Naturally Occurring Human Antiglobulins with Specificity for γE

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Abstract Human sera have been examined for antibodies with specific reactivity for γE using the tanned cell hemagglutination test. Cells tanned with three different γE myeloma proteins provided a reproducible test system. Inhibition of agglutination reactions by γE proteins, but not by γG, γA, γM, or γD confirmed the specificity of these reactions. 8.5% of 304 serial serum samples obtained from miscellaneous hospitalized patients showed clear-cut anti-γ-globulins with specificity for γE. In most of these instances no definite clinical history of concomitant allergic disorders could be obtained. 53% of 73 patients with well-established allergic disorders (hay fever, extrinsic asthma) showed serum anti-γ-globulins with reactivity for γE. Some patients studied before and after desensitization to Bermuda grass allergen showed an increase in titer or a conversion from negative to positive reactions for anti-γE antibodies following several month courses of progressive desensitization. Gradient and gel filtration studies indicated that anti-γE globulins were 19S γM in all instances. No clear correlation was noted between quantitative serum γE levels and titer of anti-γE antibodies.

19S serum fractions with anti-γE antibody activity did not release histamine from normal human peripheral blood leukocytes, whereas specific rabbit anti-γE antisera consistently induced leukocytic histamine release. Moreover, macroglobulin fractions with anti-γE activity did not block allergen-specific leukocyte histamine release induced by in vitro leukocyte challenge with allergens such as Bermuda grass and leukocytes from allergic donors. In some instances 19S human serum fractions with anti-γE activity appeared to potentiate histamine release when incubated concomitantly with specific allergen and leukocytes from allergic individuals.

INTRODUCTION

The discovery of the original γE myeloma (IgND) by Johansson and Bennich (1, 2) and subsequent structural studies characterizing this interesting new class of immunoglobulins have paved the way to a clearer understanding of some of the molecular events involved in allergic reactions. Studies by K. Ishizaka and coworkers (3–9), Stanworth, Humphrey, Bennich, and Johansson (10, 11), Osler and others (12, 13) have provided a rapidly accumulating body of knowledge concerning the relationship between histamine release by basophils or mast cells and reaginic γE antibody. Recently in vitro studies by Ishizaka have indicated that rabbit antibody to γE is capable of releasing histamine or degranulating mast cells (14). It occurred to us that naturally occurring human antiglobulins—perhaps first cousins to the family of human anti-γ-globulins or rheumatoid factors—might exist in some human sera. If such were the case, they might function as histamine-releasing boosters in some allergic patients, by reacting with γE reaginic antibody fixed to circulating basophils or tissue mast cells. Another possible physiologic role for such human antiglobulins might conceivably be to block allergen specific leukocyte histamine release by sterically interfering with the reaction between leukocyte-fixed reagin and allergen. Finally, a third possibility might be postulated. If human anti-γE antibodies showed specificity for Fc γE determinants, they might be capable of interacting with circulating γE reagin and preventing its

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subsequent attachment to basophilic leukocytes. These possible ways of interaction are shown in Fig. 1.

The present study clearly documents the occurrence of antiglobulins with γE specificity found in some human sera and attempts to relate their activity to clinical findings in patients with various allergic disorders.

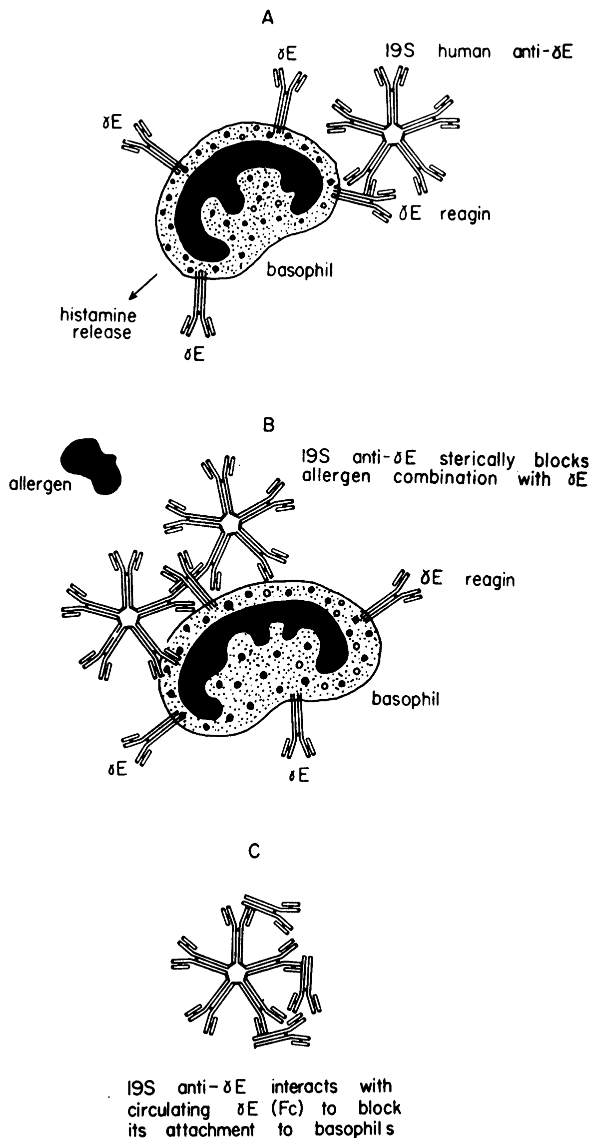

FIGURE 1 Three possible mechanisms of interaction between human anti-γE antibodies and leukocytes. A. Indicates reaginic γE antibody molecules affixed through their Fc regions to the surface of a basophil. Interaction of human 19S anti-γE globulin with basophil-fixed γE might result in cell membrane perturbations or other intracellular events capable of inducing histamine release.

