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DEMONSTRATION OF Fc_γ RECEPTORS ON HUMAN BASOPHIL GRANULOCYTES¹

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Fc_γ receptors were detected on human basophil granulocytes. The mononuclear cell fraction of human peripheral blood was incubated with heat-aggregated human IgG (HGG) followed by ¹²⁵I-anti-HGG. Autoradiography of the cells showed that the majority of basophil granulocytes gave a significant number of grains. Basophils were not labeled by preincubation of the same cells with monomeric HGG followed by ¹²⁵I-anti-HGG. However, the binding of aggregated HGG to basophils was inhibited by the presence of a high concentration of monomeric HGG or its Fc fragment but not by the Fab fragment. Evidence was obtained that Fc_γ receptors are distinct from IgE receptors on the same cells: i) Saturation of basophils with IgE did not affect the binding of aggregated HGG to the cells. ii) Preincubation with and the presence of aggregated HGG failed to affect the binding of ¹²⁵I-IgE to basophils, or to block passive sensitization of the cells with IgE antibodies. iii) The Fc_γ receptors did not co-cap with IgE receptors. Aggregated HGG failed to induce histamine release from basophils even in the presence of D₂O. It was also found that the presence of aggregated HGG on basophils did not modulate IgE-mediated histamine release from the cells.

It is well-established that basophil granulocytes and mast cells bear receptors for IgE (1-4), and that the binding of IgE antibodies to the receptors results in the sensitization of the cells for antigen-induced mediator release. Passive sensitization of rodents for anaphylactic reactions by antibodies of a certain IgG subclass, however, suggests strongly that mast cells may have receptors for this immunoglobulin. Indeed, Tigelaar and Ovary (5) reported that mouse mast cells form rosettes with IgG-coated erythrocytes, and Halper and Metzger (6) have shown that rat mast cells bind antigen-IgG2a antibody complexes. However, the relationship between the Fc_γ receptors and IgE-receptors on the same cells has not been elucidated. The present experiments were undertaken to answer this question by using human basophil granulocytes. The results will show that human basophils bear Fc_γ receptors that combine aggregated human IgG and that these receptors are distinct from IgE-receptors on the same cells.

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MATERIALS AND METHODS

Source of basophil granulocytes. Blood was obtained from three normal and two ragweed-sensitive atopic individuals. All of the three normal donors had less than 60 ng/ml of serum IgE. As reported in previous articles (7, 8), less than 1/3 of IgE-receptors on their basophil granulocytes were occupied by IgE. Atopic donors had 5.9 μg/ml and 2.9 μg/ml of serum IgE. The mononuclear cell fraction was obtained from their peripheral blood by differential centrifugation on a Hypaque-Ficoll layer (9). The fraction consisted of 1.8 to 2.9% basophils, 16 to 22% monocytes, and the rest lymphocytes. The fraction from one of the atopic patients contained 1 to 2% neutrophil granulocytes. The percentage of basophils in the preparations was assessed by supravital staining with 0.03% toluidine blue (7).

Immunoglobulins. E myeloma protein was isolated from the serum of patient P.S. by chromatography on a diethylaminoethyl (DEAE) cellulose column followed by gel filtration through a Sephadex G200 column (10). The purity of the protein was 99.8%. A portion of E myeloma protein was labeled with ¹²⁵I (high specific activity, New England Nuclear, Boston, Mass.) by the method of Klinman and Taylor (11) with chloramine T. Normal human IgG (HGG)² was isolated from a pooled serum by ammonium sulfate precipitation and DEAE cellulose column chromatography. Protein eluted from the column with 0.005 M phosphate buffer, pH 8.0, was concentrated and passed through a Sephadex G200 column to remove aggregates. HGG was digested with crystalline papain by the method of Porter (12). The Fab and Fc fragments in the digest were isolated by ammonium sulfate precipitation, followed by Pevicon block electrophoresis (13). The purity of the fragments was confirmed by immunodiffusion with antisera specific for the Fc or Fab fragments.

