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Kimishige Ishizaka, Teruko Ishizaka and Margaret M. Hornbrook

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PHYSICO-CHEMICAL PROPERTIES OF HUMAN REAGINIC ANTIBODY

IV. Presence of a Unique Immunoglobulin as a Carrier of Reaginic Activity¹

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In previous articles (1-3), it was reported that the reaginic activity in chromatographic fractions and γ A-globulin fractions of sera from atopic subjects did not diminish after the precipitation of γ A- and/or γ G-globulins in the fractions. It was also found that the precipitation of γ D-globulin in patients' sera was not accompanied by any detectable decrease of reaginic activity (2). As reported by Anderson and Vannier (4), as well as by Rockey and Kunkel (5), the sedimentation coefficient of the reaginic antibody was about 8 S (2), and the reaginic activity was not coprecipitated with γ M-globulin-anti- γ M-globulin complexes (6). The possibility of the association of reaginic activity with one of the minor subclasses of γ G-globulins, i.e., γ_{2a} , γ_{2c} , was also excluded (6). These results highly suggest that human reaginic antibody is associated with a unique immunoglobulin other than γ G-, γ A-, γ M- and γ D-globulins. The present experiments were undertaken to study this possibility. Rabbits were immunized with chromatographic fractions of normal human serum in order to obtain an antiserum which was capable of precipitating reaginic antibodies. The antisera obtained were absorbed with normal γ G-globulin and γ A myeloma proteins. The reaginic antibody was precipitated with one of the antisera which contained none of the antibodies specific for γ A-, γ M- or γ G-globulins and those specific for κ and λ chains in immunoglobulins. The results suggest the presence of a unique immunoglobulin as a carrier of reaginic activity.

MATERIALS AND METHODS

Human sera from atopic patients and a normal individual. Four serum specimens from ragweed sensitive patients were used. Serum A was ob-

tained from a patient in residence at the Children's Asthma Research Institute and Hospital. Serum P was supplied by Drs. L. Perelmutter and L. Goodfriend, Montreal, Canada. The other two serum pools, one from untreated patients (U) and the other from treated patients (T), were kindly supplied by Dr. L. M. Lichtenstein, Johns Hopkins University. A normal serum was purchased from Bonfils Blood Bank, Denver Colorado.

Myeloma proteins. Three preparations of γ A myeloma protein and γ_{2a} - (Type K), γ_{2b} - (Type L), γ_{2c} - (Type L) globulins were kindly supplied by Dr. H. Fudenberg, University of California, San Francisco. These were isolated from patients' sera by starch block electrophoresis. One serum sample from a γ A myeloma patient was supplied by Dr. H. Claman, University of Colorado. The myeloma protein was purified by block electrophoresis in Pevicon C-870 (Fosfatbolaget, Sweden) (7) followed by chromatography on a diethylaminoethyl (DEAE) cellulose (Mann Research Lab, New York, N. Y.) column. A preparation of γ_{2d} -globulin was supplied by Dr. W. D. Terry, National Cancer Institute.

Fractionation of human sera. The globulin fraction of a normal serum and a patient's serum was obtained by precipitation with half saturation of ammonium sulfate followed by chromatography on DEAE cellulose columns (1). Proteins were eluted stepwise with 0.005 M, 0.01 M, 0.02 M, 0.035 M and 0.05 M phosphate buffers of pH 8.0 and the fractions obtained with each buffer were pooled separately (Frs. I-V). After concentration by negative pressure filtration, the fractions from the normal serum were used for immunization of rabbits.

The γ A-globulin fraction of reaginic sera was obtained by the zinc precipitation method described by Vaerman *et al.* (8) with slight modifications. Details of the method were described in previous articles (2, 9). The γ A-globulin prepara-

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tions gave a single precipitin band with anti-human serum in immunoelectrophoresis but no precipitin band was obtained with either anti- γ G or anti- γ M antibodies in immunodiffusion (3). The γ A-globulin preparations were further fractionated into three subfractions by chromatography on DEAE cellulose columns (3). The preparations were dialyzed against 0.02 M phosphate buffer (pH 8.0) and applied to the column (2 by 75 cm). Proteins were eluted stepwise with 0.02 M, 0.035 M and 0.08 M phosphate buffers of pH 8.0, and the fractions eluted with each buffer were pooled separately (Frs. γ A₁, γ A₂, γ A₃).

Antibodies specific for human immunoglobulins. Rabbit antisera specific for γ G-, γ A- or γ M-globulins were the same preparations described in the previous articles (1-3, 9). They were obtained by immunizing rabbits with one of the three immunoglobulins, i.e., γ G-, γ A- and γ M-globulins, from normal human sera followed by the absorption of cross-reacting antibodies with appropriate purified immunoglobulins. The goat antiserum specific for γ A-globulin was purchased from Hyland Laboratories, Los Angeles, California. The γ -globulin fraction of the antisera was obtained by chromatography on DEAE cellulose columns. The rabbit antiserum specific for γ D-globulin was kindly supplied by Drs. D. S. Rowe and J. L. Fahey, National Cancer Institute, Bethesda, Maryland (10).

Immunodiffusion. Double diffusion in agar was performed by the method of Ouchterlony (11). Immunoelectrophoresis was carried out on microscopic slides by the modifications of Scheidegger (12). The plates were incubated at room temperature for 24 to 48 hr and washed, and the precipitin bands were stained with Amidoschwartz 10B. The goat anti-human serum used in the immunodiffusion experiments was purchased from Hyland Laboratories, Los Angeles, California.

