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BIOLOGICAL FUNCTION OF γE ANTIBODIES AND MECHANISMS OF REAGINIC HYPERSENSITIVITY

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SUMMARY

Human reaginic antibodies in sera of atopic individuals are associated with immunoglublin E which represents a distinct immunoglobulin class. The γE antibody agglutinated red cells coated with antigen, indicating that the antibodies are probably divalent. However, the antibodies do not have complement-fixing activity. The antibodies are responsible for P–K reactions in humans, passive cutaneous anaphylaxis reactions in monkeys and sensitize human leucocytes and monkey lung tissues, but not the guinea-pig skin. Immunoglobulin E combines with the tissues which are involved in the reaginic hypersensitivity reactions through the Fc portion of the molecules.

The initial step in hypersensitivity reactions is probably bridging of cell-bound γE molecules which induce structural changes in these molecules. These changes may induce enzymatic sequences leading to the release of histamine and/or slow reacting substance of anaphylaxis depending on the cells involved.

Studies on the distribution of γE forming cells indicated that these cells localized in respiratory and gastro-intestinal tracts. The results suggested that locally formed γE may play an important role in allergic diseases.

INTRODUCTION

Physico-chemical properties of human reaginic antibodies have been studied using various fractionation methods (cf. Stanworth, 1963). Since the concept of 'immunoglobulin' was introduced, the correlation between reaginic antibodies and known immunoglobulins have been investigated. When reaginic sera were fractionated by electrophoresis, by ion-exchange column chromatography, or by the zinc precipitation method, skin-sensitizing activity of the sera was usually detected in γA fraction (Heremans & Vaerman, 1962). These findings suggested the possibility that reaginic activity may be associated with γA. However, some findings could not be explained by γA globulin hypothesis. For example, γA antibodies

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against blood group substance did not have skin-sensitizing activity (Ishizaka *et al.*, 1965). As the immunological properties of antibodies differ depending on the immunoglobulin class to which the antibodies belong (Franklin, 1964; Ishizaka *et al.*, 1965), the possibility was considered that the reaginic antibodies in the γA globulin fraction might be associated with a small amount of protein, which contaminated the fraction. Indeed, subsequent studies on antigenic structure of reaginic antibodies indicated that the antibodies do not belong to any of the γG, γA, γM and γD immunoglobulins (Ishizaka & Ishizaka, 1966; Ishizaka, Ishizaka & Lee, 1966d). Finally, a new immunoglobulin, γE,* was detected in a reagin-rich fraction and the protein was identified as a carrier of reaginic activity. Identification of reaginic antibody as γE immunoglobulin provided new directions for the immunological studies on reaginic hypersensitivity. In this symposium, we would like to summarize the nature of γE antibodies and discuss the immune mechanisms of reaginic hypersensitivity reactions induced by γE antibody systems.

CORRELATION BETWEEN γE ANTIBODIES AND REAGINIC ACTIVITY

In order to prove the hypothesis that reaginic antibodies belong to a unique immunoglobulin class, attempts were made to prepare rabbit antibodies which react with the carrier protein of reaginic activity. A rabbit antiserum against a reagin-rich fraction of an atopic patient's serum was prepared, and absorbed with known immunoglobulins and myeloma proteins. The

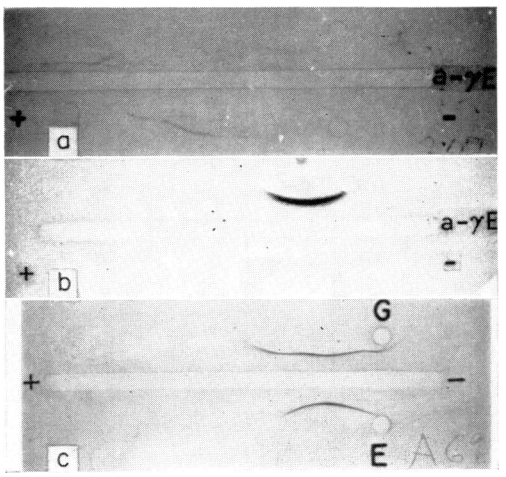

FIG. 1. (a) and (b) Radioimmunoelectrophoresis of a reagin-rich fraction. Both the stained slide (a) and the radioautograph (b) are shown. The antibody trough was filled with anti-γE. The γE band combined radioactive ragweed antigen in (b). (c) Fresh human serum was incubated with aggregated γG or aggregated γE and analysed by immunoelectrophoresis with anti-C3. Aggregated γG but not aggregated γE formed C3a.

antiserum did not give a precipitin band with any of the γG, γA, γM and γD. However the antiserum gave a γ_1 precipitin band with a reagin-rich fraction from the serum of a ragweed-sensitive patient serum and the γ_1 precipitin band combined radioactive ragweed allergen in a radioautograph (Fig. 1a and b). As this finding showed that the γ_1-globulin

* IgE, *Bull. Wld Hlth Org.* (1968) **38**, 157.

had a unique antigenic structure which is different from four immunoglobulins, i.e. γG, γA, γM and γD and that the γ_1-globulin had antibody activity, this protein was tentatively designated γE-globulin (Ishizaka, Ishizaka & Hornbrook, 1966b).

In the next step, the relationship between γE and human reaginic antibodies has been studied. It was found that the presence of reaginic activity against ragweed allergen correlated

