The results are evidence that neutrophils can secrete peptide products regulating the function of immunocompetent cells. It is well known that neutrophils penetrate more rapidly than other cells into a focus of injury and they are the first to respond to injection of most antigens [5]. Accordingly, it is logical to postulate that activation products of neutrophils may serve as initiating factors in the development of cellular cascade reactions, involved in the development of inflammation and the immune response. Under normal conditions peptide products of intact neutrophils may act as the source of suppressor regulatory factors of immunocompetent cells.

LITERATURE CITED

1. B. M. Val'dman, I. A. Volchegorskii, and R. I. Lifshits, Patol. Fiziol., No. 2, 36 (1985).
2. V. G. Galaktionov and T. V. Anfalova, Zh. Obshch. Biol., 35, No. 3, 365 (1974).
3. I. I. Dolgushin, A. V. Zurochka, and S. I. Marachev, Immunologiya, No. 2, 79 (1986).
4. I. I. Dolgushin, L. Ya. Ébert, A. V. Zurochka, et al., Author's Certificate 12877006 USSR, Otkrytiya, No. 4, 181 (1987).
5. R. B. Colvin and N. E. Dvorak, Mechanisms of Immunopathology [Russian translation], Moscow (1983), pp. 85-108.
6. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
7. R. Röbler, S. Thiefedler, and W. Ruppelt, Blut, 31, No. 3, 149 (1975).
8. L. Wong and J. D. Wilson, J. Immunol. Meth., 7, 69 (1975).

NATURE OF A SERUM FACTOR PRECIPITATING AUTOLOGOUS ALPHA-GLOBULINS IN RABBITS

L. I. Krutitskaya and M. V. Polosatov

UDC 612.017.1:615.373.34

KEY WORDS: antibodies to human albumin, autologous alpha-globulins, autoimmune response.

One of the principal phenomena in which specific interaction of serum antibodies with antigens against which they are directed is manifested is the precipitation reaction. The writers showed previously that on immunization of rabbits with human albumin, starting with the 7th day and throughout the period of the immune response precipitating activity is found in the blood serum, directed both against human albumin and against autologous alpha-globulins (AAG) of intact rabbit serum. The reaction between proteins of immune and intact blood sera was recorded by immunodiffusion and immunoelectrophoresis in agarose [2]. The appearance of precipitating activity was observed in 100% of animals immunized with human albumin in a dose of 100 mg, and was absent in intact rabbits and rabbits immunized with other antigens tested (human transferrin and IgG, bovine albumin, rabbit albumin, Vi-antigen, sheep's red blood cells).

The aim of this investigation was to study the nature of a factor in rabbit immune blood serum precipitating autologous serum alpha-globulins.

EXPERIMENTAL METHOD

Blood serum from rabbits immunized intravenously by a single injection of human albumin ("Reanal," Hungary) in a dose of 100 mg per animal was used.

Antibodies in the immune serum were determined quantitatively by the passive hemagglutination test (PHT) using sheep's red blood cells sensitized with human albumin [1]. The AAG-precipitating factor was isolated by ion-exchange and immunoaffinity chromatography.

Group for Biochemical Methods of Analysis, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 9, pp. 331-334, September, 1988. Original article submitted January 22, 1988.

0007-4888/88/0009-1304$12.50

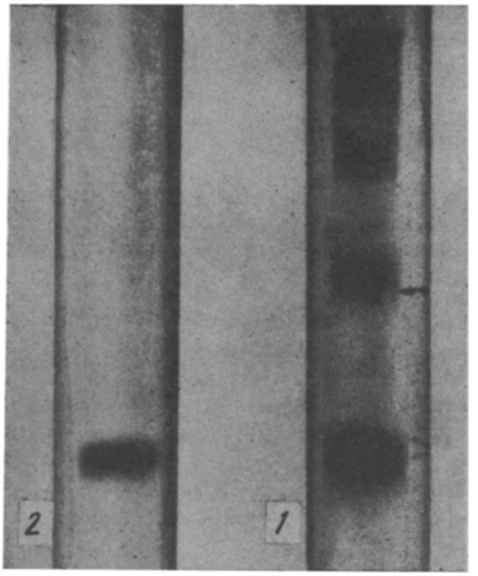

Fig. 1. Disk electrophoresis of human albumin preparations. 1) Human albumin ("Reanal"); 2) human albumin preparation after purification, 7.5% polyacrylamide gel. Stained with Amido Black.

