

STUDIES ON REACTIONS OF HUMAN ALLERGIC SERUM WITH PROTEIN ANTIGENS

I. Methods of Passive Immune Elimination and Gel Diffusion Autoradiography

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THE SERUM FACTOR in man which has been termed atopic reagin or skin-sensitizing antibody is presumed to be of importance in the pathogenesis of the wheal and erythema type of skin reaction and the symptoms of allergic disease occurring as a result of the reaction of this antibody with its specific antigen. Some characteristics of this antibody that have been demonstrated are its ability to passively transfer the wheal and erythema type of skin reactivity to normal individuals, its ability to persist at a passively sensitized skin site, its marked susceptibility to heating at 56° C., and its failure to react with antigen in a visible precipitin reaction.

A serum factor with these characteristics occurs in dogs and is capable of passively transferring not only skin reactivity, but also nasal symptoms, asthma, and anaphylaxis to normal dogs,¹ which provides additional evidence of the importance of this serum factor in the pathogenesis of allergic manifestations.

Antigens which are major causes of respiratory symptoms in allergic patients, such as pollen antigens, mold antigens, or house dust antigens, are mixtures of materials from which the major antigenic fractions have not been obtained in pure form with sufficient certainty or in sufficient quantity to provide a system for general use in the study of a single antigen antibody system. Skin-sensitizing antibodies against insulin,² ovalbumin,³ and diphtheria toxoid⁴ have provided systems for study of these antibodies against more purified antigens, but, except for ovalbumin, these sensitivities do not occur spontaneously but as the result of injection of the antigen as prophylactic or therapeutic measures.

It is well known that some patients with a clinical history of sensitivity to animal dander have positive wheal- and erythema-type skin reactions to the serum of animals to which they are sensitive, and that this sensitivity is of great importance in evaluation of such patients prior to the administration of foreign

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serum used as antitoxin. Since an individual sensitive to a foreign serum must be sensitive to at least one component of that serum, these patients may be used as sources of allergic antibody against foreign serum protein antigens. Since many of these serum proteins are readily obtainable in reasonably pure form, this system provides a means of study of a single, spontaneously occurring, allergic antibody-antigen reaction. In addition, these foreign serum protein antigen fractions have been studied as antigenic materials in animals and are characterized in such respects as molecular weight and electrophoretic migration.

The studies to be reported describe the occurrence of wheal and erythema sensitivity in atopic individuals against foreign serum protein fractions, and various immunologic studies with the sera of these patients and their homologous antigen. The demonstration of serum protein antigens in animal dander extracts suggests one explanation of foreign serum sensitivity in patients who have not been injected with foreign serum.

METHODS AND MATERIALS

Foreign Serum Protein Antigens.—Horse serum albumin (HSA) and dog serum albumin (DSA) were Cohn Fraction V and horse serum gamma globulin (HGG) was Cohn Fraction II prepared from the corresponding sera.* Guinea pig serum albumin (GPSA) and guinea pig gamma globulin (GPGG) for skin testing were obtained by elution of these fractions from paper after electrophoretic migration of guinea pig serum with the use of a Spincro Model R electrophoresis apparatus. Filter paper electrophoresis of a serum sample was carried out in the usual manner and stained. A duplicate sample with 10 applications of the serum was run simultaneously and portions of this paper strip corresponding to the area of migration of albumin and gamma globulin of the stained strip were cut out and eluted with 0.2 ml. of 0.15 M NaCl.

I¹³¹ Trace Labeling of Antigen.—The method of Talmage⁵ for trace labeling of proteins with I¹³¹ (I*) was used to prepare I* HSA, I* HGG and I* DSA. Radioactivity in experiments using labeled proteins was counted in a NaI scintillation counter.

Autoradiographs.—Autoradiographs of gel diffusion reactions using I* antigens were prepared in the manner previously described.⁶ Wells in gel diffusion slides were charged with I* antigen and the respective human anti-serum and were incubated for 48 hours at 4° C. The slides were washed, dried, and placed in contact with Kodak lantern slides. Several duplicate slides were prepared and developed at varying periods of time to determine the appropriate exposure time.

Double Gel Diffusion Precipitin and Immuno-electrophoretic Reactions.—These procedures were carried out using the method of Ouchterlony⁷ and Grabar and Williams,⁷ respectively.

