INVESTIGATION OF THE BIOLOGICAL ACTIVITY OF PEPSIN Fab^1-FRAGMENTS OF HOMOLOGOUS AND AUTOLOGOUS γG-GLOBULIN

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UDC 615.373.6:547.962.4].015.4

Intravenous injection of 0.5-5 mg of pepsin Fab^1-fragments of homologous and autologous γG-globulin, and also of homologous γG-globulin (IgG) into a rabbit lowers the level of endogenous complement, and this is accompanied by a transient increase in the body temperature and marked leukopenia. The biological action of the Fab^1-fragments is similar to that of aggregated homologous and heterologous IgG and correlates with their ability to fix complement nonspecifically. Unlike aggregated IgG, the Fab^1-fragment exhibits its activity only in a homologous system, which is determined by its ability to react with IgG obtained from the same species.

Recent observations have determined new approaches to the study of biological properties of γG-globulin (IgG) and its fragments. Besides IgG, fragments of it with identical immunochemical properties to those of the pepsin Fab^1-fragment [2, 4], have also been shown to circulate in the body. These fragments are formed during catabolic breakdown of homologous and endogenous IgG in the lysosomal system of the phagocytes, and they are relatively highly resistant to decomposition by tissue cathepsins [9, 10]. It has also been demonstrated that fragments from the Fab-segment of the IgG molecule can react with homologous and autologous IgG [11, 14, 15], and that both normal IgG as well as γG-antibodies against heterologous antigens (even after blocking of their active centers [5]), can take part equally in the reaction. Although on this basis this reaction cannot be regarded as one of antigen–antibody type, it is significant that interaction between IgG and the homologous (autologous) Fab^1-fragment is accompanied by complement fixation [5]. This fact suggested that fixation of endogenous complement may take place during the reaction in vivo between IgG and fragments of the Fab^1 type formed during its catabolism, in conjunction with development of pathophysiological reactions accompanying this process.

In the investigation described below, the effect of Fab^1-fragments of autologous and homologous IgG on the level of endogenous complement and on some physiological indices was studied in rabbits.

EXPERIMENTAL METHOD

IgG was isolated from rabbit serum by means of ion-exchange chromatography on DEAE-Sephadex as described previously [3]. IgG was isolated in the same way from normal bovine serum [6]. Fab^1-fragments were obtained from pepsin hydrolysates of rabbit and bovine IgG by the method of Mandy et al. [12]. Aggregate-free (AF) and aggregated (A) fractions of IgG were obtained exactly in accordance with the method described by Abdou and Richter [8]. Complement was titrated by Mayer's method [7]. Acid phosphatase in the blood serum was determined by Asatiani's method [1].*

*The authors are grateful to Dr. V. A. Toder for his help with the determinations of acid phosphatase.

Laboratory of Immunochemistry and Radiation Immunology, 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. 72, No. 7, pp. 64-68, July 1971. Original article submitted December 28, 1970.