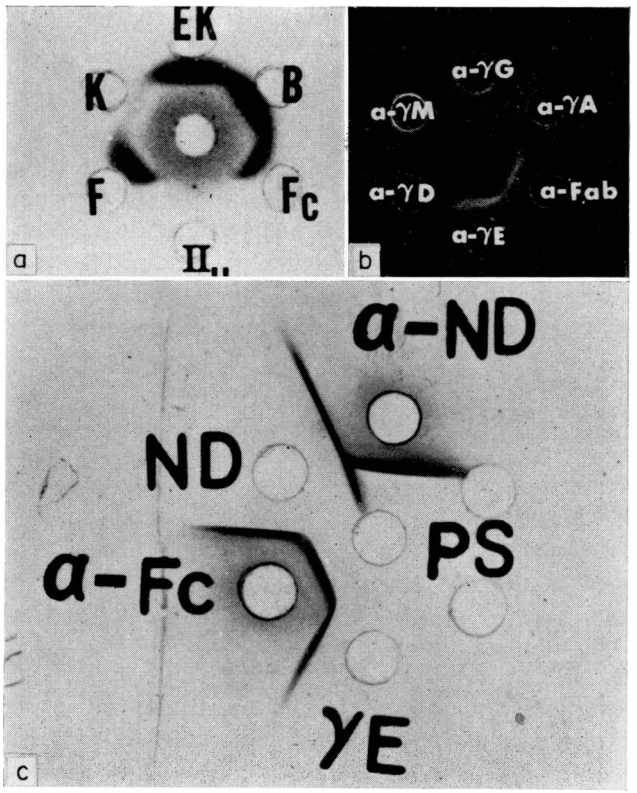

FIG. 2. (a) Radioimmunodiffusion of reaginic sera. The centre well was filled with the mixture of anti-γE and radioactive antigen E. Peripheral wells were filled with reaginic sera, serum K and a reagin-rich fraction (Fraction II_{II}) of another serum did not contain reaginic antibody against antigen E. (b) Radioimmunodiffusion of γE preparation placed in the centre well. Peripheral wells were filled with antisera specific for one of the immunoglobulin classes (γG, γA, γM, γD, and γE) and the antibody against Fab portion of γG. After washing the slide, peripheral wells were filled with radioactive antigen E. (c) Antigenic structure of E myeloma proteins. The precipitin band between ND protein and the antiserum specific for ND showed a spur over the precipitin band with PS protein. The antiserum specific for Fc portion of ND protein (a-Fc) showed a precipitin band of identical specificity with ND protein, PS protein and normal γE.

with the presence of γE antibody against the allergen (Ishizaka, Ishizaka & Hornbrook, 1966c). In radioimmunodiffusion in which radioactive ragweed allergen was employed, all of the sera containing high reaginic antibody against the purified ragweed allergen gave the radioactive γE band, whereas the patient's sera containing the antibodies against the other allergens did not (Fig. 2a). γE antibodies against rye-grass allergen and horse dandruff

have also been detected by radioimmunodiffusion in the sera of atopic patients who are sensitive to thse allergens.

The second evidence for the correlation between γE and reaginic activity is that the skin-sensitizing activity of patients' sera was precipitated by the antibodies specific for γE (Ishizaka *et al.*, 1966b, c). So far, skin sensitizing antibodies against ragweed, grass pollen, horse dandruff, egg white, house dust and penicillin have been studied. In all these cases, the skin-sensitizing activity was precipitated by anti-γE. These findings indicated that association of reaginic activity with γE is not only in ragweed system but also in the other allergen–reagin systems (Ishizaka & Ishizaka, 1968c).

The third evidence is that the P–K titre of ragweed sensitive patients' sera quantitatively paralleled γE antibody concentration to the allergen. A method was devised for measuring antigen-binding activity of the antibodies associated with each immunoglobulin class and this was applied to atopic patients' sera. The results showed that the skin-sensitizing activity of the patients' sera correlated with γE antibody concentration as measured by antigen binding activity. On the other hand, no correlation was obtained between the P–K titre and any of the γG, γA or γM antibody concentration in the patients' sera. The complete correlation between γE antibody concentration and reaginic activity indicated that at least the major part of the skin-sensitizing antibody in the patient's sera belonged to γE (Ishizaka, Ishizaka & Hornbrook, 1967b).

The fourth evidence is similarities between reaginic antibody and γE antibodies in their physico-chemical properties. When atopic patients' sera were fractionated by ion exchange column chromatography, gel filtration, sucrose density gradient ultracentrifugation and by gel electrophoresis, the distribution of reaginic activity paralleled that of γE antibody detected by radioimmunodiffusion (Ishizaka *et al.*, 1966c).

Direct proof for γE as a carrier of reaginic activity was obtained after purification of the protein from reaginic sera. The serum samples were fractionated successively by salt precipitation, DEAE-cellulose column chromatography and recycling gel filtration followed by DEAE-Sephadex column chromatography. The γG and γA globulins which remained in the γE-rich fraction were removed by immunosorbents. On a weight basis, reaginic activity in the final preparations was about 1000 times more active than the original serum. The preparation gave a positive P–K reaction with 0·001 μg N, while the P–K titre of the preparation was 1:80,000. The presence of anti-ragweed antibodies was analysed by radio immunodiffusion. As shown in Fig. 2(b), only γE antibody but none of γG, γA, γM or γD antibody was detected in the preparation. The radioactive band formed by anti-Fab is due to the presence of light chains in γE. Furthermore, both reaginic activity and γE antibody in the active preparation were completely precipitated by anti-γE. These results clearly show that γE antibodies are responsible for the reaginic activity in the purified preparation and thus identified γE as a carrier of reaginic activity (Ishizaka & Ishizaka, 1967).

CHARACTERIZATION OF γE IMMUNOGLOBULIN

In order to prove that γE represents a distinct immunoglobulin class, the antigenic structure of the protein was studied (Ishizaka, Ishizaka & Terry, 1967d). It was found that γE has κ and λ light chain antigenic determinants which are common for all the immunoglobulins described. In addition, γE has antigenic determinants which are not shared by any of the

immunoglobulins of known classes and subclasses. Thirdly, γE does not possess any of the major antigenic determinants present in the other four known immunoglobulin classes, indicating that γE is not a subclass of the other immunoglobulin. This information together with the presence of antibody activity associated with γE are sufficient for the conclusion that γE represents a distinct immunoglobulin class. Furthermore, myeloma protein ND,* described by Johansson & Bennich (1967) was proved to be an E myeloma protein (Bennich *et al.*, 1969). Quite recently, another E myeloma patient (PS) was found in Hanover, New Hampshire (Ogawa *et al.*, 1969). The protein isolated from the serum as well as ND protein had γE antigenic determinants in the Fc portion of the molecule and belonged to λ type protein. The only antigenic difference between ND protein and the new myeloma protein (PS) was idiotypic specificity present in the Fd portion of the molecules (Fig. 2c). The presence of myeloma protein having the same antigenic structure as normal γE indicates that the protein represents a distinct immunoglobulin class.