B. Middle diagram indicates possibility of steric blockade by 19S anti-γE antibody of effective reaction between basophil-fixed γE and approaching allergen.

C. Lower diagram indicates possibility of reaction between 19S anti-γE antibody and γE reagin, thereby tying up the Fc portion of γE essential for fixation of γE to basophil.

METHODS

Sera were collected from 40 normal healthy donors (1st and 2nd yr medical students) as well as from 304 hospitalized patients at the Bernalillo County Medical Center and Albuquerque Veterans Administration Hospital. In addition, several special groups of patients' sera were studied which included 90 sera from patients with rheumatoid arthritis, 35 sera from patients with active systemic lupus erythematosus, 50 serum samples from heroin addicts under treatment by the Albuquerque NARA methadone program, 73 sera from allergic patients obtained from individuals with well-documented atopic eczema, hay fever, or asthma seen by several practicing allergists, and 15 sera from patients with visceral worm infestations (6 with *Ascaris* infestation and 9 with visceral larva migrans).

In addition a group of patients undergoing 3–4 month courses of desensitization to Bermuda grass or Russian thistle were studied before and after courses of desensitization.

Antibody to γE was measured by agglutination of sheep erythrocytes tanned (15) with preparations of isolated γE myeloma protein (PS) obtained through the courtesy of Dr. O. R. McIntyre, Dartmouth Medical School, Hanover, N. H. In addition a second γE myeloma protein (H) used in inhibition and tanned cell agglutination experiments was the generous gift of Dr. Gerald A. Penn, U. S. Naval Medical Center, Bethesda, Md. The third γE myeloma protein used to test specificity in tanned cell agglutination and inhibition reactions was IgND kindly furnished by Dr. S. G. O. Johansson. γE myeloma PS and the original IgND were of lambda type; the H γE myeloma possessed kappa light-chains. Sheep cells were tanned using myeloma proteins isolated by starch block electrophoresis and repeated gel filtrations on Sephadex G-200. Such preparations showed only γE by immunoelectrophoresis analysis of purity using monospecific antisera to γG, γA, γD, γE, and γM. No more than 1% contamination by background γG was detectable by radial diffusion quantitative estimation in the myeloma preparations used for tanning cells. All human sera to be tested were absorbed with packed washed sheep cells before testing to remove heterophil antibody. A concentration of γE myeloma of 1–2 mg/ml was used for tanning cells. Appropriate controls for tanned cells alone and nonspecific agglutination were included in all procedures. Specificity of agglutination reactions for γE in all sera scored as positive was confirmed by inhibition of 2–3+ agglutination by isolated preparations of γE myeloma proteins tested in parallel with isolated γG, γA, γM, and γD paraproteins. Inhibiting preparations of myeloma proteins were tested as doubling dilutions beginning at concentrations of 0.5 mg/ml. Specific inhibition could often be recorded for γE inhibiting proteins at concentrations of 0.004–0.001 mg/ml. If myeloma PS was used to tan cells, specificity of inhibition was checked not only with PS γE but also with one of the other two myelomas ND or H as well.

Physical studies. Molecular distribution of human anti-γE antibodies was studied using separations by sucrose density gradient ultracentrifugation as previously described (16). In some instances separation by Sephadex G-200 gel filtrations was utilized to confirm the molecular class of antibodies present in some sera.

Assay for physiologic activity of anti-γE antibody. An indication of the physiologic significance of human anti-γE antibodies was sought using a modification of the in vitro histamine release assay as outlined by T. Ishizaka Tomioka, and K. Ishizaka (14). The method recently described by May, Lyman, Alberto, and Cheng (17) was utilized for histamine release determinations in all of our studies. In some of these experiments, serum fractions containing strong agglutinating activity for γE were added to normal donor leukocytes and subsequent release of histamine measured by the fluorometric technique. As a positive control, rabbit antibody to γE was utilized in order to compare amount and rapidity of histamine release under the same experimental conditions using a heterologous antibody. In addition, leukocytes were obtained from known allergic individuals and effects of allergen (Bermuda grass) induced histamine release studied in the presence of serum or fractions containing anti-γE activity.