Aggregated HGG was freshly prepared for each experiment by heating. A 2% solution of HGG in borate-buffered saline (BBS), pH 8.0, was heated for 25 min at 63°C. The preparation was diluted 10-fold with BBS and centrifuged at 15,000 × G for 30 min to remove large aggregates. The supernatant was kept at 37°C or room temperature until use.

Anti-immunoglobulins. Rabbit antibodies specified for human IgE were obtained by immunization with the Fc fragments of E myeloma protein, included in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The antiserum was absorbed with the Fab fragment of the myeloma protein and passed through a Sepharose-immunosorbent column coated with a normal human serum. The antiserum was then passed through a Sepharose column coated with the E myeloma protein, and antibodies were eluted from the column with glycine-HCl buffer at pH 3.0. Protein concentration of the final prepa-

² Abbreviations used in this paper: HGG, human IgG; RGG, rabbit IgG; BBS, borate-buffered saline; BSS, balanced salt solution; HSA, human serum albumin; EDTA, ethylene diaminetetraacetic acid.

ration was 6 mg/ml, of which 2.8 mg were associated with the precipitating antibody against IgE. Monospecific antibodies for HGG were prepared from a rabbit antiserum against HGG. The antiserum was absorbed extensively with a Sepharose-immunosorbent coated with the Fab fragments of HGG. The antibodies specific for the Fc portion of HGG were then purified with a Sepharose column coated with HGG. The final antibody preparation (anti-HGG) contained 3.1 mg protein/ml, and 60% of the total protein was associated with the precipitating antibody. A portion of the specifically purified anti-HGG was labeled with ¹²⁵I by using chloramine T (11). Fluoresceinated goat anti-rabbit IgG (RGG) antibody was purchased from Meloy Laboratories, Springfield, Va.

Immunofluorescence. Cell-bound IgE on basophil granulocytes was stained by indirect immunofluorescence. Unless otherwise specified, all procedures were carried out at 0°C in the presence of 10 mM sodium azide. A suspension of mononuclear cells in Hanks' balanced salt solution (BSS; Microbiological Associates, Walkersville, Md.) was incubated with an equal volume of rabbit anti-IgE antibody for 30 min. The final concentration of the antibody in the cell suspension was 20 µg/ml. After washings, the cells were stained with an appropriate concentration of fluoresceinated anti-RGG for 30 min. In order to induce capping of cell surface IgE, mononuclear cells were incubated at 37°C for 60 min in 100 µg/ml of anti-IgE. Cells were washed three times with Hanks' BSS and then treated with fluoresceinated anti-RGG at 0°C. Fluorescent staining was examined with a Leitz Ortholux microscope (E. Leitz Inc., Rockleigh, N. J.) equipped with an Osram HG 200 mercury lamp, BG 38 and KP 490 excitation filters, and a K 530 suppression filter.

Autoradiography. Mononuclear cells were suspended in Tris-A EDTA² buffer, pH 7.6, which contains 0.025 M Tris, 0.12 M NaCl, 0.005 M KCl, 0.01 M EDTA, and 0.5 g human serum albumin (HSA) per liter (ICN Pharmaceuticals Inc., Cleveland, Ohio). One-half milliliter of the cell suspension containing 3 to 7 × 10⁷ cells was incubated with an equal volume of monomeric HGG or aggregated HGG at 37°C for 60 to 90 min. After extensive washings, these cells as well as untreated cells were incubated in 0.2 to 1.0 µg/ml of ¹²⁵I-anti-HGG at 0°C for 30 min. Cells were centrifuged at 1000 RPM for 10 min through a layer of heat-inactivated fetal calf serum, and cell pellets were washed three times with Tris-A EDTA buffer. Smears of the cells were stained with Wright's stabilized staining solution and their autoradiographs were taken by the procedures previously described (1, 7). After 12 to 21 days exposure, slides were developed and the number of radioactive grains on the cells was counted under a light microscope. At least 200 basophils, 500 monocytes, and 1000 lymphocytes per slide were examined to evaluate the binding of aggregated HGG to the cells. In order to examine the binding of IgE to basophils, mononuclear cells were incubated in 1 to 4 µg/ml of ¹²⁵I-IgE at 0°C for 30 min. After washing, smears of the cells were stained and their autoradiographs were prepared by the same procedures described above.

