ADJUVANT ACTION OF FRAGMENTS OF THE IgG MOLECULE PRESENT IN THE SERUM OF PARTIALLY HEPATECTOMIZED RABBITS

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Fragments of the IgG molecule, related to F*ab*, present in the blood serum of partially hepatectomized rabbits, have the property of intensifying the immune response of rabbits through xenogeneic erythrocytes. After simultaneous injection of sheep's erythrocytes and the F*ab*-like fragment into rabbits there was an increase in the number of antibody-producing cells in the spleen and in the titers of 19S and 7S hemagglutinins.

KEY WORDS: partial hepatectomy; $F(ab')_2$ fragment of autologous IgG; immune response.

The writers showed previously that the blood serum of rabbits 4 h after partial hepatectomy contains fragments of the IgG molecule with a sedimentation constant of 5.2S, similar in their antigenic structure to the F*ab*' and $F(ab')_2$ fragments obtained with pepsin [1]. It was also shown that at the same times after partial hepatectomy the serum of the animals contains a factor capable of stimulating the immune response of homologous recipients to various antigens [1, 2, 5]. Since model experiments have shown that pepsin F*ab*' fragments of homologous IgG possess the function of an adjuvant [6], the ability of the serum of partially hepatectomized animals to intensify the immune response may be attributable to the F*ab* fragments of IgG which it contains.

In the investigation described below the adjuvant function of the F*ab*-like fragments of IgG isolated from the serum of partially hepatectomized rabbits was investigated.

EXPERIMENTAL METHOD

Partial hepatectomy was performed on rabbits weighing 2.3-2.5 kg and the left lobe of the liver (35-40% of the weight of the organ) was removed. The animals were exsanguinated 4 h after the operation by cannulation of the common carotid artery. Pooled sera from five to eight partially hepatectomized rabbits were used in the experiments.

The method of isolation of the $F(ab')_2$ fragments of IgG from the serum of partially hepatectomized rabbits on an immunosorbent obtained by a combination of Sepharose 4B activated with cyanogen bromide and donkey antibodies against rabbit IgG, was described by the writers previously [1]. To remove IgG from the serum of the partially hepatectomized rabbits, it was dialyzed against 0.01 M acetate buffer, pH 5.76, for 48 h in the cold, the residue of euglobulins was discarded, and the supernatant was passed through a column with carboxymethylcellulose CM-11, equilibrated with the above-mentioned buffer. The eluate was concentrated by ultrafiltration through a Diaflo PM-10 membrane to the initial volume.

Rabbits were immunized with 5×10^6 sheep's erythrocytes intravenously. The $F(ab')_2$ fragment of IgG isolated from the serum of the partially hepatectomized rabbits was injected into intact rabbits (group 1) simultaneously with sheep's erythrocytes in a dose of 0.5 mg (this dose, according to calculations, is

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TABLE 1. Titers of Hemagglutinins in Rabbits after Injection of Antigen Combined with Serum of Partially Hepatectomized Rabbits or Various Preparations Obtained from that Serum

Group No. of animals	Number of animals	Titer of hemagglutinins (in $\log_2$) and of 7S antibodies (in parentheses) at different times after injection of antigen				
		initial	7th day	11th day	15th day	19th day
1	6	2,5±0,2 (0)	7,7±0,2 (2,3±0,2)	9,0±0,0 (3,0±0,0)	8,0±0,0 (3,3±0,3)	7,3±0,3 (4,0±0,0)
2	6	2,2±0,3 (0)	7,3±0,2 (2,2±0,17)	8,3±0,3 (2,7±0,3)	7,7±0,3 (3,3±0,3)	7,0±0,57 (3,7±0,3)
3	9	2,1±0,26 (0)	7,7±0,37 (2,1±0,1)	8,8±0,26 (3,0±0,3)	7,8±0,37 (3,6±0,24)	7,0±0,3 (3,8±0,37)
4	6	2,2±0,3 (0)	4,5±0,34 (0)	4,7±0,3 (0)	4,3±0,3 (1,3±0,89)	4,0±0,0 (2,0±0,0)
5	6	2,2±0,25 (0)	4,2±0,3 (0)	5,3±0,3 (0)	4,3±0,3 (1,3±0,89)	4,3±0,3 (2,3±0,3)
6	5	2,2±0,2 (0)	7,2±0,37 (2,2±0,2)	8,6±0,5 (3,0±0,3)	7,8±0,37 (3,4±0,5)	6,8±0,37 (3,8±0,58)
7	5	2,4±0,24 (0)	3,2±0,4 (0)	4,2±0,2 (0)	3,8±0,2 (0)	3,6±0,24 (2,0±0,0)
8	5	2,4±0,24 (0)	3,4±0,24 (0)	4,0±0,3 (0)	3,6±0,24 (1,6±0,4)	3,4±0,24 (2,2±0,2)