Precipitating antibody levels of rabbit anti-HSA serum were determined by quantitative precipitin reactions.⁷ Nitrogen determinations were made by the Markham modification of the micro-Kjeldahl reaction.⁷ Dog and guinea pig

*Obtained from Pentex Corp., Kankakee, Illinois.

dander extracts were prepared by extraction of dog and guinea pig hair in 0.15 M NaCl with 1:10,000 Merthiolate. Dog and guinea pig hair were collected by clipping the animals without trauma to the skin to avoid contamination of hair with blood. Passive cutaneous anaphylaxis in the guinea pig was done using the method of Ovary.⁷

Human Sera.—Patients with positive cutaneous reactions to dander extracts were tested with the serum protein fractions of the species to which they were sensitive. Skin testing antigens contained 4 mg. of protein per milliliter. Intracutaneous testing was not done, consequently no correlation between the incidence of dander sensitivity and serum protein sensitivity is available. The sera collected for study, degree of cutaneous reactivity, and brief clinical history of the subjects are shown in Table I. Cutaneous tests were performed and interpreted in the customary manner.⁸ The serum of patient G. A. U., whose sensitivity to multiple animal danders and sera was described by Arbesman and associates,¹⁰ was obtained for study in our laboratory. G. A. U. was not available for retesting.

RESULTS

The human antisera against serum protein antigens shown in Table I were studied by the various techniques with the results shown in Table II.

Immune Elimination of I Labeled Foreign Serum Proteins From Guinea Pigs.*—The immune elimination of I* labeled foreign serum protein antigens in passively sensitized animals was previously described as a sensitive method of demonstrating small amounts of antibody.⁹ Two slightly modified methods were

TABLE I. SOURCE OF HUMAN ANTISERA AGAINST FOREIGN SERUM PROTEIN ANTIGENS

SERUM DONOR	SERUM PROTEIN ANTIGEN	CUTANEOUS REACTION	CLINICAL HISTORY OF SERUM DONOR
J. P.	HSA HGG	++++ ++	Respiratory symptoms after exposure to several animal species Serum sickness after injection of horse serum 27 years previously
G. S.	HSA	++++	Respiratory symptoms following laboratory exposure to dried horse serum
E. C.	DSA	++	Respiratory symptoms after exposure to dogs
D. R.	DSA	++	Respiratory symptoms after exposure to dogs
C. F.	DSA	+++	Respiratory symptoms after exposure to dogs
L. B.	DSA	++	Respiratory symptoms after exposure to dogs
M. H.	GPSA GPGG	+++ +	Respiratory symptoms after exposure to guinea pigs
G. A. U.	HSA HGG DSA	Not tested	Previously described sensitivities and tests (Refs. 10 and 11)

TABLE II. SUMMARY OF IMMUNOLOGIC STUDIES WITH SERA OF PATIENTS SENSITIVE TO FOREIGN SERUM PROTEIN FRACTIONS

SERUM	ANTIGEN	DOUBLE GEL DIFFUSION PRECIPITIN REACTION	DOUBLE GEL DIFFUSION AUTORADIOG- RAPHY	IMMUNE ELIMINATION OF I* ANTIGEN IN GUINEA PIGS	PASSIVE CUTANEOUS ANAPHY- LAXIS	PASSIVE TRANSFER TO HUMAN SKIN
J. P.	HSA	Negative	Unheated, positive Heated, positive	Unheated, positive Heated, positive	Unheated, positive† Heated, positive†	Unheated, positive Heated, negative
J. P.	HGG	Negative	Not done	Unheated, positive Heated, positive	Unheated, negative Heated, negative	Not done
G. S.	HSA	Negative	Unheated, positive Heated, positive	Unheated, positive Heated, positive	Unheated, negative Heated, negative	Not done
G. S.	HGG	Negative	Not done	Unheated, positive Heated, positive	Unheated, negative Heated, negative	Not done
G. A. U.	HSA	Negative	Unheated, positive Heated, positive	Unheated, positive Heated, positive	Unheated, negative Heated, negative	Not done
D. R.	DSA	Negative	Negative	Negative	Negative	Not done
C. F.	DSA	Negative	Negative	Negative	Negative	Not done
E. C.	DSA	Negative	Negative	Negative	Negative	Not done

†Faint positive: 7 mm. in diameter.

