THE HOMOREAGENT SYSTEM AND IMMUNOBIOLOGICAL PROPERTIES OF THE PRODUCTS OF PROTEOLYTIC HYDROLYSIS OF THE ANTIGEN – ANTIBODY COMPLEX

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The complex formed by egg albumin with monovalent Fab'-fragments of rabbit antibody interacts with the homoreagent from homologous (rabbit's) serum and, if injected into a rabbit, causes considerable transient disturbances of homeostasis. Water-insoluble complexes of antigen with bivalent $F(ab')_2$-fragments of the antibodies do not possess these properties.

Previous work has shown that 5S- and 3.5S-fragments from the Fab'-segment of the γG-globulin (IgG) can interact with autologous or homologous IgG and with purified G-antibodies against heterologous antigen [3, 9, 11]. This interaction, ascribed to a particular fraction of immunoglobulins known as homoreagents [9], is accompanied by the consumption of complement [3], and this is presumably responsible for the appearance of marked transient disturbances of homeostasis when animals are injected with 3.5S-Fab'-fragments of autologous and homologous IgG [4].

When the immunobiological role of the homoreagent system is assessed it must be remembered that the Fab'-segment of the IgG molecule has considerable resistance to hydrolysis by tissue cathepsins [6, 8], by virtue of which fragments of the Fab' type are intermediate products of the catabolism of IgG and γG-antibodies [1, 2]. For the same reason it must be expected that 5S and 3.5S-active fragments [of the $F(ab')_2$ or Fab' type] exist among the intermediate products of catabolism of the antigen – antibody complex and that they accumulate in the body after reimmunization. Since these products can interact with the homoreagent in vivo, it is very interesting to discover whether this interaction can make a contribution to the series of pathophysiological reactions arising after reinjection of an antigen.

TABLE 1. Complement Consumption in vitro in Homologous (Rabbit) and Heterologous (Guinea Pig) Sera under the Influence of Immune Precipitate, ETP, SETP, and Pepsin Fab'-Fragment of Normal IgG

Preparation	Serum	
	rabbit	guinea pig
Precipitate	+	+
ETP	–	–
SETP	+	–
Fab-fragment	+	–

In the investigation described below the biological activity of soluble complexes of egg albumin with monovalent antibody fragments of the Fab' type was studied.

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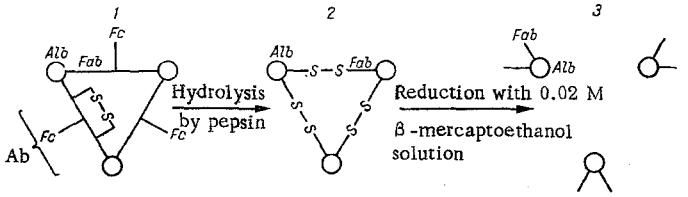

Fig. 1. Scheme of preparation of ETP (2) and SETP (3) from immune precipitate (1). Alb) egg albumin; Ab) γG-antibodies against egg albumin; Fab and F) corresponding fragments of γG-antibody molecule.

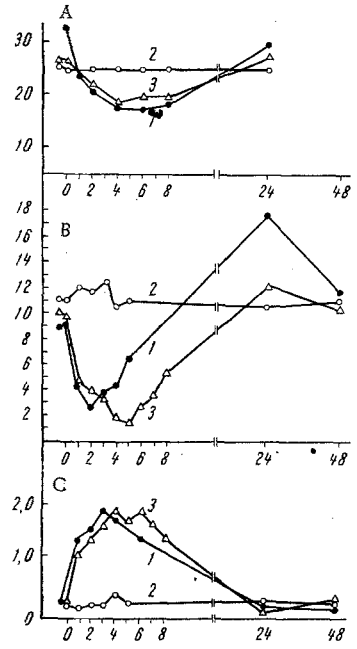

Fig. 2. Changes in levels of endogenous complement (a), blood white cell count (b), and body temperature (c) of rabbits under the influence of pepsin Fab'-fragment of IgG (1), ETP (2), and SETP (3). Each preparation injected intravenously in dose of 0.5 mg. Abscissa, time (in h); ordinate: in A) complement, in 50% hemolytic units, in B) total white cell count (1000/mm^3), in C) change in temperature (in °C).