Histamine release. Mononuclear cell fraction was suspended in Tris-ACM buffer containing 0.025 M Tris, 0.12 M NaCl, 0.005 M KCl, 6 × 10⁻⁴ M CaCl₂ and 10⁻³ M MgCl₂, and 0.5 g HSA per liter, and 0.5 ml each of the cell suspension was incubated with an equal volume of aggregated HGG, anti-HGG, or anti-IgE of appropriate concentrations. After incubation for 40 min at 37°C, the cells were centrifuged and histamine content in the supernatants was assayed by the automated technique described by Siraganian (14). In order to enhance histamine release, some

experiments were carried out in Tris-ACM buffer containing 50% deuterium oxide (D₂O) (15).

Passive sensitization of leukocytes with IgE antibodies was carried out by the procedures previously described (16), with a serum from a ragweed-sensitive patient. The concentrations of total IgE and IgE antibodies specific for antigen E in the serum were 6 µg/ml and 1.5 µg/ml, respectively.

RESULTS

Demonstration of Fc_γ receptors on basophil granulocytes. Possible binding of aggregated HGG to basophils was studied by autoradiography. The mononuclear cell fraction was obtained from three normal individuals, and aliquots of the fraction were incubated in 100 µg/ml of either heat-aggregated HGG or monomeric HGG or kept in Tris-A-EDTA buffer as a control. After washings, all of the cell preparations were incubated with ¹²⁵I-anti-HGG. Representative results of autoradiography are shown in Table I. In control cell preparations, only a small fraction (1 to 3%) of basophils and lymphocytes, and 6 to 15% of monocytes gave a significant number of grains. Pretreatment of the cells with monomeric HGG failed to increase the proportion of labeled cells. In all of the cell preparations treated with aggregated HGG, however, 43 to 83% of basophils, 15 to 22% of lymphocytes, and 28 to 45% of monocytes gave more than 10 grains per cell. The results indicated that aggregated HGG bound with basophils. A separate experiment was carried out to confirm the proportion of Fc_γ receptor-bearing cells. Aliquots of the mononuclear cell fraction were treated with either 100 µg/ml of 1 mg/ml or aggregated HGG followed by ¹²⁵I-anti-HGG. A 10-fold increase in the concentration of aggregated HGG for the pretreatment resulted in an increase in labeled monocytes from 32 to 51%, but failed to affect the proportion of basophils and lymphocytes labeled.

In order to confirm the specificity of the binding of aggregated HGG to basophils, attempts were made to block the binding with monomeric HGG or its fragments. Aliquots of mononuclear cell fraction were preincubated in 2 mg/ml of monomeric HGG, Fc fragments, or Fab fragments at 37°C for 30 min. To each cell suspension, as well as control cells, which was kept in Tris-A-EDTA buffer, 50 µg/ml of aggregated HGG were added. After further incubation for 1 hr at 37°C, cells were washed and

TABLE I
Demonstration of Fc_γ receptors on mononuclear cells by autoradiography^a

Incubated with	Cell Type	No. of Grains per Cell ^b			
		<10	11-30	31-50	>50
Agg-HGG	Basophils	34	56	8	2
	Lymphocytes	84	13	3	0
	Monocytes	64	21	11	4
Monomeric HGG	Basophils	98	2	0	0
	Lymphocytes	97	3	0	0
	Monocytes	84	16	0	0
Buffer	Basophils	98	2	0	0
	Lymphocytes	98	2	0	0
	Monocytes	86	14	0	0

^a A mononuclear cell preparation was incubated with 100 µg/ml of aggregated HGG or monomeric HGG at 37°C for 90 min. After washings, these cells, as well as untreated cells, were incubated with ¹²⁵I-anti-HGG at 0°C.