Relation of human anti-γE antibody to quantitative estimation of γE levels in serum. It was felt important to study any possible direct relationship between the occurrence of human anti-γE antibody and quantitative levels of γE estimated in the same serum. The indirect Mancini technique described by Rowe (18) was employed utilizing a serum pool standard containing 10,000 ng of γE/ml and several different rabbit antisera to γE, ^{131}I-labeled goat anti-rabbit γ-globulin was added as an amplification system, and radial diffusion diameters were determined after radioautography.

RESULTS

Initial screening of 40 normal healthy adult sera revealed one subject with clear-cut antiglobulin activity for γE. This individual gave a history of repeated episodes of urticaria during childhood and early adult life. In addition, the survey of 304 sera obtained from miscellaneous hospitalized patients showed that 8.5% contained antibody with clear-cut specificity for determinants present on γE, but not shared by γG, γA, γM, or γD. The hemagglutinin test was arbitrarily scored as significantly positive if agglutination was noted in a titer of 1:8 or more. The titers of these antibodies showing anti-γE reactivity among the miscellaneous hospitalized patients are shown in Table I.

TABLE I

Tanned Cell Hemagglutinin Titers of Anti-γE Antibodies as Recorded among Various Groups of Patients

Reciprocal titers	Miscellaneous hospital patients (304)	Allergic patients (73)	Rheumatoid arthritis (90)	Heroin addicts (50)	Worm infestation (15)
8*	11	19	8	3	4
16	9	8	1	1	1
32	4	6	0	0	2
64	1	0	0	0	2
128	1	0	0	0	0
256	0	5	0	0	0
512	0	1	0	0	0

* Titers of 1:8 or greater were considered positive in the tanned cell test.

An indication of the specificity of the anti-γE antibodies found in these human sera was shown by the clear inhibition of agglutination reactions by isolated γE myeloma proteins but not by γG, γA, γM, or γD. An example of this specificity as demonstrated by inhibition is shown in Table II. In addition, specificity of anti-γE antibodies was tested using Fc and Fab fragments isolated after papain digestion of γE (PS) using the conditions previously described by Bennich and Johansson (19). In general, specific inhibition of tanned cell hemagglutination was clearly evident using whole γE myeloma protein but not with isolated Fc or Fab fragments of papain-digested γE myelomas. However, many sera containing anti-γE antibodies showed weak agglutination (1:2–1:4) of cells tanned with Fc fragments of papain digested γE myeloma. If whole γE myelomas

TABLE II

Specificity of Human Antibodies to γE as Demonstrated by Inhibition of Agglutination of Cells Tanned with γE Myeloma Protein (PS)

	Agglutinating serum Ho. 1:16—2+ Dilution of inhibiting immunoglobulin											
	2	4	8	16	32	64	128	256	512	1024	2048	4096
IgE*	0	0	0	0	0	0	0	0	0	0	1+	2+
IgA*	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
IgG*	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
IgM*	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
IgD*	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+

* All inhibiting immunoglobulin preparations were free from cross-immunoglobulin contamination as judged by immunoelectrophoresis against monospecific anti-γG, γA, γM, γD, and γE antisera; the 1:2 dilution of inhibitor represented a concentration of 0.5 mg/ml.

TABLE III

Tanned Cell Hemagglutination Titers Using Cells Tanned with Three Different γE Myeloma Proteins

Human serum tested*	Reciprocal titers using cells coated with		
	Sh (γE,λ)	H (γEK)	ND (γE,λ)
1. 0900	16	0	0
2. 0920	32	4	256
3. 0952	8	2	2
4. 0966	16	4	0
5. 1005	256	4	4
6. Ho.	16	16	2
7. Jac.	512	32	16
8. Sn.	8	8	2
9. D-2	16	16	8
10. D-1	8	8	0
11. M.W.	0	8	8
12. B.W.	0	256	16
13. A.B.	0	128	0
14. N.A.	16	32	8
15. D.P.	16	16	16
16. Rabbit anti-human γE 1:100	1024	1024	1024

* Sera 1-5 chosen from miscellaneous hospitalized patients; sera 6-15 represent sera from known allergic patients.

were heated to 60°C for 10 min in an attempt to produce γE aggregates, inhibitory capacity for specific anti-γE agglutinators was not increased. However, if γE myelomas were similarly heated before affixation to cells by tanning, titers of agglutination markedly increased in some instances. Using a panel of 10 different sera with clear-cut anti-γE antibody reactivity, clear inhibition by 8S γE preparations isolated after sucrose gradient ultracentrifugation presumably free of γE aggregates, could be demonstrated. This specificity or apparent primary activity for determinants on whole γE provided a distinct difference from the usual variety of 19S human anti-γ-globulins which generally show strong inhibition by aggregates of γG. Clinical inquiry among the group of miscellaneous hospital patients showing anti-γE antibodies did not elicit a clear-cut history of allergy except in a minority of instances. Of interest was the finding of recent cerebral or central nervous system trauma, alcoholism, or hepatitis in a number of miscellaneous hospital patients showing serum anti-γE activity.

