host reaction (GVHR) and 2 and 10 days after injection of parental immunocompetent cells. Recipients with an intact spleen, and those undergoing total splenectomy or a mock operation served as the control. Hybrids with a regenerating spleen up to 12 days of regeneration were shown to have increased resistance to GVHR, whereas splenectomy increased the resistance of the hybrids to GVHR only if carried out 2 days before induction of GVHR or 2 days after it.

KEY WORDS: graft versus host reaction; partial splenectomy; regeneration.

In the early stage of the systemic graft versus host reaction (GVHR) splenomegaly is known to develop, and this may indicate a role of the spleen in the pathogenesis of the GVHR. Removal of the spleen 2 days after injection of donors' immunocompetent cells has been shown experimentally to cause weakening of this reaction [5, 6]. However, this hypothesis is contradicted by the fact that splenectomy, if performed 1, 2, or 7 days before induction of the GVHR, had no significant effect on the intensity of the reaction. Admittedly the same operation, if performed 12 days before induction of GVHR, aggravated its course [5-7].

No reference to the study of the effect of partial splenectomy (PS) on the course of the GVHR could be found in the accessible literature. However, such an investigation appeared interesting because during regeneration of the spleen immunological changes are observed in the animal, affecting both its cellular and its humoral immunity [1-4].

The object of this investigation was to study the effect of PS, performed at different times before and after induction of the GVHR, on the course of this reaction.

METHODS

Experiments were carried out on 524 female (CBA × C57BL/6)F_1 hybrids weighing 19-22 g, obtained from the "Stolbovaya" Nursery of Inbred Animals, Academy of Medical Sciences of the USSR. Under superficial ether anesthesia, two-thirds of the tissue of the spleen was removed from the recipients of the experimental group by the usual method [2, 4] or total splenectomy (TS) was performed 2, 12, 15, and 20 days before induction of GVHR and 2 and 10 days after injection of parental immunocompetent cells. The control recipients underwent either a mock operation or no operation at all.

C57BL/6 female mice served as donors for induction of GVHR. A suspension of spleen cells was prepared by homogenization in medium 199 and filtered twice through a fine Kapron filter. Viable cells were counted with the aid of trypan blue. A systemic GVHR was induced by injection of $75 \cdot 10^6$ living parental spleen cells in a volume of 0.4 ml into the retro-orbital venous sinus. Development of the GVHR was assessed clinically from the reduction in weight of the recipients, falling out of the hair, the stooping posture, diarrhea, and death of the animals.

Animals of each group were killed five at a time on the 10th and 25th day after induction of the GVHR and the weight of the spleen and body weight were determined.

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