EXPERIMENTAL METHOD

Rabbit antiserum against egg albumin was used. After equivalent proportions of antigen and antibody had been chosen in the quantitative precipitation test [5] an immune precipitate was obtained by adding 6 mg egg albumin to 20 ml antiserum. After formation of the precipitate it was thoroughly washed by centrifugation with cold physiological saline. The subsequent treatment of the precipitate followed the scheme shown in Fig. 1. Washed precipitate containing 30 mg protein was suspended in 6 ml 0.1 M acetate buffer, pH 4.1, 1 mg crystalline pepsin was added, and the sample was incubated at 37°C for 20 h. The pH was then adjusted to 7.2 and the enzyme-treated precipitate (ETP) was washed repeatedly by centrifugation with large volumes of cold physiological saline. To obtain a soluble complex of egg albumin with monovalent Fab'-fragments of antibody (SETP) part of the ETP suspension in phosphate buffer, pH 7.2, was incubated at room temperature with 0.04 M β-mercaptoethanol solution. After the precipitate had dissolved (10-15 min) sodium monoiodoacetate was added up to a final concentration of 0.08 M in order to block the liberated SHA-groups, and after 30 min the sample was dialyzed in the cold against physiological saline for 48 h.

The pepsin Fab'-fragment from normal rabbit IgG was obtained by the method of Nisonoff et al. [10]. The complement fixation test and the experiments in vivo were performed as described previously [4].

EXPERIMENTAL RESULTS

On hydrolysis of the immune precipitate by pepsin under the conditions described above, the Fc-fragments are separated from the antibody molecules. This was confirmed by experiments in which ETP was used, together with the original precipitate, to adsorb ass antiserum against rabbit IgG. Whereas the original precipitate extracted antibodies against the Fab'- and Fc-fragments of the IgG molecule, ETP bound antibodies against the Fab'-fragment only. The fact that ETP dissolved in the presence of a reducing agent (β-mercaptoethanol) also confirmed that the ETP contained Fab'-fragments of the antibodies linked by disulfide bridges (Fig. 1).

Immunochemical differences between the precipitate and ETP were clearly revealed by a study of the effect of these preparations on the complement level in fresh rabbit and guinea pig sera (Table 1). The Fab'-fragment of normal IgG, which, as previous investigation [3, 4] showed, can give rise to complement consumption only after reaction with homologous IgG, possesses the same properties. The coincidence between the properties of the Fab'-fragment and SETP accordingly indicates that the ability of the SETP to lower the complement level only in homologous serum is explained by its interaction with the homologous IgG contained in this serum (the homologous "homoreagent"). The fact that ETP does not possess these properties may mean

that the Fab'-fragments contained in it do not react with homoreagent because of stereochemical screening of the determinant groups on the surface of the Fab'-fragments responsible for this interaction.

As was demonstrated previously [4], homologous and autologous Fab'-fragments, when injected intravenously into a rabbit, cause a transient lowering of the endogenous complement level as well as changes in the white cell count of the blood and in the temperature. The action of the Fab'-fragment is species-specific and correlates with its ability to lower the complement level in homologous serum by interaction with the homoreagent. Investigation of the biological properties of ETP and SETP confirmed this rule and demonstrated similar properties of the SETP and Fab'-fragment of normal IgG. In fact, ETP did not cause the consumption of complement when added to the serum in vitro (Table 1), and in turn, if injected intravenously into rabbits, it did not affect their endogenous complement level, body temperature, or white cell count in the blood of the experimental animals (Fig. 2). Meanwhile, SETP, which, like Fab'-fragment, causes the consumption of complement in homologous serum in vitro, produced changes similar to those as a result of the action of Fab'-fragment in the experimental animals (Fig. 2). The only very slight differences between the biological properties of SETP and Fab'-fragment were that, for identical weight of the two substances, the action of SETP was more prolonged. This can be attributed to the longer duration of circulation of the antigen–Fab'-fragments in the body than of the free Fab'-fragment [12]. Egg albumin itself, even in a dose of 10 mg, when injected into rabbits caused no changes in the indices studied.

It can be concluded from these results that soluble products of catabolism of the antigen–antibody complex, including Fab'-like fragments of antibodies resistant to the action of cathepsins, can play an important role in the development of pathophysiological reactions to reinjection of an antigen. The activity of this complex is due to its ability to cause complement consumption through interaction between products of the SETP type with homoreagent circulating in the blood stream. The biological activity of the soluble intermediate products of breakdown of the antigen–antibody complex can thereby add to the biological effect of direct formation of the antigen–antibody complex in the circulation, the pathophysiological activity of which has now been well studied.

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