Radioimmuno-electrophoresis was carried out by the method of Yagi *et al.* (13). Antigen E from ragweed pollen was kindly supplied by Dr. M. W. Chase, Rockefeller Institute. The antigen was labeled with I^{131} by the method described by Yagi *et al.* (14) using chloramin T. Approximately 0.5 μ g/ml of the labeled antigen E was included in appropriate rabbit antisera. Immunoelectrophoresis and immunodiffusion on Ouchterlony plates were carried out using the

mixture of the labeled antigen E and antisera instead of antisera alone. The plates were washed for 2 to 3 days, dried and wrapped with a plastic film (Saran-wrap). Radioautographs were obtained by placing x-ray film (Kodak Industrial X-ray film, Type KK) in contact with the plates.

Prausnitz-Küstner reactions. The tests were carried out on individuals who were not sensitive to the test allergen. One-twentieth milliliter of test samples was injected intracutaneously (i.c.). Twenty-four hours later, the sites received an i.c. injection of 0.02 ml of ragweed allergen extract (Center Lab., New York, N. Y.) containing 10 protein nitrogen units (PNU). Erythema-wheal reactions, which appeared 15 to 20 min after the injection of allergen, were traced on a transparent plate which was applied to the area. When the reaginic activity of several samples was compared, the samples were tested in the same individuals.

EXPERIMENTAL RESULTS

Two rabbits were immunized with each chromatographic fraction of normal human globulin. The fractions, containing 5 mg protein, were included in complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan) and injected into the footpads. The rabbits were then given three intramuscular (i.m.) injections of an emulsion of 5 mg of protein in Freund's adjuvant at weekly intervals. Two weeks after the last injection, 30 to 40 ml of blood were obtained from the rabbits, and bleeding was repeated at 2-week intervals. Immunization was continued by another i.m. injection of the same emulsion. The final bleedings were made 10 weeks after the first injection. The antisera were tested for the presence of antibodies against immunoglobulins by immunoelectrophoresis. The antibody against γ G-globulin in the antisera was absorbed with normal γ G-globulin and the antibody specific for γ A-globulin was then absorbed with a γ A myeloma protein at the equivalence zone. The γ -globulin in the supernatants was precipitated with $\frac{1}{3}$ saturation of ammonium sulfate, dissolved in $\frac{1}{4}$ to $\frac{1}{6}$ of the original volume of saline, and tested by immunoelectrophoresis for the presence of antibody against Fr. III of serum U which contained high reaginic activity. Only the antisera obtained by immunization with Fr. III gave definite precipitin bands in the β -globulin region when tested against the same fraction

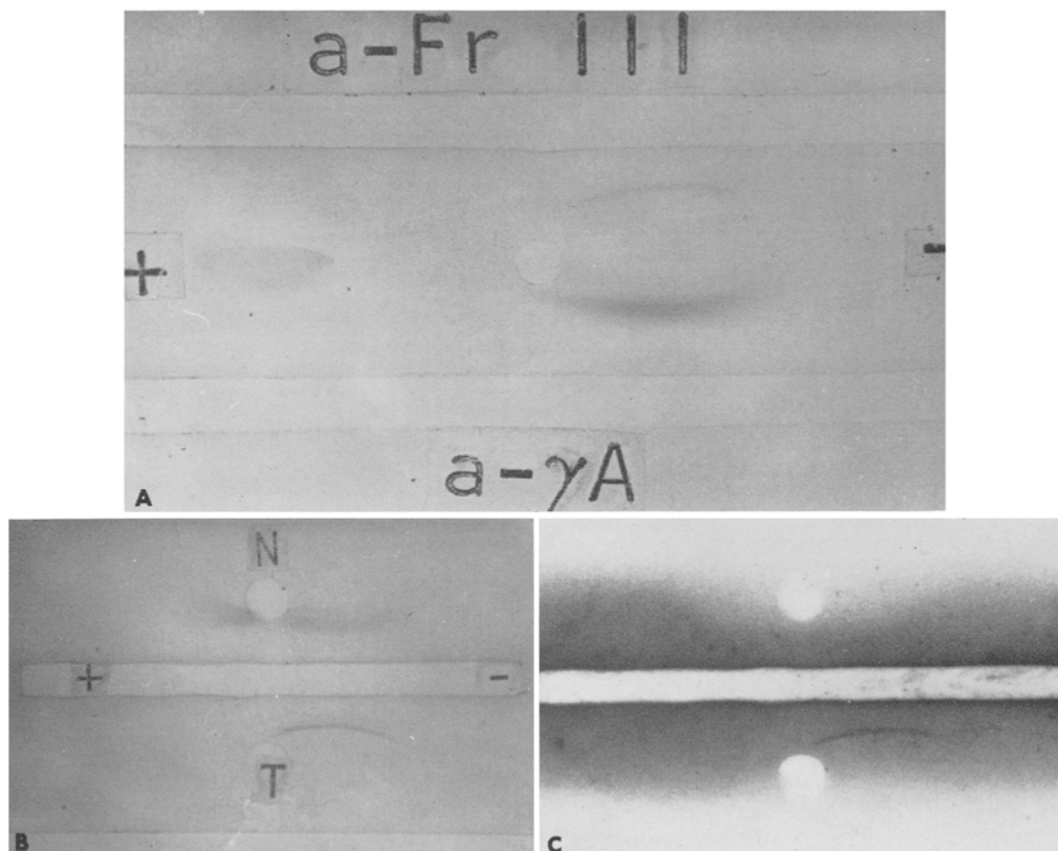


Figure 1. A, upper, Immunoelectrophoresis of Fr. γA_1 (serum U) against anti- γA (rabbit) and anti-Fr. III. B, lower left and C, lower right, Radio-immunoelectrophoresis of Fr. γA_1 from normal serum (N) and from serum T. The trough was filled with anti-Fr. III mixed with I^{131} -labeled antigen E. Both the stained slide (B) and the corresponding radioautogram (C) are shown.