^b Numerals in the table represent the percentage of cells giving a certain number of grains.

incubated with 1 $\mu\text{g}/\text{ml}$ of ^{125}I -anti-HGG. Representative results of autoradiography are shown in Figure 1. It is apparent that monomeric HGG and its Fc fragment significantly inhibited the binding of aggregated HGG to basophils, whereas the Fab fragments failed to do so. Experiments of the same design were carried out with the mononuclear cell fraction from another normal individual with comparable results. The results indicated that aggregated HGG bind with basophils through the Fc portion of HGG molecules.

A possible relationship between IgE-receptors and Fc γ receptors was explored. As a preliminary experiment, mononuclear cells from normal individuals were divided into two portions and one portion was incubated with 100 $\mu\text{g}/\text{ml}$ of IgE for 30 min. After washings, both the IgE-treated and untreated cells were incubated with 1 $\mu\text{g}/\text{ml}$ of ^{125}I -IgE and their autoradiographs were taken. The results showed that basophils treated with unlabeled IgE failed to bind a detectable amount of ^{125}I -IgE, whereas more than 90% of untreated basophils gave a significant number of grains. On the basis of these results, we examined whether the saturation of basophils with IgE may affect the binding of aggregated HGG. Thus, an aliquot of mononuclear cells from the same donors was treated with 100 $\mu\text{g}/\text{ml}$ of IgE for 1 hr. After washing, both the treated and untreated cells were incubated with 100 $\mu\text{g}/\text{ml}$ of aggregated HGG, followed by ^{125}I -anti-HGG. Results of autoradiography are summarized in Table II. It is apparent that the binding of aggregated HGG with basophils was not affected by saturation of IgE-receptors with IgE.

Since basophils from some atopic patients are saturated with IgE (7, 8), experiments were carried out to study whether the aggregated HGG may bind with such basophils. The mononuclear cell fraction was obtained from peripheral blood of two atopic patients who have a high serum IgE level. An aliquot of the cell preparations was incubated with 1 $\mu\text{g}/\text{ml}$ of ^{125}I -IgE to confirm that IgE-receptors on basophils are saturated. Indeed,

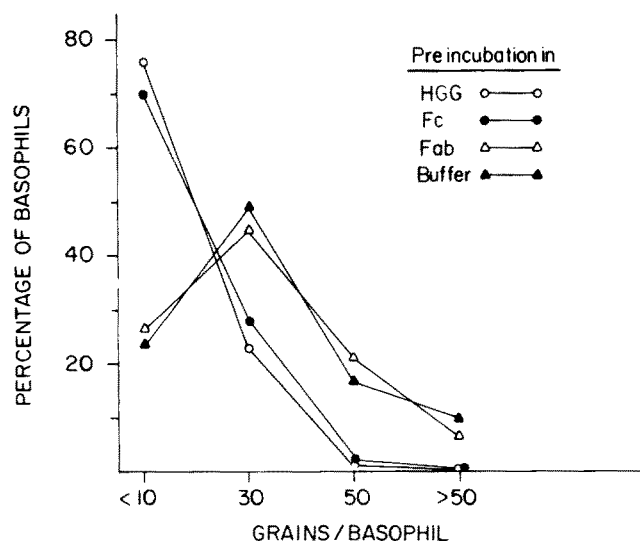


Figure 1. Inhibition of the binding of Agg-HGG to basophils by monomeric HGG or Fc fragments. Aliquots of mononuclear cells were preincubated with 2 mg/ml of monomeric HGG, Fc fragments, Fab fragments of HGG, or kept in Tris buffer. Fifty micrograms per milliliter of Agg-HGG were then added to each cell suspension. After washing, cells were treated with ^{125}I -anti-HGG, and autoradiography was performed. The ordinate indicates the percentage of basophils giving a certain number of grains per cell. The abscissa represents the number of grains per cell: 30 represents 11 to 30 grains and 50 represents 31 to 50 grains per basophil.