In general, some variability in reactivity for determinants on γE was noted when a representative panel of sera were tested for their agglutination titers of cells coated individually with the three different γE myeloma proteins. The pattern of variability recorded in some agglutinators (Table III) suggested the type of reaction which has been noted when several individual

TABLE IV

Occurrence of Anti-γE Antibodies among Eight Allergic Subjects Desensitized to Bermuda Grass for Periods from 3–4 Months

Patient		Titer of anti-γE antibody as measured by tanned cell agglutination									
		2	4	8	16	32	64	128	256	512	102
1. Ho.	Before	0	0	0	0	0	0	0	0	0	0
	After	4+	4+	4+	3+	1+	0	0	0	0	0
2. De.	Before	0	0	0	0	0	0	0	0	0	0
	After	0	0	0	0	0	0	0	0	0	0
3. Ja.	Before	4+	4+	4+	4+	4+	4+	4+	4+	±	0
	After	4+	4+	4+	4+	4+	4+	4+	4+	3+	1+
4. Ne.	Before	4+	4+	4+	3+	2+	1+	0	0	0	0
	After	4+	4+	4+	3+	2+	1+	0	0	0	0
5. Cl.	Before	4+	4+	±	0	0	0	0	0	0	0
	After	4+	1+	0	0	0	0	0	0	0	0
6. Co.	Before	0	0	0	0	0	0	0	0	0	0
	After	0	0	0	0	0	0	0	0	0	0
7. Cl.	Before	0	0	0	0	0	0	0	0	0	0
	After	0	0	0	0	0	0	0	0	0	0
8. Ja.	Before	0	0	0	0	0	0	0	0	0	0
	After	0	0	0	0	0	0	0	0	0	0

human anti-Rh incomplete antibodies coating Rh-positive cells are compared with a panel of human anti-γ-globulin containing sera (20). No evidence of distinct specificity for γE λ molecules (PS or ND) was apparent among any of the agglutinating antibodies studied. Sera which showed agglutination of cells coated with γE myeloma PS (γE λ) could be inhibited in many instances by the γEK myeloma H; in some instances this could be shown although the agglutinating serum would not react directly to agglutinate cells coated with the latter myeloma. Preliminary data on inhibition of hemagglutination reactions indicated that many of the human sera containing agglutinating activity for γE apparently reacted with multiple γE determinants. For instance serum Jac. (Table III) which showed a titer of 1:512 with Sh-tanned cells could be inhibited by Sh and H γE myelomas to a greater extent than by γE ND when all were tested at equal concentrations of inhibitor. In reverse fashion serum 0920 showing a titer of 1:256 with ND-coated cells could be inhibited by lower concentrations of γE Sh (0.016 mg/ml) than by γE H which inhibited only to concentrations of 0.06 mg/ml. The inhibition by several different samples of γE myeloma proteins served to reinforce the impression of specificity for determinants peculiar to γE; however, the pattern of variability in agglutination patterns suggested that these human antisera contained antibodies against multiple determinants present on γE.

The incidence of positive antiglobulins with reactivity for γE was no higher among patients with rheumatoid arthritis, systemic lupus erythematosus, or narcotic addiction than was noted in the general hospitalized population studied (Table I).

When the sera from 73 known allergic patients were examined, a much higher incidence of positive tests for anti-γE antibodies was recorded. 53% of these individuals showed clear agglutinating reactivity for cells coated with γE. In general, however, titers of hemagglutinating antibody were not higher than the positive sera found among the general hospital population although some sera with extremely high titers (1:256–1:512) were noted among allergic patients (Table I). Among the eight patients studied before and after several months of desensitization to Bermuda grass, three showed strong reactivity before desensitization, and one developed anti-γE antibody after the course of injections (Table IV). No clinical correlations could be drawn, however, between the objective or subjective results of such desensitizations and the presence of anti-γE antibodies in the serum.

In the small group of 15 worm-infested patients studied 9 (60%) showed distinct antibodies with specificity for human γE. The results of our clinical screening of these groups of patients are summarized in Table I.

It was clear that many of the human sera shown to contain anti-γE antibodies were derived from several patient groups known to show apparent quantitative elevation of γE levels—as in patients with allergic disorders or worm infestations. Radioimmunoassay was therefore utilized to study any possible relationship between quantitative elevation of serum γE levels and the titers of anti-γE antibody as established by tanned cell hemagglutination. No clear-cut direct relationship was readily apparent between tanned cell anti-γE titer and quantitative estimation of γE, although the highest level of serum γE recorded (5600 ng/ml) occurred in serum Ja. (Table IV) which showed one of the highest titers of anti-γE antibodies in tanned cell agglutination. These results are illustrated in Fig. 2.

Physical studies. Gradient separation of 12 sera containing anti-γE antibody indicated that in all instances this was high molecular weight (19S) in distribution. Absorption of 19S gradient fractions by monospecific immunoglobulin antisera confirmed the impression that anti-γE antibody was 19S γM in all instances studied.