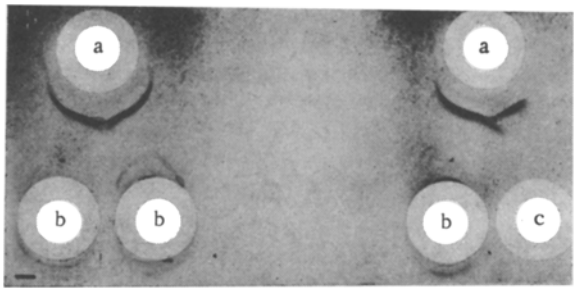

Fig. 2. Comparative analysis of precipitation arcs formed by human albumin and intact serum factor with antibodies to human albumin (countercurrent immunoelectrophoresis in agarose). a) Antibodies to human albumin (2 μg/ml); b) human albumin (1.5 μg/ml); c) serum of intact rabbit (dilution 1:2).

For this purpose the IgG fraction precipitated by ammonium sulfate at 33% saturation was dialyzed against 0.05 M sodium acetate buffer (pH 5.5) and chromatographed on a column (2.0 × 10 cm) with CM-Sephadex C-50 ("Pharmacia," Sweden) in a linear gradient of 0.05 M-0.6 M sodium acetate buffer (pH 5.5) [6]. The rate of elution was 30 ml/h. Further purification of the pooled and concentrated IgG fractions was carried out on a column (1.6 × 4 cm) of Sepharose 4B with immobilized human albumin. The immunosorbent was prepared by the method in [3]. Immune fractions of IgG were adsorbed in 0.05 M sodium acetate buffer (pH 5.5) containing 0.5 M NaCl. Bound protein was desorbed with 0.1 M acetic acid. Fractions containing AAG-precipitating factor were identified by Ouchterlony's immunodiffusion method and by immunoelectrophoresis and countercurrent immunoelectrophoresis in 0.7% agarose gel ("Serva," West Germany), made up in 0.025 M veronal-medinal buffer (pH 8.6) [1]. To study precipitating activity of the immune rabbit serum, it was adsorbed with human albumin in the proportion of 6-30 μg albumin to 1 ml of immune serum. Completeness of adsorption was monitored in the precipitation reaction by countercurrent immunoelectrophoresis in agarose. A preparation of human albumin purified from impurities was used for adsorption. The albumin was purified by methods of anion- and cation-exchange chromatography [4] and preparative electrophoresis in 7.5% polyacrylamide gel (PAG) by the method in [5]. IgG isolated from the immune serum were fragmented with papain ("Merck," West Germany), with enzyme and protein in the ratio of 1:50 by the method in [7]. Fab- and Fc-fragments were separated on a column (0.4 × 1 cm) with protein A-CI-Sepharose 4B ("Pharmacia"), equilibrated with 0.1 M

TABLE 1. Inhibition of Precipitation Reaction between Intact and Immune Rabbit Sera by Fab-Fragment of Rabbit Antibodies to Human Albumin

Experimental conditions				Precipitation reaction with immune serum
Intact rabbit serum, µl	0.1 M Tris-HCl buffer (pH 6.5), µl	Fab-fragment, µg	Fc-fragment, µg	
20	80	—	—	+
20	60	140	—	+
20	55	175	—	Inhibition
20	50	210	—	»
20	45	245	—	»
20	40	280	—	»
20	60	—	76	+
20	40	—	152	+
20	20	—	304	+
20	—	—	380	+

Legend. Final volume of each sample was 100 µl.

Tris-HCl buffer solution (pH 6.5). The Fc-fragment was desorbed with 0.1 M acetic acid. Activity of the isolated Fab- and Fc-fragments was estimated by ability to inhibit the precipitation reaction between alpha-globulins of intact serum and of the immune serum. For this purpose, various quantities of Fab- and Fc-fragments (the final volume of each sample was 100 µl) were added to intact rabbit serum (20 µl) and, after incubation for 18 h at room temperature, the ability of the intact serum to give a positive precipitation reaction with immune rabbit serum was investigated (countercurrent immunoelectrophoresis in agarose).

