ISOLATION AND IMMUNOCHEMICAL ANALYSIS OF HYDROLYSIS PRODUCTS OF γG-GLOBULIN IN THE SERUM OF PARTIALLY HEPATECTOMIZED RABBITS

D. N. Evnin, I. A. Tarkhanova, and A. Ya. Kul'berg

UDC 616.36-089.873-092.9-07:616.15-097.5-078

A fragment of the IgG molecule related to Fab was discovered in the serum of partially hepatectomized rabbits by immunochemical analysis. Subsequent fractionation of the serum on carboxymethylcellulose, with the use of an immunosorbent, led to the isolation of this fragment in a purified form. Its sedimentation constant was found to be 5.2S. It thus corresponds to a bivalent IgG fragment. If partial hepatectomy was performed on animals receiving trasylol, a polyvalent proteinase inhibitor, before the operation and during the next 4 h, the presence of the Fab fragment could not be demonstrated. It is concluded that the Fab fragment appears as a result of hydrolysis of IgG by tissue proteases liberated from the liver cells damaged during operation.
KEY WORDS: *partial hepatectomy; immunochemical analysis; endogenous F(ab')$_2$ fragment of IgG.*

A previous investigation showed that intravenous injection of Fab fragments of homologous or autologous IgG into rabbits is followed by a variety of pathophysiological responses [6] and leads to increased immunological reactivity of the animals [7]. Since these findings point to an important role for Fab fragments of IgG in the regulation of homeostasis, it is interesting to discover under what conditions the content of these IgG breakdown products can be increased in the body.

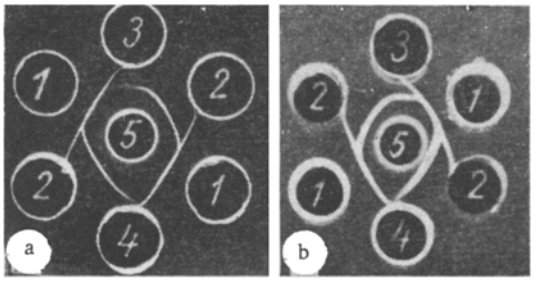

Fig. 1. Agar-diffusion test on third protein peak (a) and GFS fraction (b) of serum of partially hepatectomized (3) and intact (4) rabbits, of IgG (1) and Fab' fragment (2) with anti-IgG serum (5).

Fab fragments are formed by hydrolysis of IgG by various proteinases, including tissue cathepsins [9, 10, 12]. It is therefore logical to suppose that if cells with a high content of intracellular proteinases are damaged, the enzymes may appear in the circulation and cause hydrolysis of IgG to Fab fragments.

The object of the investigation described below was to demonstrate hydrolysis products of autologous IgG in the blood serum of rabbits after partial hepatectomy.

EXPERIMENTAL METHOD

Partial hepatectomy was performed on rabbits weighing 2.3-2.5 kg, during which the left lobe of the liver (35-40% of the

Kursk Medical Institute, 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. 81, No. 3, pp. 328-330, March, 1976. Original article submitted February 27, 1975.