TABLE 2. Number of AFCs in Spleen of Rabbits on Seventh Day after Injection of Antigen Combined with Serum of Partially Hepatectomized Rabbits or Various Preparations Obtained Therefrom

Group No. of animals	Number of AFC per 10^6 spleen cells	Index of significance
1	699±6,5	P_{1-5} <0,001
2	694±7,4	P_{2-5} <0,001
3	681±3,7	P_{3-5} <0,001
4	156±5,5	P_{4-5} >0,5
5	155±3,7	—
6	669±26,0	P_{6-5} <0,001
7	155±3,0	P_{7-5} >0,5

equivalent to the content of $F(ab')_2$ fragments in 5 ml of serum of the partially hepatectomized animals). Simultaneously with the antigen, other groups of rabbits received injections of 0.5 mg of pepsin $F(ab')_2$ fragment of IgG (group 2), a fraction of the serum of partially hepatectomized rabbits freed from IgG (designated SHS; group 3), the SHS fraction exhausted on immunosorbent (group 4), physiological saline (group 5), the serum of partially hepatectomized rabbits (group 6), the serum of rabbits undergoing a mock operation (group 7), and normal rabbit serum (group 8). All the preparations were injected in a volume of 5 ml.

Hemagglutinins were determined by a microtitrator of the Takachi system. To determine 7S hemagglutinins the serum was first incubated for 1 h at 37°C with 0.1 M 2-mercaptoethanol.

The number of antibody-forming cells (AFC) in the spleen was determined by Jerne's direct method [9].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that both the purified $F(ab')_2$ fragment contained in the serum of the partially hepatectomized animals and the pepsin $F(ab')_2$ fragment considerably increased the production of 19S and 7S hemagglutinins. Their stimulating action was comparable with that of the unfractionated serum of partially hepatectomized animals and with the activity of the SHS fraction. The SHS fraction, exhausted on immunosorbent, lost its ability to stimulate the immune response (Table 1).

The increase in the hemagglutinin level produced by the $F(ab')_2$ fragment was the result of a general increase in the number of AFC, as shown by Jerne's direct plaque method (Table 2).

This investigation thus showed that the stimulating action of the serum of partially hepatectomized animals on the immune response is due exclusively to related $F(ab')_2$ fragments present in that serum in relatively large quantities. The fact that the serum of partially hepatectomized animals possesses a stimulating action on the immune response only if obtained during the first 4 h after the operation [2, 5] can be attributed to the high rate of excretion of the Fab fragment from the body [7, 10], on the one hand, and to effective neutralization of tissue proteinases by inhibitors present in the serum, so that the formation of fresh quantities of Fab fragments is prevented.

The biological significance of this stimulating action of Fab fragments of autologous IgG on immunoglobulin synthesis is evidently extremely important, if it is remembered that these fragments are inter-

mediate products of IgG catabolism [3, 4, 8, 11]. In this way a product of IgG catabolism, which stimulates antibody synthesis by a feedback mechanism, is able to maintain the constancy of the immunoglobulin level in the body.

LITERATURE CITED

1. D. N. Evnin, in: Proceedings of an All-Union Conference on General and Applied Immunology [in Russian], Part 1, Moscow (1974), pp. 53-54.
2. D. N. Evnin and A. Ya. Kul'berg, Dokl. Akad. Nauk SSSR, 197, 711 (1971).
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, M. N. Svirezheva, et al., Vestn. Akad. Med. Nauk SSSR, No. 7, 15 (1970).
5. A. Ya. Kul'berg and D. N. Evnin, Zh. Obshch. Biol., No. 5, 626 (1972).
6. M. N. Svirezheva and A. Ya. Kul'berg, Zh. Mikrobiol., No. 10, 76 (1974).
7. J. L. Fahey and A. G. Robinson, J. Exp. Med., 118, 845 (1963).
8. V. Ghetie and A. Sulica, Immunochemistry, 7, 175 (1970).
9. N. K. Jerne, A. A. Nordin, and C. Henry, in: Cell Bound Antibodies (ed. by B. Amos and H. Koprowski), Philadelphia (1963), p. 109.
10. H. L. Spiegelberg and W. O. Weigle, J. Exp. Med., 121, 323 (1966).
11. F. I. Tsuji, D. L. Davis, and D. H. Donald, J. Immunol., 102, 519 (1969).