COMPARATIVE ANALYSIS OF ADJUVANT ACTION OF Fab FRAGMENTS OF NORMAL RABBIT IgG OBTAINED WITH PEPSIN AND PAPAIN

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A comparative study of the adjuvant action of monovalent and bivalent Fab fragments obtained with the aid of pepsin and of the pepsin Fab fragments of normal rabbit IgG showed that their property of intensifying the immune response of rabbits to sheep's red cells is due to the structure of the C-terminal regions of the Fd fragment of the heavy chain. Pepsin $F(ab')_2$ and Fab' fragments stimulated hemagglutinin production and proliferation of antibody-producing cells in the spleen considerably; papain Fab fragments in the same dose had only a very slight effect on the immune response. The ability of fragments obtained with pepsin and papain to stimulate the immune response is inversely proportional to the concentration of pepsin and papain homoreactants in the rabbits' serum. It is postulated on the basis of these findings that the target cells for Fab fragments are lymphocytes with cytophilic homoreactants as their receptors.

KEY WORDS: regulation of the immune response; Fab fragments; serum and cytophilic homoreactants.

It was demonstrated previously that $F(ab)_2$-like fragments contained in the serum of partially hepatectomized rabbits have the property of stimulating the immune response to sheep's red cells in homologous recipients; the pepsin fragment of normal rabbit IgG gives a similar effect [1, 5]. Together with data indicating that fragments of the Fab type are intermediate products of immunoglobulin catabolism [2, 4, 5, 14], these facts pointed to the existence of a hitherto unknown mechanism of nonspecific regulation of immunoglobulin biosynthesis with the aid of hydrolysis products of autologous IgG.

The object of this investigation was to study the mechanism of the adjuvant action of the Fab fragments of normal rabbit IgG.

EXPERIMENTAL METHOD

Chinchilla rabbits weighing 2.5-3.0 kg were used. Rabbit IgG (Calbiochem), hydrolyzed with pepsin by the method of Nisonoff et al. [10], was used to obtain the $F(ab')_2$ and Fab' fragments. The hydrolyzed protein was passed through a Sephadex G-200 column (2.5 × 145 cm) equilibrated with physiological saline, pH 7.2; the 5.0S peak was collected, concentrated by ultrafiltration through a Diaflow PM-10 (Amicon) membrane, and passed again through the same column to purify the $F(ab')_2$ fragments further from contamination by unhydrolyzed IgG and low-molecular-weight products. The Fab' fragment was obtained by incubating the $F(ab')_2$ fragment in 0.01 M 2-mercaptoethanol for 5 h at 37°C. After the addition of iodoacetate to block free SH groups the preparation was passed through a Sephadex G-200 column and the 3.5S peak was collected and concentrated by ultrafiltration.

The papain Fab fragment of rabbit IgG was obtained by Porter's method [11]. All preparations of the fragments were tested by the double diffusion test in agar with donkey serum against rabbit globulins. They were found to be homogeneous and were partly identical with rabbit IgG. The homogeneity of the preparations

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TABLE 1. Effect of Papain Fab Fragment and Pepsin Fab' and $F(ab')_2$ Fragments of Normal Rabbit IgG on Proliferation of AFC in Spleen of Rabbits Immunized with Sheep's Red Cells

Group No.	Number of animals	Preparation	No. of AFC per 10^6 viable spleen cells on 7th day	Significance of difference
1	4	Physiological saline	172±23	—
2	4	Papain Fab fragment	284±32	$P_{1-2}<0,05$ $P_{2-3}<0,001$ $P_{2-4}<0,001$
3	4	Pepsin Fab' fragment	708±31	$P_{1-3}<0,001$ $P_{3-4}>0,05$
4	4	Pepsin $F(ab')_2$ fragment	633±36	$P_{1-4}<0,001$

Legend. Animals of experimental groups received $5 \cdot 10^6$ sheep's red cells simultaneously with 0.5 mg of the preparation of the corresponding fragment in 5 ml physiological saline intravenously. Animals of the control group received the same dose of sheep's red cells in 5 ml physiological saline.

TABLE 2. Effect of Papain Fab Fragment and Pepsin Fab' and $F(ab')_2$ Fragments of Normal Rabbit IgG on Production of Hemagglutinins against Sheep's Red Cells in Rabbits