from the patient's serum. The γ -globulin fractions of the antisera were pooled, dialyzed against 0.01 M phosphate buffer, pH 8.0, and applied to a DEAE cellulose column which was equilibrated with the buffer. The protein eluted from the column with the same buffer was concentrated by negative pressure filtration. In immunoelectrophoresis, the antibody preparation gave four precipitin bands with the serum from a ragweed sensitive patient (Serum P). One of these bands was identified as a transferrin-anti-transferrin precipitin band by Osserman's technique (15). The quantitative precipitin reaction was set up with transferrin (Nutritional Biochem. Corp., Cleveland, Ohio), and anti-transferrin in the antibody preparation was absorbed at the equivalence zone. The presence of anti- γA -globu-

lin antibody in the supernatant was tested again in Ouchterlony plates using four preparations of γA myeloma protein. One of the myeloma proteins gave a precipitin band, whereas the other three preparations did not. Using the protein which gave a precipitin band, the supernatant was absorbed at the equivalence point. The final supernatant (anti-Fr. III) did not form a precipitin band with any of the four myeloma proteins, but gave three precipitin bands with serum P in immunoelectrophoresis. Since it was reported in previous experiments (3) that a subfraction of γA -globulin, i.e., Fr. γA_1 , from atopic patients' sera contained high reaginic activity, immunoelectrophoresis was set up between Fr. γA_1 and anti-Fr. III. It was found that Fr. γA_1 gave a single precipitin band with anti-Fr. III in the

γ_1 -globulin region (Fig. 1A). The position of the precipitin band was similar to that of the γ A-globulin band. Specificity of the precipitin band formed between Fr. γA_1 and anti-Fr. III was studied by immunodiffusion. The center well in an Ouchterlony plate was filled with Fr. γA_1 and the peripheral wells were filled with four antibody preparations, i.e., anti-Fr. III, anti- γ A, anti- γ G and anti-human serum, and a myeloma γ A-globulin. As shown in Figure 2A, Fr. γA_1 gave a single precipitin band with anti- γ A and with anti-Fr. III, and the specificities of the bands were entirely different from each other. The presence of the antibody specific for the known immunoglobulins in anti-Fr. III was also studied using myeloma proteins. It is apparent in Figure 2B that the antibody preparation gave no precipitin band with any of the myeloma proteins of γ_{2a} - (Type K), γ_{2b} - (Type L), γ_{2c} - (Type L), γ A- and γ M-globulin. It was also found that anti-Fr. III did not give a precipitin band with γ_{2d} -globulin. These findings indicate that anti-Fr. III contains none of the anti- γ G, anti- γ_{2a} , anti- γ_{2b} , anti- γ_{2c} , anti- γ_{2d} , anti- γ A, anti- γ M, anti- κ and anti- λ chains. The presence of the antibody specific for γ D-globulin in anti-Fr. III was studied using anti- γ D-globulin and a normal serum containing a relatively high concentration of γ D-globulin. In an Ouchterlony plate, anti-Fr. III gave three precipitin bands with human serum; one of them had identical specificity with the precipitin band formed between the serum and anti- γ D-globulin (Fig. 2C). However, Fr. γA_1 gave no precipitin band against anti- γ D-globulin and the single precipitin band formed between Fr. γA_1 and anti-Fr. III fused to a band between anti-Fr. III and human serum. Lack of γ D-globulin in Fr. γA_1 was confirmed in immunoelectrophoresis. It is evident that the γ_1 precipitin band formed between Fr. γA_1 and anti-Fr. III is not due any of the known immunoglobulins. The γ_1 -globulin was, therefore, tentatively called γ E-globulin.

In order to study the correlation between the γ E-globulin and reaginic activity, the presence of this protein in the subfractions of γ A-globulin preparations was investigated. The skin-sensitizing activities of the three subfractions from sera U and T were determined by Prausnitz-Küstner (P-K) reactions. As reported previously (3), Fr. γA_1 had the highest reaginic activity (Table I). The γ A subfractions were placed in the peripheral wells of an Ouchterlony plate and anti-Fr. III or anti- γ A-globulin in the center well. The protein concentration and P-K titers of the subfractions used in the plate are shown in Table I. Only Fr. γA_1 from both sera gave a definite precipitin band with anti-Fr. III, whereas all of the fractions gave a precipitin band with anti- γ A. It seems that the presence of γ E-globulin in the subfractions has some correlation with the reaginic activity.