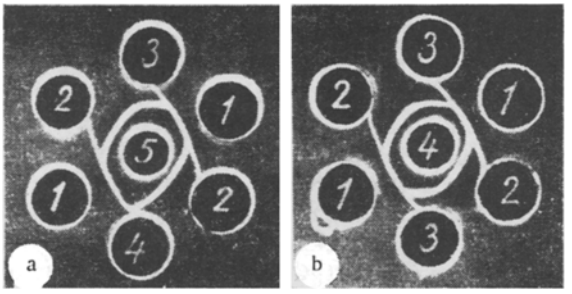

Fig. 2

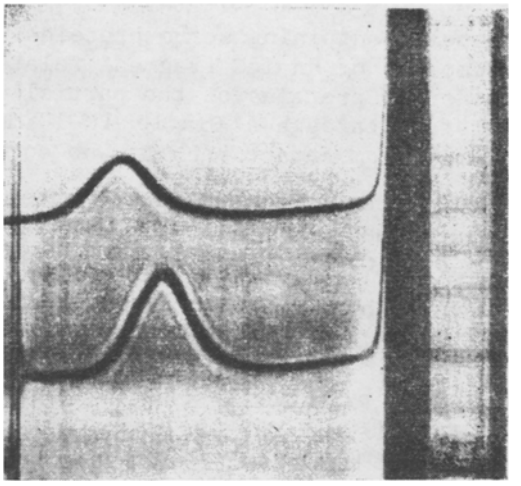

Fig. 3

Fig. 2. Agar-diffusion test on GFS fraction, on GFS fraction exhausted on immunosorbent, on protein eluted from immunosorbent, and on IgG and Fab' fragment with anti-IgG serum. In a: 1) IgG; 2) Fab' fragment; 3) GFS fraction; 4) GFS fraction exhausted on immunosorbent; 5) anti-IgG serum. In b: 1) IgG; 2) Fab'-fragment; 3) protein eluted from immunosorbent; 4) anti-IgG serum.

Fig. 3. Sedimentation diagram of Fab-like fragment purified on immunosorbent (above) and of pepsin $F(ab')_2$ fragment (below).

mass of the organ) was removed. The animals were exsanguinated 4 h after the operation by cannulation of the common carotid artery. Pooled sera from 5 to 8 partially hepatectomized rabbits were used in the experiments.

IgG was obtained from normal rabbit serum by the method described in [11]. Rabbit IgG was hydrolyzed by pepsin (Koch-Light, England) [14]. The $F(ab')_2$ fragment was isolated on carboxymethylcellulose CM-11 (Whatman, England) [13]. The monovalent Fab'fragment was obtained by the method described previously [4].

An immunosorbent with donkey antibodies against rabbit IgG was obtained on the basis of sepharose 4B activated by cyanogen bromide [8]. The globulin fraction was obtained from the donkey serum against rabbit IgG by precipitation with ammonium sulfate at 50% saturation, and mixed with the activated sepharose in physiological saline, pH 7.0. For every 10 ml of activated sepharose, 100 mg of immunoglobulin was added and the mixture was incubated with constant mixing for 20 h at 4°C. The immunosorbent was then thoroughly washed in a column with a large volume of cold physiological saline. It was found that 96% of the protein was fixed to the sepharose. The resulting immunosorbent specifically bound rabbit IgG (0.8 mg/ml sorbent) and the pepsin Fab' fragments (0.19 mg/ml sorbent) and $F(ab')_2$ fragments (0.38 mg/ml sorbent) of rabbit IgG, but did not fix detectable amounts of egg albumin.

Gel chromatography was carried out on Sephadex G-200 in column measuring 2.5 × 120 cm. Samples were applied in a volume of 5 ml. Fractions of eluate measuring 4 ml were collected automatically and their protein content was estimated from their absorption at 280 nm.

The double diffusion test in agar was carried out in the micromodification described in [1]. The Spinco model E analytical ultracentrifuge was used for sedimentation analysis (52,600 rpm, 20°C).

EXPERIMENTAL RESULTS

To detect Fab-like fragments in the serum of the partially hepatectomized animals, two methods were used. One consisted of fractionating the serum proteins by gel chromatography on Sephadex G-200, followed by analysis by the double diffusion in agar technique,

of the third peak containing serum proteins with molecular weights of 50,000-100,000. Appreciable amounts of an IgG fragment related to the Fab' fragment were found in the third peak of serum proteins of the partially hepatectomized animals after concentration by ultrafiltration through a Diaflo PM-10 membrane. Meanwhile, in the serum fraction obtained similarly from intact rabbits, no corresponding fragment could be detected (Fig. 1a).

The second method was preliminary absorption of IgG from the serum of hepatectomized rabbits by ion-exchange chromatography on carboxymethylcellulose followed by detection of Fab fragments of IgG in the resulting preparation.