The physico-chemical properties of immunoglobulin E are summarized in Table 1 as compared with the other immunoglobulins. Briefly, the protein is a γ_1 glycoprotein of which the molecular weight is 200,000 (Bennich & Johansson, 1968). The physico-chemical properties of γE are clearly different from the other immunoglobulins, with respect to the sedimentation coefficient, molecular weight and carbohydrate content. The chromatographic properties of γE in Table 1 represent the molarity of phosphate buffer (pH 8·0) by which

TABLE 1. Physico-chemical properties of immunoglobulin E in comparison with the other immunoglobulins

Properties	γG	γM	γA	γD	γE
Electrophoretic mobility	γ_1–γ_2	γ_1	γ_1	γ_1	γ_1
$S^{\circ}_{20,w}$>	6·6	18	6·6	7·0	8·2
DEAE-cellulose chromatography (M/1)	0·005	0·15	0·035	0·035	0·02
Molecular weight	150,000	900,000	180,000	—	200,000
Carbohydrate (%)	2·9	11·8	7·5	—	10·7

the major part of normal γE was eluted from a DEAE-cellulose column (Ishizaka *et al.*, 1966c). Indeed, more than 99% of E myeloma protein PS was eluted between 0·005 and 0·025 M phosphate buffer. However, normal γE is chromatographically heterogeneous. When the γE-rich fraction of a ragweed-sensitive serum was applied to a DEAE-Sephadex column and eluted by increase of molarity of Tris–HCl buffer, γE antibody was detected in all fractions shown in Fig. 3. As expected, all fractions had reaginic activity which paralleled the intensity of radioactivity of the γE precipitin band and precipitation of γE in the different fractions with anti-γE was accompanied by loss of reaginic activity (Ishizaka & Ishizaka, 1967). The chromatographic heterogeneity of γE might explain heterogeneity of reaginic antibodies observed by other investigators (Reid, Minden & Farr, 1966; Fireman, Boesman & Gitlin, 1967).

IMMUNOCHEMICAL PROPERTIES OF γE ANTIBODIES

Our original purpose in studying human reaginic antibodies was not only to identify the carrier of the antibodies but also to study the mechanisms of reaginic hypersensitivity

* Johansson & Bennich called their myeloma protein ND or IgND. ND and PS are initials of patients.

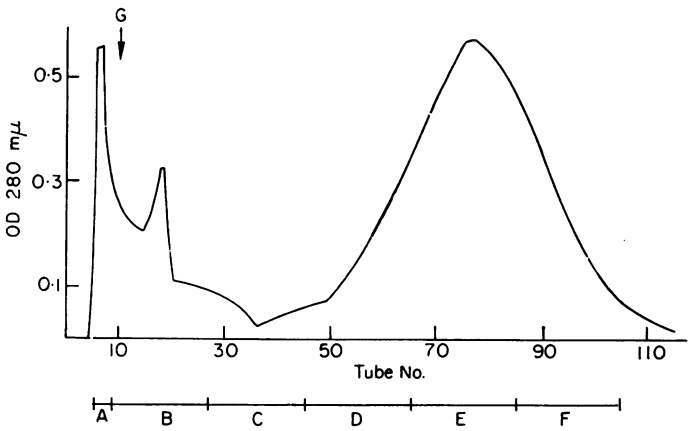

FIG. 3. DEAE-Sephadex chromatography of a reagin-rich fraction. The starting buffer was 0·1 M Tris–HCl buffer, pH 8·0. Gradient elution was made with 0·5 M Tris buffer. All fractions A–F contained the γE antibody. The highest activity was in Fraction B.

reactions. For this purpose the immunochemical properties of γE antibodies have to be studied. As already described, γE antibodies give a P–K reaction in normal individuals. Assuming that anti-ragweed γE antibody in the patient's sera and rabbit γG antibody against the same antigen have comparable avidity, the minimal dose of γE antibody required for a positive P–K reaction was calculated from the antigen-binding activity of the antibodies. The calculated dose in six patients' sera studied was very close to one another and of the order of 4×10^{-5} μg N/ml (Table 2) (Ishizaka *et al.*, 1967b). The IgE antibodies are also responsible for the PCA reactions in monkeys given atopic patients' sera. When *Macaca irus* was used as an experimental animal, about thirty times as much γE antibody was required to sensitize monkey skin, as compared with the P–K reactions in humans (Ishizaka, Ishizaka & Arbesman, 1967a). Evidence was obtained in this experiment that neither γG nor γA antibodies sensitized monkey skin for PCA reactions. On the other hand, γE anti-

TABLE 2. Estimation of the minimum sensitizing dose of γE antibody in Prausnitz–Küstner reactions

Serum specimen	P–K titre	Allergen-binding value of γE antibody (μg/ml)	Rabbit antibody concentration (μg N/ml)*	Rabbit antibody corresponding to minimum P–K dose ($\times 10^{-6}$ μg N)
EK	1600	0·23	0·065	2·0
Fc	800	0·14	0·038	2·4
F	600	0·097	0·026	2·1
A	400	0·10	0·028	1·8
W	600	0·099	0·027	2·2
P	400	0·07	0·019	2·4

* The concentration of rabbit antibody which is comparable to γE antibody with respect to allergen-binding activity.

body did not give a PCA reaction in the guinea-pig, even when a reaginic fraction having the P–K titre of 1:10,000 was used for passive sensitization. Recently, we have studied the possibility whether E myeloma protein can sensitize guinea-pig skin for reversed PCA reactions. The E myeloma protein was injected intracutaneously into guinea-pigs which then received an intravenous injection of anti-γE 3 hr later. The results showed that even 100 μg N/ml of E myeloma protein failed to sensitize guinea-pig skin. It is clear that immunoglobulin E can sensitize both human and monkey skin but not guinea-pig skin (Table 3).