In view of these findings, separate aliquots of Fr. γA_1 from sera U and T were absorbed with an equal volume of anti-Fr. III or anti- γ A-globulin. After incubation for 24 hr, the precipitates were removed by centrifugation, washed three times with cold saline and the precipitated nitrogen measured. The amount of protein precipitated with anti-Fr. III was less than that precipitated with anti- γ A antibody (Table II). In immunodiffusion experiments using the supernatants, it was shown that the γ A-globulin in Fr. γA_1 was completely precipitated with anti- γ A-globulin but not with anti-Fr. III. The supernatant remaining after the precipitation of γ A-globulin gave a precipitin band with anti-Fr. III. It was also found that the supernatant obtained after the precipitation with anti-Fr. III gave no precipitin band with anti-Fr. III, but a definite band formed with anti- γ A (Fig. 4A, 4B). The latter supernatant also gave a precipitin band with unabsorbed Fr. γA_1 , indicating the presence of excess antibody. Serial twofold dilutions of the supernatants were injected i.c. into normal individuals for passive sensitization, and the skin

Figure 2. Immunodiffusion analyses of anti-Fr. III. *A, upper,* Fr. γA_1 (serum U) was placed in the center well against anti-Fr. III (a-Fr. III), anti- γ A (a- γ A), anti- γ G (a- γ G) and anti-human serum (a-HS). M- γ A represents γ A myeloma protein. The precipitin lines between the center well and one of the a- γ A wells are due to the reaction between anti-HS and human serum proteins present in a-Fr. III. *B, center,* anti-Fr. III in the center well gave no precipitin band with any of the myeloma proteins in peripheral wells. γ_{2a} -, γ_{2b} -, γ_{2c} -globulins were type K, L and L, respectively. The concentration of the myeloma proteins placed in the wells were 100 μ g N/ml. *C, lower,* Wells were filled with anti- γ D (a- γ D), human serum (HS) anti-Fr. III and Fr. γA_1 . γ D-Globulin is not involved in the formation of the precipitin band between Fr. γA_1 and anti-Fr. III.

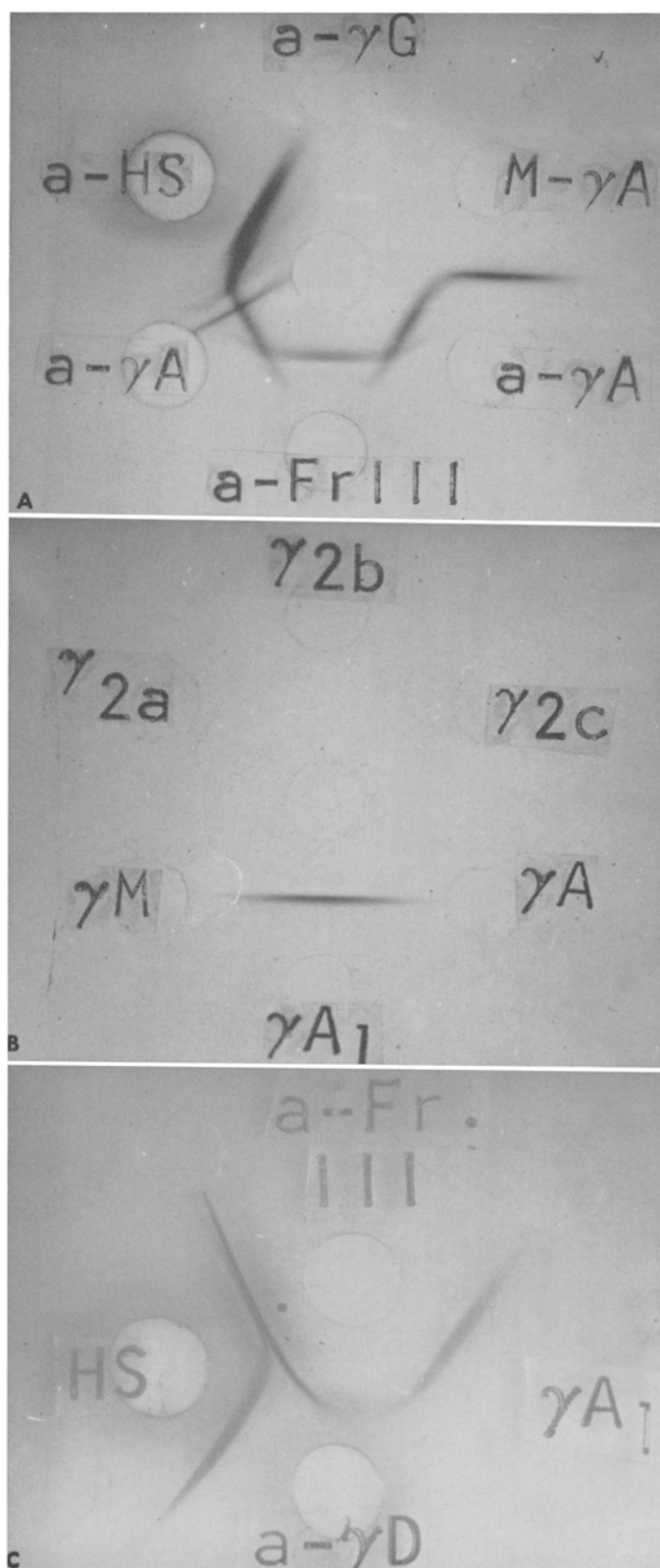


Figure 2

sites were challenged with 10 PNU of ragweed pollen extract. The supernatant obtained after the precipitation of γ A-globulin gave positive P-K reactions, whereas the supernatant from the anti-Fr. III mixture did not (Table II). It

was also found that the P-K titers of the fractions did not decrease by the precipitation of γ A-globulin. Representative results are shown in Figure 3. The absorption experiments with anti-Fr. III were repeated using sera A and P. One tenth to 0.3 ml of anti-Fr. III was incubated with 0.1 ml of each serum and specific precipitates were removed by centrifugation. The P-K titers of the supernatants and of the unabsorbed sera were determined. The results shown in Table II indicate that reaginic activity was lost after the precipitation with 0.3 ml of anti-Fr. III.

