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APPEARANCE OF AN ADJUVANT-LIKE FACTOR IN THE SERUM OF RATS AFTER UNILATERAL AND BILATERAL NEPHRECTOMY

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A factor capable of stimulating the immune response to heterologous red blood cells in syngeneic recipients was shown to be present in the serum of rats after unilateral and bilateral nephrectomy. This factor is similar in its adjuvant activity to the factor appearing in the serum after partial hepatectomy.

KEY WORDS: nephrectomy; immune response; adjuvants.

It was shown previously that after partial hepatectomy a factor stimulating the immune response to thymus-dependent antigens appears in the blood serum of rabbits [1]. An active factor was isolated from the serum by ion-exchange chromatography and a technique of immunoadsorption. In its immunochemical properties it is identical with the $F(ab')_2$ fragment of IgG and is formed by hydrolysis of this protein in vivo under the influence of neutral serine proteinases [2, 9]. It has also been shown that the adjuvant properties of the Fab fragments of homologous IgG are determined by the structure of the regions of the molecule located outside the combining sites of the antibodies [8].

Since the walls of large blood vessels contain considerable quantities of various proteinases [7], it might be supposed that during operations associated with ligation of the large arteries, breakdown products of IgG possessing adjuvant properties may appear in the blood stream.

In this investigation the appearance of an adjuvant-like factor in the serum of rats after nephrectomy was studied.

EXPERIMENTAL METHODS

Experiments were carried out on Wistar rats weighing 120-140 g. Unilateral and bilateral nephrectomy were performed under ether anesthesia by the usual method, and partial hepatectomy by the method of Higgins and Anderson [5]. The rats were exsanguinated 4 h after the operation. The serum obtained was injected intraperitoneally into syngeneic recipients, in a volume of 1 ml simultaneously with sheep's red blood cells

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TABLE 1. Effect of Serum from Rats Subjected to Unilateral and Bilateral Nephrectomy or Partial Hepatectomy on Primary Immune Response of Syngeneic Recipients to Sheep's Red Blood Cells (M ± m)

Group of animals	Number of animals	Hemagglutinin titer, $\log_2$		Number of AFC in spleen		Number of RFC in spleen	
		total	IgG	per 10^6 cells	per spleen	per 10^6 cells	per spleen ($\times 10^3$)
1	7	5,9±0,3	3,4±0,37	6,1±1,86	2 806±860	786±127	344±50
2	11	5,3±0,3	2,8±0,3	9,4±2,5	3 506±797	705±116	294±55
3	11	9,1±0,2	6,5±0,3	27±6,5	12 966±2850	1523±104	789±74
4	7	8,7±0,18	5,6±0,48	32,5±5,0	13 343±4323	1393±236	581±102
5	10	8,5±0,26	5,7±0,3	19,4±2,9	10 908±1638	1275±87	727±105
	P_{1-3}	<0,001	<0,001	<0,001	<0,01	<0,001	<0,001
	P_{2-3}	<0,001	<0,001	<0,05	<0,01	<0,001	<0,001
	P_{1-4}	<0,001	<0,01	<0,002	<0,05	<0,05	>0,05
	P_{2-4}	<0,001	<0,001	<0,001	<0,05	<0,02	<0,05
	P_{1-5}	<0,001	<0,001	<0,002	<0,001	<0,01	<0,01
	P_{2-5}	<0,001	<0,001	<0,02	<0,001	<0,001	<0,002

Legend. Animals of group 1 received injection of 1 ml physiological saline, group 2 1 ml serum of normal rats, group 3 1 ml serum of unilaterally nephrectomized rats, group 4 1 ml serum of bilaterally nephrectomized rats, group 5 1 ml serum of partially hepatectomized rats.

($5 \cdot 10^6$ cells). Simultaneously with the antigen, the animals of the control groups received an injection of serum from intact rats or physiological saline. The number of antibody-producing cells (AFC) [6] and the number of rosette-forming cells (RFC) [12] in the spleen and the titers of IgM and IgG hemagglutinins in the blood serum [4] were determined on the 7th day after immunization. Statistical analysis was carried out by Student's t-test.