Immunochemical properties of γE antibodes have also been studied *in vitro*. It was found that γE antibodies against ragweed antigen agglutinated the red cells coated with the antigen (Ishizaka & Ishizaka, 1968c). On the basis of antigen-binding activity, the γE and γG antibodies obtained from the same serum had comparable haemagglutinating activity. It was also found that the agglutination of the antigen-coated cells by γE antibody preparation was enhanced if the sensitized cells were incubated with anti-γE. The enhancement

TABLE 3. Immunological properties of γE

Reactions	Minimum concentration of antibody (μg N/ml)	Activity
In vivo		
P–K in human	4×10^{-5}	+
PCA in monkey	10^{-3}	+
PCA in guinea-pig	> 100	–
In vitro		
Agglutination	10^{-2}	+
C-fixation	> 800*	–

* Aggregated γE.

was specific because both anti-γG and anti-γA failed to enhance the haemagglutination. The results showed that γE antibodies were actually responsible for the haemagglutination and indicated that the antibodies are multivalent with respect to their antibody combining sites. Bennich & Johansson (1968) indicated that γE is composed of two heavy and two light chains. If the antibody combining sites of γE antibody are located in the Fab portion of the molecules, one can speculate that γE antibodies are probably divalent.

Participation of complement (C) in anaphylactic reactions and reaginic hypersensitivity has been discussed for a long time. So far, C fixation by γE antibodies has not been detected. However, the concentration of γE antibodies in reagin-rich fractions was too low to exclude the possibility that γE antibodies may have weak C-fixing activity. Therefore, the C-fixing activity of non-specifically aggregated γE was studied instead of antigen–γE antibody complexes. As we have shown many years ago, aggregated γG fixed C in similar mechanism as antigen–γG antibody complexes (Ishizaka, Ishizaka & Borsos, 1961). It was also found in subsequent studies that non-specifically aggregated γM fixed C, whereas aggregated γA did not (Ishizaka *et al.*, 1967e). These results agree with the facts that γM antibodies fix C with antigen, whereas γA antibodies fail to do so (Ishizaka *et al.*,

1966a). In view of this, studies on C-fixing activity of aggregated γE will give us the conclusion as to whether the γE antibodies fix C or not. Our recent studies on aggregated E myeloma protein in collaboration with Dr Bennich have shown that even 800 μg N of aggregated γE does not fix any significant amount of C. Under the same condition, 5 μg N of aggregated γG fixed 50 CH_{50} units of C out of 100. Lack of C fixation by aggregated γE was confirmed by Cla fixation test which is more sensitive than C fixation. It was also found that aggregated γE failed to form anaphylatoxin from C3. When fresh human serum was incubated with aggregated γG or aggregated γE at 37°C for 20 min and then analysed by immunoelectrophoresis using the antiserum specific for C3, anaphylatoxin (C3a) was detected in the mixture with aggregated γG but not in that with aggregated γE (Fig. 1c). It seems that γE immunoglobulin lacks C-fixing activity and that anaphylatoxin formation is not involved in the mechanisms of reaginic hypersensitivity.

SENSITIZING ACTIVITY OF γE

Among the immunochemical properties of γE antibodies, the most important characteristics are the activity to sensitize homologous species. This property is probably due to affinity of γE molecules for target cells which are involved in reaginic hypersensitivity. It was found that non-antibody γE blocked passive sensitization with reaginic antibodies (Ishizaka *et al.*, 1967d). When a reaginic serum was mixed with non-antibody γE and injected into normal individuals, skin sites were not sensitized for P–K reactions. Stanworth *et al.* (1967) showed that E myeloma protein also blocked passive sensitization. The blocking of passive sensitization is one of the characteristic properties of γE because myeloma proteins of the other four immunoglobulin classes and subclasses did not show the blocking effect. More recently, Stanworth *et al.* (1968) showed that Fc fragments of E myeloma protein ND blocked passive sensitization of human skin and we have found that the fragments of another myeloma protein PS blocked the sensitization of both human and monkey skin with reaginic antibodies. The results indicated that structures essential for tissue affinity are present in the Fc portion of the molecules.

It is well known that reaginic antibody is inactivated by heating at 56°C for 4 hr. In fact, skin-sensitizing activity of γE antibodies was lost by the heat treatment. Our studies also indicated that affinity of γE molecules for target cells was lost by heating. After heating, the non-antibody γE lost the activity to block passive sensitization with reaginic antibodies. Further studies on the effect of heating on γE antibodies have shown that antibody combining sites in the γE antibodies remained after heating, whereas the structures essential for passive sensitization as well as γE antigenic determinants were destroyed by the heat treatment (Ishizaka, Ishizaka & Menzel, 1967c).

It is also known that reaginic antibody is inactivated by reduction in 0·1 M mercaptoethanol followed by alkylation. Our recent studies have shown that skin-sensitizing activity of γE antibodies greatly diminished by reduction in 0·1 M mercaptoethanol followed by alkylation. By this treatment, however, the antigenic determinant in the γE molecules was not destroyed. Both the antigen-binding activity of anti-ragweed γE antibodies and affinity of the molecules for tissues diminished by the treatment (Table 4) (Ishizaka & Ishizaka, 1969a).

Further evidence for the affinity of γE molecules for target cells is reversed type allergic reactions. As normal individuals have γE, one can expect that normal γE may be present on target cells which are involved in reaginic hypersensitivity. If this is the case, an intradermal

TABLE 4. Effect of heat and reduction-alkylation on γE antibodies

Structure	Heat (56°C, 4 hr)	Reduction-alkylation
Antibody combining sites	Intact	Diminished
Antigenic determinant	Destroyed	Intact
Affinity for target cells	Lost	Diminished

injection of anti-γE to normal individuals should result in induction of a skin reaction. Indeed, anti-γE gave erythema–wheal reactions (Fig. 4a). The minimum dose of anti-γE required for the reaction was only 10^{-5} μg Ab N. On the other hand, even 0·01 μg N of the antibodies specific for γG, γA, γM and γD failed to give an erythema–wheal reaction (Ishizaka & Ishizaka, 1968a).