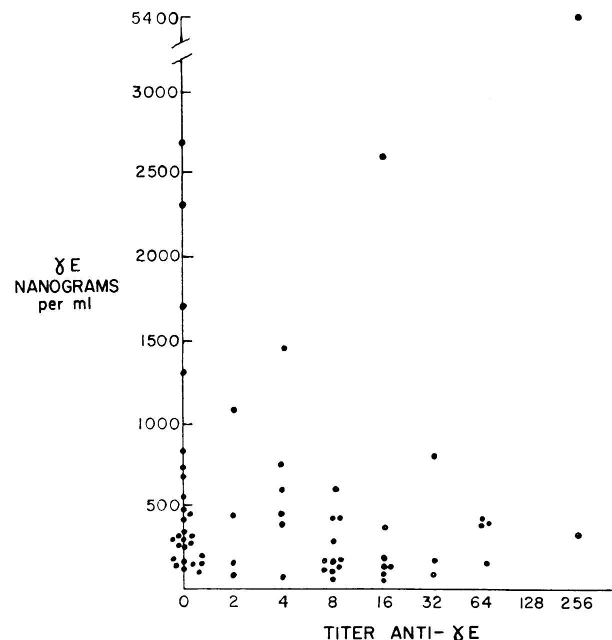

FIGURE 2 Anti-γE antibody titers of representative sera from miscellaneous hospitalized and allergic patients are plotted on abscissa while parallel quantitative γE level estimations are shown on ordinate. No clear relationship between titer of anti-γE antibody and serum level of γE is apparent. The anti-γE antibody titers shown are those using γE PS tanned cells. Although titers using the two other γE myelomas affixed to cells were sometimes markedly different (Table III), when the data were replotted in relationship to γE quantitative estimations, again no correlation was noted.

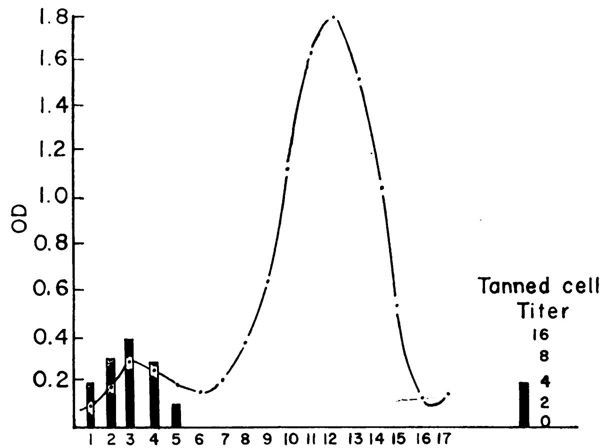

FIGURE 3 Sucrose density gradient separation of serum 3378 from a patient hospitalized with diagnosis of bacterial pneumonia. No definite allergic history could be obtained from this patient. The top of the gradient is shown to the right and the bottom (19S) fractions to the left. The hemagglutinating antibody activity for cells tanned with γE myeloma protein was found only in 19S fractions 1–5.

A representative gradient separation result is shown in Fig. 3.

Histamine release studies. A direct examination was performed to determine if serum or isolated 19S fractions from sera of patients with anti-γE antibody affected histamine release by human peripheral blood leukocytes. 19S fractions similarly obtained from normal sera were used as negative controls and specific rabbit anti-γE antisera (14) were employed as positive controls in many experiments. The 19S fractions of all sera studied were separated by Sephadex G-200 gel filtration and subsequently tested to insure the retention or absence of anti-γE antibody. The results of two such tests are shown in Table V; it can be seen that using normal leukocyte donors and a wide range of dilutions of reagent 19S fractions, no direct histamine release by the human 19S anti-γE materials could be documented. However, rabbit antiserum containing anti-human γE reactivity clearly produced histamine release. These experiments were repeated on many occasions and were closely reproducible.

A more direct approach to the possible relationship of 19S human anti-γE antibodies and histamine release by human leukocytes was next undertaken using peripheral blood leukocytes from known allergic donors challenged in the presence of varying concentrations of specific allergen. In all instances leukocytes from allergic patients known to be sensitive to Bermuda grass were studied using varying concentrations (0.001 to 0.1 mg/ml) of Bermuda grass allergen. An attempt was made to see if preincubation of leukocytes with human anti-γE (tested as 19S fractions isolated by Sephadex G-200 gel filtration or as whole serum) was capable of inhibiting subsequent histamine release by these leukocytes when exposed to specific allergen. No inhibition of such histamine release was noted with 19S anti-human γE antibody containing fractions; however, in some instances distinct inhibition was noted when similarly isolated 19S fractions from one normal nonallergic human serum were preincubated with allergic patients' leukocytes before exposure to varying amounts of known allergen (Table VI). Nine different experiments utilizing 19S fractions from five normal nonallergic subjects showed no such blockade; on the other hand five other experiments utilizing 19S fractions from one particular normal subject consistently showed histamine release blockade with five different allergic donor leukocytes. Immunoelectrophoresis of this particular 19S fraction from normal human serum showed

TABLE V

In Vitro Histamine Release Studies Using Human and Rabbit Serum Fractions Containing Anti-γE Antibody and Normal Human Leukocytes

Fraction or sample studied	Basic anti-γE titer	Total cell per cent histamine released§
		%
1. Jac 19S* (undiluted)	1:32	0
" " 1:2		0
" " 1:10		0
" " 1:100		0
2. FL 19S* (undiluted)	1:16	5.7
" " 1:2		2.4
" " 1:10		9.2
" " 1:100		3.5
3. Kei 19S* (undiluted)	1:8	8.9
" " 1:2		0.7
" " 1:10		0.8
" " 1:100		0
4. Normal 19S A (undiluted)	0	0
" " 1:2		2.1
" " 1:10		0
" " 1:100		0
5. Normal 19S B (undiluted)	0	1.8
" " 1:2		9.5
" " 1:10		5.0
" " 1:100		2.1
6. Rabbit anti-γE 1:100	1:32	66.5

* Samples obtained from sera of patients showing anti-γE antibody activity.