Group No.	Number of animals	Preparation	Hemagglutinin titer ($\log_2$)					
			7th day		11th day		15th day	
			total	IgG	total	IgG	total	IgG
1	12	Physiological saline	4,25±0,2	0	5,4±0,18	1,2±0,48	5,0±0,2	2,0±0,3
2	14	Papain Fab fragment	5,1±0,2	0	6,0±0,2	2,5±0,17	5,7±0,15	2,9±0,18
3	7	Pepsin Fab' fragment	7,5±0,3	2,9±0,26	8,7±0,3	3,7±0,3	8,3±0,3	4,0±0,0
4	7	Pepsin $F(ab')_2$ fragment	6,9±0,26	2,48±0,28	8,3±0,3	3,7±0,3	7,7±0,3	3,7±0,3
		P_{1-2}	<0.01	>0,05	<0,05	<0,05	<0,02	<0,05
		P_{1-3}	<0,001	<0,001	<0,001	<0,002	<0,001	<0,001
		P_{1-4}	<0,001	<0,001	<0,001	<0,002	<0,001	<0,01
		P_{2-3}	<0,001	<0,001	<0,001	<0,01	<0,001	<0,001
		P_{3-4}	>0,05	>0,05	>0,05	>0,05	>0,05	>0,05

of fragments also was established by analytical ultracentrifugation. The sedimentation constant of the $F(ab')_2$, Fab', and Fab fragments were 5.0, 3.5, and 3.4S, respectively.

Rabbits were given intravenous injections of $5 \cdot 10^6$ sheep's red cells and 0.5 mg of one of the test preparations of Fab fragments. The animals of the control group received sheep's red cells only, in the same dose.

The number of antibody-forming cells (AFC) in the spleen was determined by the direct method of Jerne et al. [6]. Hemagglutinins were titrated by means of a microtitrator of the Takachi system (Labor). The titer of IgG hemagglutinins was determined after inactivation of the IgM hemagglutinins by 2-mercaptoethanol. The double diffusion test in agar was carried out in the micromodification. Pepsin and papain homoreactants in the rabbits' sera were titrated by the passive hemagglutination method described previously [3].

EXPERIMENTAL RESULTS

On immunization of the rabbits with $5 \cdot 10^6$ sheep's red cells simultaneously with $F(ab')_2$- and Fab'-pepsin fragments of normal rabbit IgG (each in a dose of 0.5 mg) marked stimulation of the immune response took place, as reflected in an increase in the number of AFC in the spleen and increased production of 19S and 7S hemagglutinins (Tables 1 and 2).

The degree of stimulation of the immune response by the fragments compared was identical. Meanwhile, the same dose of papain Fab fragment (0.5 mg) had significantly less effect on the immune response of the rabbits to sheep's red cells (Tables 1 and 2). Differences in the immune response of the animals of the various groups were statistically significant for all times of observation.

Normal rabbit serum is known to contain naturally produced antiglobulin factors, known as homoreactants, which interact specifically with fragments from the Fab region of the IgG molecule [9, 13]. The determinants recognized by the homoreactants are located in the C terminal regions of the Fd fragments of the heavy chain [12, 13], and for that reason the pepsin homoreactant does not react with papain Fab fragment and vice versa [8]. In the light of these findings it might be supposed that the adjuvant activity of the pepsin and papain fragments of normal rabbit IgG is dependent on the concentration of homoreactants against these fragments in the rabbits' serum.

Sheep's red cells sensitized by equal quantities of monovalent pepsin and papain Fab fragments of rabbit IgG antibodies against sheep's red cells were used to titrate the homoreactants in the passive hemagglutination test (PHT). The quantity of fragments fixed on the red cells was judged from the results of the PHT with donkey serum against rabbit IgG. All the sera tested were first heated to 56°C and exhausted by adsorption with sheep's red cells.

Investigation of the pool of normal rabbit sera showed that the titer of the papain homoreactant was significantly higher than the titer of the pepsin homoreactant (1:1024 and 1:128, respectively), whereas the quantity of papain and pepsin Fab fragments fixed on the red cells was the same (the hemagglutination titer with donkey serum against rabbit IgG was 1:3000 in both cases. These results were in agreement with those obtained by other workers [15]. The ability of the corresponding Fab fragments to stimulate the immune response was thus shown to be inversely proportional to the concentration of homoreactants against that fragment in the normal rabbit serum. Since homoreactants, as naturally produced antiglobulin factors, specifically recognize C-terminal regions of Fd fragments of the heavy chain which differ in their amino acid sequence [12, 13], it can be postulated that the absence of any marked adjuvant action of the papain Fab fragments is due to specific blocking of the fragments by the papain homoreactants on account of the high concentration of the latter in rabbit serum.

In the light of these observations it is reasonable to assume that it is the determinants formed by the C-terminal regions of the Fd fragment of the heavy chain which are responsible for the adjuvant properties of the Fab fragments; moreover, receptors similar in specificity to serum homoreactants are present on lymphocytes. The existence of such receptors on mouse lymphocytes has recently been demonstrated by Johnson et al. [7]. With these observations in mind it can be postulated that the target cells for Fab fragments are lymphocytes with cytophilic homoreactants as their receptors; under these circumstances the serum homoreactants perform the role of specific inhibitors, preventing the adjuvant action of the hydrolysis products of IgG arising from its Fab region from taking place.

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