used to determine whether these human antisera were capable of producing immune elimination of I* antigen in guinea pigs. Female guinea pigs, weighing approximately 250 grams, were injected intravenously with mixtures of the human antisera and a known amount of I* antigen prepared in vitro, and the rate of elimination of circulating radioactivity from the guinea pig was determined. A second method consisted of injection of I* antigen intravenously in the guinea pig. After 10 minutes, blood was withdrawn by cardiac puncture and the radioactivity of 1 ml. of blood was determined. The animals were then injected intravenously with serum from patients sensitive to the respective I* protein, and the radioactivity of the blood was determined at intervals. Negative control serum consisted of normal human serums (NHS), and positive control serum was rabbit anti-HSA precipitating serum. Comparative results of elimination of I* HSA by rabbit anti-HSA antibody and serum J.P., using varying amounts of I* HSA, are shown in Table III. The amounts of rabbit anti-HSA used resulted in rapid immune elimination of circulating I* antigen. One milliliter of serum J.P. resulted in a slightly increased rate of elimination of the 4 and 8 μ g of I* antigen used in this experiment.

Table IV shows the rates of immune elimination of I* antigen from the circulation of guinea pigs after injection of a constant amount of I* HSA

TABLE III. IMMUNE ELIMINATION OF I* HSA FROM GUINEA PIGS BY HUMAN AND RABBIT ANTI-HSA

ANIMAL NO.	I.V. INJECTION AT 0 TIME WITH MIXTURE OF:	PER CENT OF I* HSA REMAINING IN BLOOD†	
		10 MIN.	30 MIN.
1	60 µg rabbit anti-HSA N + 4 µg I* HSA N	10.5	7.5
2	120 µg rabbit anti-HSA N + 8 µg I* HSA N	12.2	10.2
3	4 µg I* HSA N	85.4	69.4
4	8 µg I* HSA N	88.0	74.5
5	1 ml. serum J. P. + 4 µg I* HSA N	62.5	47.3
6	1 ml. serum J. P. + 8 µg I* HSA N	74.8	60.6
7	1 ml. serum J. P. (heated) + 8 µg I* HSA N	73.7	59.0

†Recorded as $\frac{\text{c. p. m./ml. blood remaining}}{\text{c. p. m./ml. blood injected}} \times 100$. C. p. m./ml. injected = $\frac{\text{total c. p. m.}}{\text{blood volume}}$ (c. p. m.: counts per minute.)

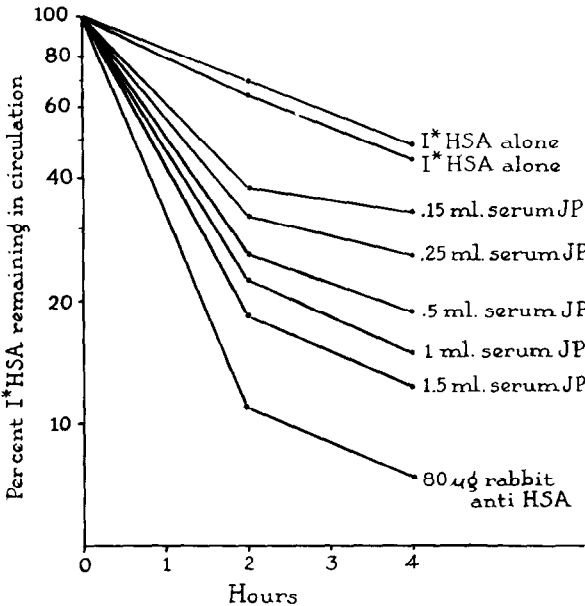


Fig. 1.—Per cent of I* HSA remaining in circulation of guinea pigs after injection of 0.5 µg I* HSA N followed by administration of human or rabbit anti-HSA.

followed by increasing amounts of serum J.P. These rates of elimination of I* HSA are shown graphically in Fig. 1. As shown in Table IV, serum J.P. heated to 56° C. for 4 hr. also resulted in immune elimination of I* HSA in guinea pigs. Serum G.S. resulted in immune elimination of circulating I* HSA in guinea pigs as shown in Table V. It was necessary to use smaller amounts of I* HSA with serum G.S. than with serum J.P. to demonstrate the antibody activity of serum G.S. with this method. The ability of serum G.S. to result in immune elimination of I* HSA in guinea pigs was also stable to heat at 56° C. for 4 hr. The four human antisera against dog serum albumin (DSA) gave no evidence of immune elimination of I* DSA in guinea pigs.

All double gel diffusion precipitin reactions were negative using human sera against the foreign serum protein antigens to which the patients gave positive

TABLE IV. IMMUNE ELIMINATION OF I* HSA IN GUINEA PIGS BY VARYING AMOUNTS OF SERUM J. P.