Ion-exchange chromatography was carried out under conditions (acetate buffer, pH 5.76, ionic strength 0.01) at which no binding of Fab fragments of IgG obtained with the aid of pepsin or papain [13, 15] took place. Proteins eluted from the column were concentrated to the initial volume by ultrafiltration through a Diaflo PM-10 membrane. No sign of IgG was found in the resulting preparation by the double diffusion in agar test with goat antiserum against the Fc fragment of rabbit IgG. Meanwhile, when the eluate was tested with donkey antiserum containing antibodies against the Fab portion of the IgG molecule, it was found to contain a component antigenically identical with the pepsin Fab fragment of IgG. In the preparation obtained similarly from the serum of normal rabbits or rabbits undergoing a mock operation, no antigenic component related to the Fab fragment of IgG could be found (Fig. 1b).

For a closer physicochemical and antigenic analysis of the fragment related to Fab', the IgG-free fraction of serum of partially hepatectomized rabbits (designated GFS) was used to isolate the above-mentioned IgG fragments on immunosorbent with fixed donkey antibodies against rabbit IgG. The experimental method was as follows. The GFS fraction was passed through a column containing the immunosorbent gel. The filtrate was collected, the immunosorbent was washed with cold physiological saline (pH 7.0), and the protein fixed on the sorbent was eluted with glycine-HCl buffer, pH 2.8. After analysis against physiological saline, the eluted protein was concentrated and investigated in the double diffusion in agar test. At the same time, a GFS preparation exhausted on the immunosorbent was tested. As Fig. 2b shows, donkey serum against rabbit IgG revealed one antigenic component in the eluate completely identical with the pepsin Fab' fragment of IgG. This fragment was not found in the GFS preparation exhausted on the immunosorbent (Fig. 2a). Calculations showed that each milliliter of serum of partially hepatectomized rabbits contained 100 μg of the fragment related to Fab'.

The results of analytical ultracentrifugation showed that the fragment related to Fab', purified on the immunosorbent, settled as a single peak with sedimentation coefficient of 5.2S (Fig. 3). The sedimentation constant of the pepsin $F(ab')_2$ fragment used as the control was 5S. It can accordingly be concluded that the IgG fragment present in the serum of the partially hepatectomized rabbits corresponded to the $F(ab')_2$ fragment of rabbit IgG.

The appearance of considerable amounts of $F(ab')_2$ fragments in the blood serum of partially hepatectomized animals can be connected with the liberation of proteinases from the injured liver cells. This hypothesis confirms observations showing the effect of the polyvalent proteinase inhibitor on the appearance of $F(ab')_2$-like fragments in partially hepatectomized animals. Experiments were carried out in accordance with the following scheme. Five intravenous injections, each of 5000 k.i.u. (kallikrein inactivator units) of trasylol were given to rabbits at intervals of 1 h. Partial hepatectomy was performed 1 h after the first injection, as described in the section "Experimental Method," and the animals were exsanguinated 4 h after the operation. No fragments of IgG related to $F(ab')_2$ were found in the serum obtained. It can accordingly be concluded that fragments related to $F(ab')_2$ were formed as the result of hydrolysis of IgG by proteinases liberated from liver cells damaged at operation.

As was stated above, autologous and homologous Fab fragments, if injected into the blood stream, can induce substantial transient disturbances of homeostasis. The appearance of large quantitities of fragments related to $F(ab')_2$ in the blood stream after operations on a parenchymatous organ such as the liver, discovered in the present experiments, suggests that these fragments play an important role in the development of pathophysiological reactions which accompany injury to the cells of parenchymatous organs. When the results of these experiments are evaluated it must also be remembered that the serum of partially hepatectomized animals obtained 2-4 h after the operation can potentiate the immune response to different antigens in homologous recipients [2, 3, 5]. This effect of the serum of partially

hepatectomized animals can be associated with the presence of IgG fragments related to $F(ab')_2$ in its composition, for model experiments have shown that pepsin Fab' fragments of IgG possess adjuvant function [7]. The authors are at present engaged on research to demonstrate that the adjuvant properties of the serum of partially hepatectomized animals are due to $F(ab')_2$-like fragments of IgG present in it.

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