The inactivation of reaginic activity following absorption with anti-Fr. III suggests that the reaginic antibody was precipitated. In order to confirm this possibility, radioimmuno-electrophoretic experiments were performed. The γ A₁ fractions from sera U and T and from a normal serum were subjected to electrophoresis on agar-coated slides and channels were filled with a mixture of I¹³¹-labeled antigen E and either anti- γ A or anti-Fr. III. It was found that Fr. γ A₁ from reaginic sera gave a radioactive precipitin

TABLE I
Skin-sensitizing activity of γ A subfractions

Serum	Subfractions	Protein Concentration	P-K Titer ^a
		μ gN/ml	
U	γ A ₁	72	144
	γ A ₂	77	15
	γ A ₃	92	9
T	γ A ₁	69	137
	γ A ₂	163	20
	γ A ₃	116	4

^a Maximal dilutions of samples still giving positive Prausnitz-Küstner (P-K) reactions. Five hundredths milliliter of the dilutions were injected for passive sensitization.

TABLE II
Effect of absorption of reagin-containing fraction with anti-Fr. III and anti- γ A on skin-sensitizing activity^a

Serum	Fraction	Absorption With ^b	Pptd N ^c	P-K ^d Reaction: Dilutions Injected			
				18×	36×	72×	144×
U	γ A ₁	— ^e	μ g				
		Anti-Fr. III	11.8	0	0	0	0
		Anti- γ A	28.0	10 (35)	9.5 (32)	8.5 (28)	7 (27)
		— ^e			10 (35)	9 (32)	8 (27)
T	γ A ₁	— ^e		17.2×	34.4×	68.8×	137×
		Anti-Fr. III	9.1	0	0	0	0
		Anti- γ A	20.4	10 (35)	9.5 (33)	9 (31)	8.5 (30)
		— ^e			10 (36)	9 (28)	7 (20)
A		— ^e		400×	800×	1600×	3200×
		Anti-Fr. III		7.5 (27)	0	0	0
		— ^e		14.5 (50)	13 (41)	10 (37)	7.5 (31)
		— ^e		100×	200×	400×	800×
P		Anti-Fr. III		0	0	0	0
		— ^e		11 (37)	10 (34)	8.5 (33)	7 (26)

^a Numerals in this table represent average diameters of wheal in mm; those in parentheses () represent the average diameter of erythema in P-K reactions.

^b The γ A₁ fraction from sera U and T was absorbed with an equal volume of either anti-Fr. III or anti- γ A. One volume of whole serum (A and P) was absorbed with 3 volumes of anti-Fr. III.

^c Precipitate obtained from 0.1 ml of Fr. γ A₁.

^d P-K = Prausnitz-Küstner

^e — = unabsorbed.

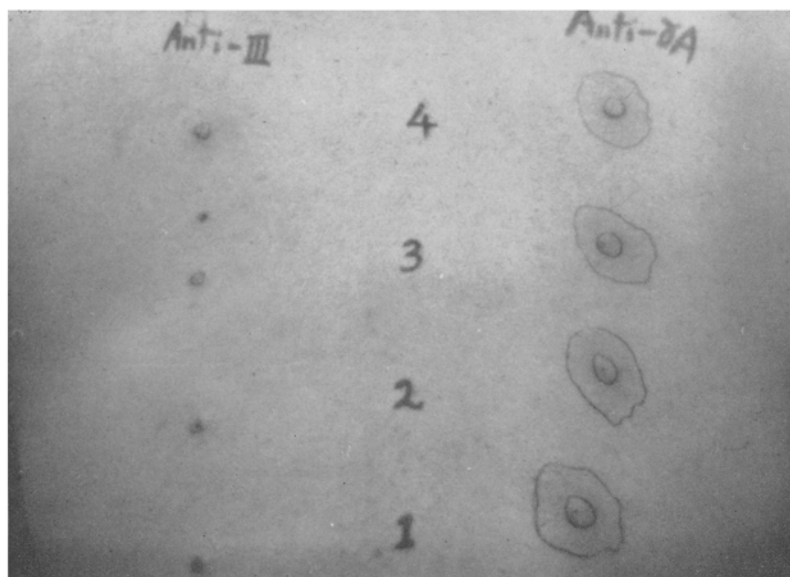


Figure 3. Prausnitz-Küstner reactions with supernatants obtained after the absorption of Fr. γA_1 (serum U) with anti-Fr. III (left) or with anti- γA (right). Skin sites 1 to 4 (bottom to top) received two-fold dilutions of the supernatants (cf. Table II).