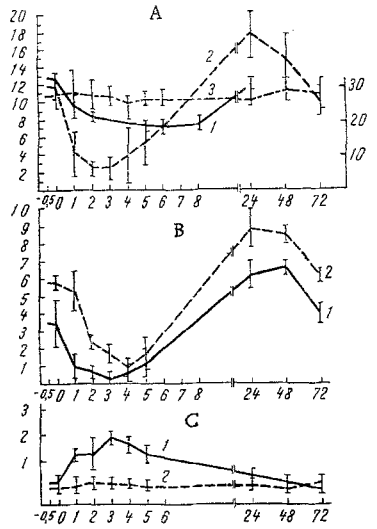

Fig. 1

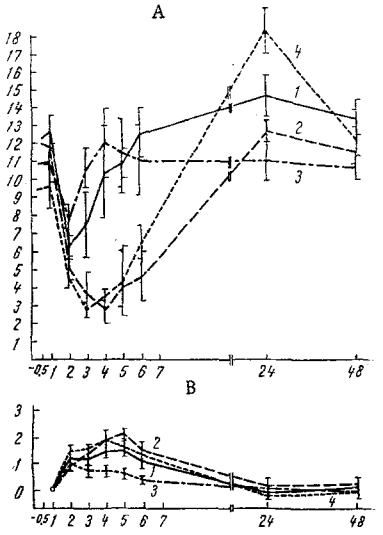

Fig. 2

Fig. 1. Change in serum complement level, blood white cell count, and body temperature of rabbits after intravenous injection of 5 mg homologous Fab[1]-fragment. A: 1) complement level, 2) total white cell count, 3) control (white cell count after intravenous injection of physiological saline). Ordinate: left – total white cell count (in thousands/mm^3), right – complement level (in 50% hemolytic units); abscissa, time (in h). B: 1) neutrophil count, 2) lymphocyte count. Ordinate, neutrophil and lymphocyte counts (thousands/mm^3); abscissa, time (in h). C: 1) body temperature, 2) control (body temperature after injection of physiological saline). Ordinate, change in temperature (Δt in deg.); abscissa, time (in h).

Fig. 2. Changes in blood white cell count (A) and body temperature of rabbits (B) after intravenous injection of 0.5 mg homologous IgG (1), A-fraction of IgG (2), AF-fraction of IgG (3), and AF-fraction of Fab[1]-fragment of homologous IgG (4). Abscissa, time (in h); ordinate: A) total white cell count (thousands/mm^3); B) change in temperature (Δt in deg).

In all the experiments in vivo, pyrogen-free physiological saline was used as the solvent for making up the protein preparations. The weight of the experimental rabbits was 2.5-3 kg.

EXPERIMENTAL RESULTS

After intravenous injection of 5 mg homologous Fab[1]-fragment into a rabbit, a marked decrease in the concentration of endogenous complement was found, its level falling to a minimum after 5-6 h (Fig. 1A). The complement level then rose, but after 24 h it was still a little below normal. Against this background of lowering of the complement level in the experimental animals, a marked leukopenia developed (Fig. 1A), with a decrease in the counts of both neutrophils and lymphocytes in the blood (Fig. 1B). The red cell count in the blood remained within normal limits. The leukopenia was transient in character, and after 24 h the white cell count was close to normal or there was even a moderate leukocytosis. Since the acid phosphatase concentration was not increased in the blood of the experimental animals during the phase of leukopenia or subsequently, this may mean that the decrease in the number of neutrophils was due to their migration into the extravascular space rather than to their destruction in the blood stream. In the period of recovery of the white cell count in the circulating blood, the number of stab cells showed a substantial increase, up to 8% (normally not more than 1-2%).

A characteristic feature of the biological action of the Fab[1]-fragment is associated with its pyrogenic effect (Fig. 1C). Measurement of the rectal temperature indicates that the greatest elevation of the temperature took place against the background of leukopenia and a lowered complement level. The temperature returned to normal in the period of restoration of the white cell count in the circulating blood.

TABLE 1. Fixation of Complement by IgG and Fab[1]-Fragments

Sample	Source of complement	
	Rabbit serum	Guinea pig serum
Rabbit Fab[1] (original)	+	−
" " (AF-fraction)	+	−
Rabbit IgG (original)*	±	±
" " (AF-fraction)	−	−
" " (A-fraction)	+	+
Bovine IgG (AF-fraction)	−	−
" " (A-fraction)	+	+
Fab[1] bovine	−	−

*Anticomplementarity of IgG is determined by the percentage content of the A-fraction.

The biological action of smaller doses of Fab[1]-fragment (0.5 and 0.005 mg) was tested. In these cases, changes similar to those described above, although less marked, were observed and their intensity was directly dependent on the dose of fragment.

Altogether 12 series of samples of Fab[1]-fragment obtained from different pools of normal rabbit serum were tested, and as a rule each sample was tested on 3 rabbits. The picture described above was observed in every case, and the differences between individual samples of the fragment were not significant and were connected entirely with the intensity of the reactions evoked by them.

The study of the biological action of Fab[1]-fragments from autologous IgG was of great interest. To obtain these specimens blood was taken from each of the 6 experimental rabbits at least 2 months before the experiment began. In every case the action of the autologous Fab[1]-fragment was qualitatively indistinguishable from the action of the homologous fragment, although there were some individual variations in the reaction of the animals to injections of the fragment.