Induction of reversed type reactions by anti-γE is not restricted to the skin. Lichtenstein & Osler (1964) have observed histamine release from isolated leucocytes of atopic patients upon exposure to allergen. Their results suggest that reaginic antibodies are involved in the reaction. If the combination of allergen to reaginic antibodies on leucocytes is actually responsible for the histamine release, γE should be present on leucocytes and, therefore

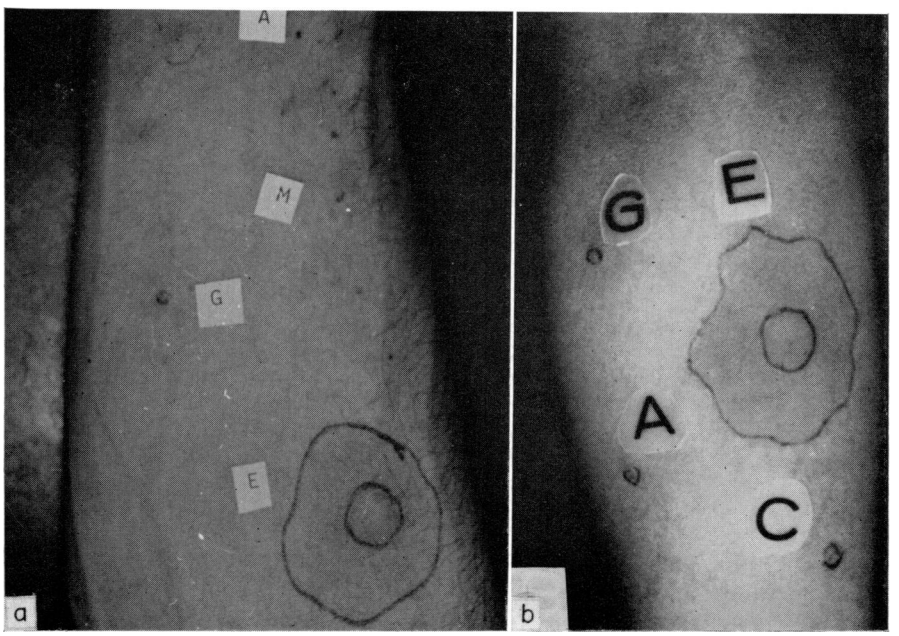

FIG. 4. (a) Induction of erythema–wheal reaction by anti-γE in human skin. Skin sites marked (G, A and M) received 0·01 μg N of anti-γG, anti-γA and anti-γM, respectively. The site E received 10^{-5} μg N of anti-γE. (b) An erythema–wheal reaction induced by antigen–γE antibody mixture (E). The skin sites which received antigen–γG antibody mixture (G), antigen–γA antibody mixture (A) and γE antibody preparation (C) failed to show any skin reaction.

one could expect a reversed type histamine release from leucocytes by the exposure to anti-γE. Our experiments actually showed that anti-γE released histamine from leucocytes of atopic patients. Furthermore, fixation of γE to leucocytes was demonstrated by passive sensitization (Ishizaka *et al.*, 1969b). The leucocytes from a normal individual were incubated with a γE-rich serum containing anti-ragweed reaginic antibody, and washed cells were exposed to either purified ragweed allergen (antigen E) or anti-γE. As demonstrated by Levy & Osler (1966), the sensitized cells released histamine upon exposure to ragweed allergen, whereas the cells before sensitization did not. At the same time, the sensitivity of the leucocytes to anti-γE significantly increased by passive sensitization. Untreated cells released 50% of total histamine by 0·08 μg N/ml of anti-γE, whereas 1/20 concentration of anti-γE was sufficient to give the same histamine release from the sensitized cells (Fig. 5).

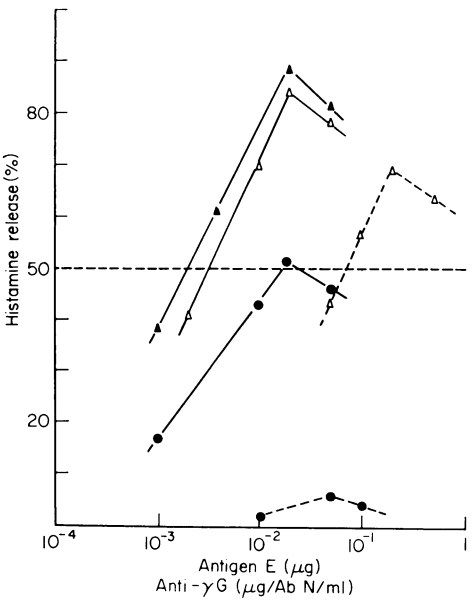

FIG. 5. Histamine release by ragweed allergen (AgE) and by anti-γE from leucocytes passively sensitized with a reaginic serum. Normal leucocytes released 50% of total histamine by 0·08 μg N/ml of anti-γE (△) but not by ragweed allergen (●- - -●). After sensitization with a reaginic serum containing 3 μg/ml of γE, 0·004 μg N/m of anti-γE (△——△) or 0·018 μg/ml of antigen (●——●) released 50% histamine. E myeloma protein, 3 μg/ml (▲——▲) had comparable sensitizing activity as the whole serum. ●, Direct (γE); △, reversed (anti-γE).

Quite recently, we have studied the ability of E myeloma protein to sensitize leucocytes from the same individual. The concentration of E myeloma protein used for passive sensitization was comparable to that of normal γE present in the dilution of whole reaginic serum which successfully established the sensitization. The results in Fig. 5 clearly show that E myeloma protein sensitized leucocytes for reversed type histamine release and that the sensitizing activity of the protein was comparable to γE present in the reaginic serum. It is evident that γE combined with leucocytes during passive sensitization. This finding supports the idea that γE antibody is responsible for antigen-induced histamine release from leucocytes.