band with anti-Fr. III, whereas the precipitin band formed with the same fraction from normal serum did not show radioactivity (Fig. 1B, C). The γA precipitin band formed with Fr. γA_1 from serum T contained radioactive antigen E, but the band formed with the fraction from serum U did not. The absence of γA anti-ragweed antibody in the latter sample was confirmed by immunodiffusion. The center well of an Ouchterlony plate was filled with Fr. γA_1 and the peripheral wells were filled with anti- γA and anti-Fr. III containing I^{31} -labeled antigen E, and a γA myeloma protein (Fig. 4A). Radioautogram of the plate revealed radioactivity on the precipitin band between Fr. γA_1 and anti-Fr. III but not on the γA band. It is evident that γE -globulin precipitated with anti-Fr. III contained anti-ragweed antibody. In order to study the correlation between the γE -globulin anti-ragweed antibody and reaginic activity in Fr. γA_1 , the samples shown in Table II were analyzed by radioimmunodiffusion. After the precipitation of γA -globulin in Fr. γA_1 from serum U, the supernatant gave a radioactive band with anti-Fr. III, whereas the supernatant obtained after the absorption with anti-Fr. III did not (Fig. 4B). The radioactive band formed between unabsorbed Fr. γA_1 and the latter supernatant may

be due to the presence of excess antibody in the supernatant. Comparisons of the radioautography with the results shown in Table II indicate that the formation of a radioactive precipitin band with anti-Fr. III correlates with the presence of reaginic activity. Fraction γA_1 from serum T gave radioactive precipitin bands with both anti- γA and anti-Fr. III (Fig. 4C). Since the radioactivity of the precipitin band between the fraction and anti-Fr. III was weak, neither of the supernatants formed a definite radioactive band with anti-Fr. III. However, the radioautography showed that the reaginic antibody is not γA -globulin. The supernatant remaining after the precipitation of γA -globulin had skin sensitizing activity but did not show a radioactive γA band, whereas the supernatant remaining after the absorption with anti-Fr. III showed the radioactive γA -globulin band and lacked skin-sensitizing activity (cf. Table II).

Radioimmunodiffusion was carried out with sera A and P. Both sera gave a radioactive precipitin band with anti- γG - and anti- γA -globulin antibodies in radioimmunoelectrophoresis, indicating the presence of γG and γA anti-ragweed antibodies. When anti-Fr. III was used in immunoelectrophoresis, γE precipitin band was not observed. None of the three precipitin

