THE IMMUNOLOGICAL CHARACTERISTICS OF SERUM PROTEINS

III. ON THE SPECIES SPECIFICITY OF IMMUNE GAMMA-GLOBULINS OF THE FIRST AND SECOND ORDER

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In the field of theoretical and practical immunology, the problem of the formation and properties of anti-antibodies had not been thoroughly investigated. There is even no agreement in the definition of anti-antibodies. According to the correct opinion of G. N. Kryzhanovskii, L. F. Fontalin, and L. A. Pevnitskii [3], true anti-antibodies must be considered such antibodies which react with specific groupings of immune protein. If there is doubt as to against which structural grouping within the antibody the antibodies are directed, the latter should be more correctly designated as antibodies of the second order [3].

According to O. E. Vyazov and P. I. Tseitlin [1], chicken immune sera, containing antibodies of the second order, showed anaphylactogenic properties of human serum albumins. In these experiments albumins were used as antigens in order to obtain antibodies of the first order. However, it was possible to isolate from precipitating antisera, after papain digestion, a peptide fraction of a specific grouping, which was not antigenic [4,9]. It was also found that the specific grouping of the antitoxin did not possess the properties of the antigenic determinant [3].

The purpose of the present investigation was to find out whether precipitating antibodies of the first and second orders acquire certain antigenic properties of those proteins with which they react specifically.

METHODS

Experimental Procedure

Animals (rabbits and chickens) were immunized with human serum albumins (HSA). Then, electrophoretically isolated immune gamma-globulins of the first order [anti-HSA of rabbit (R) and anti-HSA of chicken (C)] were used as antigens to obtain immune gamma-globulins of the second order. The latter contained antibodies directed against normal gamma-globulins, as well as against corresponding antibodies [anti-(anti-HSA C)R and anti-(anti-HSA R)C]. Guinea pigs were sensitized with immune gamma-globulins of the first and second order. Thus, two schemes of animal immunization and sensitization were tested: HSA – chickens – rabbits – guinea pigs, HSA – rabbits – chickens – guinea pigs. At the challenging injection on the 2nd or the 30th day, HSA was administered, and then, in corresponding sequence, immune gamma-globulins of rabbits and chickens. At the same time, rabbit and chicken antisera were used in quantitative precipitation reactions, in which HSA and corresponding immune gamma-globulins were used as antigens. Control antigens were horse serum albumins (HoSA) and horse gamma-globulins (HoSG).*

Immunization of Animals

Rabbits were immunized with HSA or with immune chicken gamma-globulins, according to a definite scheme [5]. Chickens were immunized with HSA or with immune rabbit gamma-globulins. Protein solutions were introduced intravenously 3 times a week during 2 weeks (5 mg of protein per dose in the first week and 10 mg in the second). The animals were reimmunized after a 2-week interval, when 10 mg of protein were injected 3 times, every other day. Animals were bled to death 7 days after reimmunization.

*Human and horse serum albumins were kindly supplied by A. E. Gurvich, to whom we wish to express our thanks for his interest in this work.

TABLE 1. Production of Anaphylaxis in Guinea Pigs by Immune Gamma-Globulins

Sensitization			Challenging injection					
Expt. No.	γ-globulin	Amt. of protein (mg)	Day of rein-jection	HSA (mg)	Reac-tion	γ-globulin or serum	Amt. of protein (mg)	Reac-tion
141	Anti-(anti-HSA R No. 40) C No. 2	1	30	12	–	Whole C No. 2	25	++++
142	The same	1	30	12	–	Anti-HSA R No. 40	26	–
156	Anti-HSA R No. 40	1	29	18	–	Whole R (standard)	30	++++
160	Anti-(anti-HSA R No. 30) C No. 7	8	30	50	–	Anti-(anti-HSA R No. 30) C No. 8	20	+++
161	The same	12	30	50	–	Anti-HSA R No. 30	30	–
164	The same	15	30	25	–	Whole anti-HSA R No. 30	28	–
166	Anti-(anti-HSA C No. 6) R No. 41	21	22	30	–	Whole C No. 6	10	–
174	The same	14	2	35	–	Anti-HSA C No. 5	12	+
172	Anti-(anti-HSA C No. 6) R No. 42	6	2	15	–	Anti-HSA C No. 6	10	++

Legend: ++++ acute anaphylactic shock ending in death in 2-5 min; +++ pronounced shock with dispnea, loss of ability to stand, followed by inhibition and death after several hours; ++ clear anaphylactic reaction with broncho-spasms; + reaction consisting of scratching, ruffling of hair, sneezing, loss of activity; – no manifestations of anaphylactic shock.