Goodfriend, Kovacs & Rose (1966) have shown passive sensitization of monkey lung by

reaginic serum for antigen-induced histamine release. In order to show that the antibodies involved in this reaction are γE, we have studied whether anti-γE releases histamine from monkey lung. Lung fragments were sensitized with an atopic patient's serum which contained high γE concentration and high reaginic activity and sensitized lung was incubated with allergen or the antibody specific for each human immunoglobulin class. The results showed that only allergen and anti-γE released histamine. Recent studies in collaboration with Dr Orange and Dr Austen showed that the slow reacting substance of anaphylaxis (SRS-A) was also released from the sensitized monkey lung by anti-γE or allergen (Ishizaka *et al.*, 1969c). The sensitizing activity in the reaginic serum for the release of both chemical mediators seems to be associated with γE. When the serum was absorbed with anti-γE-immunosorbent and the supernatant used for sensitization, the lung tissues failed to release either mediator upon exposure to either antigen or anti-γE. It was also found that the antibodies specific for the other four immunoglobulins did not release either histamine or SRS-A from the lung fragments sensitized with the original serum (Table 5). The possibility that unknown

TABLE 5. Release of histamine and SRS-A by anti-immunoglobulins

Antibody for challenge (μg Ab N/ml)		Histamine released (μg/g)	SRS-A released (μg)
Anti-γE	0·1	9·23	315
	0·01	4·74	95
	0·001	1·30	0
Anti-γG	0·1	0	0
Anti-γA	0·1	0	0
Anti-γM	0·1	0	0
Anti-γD	0·1	0	0

immunoglobulin might be involved in the release of one of the two chemical mediators was excluded by the fact that E myeloma protein, of over 99·9% purity, sensitized monkey lung and the sensitized tissues released both histamine and SRS-A upon exposure to anti-γE. Quite recently, it was also found that the Fc fragments of E myeloma protein were capable of sensitizing lung fragments for the reversed type reactions. The tissues sensitized with the Fc fragments released both histamine and SRS-A upon exposure to anti-γE. The experiments on sensitized lung tissues indicated that allergen–γE antibody reaction is responsible for the release of both histamine and SRS-A; and that γE is involved in allergic reactions not only in the skin but also in the respiratory tract.

IMMUNE MECHANISMS OF REAGINIC HYPERSENSITIVITY

The initial step of reaginic hypersensitivity is the combination of allergen to cell-bound γE antibodies. Since reversed type reactions, which are induced by the combination of anti-γE to cell-bound γE, provide an experimental model of reaginic hypersensitivity, the mechanisms of the reversed reactions were studied (Ishizaka & Ishizaka, 1969b). γ-Globulin fraction of the guinea-pig anti-γE antiserum was digested with pepsin to obtain $F(ab')_2$

of the antibody. The fragment was then split, by reduction and alkylation, to yield the Fab′ fragment. As a control, the γG fraction of the anti-γE serum was reduced and alkylated. The fragments of anti-γE antibody as well as the reduced-alkylated sample were injected intracutaneously into normal individuals. The results showed that reduced-alkylated antibody as well as $F(ab')_2$ of the antibody gave positive erythema–wheal reactions, whereas Fab′ monomer fragment did not. The activity of the fragments to release histamine from human leucocytes was also studied. As shown in Fig. 6, the original antibody preparation,

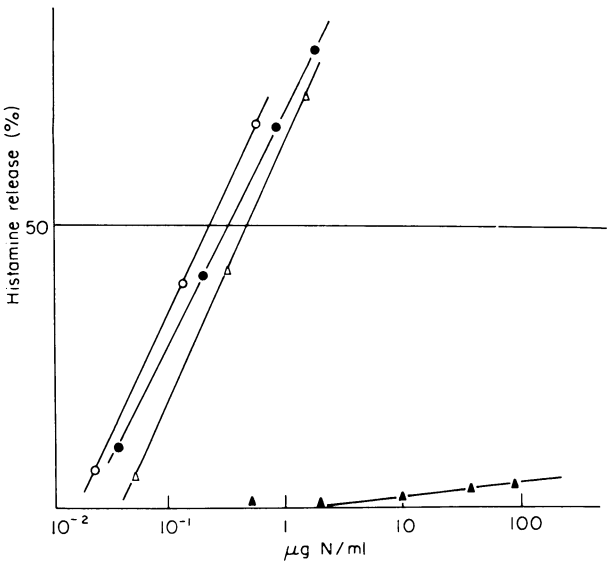

FIG. 6. The dose–response curves of histamine release from leucocytes by 7S anti-γE (●), 5S fragments (○), reduced-alkylated antibody (△) and 3·5S fragments (▲). Abscissa represents the concentration of the preparations added to leucocyte suspension. The 3·5S fragments failed to release histamine.

$F(ab')_2$ and reduced-alkylated antibody released histamine, whereas Fab′ fragment did not. On a molar basis, $F(ab')_2$ fragments and undigested antibody were comparable with respect to the ability to release histamine. The $F(ab')_2$ fragments had the activity to release both histamine and SRS-A from monkey lung which had been sensitized with reaginic serum. Again, even 100 times as much Fab′ monomer fragments failed to release both mediators (Table 6). These results strongly suggest that C is not essential for the reaction, because $F(ab')_2$ fragments having essentially no C-fixing activity had the ability to induce the reversed type reactions. Induction of the reversed type allergy by $F(ab')_2$ fragments but not by Fab′ monomer fragments indicated that bridging of two γE molecules on target cells by anti-γE is required for the induction of the reactions.

Further evidence for the requirement of two or more γE molecules for initiation of hypersensitivity reactions was obtained in the experiments on antigen–antibody complexes (Ishizaka & Ishizaka, 1968b). When ragweed allergen was mixed with γE, γG or γA antibody preparations and the mixtures were injected into normal human skin, only the mixture of γE antibody with allergen provoked erythema–wheal reactions (Fig. 4b). The intensity of the skin reaction was different depending on the antigen–antibody ratio in the

TABLE 6. Release of histamine and SRS-A by anti-γE and its fragments

Challenge (μg Ab N/ml)		Histamine released (μg/g)	SRS-A released (μg)
Undigested (7S)	0·1	1·29	250
	0·01	0·12	50
Fab′ dimer (5S)	0·1	1·74	500
	0·01	0·19	125
Fab′ monomer (3·5S)	1·0*	0·07	0
	0·1	0	0

* Estimated from the protein concentration.