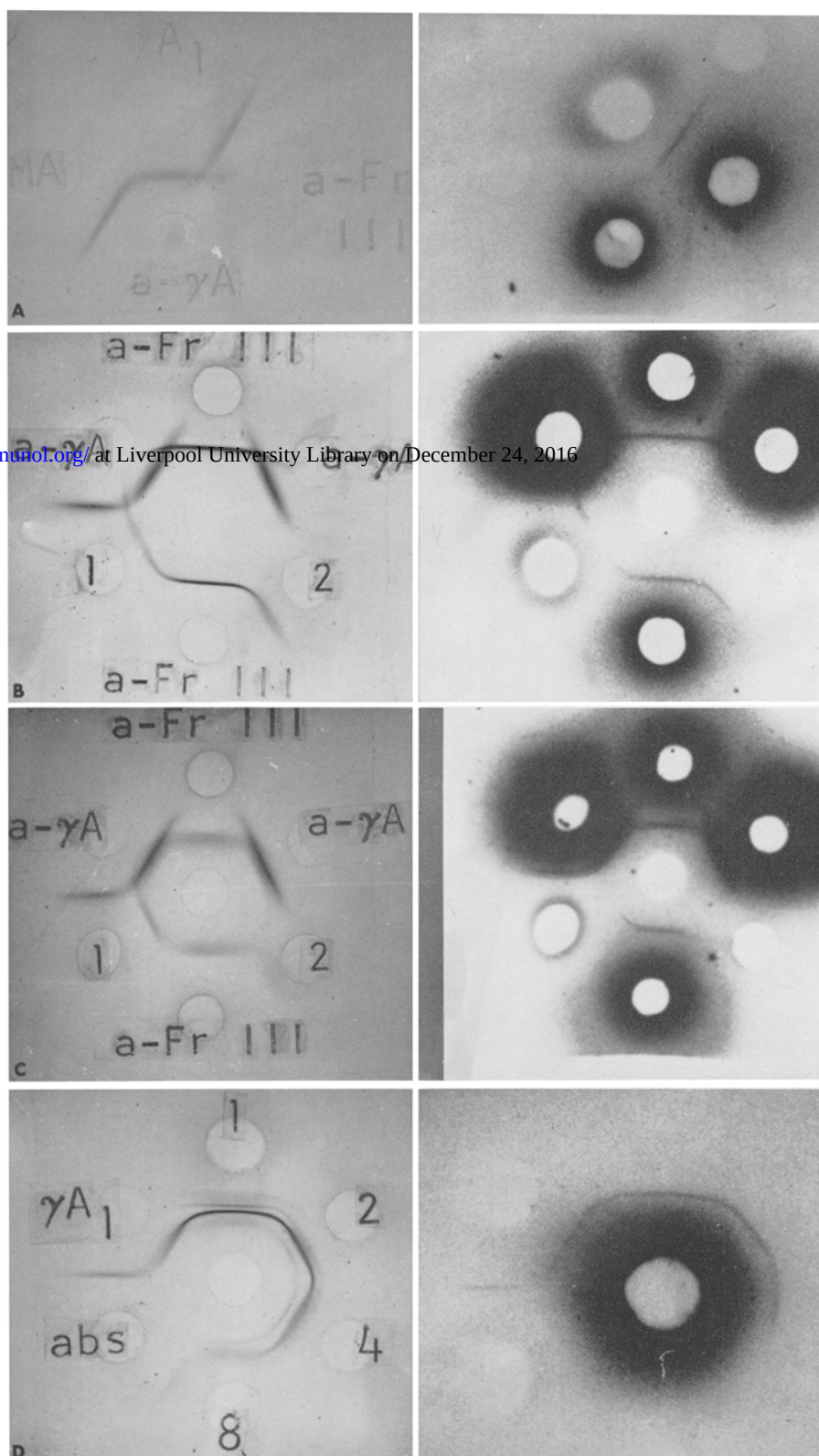


Figure 4

bands formed in the α - to β_1 -globulin region showed radioactivity. The γ E-globulin was detected in the sera by immunodiffusion. Center wells of Ouchterlony plates were filled with a mixture of anti-Fr. III and radioactive antigen E and the peripheral wells were filled with serial dilutions of the sera, and with Fr. γA_1 from sera U or T (Fig. 4D). One of the peripheral wells was filled with the serum which had been absorbed with 4 volumes of anti-Fr. III to completely precipitate reaginic antibody. Both sera A and P gave three precipitin bands with anti-Fr. III. One of these bands had identical specificity with the single band formed between the antiserum and Fr. γA_1 ; radioautography of the plates showed that only this band contained radioactive antigen E. After absorption of the sera with anti-Fr. III, the supernatants did not give any radioactive precipitin band. Representative results with serum P are shown in Figure 4D. It is clear that both sera contained anti-ragweed antibody which is associated with γ E-globulin. The absence of a radioactive γ E precipitin band in radioimmunoelectrophoresis may be due to low concentration of the anti- γ E antibody in the anti-Fr. III preparation.

DISCUSSION

Data presented in this article clearly show that the reaginic activity in a reagin-containing fraction (Fr. γA_1) and whole sera from ragweed-sensitive patients were greatly diminished by the precipitation of protein(s) with the rabbit antiserum (anti-Fr. III) which contained none of the antibodies toward γ G-, γ A- and γ M-globulins. The antibody against either κ or λ chains in immunoglobulins was lacking in the antiserum. The anti- γ D antibody was detected in the antiserum, although Fr. γA_1 did not contain a detectable amount of γ D-globulin. In immunoelectrophoresis, anti-Fr. III gave a single pre-

cipitin band in the γ_1 -globulin region with Fr. γA_1 . Furthermore, the radioimmunodiffusion experiments showed that the γ_1 precipitin band contained anti-ragweed antibody. As the γ_1 -globulin has a distinct antigenic determinant which is different from those characteristic of the known immunoglobulins, i.e., γ G-, γ A-, γ M- and γ D-globulins, and carries antibody activity, the protein is probably a unique member of the immunoglobulin family. Thus, the protein was tentatively designated γ E-globulin. The finding that the precipitation of this protein with anti-Fr. III was accompanied by the loss of reaginic activity highly suggests the association of reaginic activity with γ E-globulin.

There has been a considerable amount of evidence reported in support of the concept that reaginic antibody is γ A-globulin. These findings were 1) reaginic antibody was precipitated with anti- γ A-globulin antibody (16, 17); 2) γ A-globulin fractions of reaginic sera had reaginic activity (7, 18); 3) passive sensitization with reaginic antibody was blocked by normal γ A-globulin preparations (19) and 4) γ A-globulin antibody was detected in patients' sera by radioimmunoelectrophoresis (14). In the previous experiments of this series, we confirmed the presence of reaginic antibody in γ A-globulin preparations, however, the reaginic activity in the fraction did not diminish by the precipitation of γ A-globulin (3). Radioimmunoelectrophoretic experiments actually showed γ A anti-ragweed antibody in Fr. γA_1 from serum T and in both sera A and P, confirming the observations of Yagi *et al.* (14). The absorption of Fr. γA_1 with anti- γ A-globulin resulted in the loss of the γ A anti-ragweed antibody in the supernatant (Fig. 4C), but the reaginic activity in the supernatant was comparable to that in the unabsorbed preparation (Table II). It was also found that Fr. γA_1 from sera U and T had comparable reaginic activity on

Figure 4. Radioimmunodiffusion in Ouchterlony plates. Both the stained slides (*left*) and the corresponding radioautograms (*right*) are shown. I^{131} -labeled antigen E was added to anti- γ A and anti-Fr. III. *A, upper*, Fr. γA_1 from serum U, anti- γ A (a- γ A), anti-Fr. III and myeloma γ A (MA) were placed in wells. Only the precipitin band formed between anti-Fr. III and Fr. γA_1 showed radioactivity. *B, upper center*, and *C, lower center*, the center wells were filled with Fr. γA_1 from serum U (*B*) and T (*C*). The supernatants obtained after absorption of Fr. γA_1 with anti-Fr. III (1) and with anti- γ A (2) were placed in peripheral wells. Supernatant (2) had reaginic activity, whereas supernatant (1) did not (*cf.* Table II). *D, lower*, the center well was filled with anti-Fr. III against Fr. γA_1 (serum U), twofold dilutions of serum P and the serum which had been absorbed with anti-Fr. III (abs). Only the precipitin band, of which specificity is identical with Fr. γA_1 -anti-Fr. III band, showed radioactivity. Some of the bands in radioautograms were slightly emphasized in the figures.