TABLE II

Binding of Agg-HGG to basophils before and after saturation with IgE^a

Donor	Serum IgE	Incubation with IgE ^b	No. of Grains/Basophil ^c			
			<10	11-30	31-30	>50
<i>ng/ml</i>						
Normal						
J.H.	8	—	16	75	8	1
		+	14	75	11	0
K.I.	60	—	23	68	8	1
		+	24	65	9	2
Atopic						
R.W.	5900	—	27	63	8	2
J.E.	2890	—	17	54	25	4

^a The binding of aggregated HGG was examined by incubating cells with 100 $\mu\text{g}/\text{ml}$ of aggregated HGG at 37°C for 90 min followed by treatment with 0.5 $\mu\text{g}/\text{ml}$ of ^{125}I -anti-IgG.

^b Cells were saturated with IgE by incubation with 100 $\mu\text{g}/\text{ml}$ of E myeloma protein at 0°C for 1 hr.

^c Numerals in the table represent the percentage of basophils giving a certain number of radioactive grains in autoradiographs.

autoradiographs of the cells showed that none of the basophils gave a significant number of grains. Thus, the same cells were incubated with aggregated HGG followed by ^{125}I -anti-HGG, and their autoradiographs were examined. As shown in Table II, 73 to 83% of basophils in the cell preparations gave a significant number of grains.

Experiments were carried out to show that the binding of aggregated HGG to basophils did not affect the binding of IgE. An aliquot of the mononuclear cell fraction from two normal individuals was incubated with 2 mg/ml of aggregated HGG. After 90 min at 37°C, an equal volume of 2 $\mu\text{g}/\text{ml}$ of ^{125}I -IgE was added to the cell suspension and the mixtures were kept at 0°C for 30 min. As a control, an aliquot of the same cell suspensions was treated with ^{125}I -IgE without preincubation with aggregated HGG. Autoradiographs of the cell preparations showed that more than 90% of basophils from both donors gave a significant number of radioactive grains. It would appear that preincubation with and the presence of aggregated HGG did not interfere with the binding of ^{125}I -IgE to basophils.

Further evidence for the difference between IgE-receptors and Fc γ receptors was obtained by cap formation of IgE-receptors. The mononuclear cell fractions of two atopic donors, whose basophils are saturated with IgE, were incubated with 100 $\mu\text{g}/\text{ml}$ of rabbit anti-IgE at 37°C for cap formation. An aliquot of the same cell preparations was incubated in the same concentration of anti-IgE at 0°C. After washings, a portion of the cell preparations was stained with fluoresceinated anti-RGG to examine redistribution of cell-bound IgE. In the cell suspensions treated with anti-IgE at 0°C, 2.0 to 2.7% of the total cells showed diffuse fluorescent surface staining. The percentage of the stained cells agreed with the percentage of basophils which were enumerated by staining with toluidine blue. When the same cell suspensions were incubated with anti-IgE at 37°C, 1.8 to 2.5% of total cells showed fluorescent staining, and none of the stained cells gave a diffuse surface staining. In these cell suspensions, more than 50% of the stained cells showed capping and the rest of the cells showed patching. Some of them showed internalization of fluorescence.

The cells treated with anti-IgE either at 37°C or 0°C were then incubated with 100 $\mu\text{g}/\text{ml}$ of aggregated HGG followed by treatment with ^{125}I -anti-HGG. The results of autoradiography of the cells are shown in Table III. The percentage of basophils,

TABLE III

Binding of aggregated-HGG to basophils after capping of cell-bound IgE-molecules^a

Donor ^b	Incubation with Anti-IgE at	No. of Grains per Basophil ^c			
		<10	11-30	31-50	>50
R.W.	0°C	20	67	11	2
	37°C	25	64	10	1
J.E.	0°C	17	57	22	4
	37°C	21	59	16	4

^a Cells were incubated with 100 µg/ml of rabbit anti-IgE at 0°C or 37°C for 1 hr for capping. Control cells were incubated with anti-IgE at 0°C. After washing, cells were incubated with Agg-HGG, followed by incubation with ¹²⁵I-anti-HGG at 0°C.