mixture. The maximal reaction was obtained when a slight excess of antigen was added to the γE antibody and the activity became lower by the addition of more antigen. As this finding suggested that preformed antigen–γE antibody complexes have the skin reactivity, experiments were undertaken to prove that γE antibodies actually formed complexes with antigen *in vitro*. Purified ragweed antigen (antigen E) was labelled with ^{131}I and added to γE antibody preparation. The antigen–antibody ratio in the mixtures was in extreme antigen excess in one mixture and in moderate antigen excess in the other. The latter mixture gave a positive erythema–wheal reaction with a dilution of 1:128, whereas the mixture containing a great excess of antigen did not show the reaction even at a dilution of 1:8. Both of the mixtures were then analysed by sucrose density gradient ultracentrifugation. Free antigen gave a single peak of 3·3S and sedimentation coefficient of γE antibody was 8S. The mixture of γE antibody with a great excess of antigen gave two radioactive peaks. A slower sedimenting peak corresponded to free antigen (3·3S) and the sedimentation coefficient of the faster component was 9·7S which was higher than that of γE antibody alone. The mixture with a moderate excess antigen showed three peaks, i.e. 3·3S, 9·7S and 13·1S (Table 7). The 9·7S and 13·1S components represented antigen–γE antibody complexes because radioactive antigen in the fractions was precipitated by anti-γE. As the antigen–antibody mixture containing 13S complex had high skin reactivity while the activity of the mixture containing

TABLE 7. Sedimentation coefficient ($S_{20,w}$) of antigen, antibody and antigen–antibody complexes*

Preparations	Sedimentation coefficients			
	Antigen	Antibody	Complex 1	Complex 2
Antigen E	3·3	—	—	—
Reaginic antibody	—	8·0	—	—
Ag–Ab complex extreme Ag excess	3·4	—	9·7	—
Ag–Ab complex moderate Ag excess	3·4	—	9·7	13·1

* The values in the table were calculated in comparison with $S_{20,w}$ of rabbit γG antibody as standard. The $S_{20,w}$ of the antibody was 6·5S.

only 9·7S complex was very low, it seems that the 13S complex has the activity whereas the 9·7S complex does not.

Since γE antibody is probably divalent, one can expect that the major part of complexes formed in great antigen excess are probably composed of two antigen and one antibody molecules (Ag_2–Ab complex) of which the molecular weight is 270,000. It appears likely that the 9·7S complex represents an Ag_2–Ab complex, whereas 13S complex probably contains two antibody molecules. It seems that two or more γE antibody molecules are required for the formation of a skin reactive complex.

The requirement of two or more γE antibody molecules for the formation of a skin reactive complex is comparable to the previous observations on rabbit γG antibody complexes having skin reactivity in the guinea-pig (Ishizaka, 1963). In the case of the γG antibody complexes, it was suggested that an interaction between antibody molecules

TABLE 8. Biological activities of aggregated immunoglobulins

Immunoglobulin	Aggregation	Minimum dose for skin reaction (μgN)			C-fixing activity (μgN)	
		Human	Monkey	Guinea-pig	$C\,F_{50}$	$C\,1aF_{50}$
γE	+	0·001	0·01	>20	⩾800	⩾100
	−	>0·3	>3	>20	⩾800	⩾100
Denatured γE	+	>0·3	>3	>20	⩾800	⩾100
γG	+	0·5	2	2	5·8	1·5
	−	>8	>8	>20	>800	>100

brought into apposition by antigen and possible consequent structural changes in the Fc portion of the antibody molecules might be involved in the induction of the biological activity. The requirement of two γE antibody molecules for the formation of skin reactive complexes suggested that induction of skin reactive properties by the formation of the complexes may involve interaction between the γE antibody molecules and/or structural changes in the molecules.

If the role of antigen to form active complexes is merely to bring antibody molecules into close proximity, the possibility may be considered that non-specifically aggregated γE may have biological activity. Therefore, we have studied skin reactivity of non-specifically aggregated E myeloma protein in collaboration with Dr Bennich and Dr Johansson (Ishizaka *et al.*, 1969a). E myeloma protein was aggregated by disulphide exchange reaction or by coupling with bis-diazotized benzidine and injected intracutaneously into normal humans and monkeys. It was found that 1 ng N of the aggregated γE gave erythema–wheal reactions in humans and 10 ng N of the aggregates induced skin reactions in the monkey (Table 8). Monomer γE failed to show such skin reactions. It was also found that aggregated γE has to combine to target cells for inducing skin reactions in primates. Aggregated γE prepared by reduction in 0·1 M mercaptoethanol in the presence of guanidine hydrochloride did not give the skin reactions in the primates. Such denatured γE did not block passive sensitization with reaginic antibody, indicating that structures in γE molecules essential for fixation to target cells were destructed by the treatment. As already described, the aggregated γE, having the skin reactivity, failed to fix C and to form anaphylatoxin. It is clear that C