§ Histamine release calculated as described by May et al. (17); these experiments utilized peripheral blood leukocytes from normal nonallergic human donors.

only 19S γM and alpha-2 macroglobulins. These results appeared to be consistent with the interpretation that 19S serum fractions from occasional nonallergic patients possess the capacity to inhibit antigen-induced leukocyte histamine release whereas similarly prepared 19S fractions from several allergic patients were not capable of similar inhibition. This finding did not appear to be due to the presence of anti-γE antibody in such fractions and appeared to be related to some other properties of this preparation.

In 2 of 12 experiments performed using allergic patients' leukocytes, when specific allergen was added to leukocytes in the presence of human 19S fractions possessing anti-γE activity, apparent potentiation of histamine release was observed. In the two instances where this result was recorded, no significant histamine release was present using allergic leukocytes and allergen alone; slight release was recorded with leukocytes and rabbit anti-γE antibody. Only when 19S human anti-γE, plus allergen, plus allergic leukocytes were studied together did clearly significant histamine release occur. A representative experiment of this sort is shown in Table VII. In the two instances where such potentiation did occur, the allergic donors were different individuals. In both cases no detectable intrinsic anti-γE antibody was recorded using the allergic leukocyte donor's plasma.

TABLE VI

Studies of Leukocyte In Vitro Histamine Release Using Allergic Donors' Leukocytes in the Presence of Specific Allergen

Fraction or sample studied	Basic anti-γE titer	Total per cent histamine released
		%
1. Bermuda grass allergen–0.1 mg/ml	0	52.0
–0.01 mg/ml		16.5
2. 19S anti-human γE serum fraction (Kei) preincubated with leukocytes 30 min, with Bermuda grass allergen –0.1 mg/ml	1:8	52.3
–0.01 mg/ml		18.3
3. Kei whole serum preincubated with leukocytes 30 min with Bermuda grass allergen –0.1 mg/ml	1:16	47.5
–0.01 mg/ml		33.7
4. Normal whole serum preincubated with leukocytes 30 min., with Bermuda grass allergen –0.1 mg/ml	0	65.9
–0.01 mg/ml		35.5
5. Normal 19S human serum fraction preincubated with leukocytes 30 min, with Bermuda grass allergen –0.1 mg/ml	0	0
–0.01 mg/ml		0
6. Kei added to leukocytes	1:8	0
7. Kei whole serum added to leukocytes	1:16	0
8. Normal whole serum added to leukocytes	0	0
9. Normal 19S human serum fraction added to leukocytes	0	6.2
10. Rabbit anti-γE antiserum 1:100 added to leukocytes	1:256	87.2

TABLE VII

Apparent Potentiation of Histamine Release by 19S Human Anti-γE Fractions Using Allergic Patient's Leukocytes

Fraction or sample studied	Basic anti-γE titer	Total per cent histamine released
		%
Bermuda allergen alone, 0.001 μg	0	0
Fl. 19S	1:16	0
Bermuda allergen 0.001 μg plus Fl. 19S	1:16	39.6
Bermuda allergen 0.001 μg plus Jo. 19S	0	0
Rabbit anti-γE 1:1000	1:32	18.1

The last possibility for physiologic interactions of human anti-γE depicted in Fig. 1C was studied using preincubation of 19S human fractions containing anti-γE activity with dilutions of serum from patients allergic to Bermuda grass. After such preincubation, the mixtures were used to sensitize either normal or allergic patients' leukocytes, and subsequent histamine release measured after allergen-induced or rabbit anti-γE triggered reactions. Using these test systems, we could not obtain evidence that human 19S fractions possessing anti-γE activity interfered with passive sensitization of basophils by sera containing reaginic antibody.