ANIMAL NO.	ALL ANIMALS INJECTED I.V. WITH 0.5 μ g I* HSA N FOLLOWED IN 10 MIN. WITH I.V. INJECTION OF:	PER CENT I* HSA REMAINING IN BLOOD AT	
		2 HR.	4 HR.
8	0.15 ml. J.P. serum	37.7	33.0
9	0.25 ml. J.P. serum	32.1	25.8
10	0.5 ml. J.P. serum	25.6	18.8
11	1.0 ml. J.P. serum	22.3	15.0
12	1.0 ml. J.P. serum (heated)†	21.1	17.4
13	1.5 ml. J.P. serum	18.3	12.5
14	1.5 ml. J.P. serum	19.4	12.2
15	80 μ g rabbit anti-HSA N	10.8	7.3
16	1.0 ml. NHS	70.5	50.7
17	None	65.3	45.0
18	None	69.0	48.4

†56°C. for 4 hours.

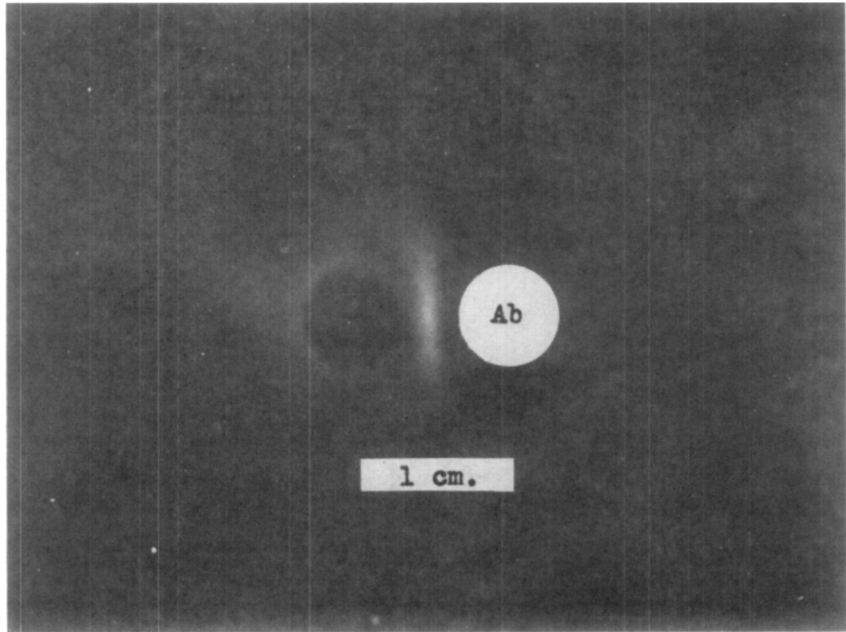


Fig. 2.—Double gel diffusion autoradiograph with serum J. P. and I* HSA. Antiserum well is on right.

cutaneous tests. Double gel diffusion autoradiographs using human sera and the appropriate I* antigens were positive using sera J.P., G.S., and G.A.U. against I* HSA. A double gel diffusion autoradiograph made with serum J.P. and I* HSA is shown in Fig. 2. The ability of these human antisera to form bands

TABLE V. IMMUNE ELIMINATION OF I* HSA IN GUINEA PIGS BY SERUM G.S.

ANIMAL NO.	ALL ANIMALS INJECTED I.V. WITH 0.1 μ g I* HSA N FOLLOWED IN 10 MIN. WITH I.V. INJECTION OF:	PER CENT I* HSA REMAINING IN BLOOD AT	
		2 HR.	4 HR.
19	0.2 ml. GS serum	45.8	35.8
20	1.0 ml. GS serum	36.3	Died
21	1.0 ml. GS serum (heated)†	34.0	28
22	3.0 ml. GS serum	23.5	15
23	1.0 ml. NHS	60.7	43

†56°C. for 4 hours.

with I* antigen in double gel diffusion autoradiographic plates was not lost by heating the sera to 56° C. for 4 hr. Double gel diffusion autoradiographs using human anti-DSA sera and I* DSA were negative.

Passive cutaneous anaphylaxis using all human sera shown in Table III and the respective antigens were negative except with serum J.P. To minimize the risk of homologous serum jaundice, passive transfer tests in normal human skin were limited to one serum, J.P. This serum gave a positive passive transfer to HSA to a nonsensitive recipient and this sensitizing ability of serum J.P. was destroyed by heating at 56° C. for 4 hr.