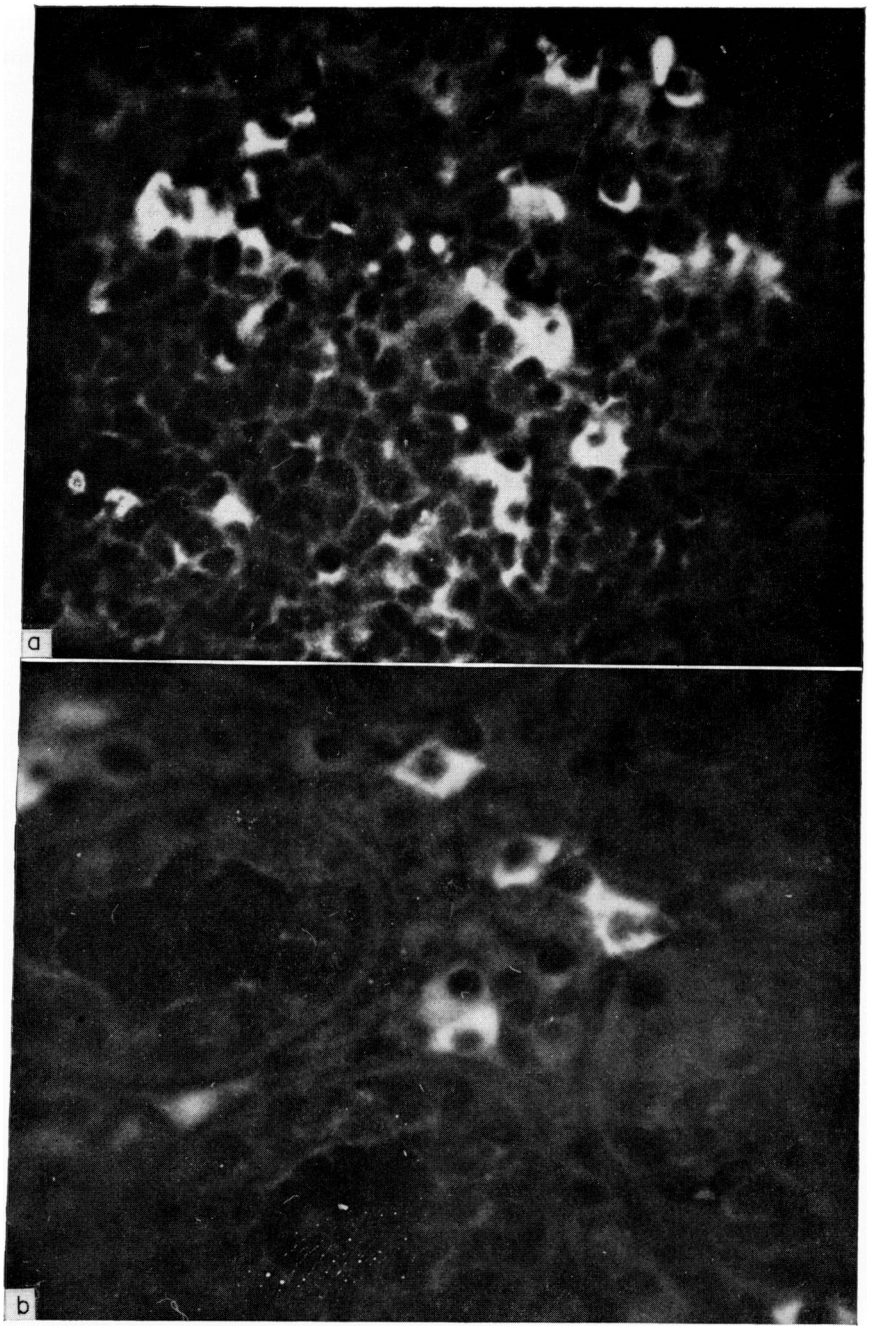

FIG. 7. Fluorescent staining of γE plasma cells in human tonsil (a). Some plasma cells under epithelial capsule and around follicles were stained by anti-γE. (b) The γE plasma cells in gastric mucosa. The cells were detected in the lamina propria.

is not involved in the induction of the skin reaction by aggregated γE. These findings indicate that interaction between γE molecules or structural changes in the molecules may be the initial step of the hypersensitivity reactions, and suggested the possibility that allergic disorders may occur without participation of allergen.

SITES OF γE FORMATION

As γE is an immunoglublin, it may be supposed the protein may be formed by plasma cells. In order to learn distribution of γE forming cells, human lymphoid tissues were studied for the presence of γE plasma cells by the fluorescent antibody technique (Tada & Ishizaka, 1969). Frozen sections from human and monkey tissues were treated with one of the guinea-pig antibodies specific for each immunoglobulin class and then stained with fluorescent antibody specific for guinea-pig immunoglobulin. Among the various lymphoid tissues studied, tonsils and adenoid tissues obtained by surgical operation possessed the greatest numbers of plasma cells stained by anti-γE. As shown in Fig. 7(a) the γE staining

TABLE 9. Distribution of γE forming cells in lymphoid tissues

Lymphoid tissues	Human		Monkey	
	Plasma cells	Germinal centre	Plasma cells	Germinal centre
Tonsil	+~+++	+~++	+	++
(Adenoid)	+~+++	+~++		
Bronchial and peritoneal	++	(+)	++	(+)
Subcutaneous lymph nodes	±~+	−	±	−
Spleen	±~+	−	+~++	±
Respiratory mucosa	+	−	+	−
GI mucosa	+~++	−	+~++	(+)*
Lung	−	−	−	−
Blood	−	−	nd	
Bone marrow	−	−	nd	

( ) = negative in some cases.
* + in Peyer's patches.

cells in tonsils were predominant around follicles under the epithelial capsules. Some germinal centres in these tissues were also stained by anti-γE. In tonsils, the number of γE staining plasma cells was about 5–6% of all plasma cells stained by anti-light chain. Bronchial and peritoneal lymph nodes also contained γE forming plasma cells. As compared with these lymph nodes, γE forming cells were very few in spleen and subcutaneous lymph nodes.

In addition to the lymph nodes, γE plasma cells were detected in respiratory and gastrointestinal mucosa. In nasal mucosa, some of the plasma cells under the epithelial cells were stained by anti-γE. The γE plasma cells were also found in bronchial and trachael mucosa especially around the mucous-serous glands. In the gastrointestinal tract, the stomach, small intestine and rectum have been studied. In all the tissues, γE plasma cells were observed in the lamina propria especially around the crypts of Lieberkühn (Fig. 7b). When gastric

mucosa and tonsils were stained by double-staining technique, it was found that γE plasma cells were entirely different from the γA plasma cells. The number of γE plasma cells in the stomach was about 3–4% of γA plasma cells, which represent more than 90% of total plasma cells. The distribution of plasma cells and germinal centres stained by anti-γE was summarized in Table 9, which also contains the distribution of γE staining cells in monkey tissues. The distribution of γE cells in the monkey tissues was very similar to that in humans. It should be noted that tonsils of normal monkeys contained germinal centres stained by anti-γE, although no sign of inflammation was detected in the tissues. More plasma cells in human tonsils than in monkeys may be related to inflammation. In addition to the lymphoid tissues, peripheral leucocytes, bone marrow cells and lung tissues from non-atopic individuals have been studied. However, the lymphoid cells in the specimens were not stained by anti-γE. The peripheral leucocytes from an atopic patient who had about fifty times as much serum γE as normal were an exception. A few medium and small sized lymphocytes were stained by anti-γE. It is clear that γE forming cells occur predominantly in respiratory and gastro-intestinal mucosa and their regional lymph nodes. Quite recently we detected γE in nasal washings and sputum from some asthmatic patients (Ishizaka & Newcomb, unpublished data). These findings strongly suggest that γE antibodies may be formed locally in the respiratory and gastro-intestinal tracts and may participate in allergic diseases affecting these organs. The presence of many γE forming plasma cells in adenoid tissues and tonsils taken at surgical operation suggests that proliferation of γE forming plasma cells may be due to antigenic stimulus.

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