EXPERIMENTAL RESULTS

As a result of fractionation of proteins of the immune rabbit serum, AAG-precipitating factor was found to be precipitated at 33% saturation with ammonium sulfate, i.e., together with IgG. On chromatography of the immune serum on CM-Sephadex C-50 this factor was eluted in fractions of the protein peak, which also included IgG. Investigation of the immune serum by immunoelectrophoresis in agarose showed that the test factor has the mobility of gamma-globulins and is found in the precipitation arc corresponding to the migration zone of IgG. Consequently, the factor responsible for precipitation of AAG in rabbits is IgG.

According to the PHT data the concentration of antibodies to human albumin in the immune serum was 1:4096. During ion-exchange chromatography of the immune serum it was found that antibodies to human albumin are present in the same fractions as the alpha-globulin precipitating factor. An attempt was made to separate them by the immunoaffinity method on a Sepharose 4B column with immobilized human albumin. The results showed that the alpha-globulin precipitating factor is bound with human albumin linked to Sepharose. Immunoelectrophoretic analysis of the fractions showed that this factor is found in material desorbed from the column by 0.1 M acetic acid, and consisting of antibodies to human albumin, but is not found among proteins not bound with the sorbent. The results suggested that antibodies to human albumin are the factor responsible for precipitating AAG. This suggestion is confirmed by the results of adsorption of immune rabbit serum by human albumin. Addition of albumin in a concentration of 15 µg/ml immune serum completely abolished its ability to bind alpha-globulins of intact rabbit serum in the precipitation test. It must be pointed out that a purified preparation of human albumin, forming one homogeneous zone on PAG electrophoresis (Fig. 1), was used for adsorption. Inhibition of the reaction after exhaustion of the immune serum with the human albumin preparation, freed from impurities, indicates that anti-albumin antibodies are involved in this phenomenon. This conclusion is supported by comparative analysis of the precipitation arcs formed by antibodies to human albumin. It will be clear from Fig. 2 that the formation of precipitation lines took place both with human albumin and with AAG and was accompanied by "spur" formation, indicating partial identity of the antigens.

The region responsible for precipitation of alpha-globulins was localized in the antigen-binding fragment of antibodies to human albumin. This was shown in experiments to study inhibition of the precipitation reaction by Fab-fragments of IgG, isolated from immune rabbit serum. It was found that the Fab-fragment completely inhibited the precipitation reaction between alpha-globulins of intact serum and of immune rabbit serum, whereas the Fc-fragment had no effect on this reaction (Table 1).

AAG-precipitating activity discovered in the blood sera of rabbits immunized with human albumin is thus associated with antibodies to human albumin cross-reacting with autologous proteins (alpha-globulins). The phenomenon of synthesis of antibodies to one autologous protein, induced by injection of a cross-reacting antigen, can be regarded as an autoimmune reaction, evidence of selective abolition of immunologic tolerance to an autologous antigen in the period of the immune response to a foreign antigen. The unusual feature of the autoimmune response is that antibodies synthesized to human albumin are directed, not against the antigen with the greatest degree of homology (rabbit albumin), but against another rabbit blood plasma protein.

LITERATURE CITED

1. H. Friemel (ed.), Immunologische Arbeitsmethoden [Russian translation], Moscow (1979), pp. 9-18, 31-37, 78-89, 108-112.
2. L. I. Krutitskaya, Mechanisms of Regulation of Physiological Functions [in Russian], Leningrad (1985), pp. 88-89.
3. J. Turkova, Affinity Chromatography [Russian translation], Moscow (1980), pp. 188-194.
4. R. Epton (ed.), Chromatography of Synthetic and Biological Polymers, Vol. 2, Chichester (1978), pp. 78-87.
5. B. J. Davis, Ann. N. Y. Acad. Sci., 121, 404 (1964).
6. D. M. Weir (ed.), Handbook of Experimental Immunology, Oxford (1978), p. 810.
7. R. R. Porter, Biochem. J., 73, 119 (1959).