Injection of fractions of an increasing dose of homologous or autologous Fab[1]-fragment into a rabbit led to the development of a state of diminished reactivity to the fragment. The scheme of the experiments was as follows: on the 1st day, 0.01, 0.02, and 0.02 mg of the fragments were injected intravenously at intervals of 30 min, on the 2nd day an extra injection (0.045 mg) of the fragment was given in addition, and on the 3rd and subsequent days the order of the injections was the same as on the previous days except that yet another injection of 4.5 mg of the fragment was given. On the first 2 days the experimental animals showed reactions to injection of the fragment, but they were substantially weaker than when a single injection of the total daily dose of the fragment was given. When fractional doses were injected, and 5 mg of the fragment was given on the 3rd day, the changes affecting the white blood cells and body temperature of the experimental animals were approximately the same as those after a single injection of 0.05 mg of the fragment. A similar decrease in reactivity was observed if autologous Fab[1]-fragment was injected in accordance with the above scheme for a period of 12 days, during the last 10 days of which daily injections of 5 mg of fragment were given. If injection of the fragment ceased even for 1 day, reactivity to it was largely restored.

The next essential step was to compare the biological action of the Fab[1]-fragment and that of intact rabbit IgG. Original IgG and two of its fractions (A and AF) were tested. At the same time, the action of the original Fab[1]-fragment and of its fraction obtained in the same way as the AF fraction of IgG was compared. Since the A-fraction amounted to not more than 5% of the total protein content in the original IgG, the dose of each sample injected intravenously was limited to 0.5 mg. The AF-fraction had a very slight effect on the body temperature and white cell count of the experimental animals compared with the A-fraction (Fig. 2). The action of the A-fraction in turn was substantially greater in degree than that of the original IgG. So far as the Fab[1]-fragment is concerned, its fraction, analogous to the AF-fraction of IgG, produced well-marked reactions indistinguishable in their intensity from those to the original Fab[1]-fragment and the A-fraction of IgG. It is thus evident that pathophysiological reactions to the Fab[1]-fragment in its monodispersed form are analogous to reactions only to aggregated IgG. The monodispersed form of IgG does not possess the biological properties of its fragment.

The appearance of reactions to aggregated IgG can be attributed to its marked anticomplementarity as regards both homologous and heterologous complement* (Table 1). In the presence of Fab[1]-fragment a decrease in the concentration of complement was observed only in the homologous serum, for this process is based on interaction between fragment and homologous IgG in the blood serum [5].

*Fresh rabbit and guinea pig sera were the source of complement.

If the results described above are borne in mind, it would be expected that the A-fraction of heterologous (bovine) IgG would elicit reactions analogous to those elicited by the A-fraction of homologous IgG and its Fab^1-fragment. This suggestion was fully confirmed. At the same time it was shown that the AF-fraction of bovine IgG and its Fab^1-fragment elicited none of the reactions described above in the rabbit, nor did they fix complement (Table 1).

Hence, by contrast with aggregated IgG, the biological activity of its Fab^1-fragment was marked by species-specificity, due to its ability to interact only with IgG of the same specific origin [5, 11, 14, 15]. From the time of formation of this complex, its biological activity, like that of aggregated IgG, was evidently determined by the reaction with endogenous complement. This hypothesis is supported by the fact that aggregate-free homologous and heterologous IgG did not fix complement spontaneously and did not elicit reactions in vivo.

Since fragments of the Fab^1-type are natural and intermediate products of IgG catabolism [2, 4], it can be concluded from the results described above that, together with IgG, they constitute a system in the body capable of fixing endogenous complement, and as a result of this process, of evoking various pathophysiological reactions [13], including those described above. Under normal conditions the functioning of this system is limited by the fact that fragments of Fab^1-type are located in the phagocytes [4]. If they enter the circulation continuously in very small quantities as the result of natural death of the cells, it is evident that the body becomes refractory to this dose of endogenous fragment, as is clear from the model experiments described above. However, in the case of a sharp increase in the level of endogenous fragment in the circulation, through an increase in permeability of the phagocytes or death of a large number of cells (for example, in acute inflammatory conditions), the conditions become ripe for fixation of endogenous complement and the development of the pathophysiological reactions accompanying this process. In this connection, our observations on the development of a pyrexial reaction to the Fab^1-fragment, as well as the well-marked migration of leukocytes under these conditions, become particularly interesting. The results described above can therefore be regarded as the basis for an investigation of the role of the products of the IgG catabolism in inflammatory processes.

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