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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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TABLE 1. Number of AFC and Hemagglutinin Titer in Rabbits after Injection of Sheep's Red Cells and Fab' Fragments from Rabbit or Human IgG

Preparation	Number of AFC per spleen (M ± m)	Antibody titer (log)	
		19S	7S
Control	1 644±264	0,54	0
Fab' from human IgG	16 789±4 960	0,79	0
Fab' from rabbit IgG	48 044±11 655	3,01	0,69

TABLE 2. Complement Fixation in Rabbit Serum by Fab' fragments from Rabbit and Human IgG

Preparation	Dose, mg	Amount of complement fixed, %
Fab' from rabbit IgG	0,2	36
	0,4	39
	0,6	44
Fab' from human IgG	0,2	12
	0,4	21,3
	0,6	36,3

The pepsin homoreactant was determined by the passive hemagglutination test [7, 8]. Sheep's red cells sensitized with Fab' fragments from rabbit IgG antibodies against sheep's red cells or human Rh-positive red cells sensitized by $F(ab')_2$ fragments from anti-Rh human antibodies were used.

EXPERIMENTAL RESULTS

To compare the adjuvant properties of homologous and heterologous Fab fragments the following experimental scheme was used. An intravenous injection of $5 \cdot 10^6$ sheep's red cells simultaneously with 0.5 mg of Fab' fragments from rabbit or human IgG was given to the rabbits. Animals of the control group were injected with the antigen only. The number of AFC at the peak of the primary immune response (the seventh day after immunization) was considerably greater in the animals receiving the Fab' fragments. The increase in proliferation of AFC under the influence of the homologous Fab' fragment was more marked (Table 1).

The existence of correlation between adjuvant activity of the homologous Fab fragment, on the one hand, and its ability to interact with homoreactant and to activate complement, on the other hand, were established previously [9]. With this fact and the data described above in mind, it was interesting to determine whether the human Fab' fragment can interact with rabbit homoreactant, and also whether it also has the property of activating the complement system in these animals.

It was shown by the use of a pool of normal rabbit serum and purified normal rabbit IgG as sources of homoreactant that both the serum and the purified IgG can effectively agglutinate red cells sensitized by rabbit or human $F(ab')_2$ fragments. Taking the results of control experiments into account, this means that human Fab' fragment can react with rabbit homoreactant.

To compare the complement-fixing properties of rabbit and human Fab fragments in experiments in vitro they were added separately in increasing doses to equal volumes (0.2 ml) of fresh rabbit serum, after which the samples were incubated for 1 h at 37°C.

As the results in Table 2 show, the complement titer in the rabbit serum was reduced by both homologous and heterologous Fab' fragments, but the homologous fragment had significantly greater activity. The same pattern was discovered when the ability of the fragments to activate the complement system was studied in vivo. Preparations of the fragments were injected intravenously into normal rabbits in a dose of 0.5 mg. The serum complement titer fell on average by 40% 4 h after injection of the homologous fragment, after which its level rose. The human Fab' fragment also caused a fall in the rabbit complement titer at these times, although by not more than 10-15%.

Hence, although the Fab' fragment of heterologous IgG can stimulate the humoral immune response in rabbits, it is less active in this respect than the Fab' fragment of homologous IgG. The weaker adjuvant activity of the heterologous Fab' fragment correlates with its weaker complement-fixing activity more than the

homologous fragment in rabbits. This fact can be regarded as evidence of correlation between the adjuvant properties of the Fab fragments and their ability to activate the complement system.

Since differences in complement-fixing activity between homologous and heterologous fragments could be detected in experiments in vitro, they are possibly attributable primarily to differences in their level of affinity for the corresponding homoreactants contained in rabbit serum. This explanation is likely to be correct because of differences in the structure of those regions of the molecules of the fragments compared that are responsible for interaction with the above-mentioned antiglobulin factors [2, 6].

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CHANGES IN THE BLOOD SERUM PROTEIN SPECTRUM OF MICE AFTER INJECTION OF A GLOBULIN PREPARATION CONTAINING HOMOLOGOUS TISSUE ANTIBODIES

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The blood serum protein spectrum of mice differing in their initial resistance to malignant tumors was investigated by disc electrophoresis in polyacrylamide gel. Differences between different strains of mice were found in the zone containing immunoglobulins. The injection of a globulin preparation containing a high titer of normal tissue antibodies induced an increase in α_2-macroglobulin and β_1-lipoprotein, containing mainly IgM and also a certain quantity of IgG, in mice of the two lines tested.

KEY WORDS: homologous globulin; tissue antibodies; blood serum protein spectrum; immunoglobulins.

Previous experimental investigations have shown that injection of a preparation of homologous globulin containing normal tissue antibodies in high titer is an effective method of increasing the resistance of the recipient to growth of malignant neoplasms [3].

In the investigation described below the effects of this preparation on the blood serum protein spectrum were studied.

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