TABLE 2. Precipitation Reaction of Antisera of Rabbits and Chickens, Immunized with Specific Gamma-Globulins

Antiserum		Antigen			Precipitate	
Volume (ml)	Designation	Volume (ml)	mg/ml	Designation	E · 100	mg/ml
0.14	Anti-(anti-HSA C No. 6)	0.14	0.6	Anti-HSA C No. 6	22.1	2.2
0.14	R No. 42	0.14	0.3	The same	30.4	3.04
0.14	The same	0.14	0.15	The same	17.6	1.76
0.14	The same	0.14	0.4	HSA	0.3	–
0.14	The same	0.14	0.2	HSA	3	0.3
0.14	The same	0.14	0.1	HSA	3.2	0.32
0.14	The same	0.14	1	HoSA	3.3	0.33
0.14	The same	0.14	0.1	HoSA	1.8	0.18
0.14	Anti-(anti-HSA C No. 6)	0.14	1.25	Anti-HSA C No. 6	25	2.5
0.14	R No. 41	0.14	1	HSA	3.2	0.32
0.14	The same	0.14	1	HoSA	3.6	0.36
0.14	The same	0.14	1	HoSG	3.1	0.31
0.14	Anti-(anti-HSA R No. 30)	0.14	1	Anti-HSA R No. 30	28.5	2.85
0.14	C No. 7	0.14	1	HSA	1.5	0.15
0.14	The same	0.14	1	HoSA	1.3	0.13

In these experiments antisera contained precipitating antibodies in the concentrations of 2.5 to 5.5 mg/ml, expressed as protein.

Isolation of Immune Gamma-Globulins

Gamma-globulin fractions were isolated from antisera by means of salting out with ammonium sulfate to a concentration of 1.34 M or by means of electrophoresis on a starch block (1 × 13 × 26 cm). Conditions of electrophoresis were as follows: veronal-medinal buffer (pH 8.6) with an ionic strength of 0.1; current 450 V, 10 mA; duration 48 h. Under these conditions, gamma-globulins migrated 2-3 cm from the point of application of the serum, in the direction of the cathode. The purity of preparations was controlled by means of paper electrophoresis.

Immunochemical Analysis

Immunochemical analysis of antisera and of gamma-globulins isolated from them was made according to the method of Heidelberger [6], modified by A. E. Gurvich and P. B. Kapner [2]. The determination consisted in sedimenting the precipitins within the optimal concentration zone of the antigen, after which the protein content of the precipitate, thoroughly washed with saline and water, was determined according to the method of Lowry et al. [7].

Production of Anaphylaxis

Guinea pigs, weighing 300 to 600 g, were sensitized once with rabbit or chicken gamma-globulins (1-15 mg of protein). The challenging doses of HSA and then of immune gamma-globulins in the corresponding sequence, were introduced intracardially. Protein concentration in 0.5-1 ml of the challenging dose constituted 10 to 50 mg. Thirty-five guinea pigs were used.

RESULTS

Similar results were obtained in all experiments with guinea pigs. As seen in Table 1, it was impossible to produce active anaphylaxis at the challenging inoculation of human albumins or of gamma-globulins of immune animals, if these proteins had not been inoculated previously in sensitizing doses. Guinea pigs underwent anaphylactic shocks only when in the sensitization and in the challenging injection immune gamma-globulins of the same species of animal were used. In addition, at the challenging injection, immune, as well as normal serum proteins of a given species of animal were equally effective (expt. No. 156).

Similar results were obtained in experiments with passive anaphylaxis. Thus, with sensitization with immune gamma-globulins of the second order, the anaphylactic reaction was noted only at the challenging injection of immune gamma-globulins of the first order (expts. Nos. 172, 174).

Thus, the method of anaphylaxis production did not reveal in the immune gamma-globulins of the second order any anaphylactogenic properties of those proteins with which antibodies of the first order reacted. Apparently, true antibodies, i.e., antibodies against specific groupings of the antibody, were not produced under the conditions of our immunological experiments.

This supposition was confirmed by the results of quantitative specific precipitation reaction, according to Heidelberger. As seen in Table 2, rabbit and chicken antisera, obtained as a result of immunization of animals with immune gamma-globulins of the first order, reacted quantitatively only with the corresponding preparations of gamma-globulins. It is true that these same antisera produced insignificant precipitations with other serum proteins, which had not been used to immunize animals. It is known, however, that the degree of achievement of cross reactions in the precipitation of serum proteins of different animal species may reach 10% of the volume of homologous reactions for albumins and 25% for gamma-globulins [8].

The results obtained by us show that a specific grouping of precipitating antibodies does not possess the properties of the corresponding antigenic determinant and, naturally, does not "transmit" these properties to antibodies of the second order. Similar conclusions were made as a result of attempts to obtain true anti-antitoxin [3].

Consequently, the known facts of the strict species specificity of serum proteins can be extended to the immune gamma-globulins, which contain precipitating antibodies of the first and the second order.

SUMMARY

Immune gamma-globulins of rabbits and chickens, containing precipitating antibodies of the first and second order, possessed strict species specificity. The results obtained pointed to the fact that the specific group of antibodies possessed no properties of the antigenic determinant of the proteins, with which this group interacted specifically.

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