Finally relative or comparative effects of human and rabbit anti-γE antibody were tested in an attempt to determine whether 30 min of preincubation of test leukocytes with 19S human fractions showing anti-γE activity influenced subsequent histamine release by rabbit anti-γE antibody. Several experiments clearly indicated that such preincubation of normal human leukocytes with 19S human preparations possessing anti-γE activity reduced subsequent histamine release when cells were then exposed to dilutions of rabbit anti-γE antiserum. A representative experiment is shown in Table

TABLE VIII

Effect of Preincubation of Normal Donor Leukocytes with 19S Human Fractions before Challenge with Rabbit Anti-γE Antiserum

Preparation tested	Basic anti-γE titer	Total cel per cent histamine released
		%
1. Rabbit anti-γE 1:100	1:256	98.9
2. Ko. 19S	1:64	0
3. Ko. 19S preincubated for 30 min with leukocytes followed by rabbit anti-γE 1:100	—	76.5
4. PL 19S (normal)	0	0
5. PL 19S preincubated for 30 min with leukocytes followed by rabbit anti-γE 1:100	—	99.0

VIII. These results provided evidence that 19S human γE was capable of combining with cell-bound γE and of modifying, at least in part, histamine release induced by rabbit anti-γE added thereafter. Control experiments using preincubation with normal human 19S fractions devoid of anti-γE activity did not modify or partially block rabbit anti-γ-E induced leukocyte histamine release.

DISCUSSION

The studies reported here clearly indicate the occurrence of 19S γM anti-γE antibodies in some human sera. Their high incidence (53%) in sera from allergic patients raises an interesting question about the possible physiologic significance of such antibodies in the body proper. Long-term follow-up of such patients correlated with variations in titer or anti-γE specificity is now needed in order to draw any final valid conclusions as to the exact in vivo function of such antiglobulins.

Further studies are also indicated to attempt to understand the exact specificity of human anti-γE antibodies. It is possible that these antibodies may be directed at relatively hidden γE determinants revealed by partial denaturation of γE molecules similar perhaps to the specificity recently described by Ito, Wicher, and Arbesman (21). The finding of naturally occurring human antibodies to γE also may make it possible eventually to define E (M) groups or genetic variations among individual patients in the same fashion as human 19S anti-γ-globulins have helped to define the GM-Inv systems previously. A recent observation by Battisto, Budman, and Freedman (22) suggests that some possible individual or genetic differences may exist with respect to capacity of homocytotropic antibodies to fix to skin.

It is also possible that the presence of anti-γE antibodies in serum from various patients may to a certain extent merely reflect quantitative levels of circulating γE active in the particular individuals. However, attempts to relate the presence of anti-γE antibody to quantitations of serum γE using radioimmunoassay radial diffusion did not indicate a clear relationship between anti-γE antibody titers and serum levels of γE.

An attempt has been underway for some time to understand the physiologic role of various types of human antiglobulin antibodies (23–27). As yet no well defined mechanism is apparent for the function which this interesting heterogeneous group of antibodies actually performs in body economy. The excellent in vitro assay system afforded by leukocyte histamine release was chosen for use in the current study since it may afford an ideal test system for elucidating the events which occur in vivo. No evidence was obtained for direct histamine release by normal or allergic human leukocytes incubated with human serum fractions containing antibody to γE except in several instances where a combination of allergen, 19S human anti-γE, and leukocytes were studied together. Under these circumstances the human anti-γ-antibody and the allergen together induced histamine release whereas no release could be detected with either alone. Rabbit serum with equal hemagglutinating anti-γE antibody activity to many of the human fractions studied showed leukocyte histamine release confirming the previous report of Ishizaka's group (14). Moreover, no protective influence of human anti-γE antibodies could be demonstrated when leukocytes from allergic patients were preincubated with 19S fractions containing human anti-γE activity before exposure to specific allergen. The most interesting finding uncovered in this series of experiments was the indication that 19S fractions without any specific anti-γE antibody activity from occasional normal nonallergic patients when preincubated with allergic patients' leukocytes, appeared to block allergen-specific induced histamine release. However, no such blockade could be demonstrated when the same normal whole serum instead of the isolated 19S fractions was used. These data could be interpreted to indicate that some protective factor is present in high molecular weight serum fractions derived from some normal human sera which is capable of inhibiting histamine release from allergic patients leukocytes when the latter are exposed to specific allergen. Further studies are needed to explore the precise mechanisms involved.

It was perhaps not surprising that the in vitro test system used in these studies did not show any direct histamine release when isolated 19S human serum fractions possessing anti-γE reactivity were added to leuko-

cytes, since it has been shown by Ishizaka and coworkers (9) that anaphylactic or γE antibody is bound to basophils via the Fc portion of the molecule. If the human 19S anti-γE antibodies show any degree of preferential reactivity for sites on γE Fc, then the antigenic sites towards which the antibodies are reactive might be covered up or sterically not exposed because they are attached to the basophil itself. The differences in reactivity recorded in this study for rabbit anti-γE antibody and human preparations showing anti-γE activity of equal hemagglutinating titers are not yet fully understood. It is conceivable that the rabbit γG antibody possesses a higher average binding constant than the 19S γM human antibody and is thus more effective in cell membrane perturbation and direct basophil histamine release.