EXPERIMENTAL RESULTS

As Table 1 shows, the serum of rats undergoing unilateral and bilateral nephrectomy, like the serum of animals subjected to partial hepatectomy, caused a marked increase in the intensity of proliferation of AFC in the spleen of syngeneic recipients immunized with sheep's red blood cells. The titers of IgM and IgG hemagglutinins in the animals of the experimental groups were significantly higher than in the controls. The number of RFC in the spleen of rats receiving serum of nephrectomized and hepatectomized rats as well as antigen also was appreciably larger.

The immune response was increased more through the action of serum from unilaterally and bilaterally nephrectomized rats than by the action of serum of partially hepatectomized animals.

A factor possessing adjuvant activity against heterologous red blood cells thus appears in the serum of rats after unilateral and bilateral nephrectomy. This factor is similar in its action to the factor which appears in the serum after partial hepatectomy. On the basis of this fact and the results of previous experiments [2, 9], it can be suggested that in both cases it is fragments of IgG resembling $F(ab')_2$ or Fab' fragments which possess the properties of an adjuvant.

When the results are assessed it must be recalled that, unlike intact IgG, its $F(ab')_2$ or Fab' fragments are not reabsorbed in the tubular apparatus of the kidneys but are rapidly excreted with the urine [3, 10, 11]. After bilateral nephrectomy it must therefore be expected that these fragments, formed during proteolysis of IgG in vivo, will accumulate in the circulation. Since reflex spasm of the vessels of the remaining kidney, accompanied by transient anuria, can develop after unilateral nephrectomy, in this case also a delay in the elimination of $F(ab')_2$ and Fab' fragments from the circulation can be expected. This evidently is the explanation of the more rapid increase in the intensity of the immune response under the influence of serum from nephrectomized rats than of serum obtained from partially hepatectomized animals.

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INVESTIGATION OF ADJUVANT PROPERTIES OF Fab FRAGMENT OF HETEROLOGOUS IgG

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The Fab fragment of human IgG can stimulate the immune response of rabbits to sheep's red blood cells. The adjuvant properties of the heterologous Fab fragment were weaker than those of the homologous Fab fragment. It was also less effective in activating the rabbit complement system both in vitro and in vivo. These results confirm the fact established previously that the adjuvant properties of Fab fragments correlates with their ability to activate the complement system.

KEY WORDS: Fab fragments; homologous and heterologous IgG; homoreactant; immune response.

Naturally arising antiglobulin factors (homoreactants or agglutinators) and the intermediate products of catabolism of homologous IgG (Fab fragments) which interact with them constitute one system for the regulation of immunologic homeostasis [1, 3]. The property of the Fab fragments of homologous IgG of nonspecifically stimulating the immune response to thymus-dependent antigens [4, 8] has also been ascribed to interaction between these IgG fragments and homoreactants [9]. It was concluded from indirect evidence that the adjuvant effect of the Fab fragments is determined by the fact that, in conjunction with homoreactants, they active the complement system [7, 10]; the active C3 products of proteolysis formed under these conditions stimulate the reaction of B lymphocytes, which have complement receptors, to the antigen [9].

The object of this investigation was to study the adjuvant properties of the Fab fragments of heterologous IgG.

EXPERIMENTAL METHODS

Rabbit IgG (from Calbiochem) and human IgG (antimeasles γ-globulin, Cohn's fraction II) preparations were used. They contained no natural hemagglutinins against sheep's or human red blood cells. Fab fragments were isolated from pepsin digests of these proteins [9]. The purity of the Fab fragments was determined by immunodiffusion analysis.

The number of antibody-forming cells (AFC) in the spleen was determined on the seventh day after intravenous immunization of rabbits with $5 \cdot 10^6$ sheep's red blood cells by the method of Jerne and Nordin [5]. The titer of antibodies against sheep's red cells was determined by the hemagglutination test.

Complement was titrated by the method described in [11] and the results expressed in 50% hemolytic units (CH_{50}).

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