Serum antigens in animal danders have been demonstrated.¹² This was confirmed in the present study. Dog and guinea pig hair was collected without trauma to the skin, extracted in 0.15 M NaCl, and concentrated by evaporation in dialysis tubing. Using the Ouchterlony technique, this dog dander extract produced a precipitin band with rabbit anti-DSA and a reaction of identity with a DSA, rabbit anti-DSA band. Using immunoelectrophoresis, the antigen in dog dander reacting with rabbit anti-DSA migrated as an albumin. Rabbit anti-guinea pig serum albumin serum resulted in a precipitin band in a double gel diffusion precipitin reaction against guinea pig dander extract.

DISCUSSION

Characterization of allergic antibody has been difficult because of the failure of this antibody to produce a precipitin reaction in any system yet demonstrable, and because many major allergens are mixtures of antigens which have not been separated either in sufficiently pure form or in sufficient quantity for extensive investigation. The availability of human antibody against foreign serum protein fractions provides a means of study of a system in which the antigens have been well characterized and are available in relatively pure form. Patients sensitive to these foreign serum protein fractions may owe their sensitivity to the injection of the antigen in the form of antitoxins, by inhalation and, possibly, by ingestion. The presence of an antigen in dog dander extract reacting with rabbit anti-dog serum albumin and migrating electrophoretically as serum albumin suggests one method by which an individual may be sensitized to serum protein antigens by inhaling the serum protein antigen in the dander. This emphasizes the caution required in treating the patient who has never had an injection of a foreign serum and requires serum of that species as antitoxin.

Three human sera produced the immune elimination of I* antigen in guinea pigs. This method has been described as a sensitive method of demonstration of antibody⁹ and the present study illustrates that human antisera are capable of combining with antigen *in vivo* and resulting in immune elimination of the antigen in guinea pigs and that this method is applicable for demonstration of small amounts of human antibody. These three sera also demonstrated positive reactions with gel diffusion autoradiography. The serum factor of patients J.P., G.S., and G.A.U. which resulted in these reactions were not evident in the remaining sera studied. The serum factor resulting in these reactions cannot be defined at present. It is possible that a small amount of precipitating antibody present in these sera resulted in these reactions. J.P. received an

injection of horse serum with subsequent serum sickness 27 years previously. If the antibody activity demonstrated in serum J.P. was the result of a small amount of precipitating antibody undetectable by gel double diffusion reaction, this would suggest that this precipitating antibody persisted 27 years after the last injection of antigen. There is no known history that either G.S. or G.A.U. received horse serum by injection. If the reactions with sera G.S. and G.A.U. are due to a precipitating antibody, sensitization probably occurred without injections of this antigen. Since these reactions did not occur with the sera from patients reacting to DSA, either these sera did not have the serum factor resulting in immune elimination or autoradiographic gel diffusion reactions or it was present in quantities insufficient to be demonstrated by these methods. The heat stability of the serum factor of J.P., G.S., and G.A.U. which produces immune elimination of I* antigen and positive gel diffusion autoradiographs suggests that it is different from the serum factor resulting in a positive Prausnitz-Küstner reaction.

Only one serum, J.P., gave a faint positive PCA reaction in a guinea pig. This reaction may have resulted from either a different type of antibody present in the serum J.P. or larger amount of antibody present in this serum.

The method of immune elimination described is useful as a qualitative demonstration of antibody. However, rough quantitative estimates of antibody content for comparative purposes can be made. It is evident from results shown in Table IV and V that serum J.P. contains more anti-HSA antibody than serum G.S., since 1 ml. of serum J.P. results in a more rapid passive immune elimination of more I* HSA than 1 ml. of serum G.S.

SUMMARY

1. Patients with positive wheal- and erythema-type reactions against foreign serum protein antigens may be used as sources of antiserum against foreign serum protein fractions. This provides a system of study of allergic antiserum against relatively pure protein antigens.

2. Human antiserum can produce immune elimination of I¹³¹ labeled serum protein antigens in guinea pigs and this method may be used for detection of small amounts of human antibody.

3. Human antiserum with I¹³¹ labeled antigen can give a positive double gel diffusion autoradiograph although gel diffusion precipitin reactions with the same sera are negative.

4. The factor in these patients' sera which resulted in immune elimination of I* labeled foreign serum protein antigens and bands on gel diffusion autoradiographs is thermostable and therefore may not be reagin.

5. Serum protein antigens are found in animal danders and this is suggested as one possible method of sensitizing human patients to foreign serum proteins in the absence of sensitizing injections.

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