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REFERENCES

1. Johansson, S. G. O., and H. Bennich. 1967. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology.* **13:** 381.
2. Johansson, S. G. O., and H. Bennich. 1967. Studies on a new class of human immunoglobulins. I. Immunological properties. Nobel Symposium 3. J. Wiley & Sons, Inc., New York. 193.
3. Ishizaka, K., and T. Ishizaka. 1968. Induction of erythema-wheel reactions by soluble antigen-γE antibody complexes in humans. *J. Immunol.* **101:** 68.
4. Ogawa, M., S. Kochwa, C. Smith, K. Ishizaka, and O. R. McIntyre. 1969. Clinical aspects of IgE myeloma. *N. Engl. J. Med.* **281:** 1217.
5. Ishizaka, T., K. Ishizaka, H. Bennich, and S. G. O. Johansson. 1970. Biologic activities of aggregated immunoglobulin E. *J. Immunol.* **104:** 854.
6. Newcomb, R. W., and K. Ishizaka. 1970. Physicochemical and antigenic studies on human γE in respiratory fluid. *J. Immunol.* **105:** 85.
7. Ishizaka, T., K. Ishizaka, R. P. Orange, and K. F. Austen. 1970. The capacity of human immunoglobulin E to mediate the release of histamine and slow reacting substance of anaphylaxis (SRF-A) from monkey lung. *J. Immunol.* **104:** 335.
8. Ishizaka, K., and T. Ishizaka. 1968. Human reaginic antibodies and immunoglobulin E. *J. Allergy.* **42:** 330.
9. Ishizaka, K., H. Tomioka, and T. Ishizaka. 1970. Mechanisms of passive sensitization: I. Presence of IgE and IgG molecules on human leukocytes. *J. Immunol.* **105:** 1459.
10. Stanworth, D. R., J. H. Humphrey, H. Bennich, and S. G. O. Johansson. 1967. Specific inhibition of the Prausnitz-Küstner reaction by an atypical human myeloma protein. *Lancet.* **2:** 330.
11. Stanworth, D. R. 1970. Immunochemical mechanisms of immediate-type hypersensitivity reactions. *Clin. Exp. Immunol.* **6:** 1.
12. Osler, A. G., L. M. Lichtenstein, and D. A. Levy. 1968. *In vitro* studies of human reaginic allergy. *Advan. Immunol.* **8:** 183.
13. Saden, N., M. B. Rhyne, E. D. Melltis, E. O. Goldstein, D. A. Levy, and L. M. Lichtenstein. 1969. Immunotherapy of pollinosis in children. *N. Engl. J. Med.* **280:** 623.
14. Ishizaka, T., H. Tomioka, and K. Ishizaka. 1971. Degranulation of human basophil leukocytes and anti-γE antibody. *J. Immunol.* **106:** 705.
15. Boyden, S. V. 1951. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. *J. Exp. Med.* **93:** 107.
16. Kunkel, H. G. 1960. Macroglobulins and high molecular weight antibodies: IV. Immunological properties. *In* The Plasma Proteins. F. W. Putnam, editor. Academic Press, Inc., New York. **1:** 292.
17. May, C. D., M. Lyman, R. Alberto, and J. Cheng. 1970. Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *J. Allergy.* **46:** 12.
18. Rowe, D. S. 1969. Radioactive single radial diffusion: A method for increasing the sensitivity immunochemical quantification of proteins in agar gel. *Bull. WHO.* **40:** 613.
19. Bennich, H., and S. G. O. Johansson. 1967. Studies on a new class of human immunoglobulins. II. Chemical and physical properties. Gamma globulins structure and control of biosynthesis. Nobel Symposium 3. J. Wiley & Sons, Inc., New York. 199.
20. Fudenberg, H., and H. Kunkel. 1961. Specificity of the reaction between rheumatoid factor and gamma globulin. *J. Exp. Med.* **114:** 257.
21. Ito, K., K. Wicher, and C. E. Arbesman. 1971. Antibodies to denatured IgE and new antigenicity of the IgE acquired during heating. *J. Immunol.* **106:** 1130.
22. Battisto, J. R., D. Budman, and R. Freedman. 1971. Specificity of fixation loci for homocytotrophic antibodies. *J. Exp. Med.* **134:** 381.
23. Kunkel, H. G., H. J. Simon, and H. H. Fudenberg. 1958. Observations concerning positive serologic reactions for rheumatoid factors in certain patients with sarcoidosis and other hyperglobulinemic states. *Arthritis Rheum.* **1:** 289.
24. Fudenberg, H. H., and E. C. Franklin. 1965. Rheumatoid factors and the etiology of rheumatoid arthritis. *Ann. N. Y. Acad. Sci.* **124:** 884.
25. Messner, R. P., T. Laxdal, P. G. Quie, and R. C. Williams, Jr. 1968. Serum opsonin, bacteria, and polymorphonuclear leukocyte interactions in subacute bacterial endocarditis anti-γ-globulin factors and their interaction with specific opsonins. *J. Clin. Invest.* **47:** 1109.
26. King, R. A., R. P. Messner, and R. C. Williams, Jr. 1969. Human lymphocyte transformation induced by anti-gamma globulin factors. *Arthritis Rheum.* **12:** 597.
27. Messner, R. P., E. W. Caperton, R. A. King, and R. C. Williams, Jr. 1969. Interactions among rheumatoid factors, G antibodies, lymphocytes, and phagocytes. *Ann. N. Y. Acad. Sci.* **168:** 93.