a weight basis; however, the fraction from serum U lacked γ A anti-ragweed antibody. These findings indicate that the γ A antibody is not responsible for reaginic activity. In regard to blocking of passive sensitization of monkey ileum with human reaginic antibody, Girard *et al.* (20) reported that not only γ A- but also γ G-globulin fraction of normal serum had a weak but definite blocking activity. This finding was confirmed in our previous experiments on passive sensitization of human skin (3). When the γ A-globulin fraction was further fractionated by chromatography on a DEAE cellulose column, the blocking activity was concentrated in a minor fraction (Fr. γ A₁) and Fr. γ A₂ containing the major part of γ A-globulin did not have much activity. Furthermore, γ A myeloma proteins lacked blocking activity (3). These findings suggested that the blocking by normal γ A-globulin fraction was due to impurities rather than to γ A-globulin itself. The distribution of reaginic activity in subfractions of a γ A-globulin preparation from a patient's serum paralleled the distribution of the blocking activity on passive sensitization with a reaginic antibody of a heterologous system, indicating that both activities are associated with the same protein which is different from γ A-globulin (3). The major discrepancy between the previous findings by others (16, 17) and our observations is that in our experiments reaginic activity was not diminished by the precipitation of γ A-globulin as reported by the other investigators. The present results showed that γ A anti-ragweed antibody was actually precipitated with our anti- γ A antibody preparation but almost all reaginic activity remained in the supernatant. It is possible that the anti- γ A-globulin preparations used by the other investigators were contaminated with anti- γ E-globulin antibody. Another possibility is that the major antigenic determinants in the protein molecules are different depending on the animal species used for immunization. Fireman *et al.* (16) used sheep anti- γ A antibody in their experiments. The possibility exists that γ E- and γ A-globulins have a common antigenic determinant in sheep and therefore, γ E-globulin can be identified as a distinct immunoglobulin only by rabbit antiserum. In any event, the present as well as the previous experiments of this series eliminate the evidence in support of the concept that reaginic antibody is associated with γ A-globulin. It was also found that the antibody is not associated

with γ G-, γ M- or γ D-globulins (1-3, 6). We, therefore, propose the hypothesis that the reaginic antibody is γ E-globulin. The possibility still exists that the anti-ragweed antibody detected in γ E-globulin does not have reaginic activity and that the reaginic antibody is associated with an entirely different protein which could not be detected so far. Another possibility is that reaginic antibody is a complex of an immunoglobulin with another serum component rather than a single protein. Stanworth (21) has reported that reagin is protected by serum α_2 glycoprotein and suggested that the complex formation between the glycoprotein and an immunoglobulin having the antigen combining site(s). If reaginic antibody is a complex in nature, the possibility may be considered that the γ E precipitin band is due to the glycoprotein moiety and the antigenic determinant characteristic for the immunoglobulin is hidden by the combination. These possibilities cannot be eliminated unless γ E-globulin is isolated or the antiserum specific for this protein is obtained.

The physicochemical properties, except electrophoretic mobility, and structure of γ E-globulin are unknown. If the reaginic antibody is actually associated with this protein, it might be expected that the sedimentation coefficient of this protein is around 8 S. In a previous article (1), it was found that reaginic antibody was coprecipitated with γ G-globulin-anti-F_{ab} (anti-L chain) antibody complexes but not with γ G-globulin-anti- γ (anti-H chain) antibody complexes. This finding suggests that γ E-globulin is composed of light chain(s), common to other immunoglobulins, and characteristic heavy chains.

Our studies in this series allow comparison to be made between human reaginic antibody and anaphylactic antibodies of other mammalian species. Recently, evidence was obtained by Oettgen *et al.* (22) that guinea pig γ_1 antibody, which is responsible for anaphylactic reactions in homologous animals, is not γ A-globulin. Barth and Fahey (23) demonstrated that mouse 7 S γ_1 -globulin antibody, but not γ A antibody, is responsible for anaphylactic reactions in mice. The rat anaphylactic antibody appears to have similar properties to that of the human reagin (24). Both of these antibodies sediment in a position intermediate between 7 S and 19 S components in ultracentrifugation and are heat labile. Furthermore, recent studies by Austen

et al. (25) on sensitization of rat mast cells with the anaphylactic antibody indicated that the sensitizing activity of the fractions from rat antiserum did not correlate with the γ G-, γ M- or γ A-globulin concentrations, and that absorption of rat antiserum with rabbit anti-rat γ A had little or no effect on the ability of the antiserum to sensitize rat skin. It seems that human reaginic antibody is not exceptional in the sense that the antibody is not associated with any of the γ G-, γ A- and γ M-globulins.

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SUMMARY

The reaginic activity in sera from ragweed-sensitive patients was inactivated after the precipitation of serum proteins with the rabbit anti-human globulin serum which did not contain any of the antibodies specific for γ G-, γ A-, γ M-globulins and for light chains of immunoglobulins. The rabbit antiserum gave a γ I-globulin precipitin band with the reagin-containing fraction which contained γ A but none of the γ G-, γ M- or γ D-globulins. The specificity of the precipitin band was different from the γ A-anti- γ A band. By radioimmuno-electrophoresis and by radioimmunodiffusion, the antibody against ragweed pollen-extract (antigen E) was detected in the γ I-globulin, indicating the presence of a unique γ I-immunoglobulin. The protein was tentatively designated γ E-globulin. Precipitation of γ E-globulin anti-ragweed antibody in the reagin-containing fraction was accompanied by the loss of reaginic activity, whereas the precipitation of the γ A-globulin antibody in the fraction did not result in any decrease of reaginic activity. The results highly suggest that the reaginic activity is associated with γ E-globulin.

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