^b Both donors were atopic patients whose serum IgE level was 5.9 µg/ml and 2.9 µg/ml, respectively. IgE-receptors on their basophils were saturated with IgE (see text).

^c Numerals in the table represent the percentage of basophils giving a certain number of grains.

showing significant number of grains, became slightly less when the cells had been pretreated with anti-IgE under capping conditions. Nevertheless, more than 75% of basophils in the preparations bound aggregated HGG. Furthermore, the distribution of radioactive grains on all of the basophils was homogeneous even after redistribution of cell-bound IgE molecules.

Failure of histamine release by aggregated HGG. Attempts were made to induce histamine release by aggregated HGG. The mononuclear cell fractions from two normal and two atopic individuals were incubated with varying concentrations of aggregated HGG in the presence of 50% D₂O. The final concentration of aggregated HGG in the cell suspensions covered the range of 0.1 µg/ml to 200 µg/ml. In all of the four cell preparations studied, no significant histamine release was obtained by aggregated HGG at any concentration tested. In order to confirm that basophils employed in the experiments were capable of releasing histamine, the same cells were suspended in Tris-ACM buffer and incubated with appropriate concentrations of anti-IgE (0.01 to 6 µg antibody/ml). In all of the four cases, 35 to 58% of total histamine was released at an optimal concentration (0.1 to 0.6 µg/ml) of the antibody. As expected, histamine release by anti-IgE was enhanced in the presence of D₂O. In two cell preparations studied, maximum histamine release obtained in the absence of D₂O was 35 and 52%, respectively. In the presence of 50% D₂O, histamine released at the same concentration of anti-IgE increased to 51 and 81%, respectively.

The possibility was considered that basophils bound with aggregated HGG might release histamine upon challenge with anti-HGG. Thus, mononuclear cell fractions from three normal individuals were first incubated with 100 µg/ml of aggregated HGG at 37°C for 90 min for sensitization. After washing, the cells were incubated in varying concentrations of anti-HGG at 37°C in the presence of D₂O. The final concentration of anti-HGG was in the range of 0.3 µg/ml to 20 µg/ml. Aliquots of the same leukocytes, which had not been treated with aggregated HGG, were challenged by the same concentrations of anti-HGG. One of the three cell preparations failed to release a significant amount of histamine upon exposure to anti-HGG with or without pretreatment with aggregated HGG. The other two cell preparations released 13 and 22% of histamine upon challenge with an optimal concentration (12.5 µg/ml to 25 µg/ml) of anti-HGG. Pretreatment of the cells with aggregated HGG, however, failed to enhance histamine release. Thus, the same cells pretreated with aggregated HGG released 15 and

20% of histamine upon exposure to the same concentration of anti-HGG.

Experiments were carried out to study whether the binding of aggregated HGG might affect the anti-IgE-induced histamine release from basophils. The mononuclear cell fraction from a normal individual was divided into three portions, and each aliquot was incubated with 100 µg/ml of aggregated HGG or the same concentration of monomeric HGG or kept in Tris-ACM buffer for 1 hr at 37°C. Anti-IgE was added to aliquots of each cell suspension for histamine release. As shown in Figure 2, dose-response curves of histamine release from the three cell suspensions were identical. Similar experiments were repeated with mononuclear cell fractions from two normal and two atopic patients with comparable results. It is apparent that the binding of aggregated HGG to basophils does not affect anti-IgE-induced histamine release.

As expected from the results of the above experiments, the binding of aggregated HGG to basophils did not affect antigen-induced histamine release. When aliquots of mononuclear cells from two ragweed-sensitive patients were treated with aggregated HGG, or kept in Tris-ACM buffer, and then challenged with antigen E, no difference in the dose-response curves of histamine release was observed between the two cell preparations.

Since the binding of aggregated HGG failed to induce histamine release or to increase antigen-induced histamine release, we have studied the effect of aggregated HGG on passive sensitization of basophils with IgE antibodies. In a preliminary experiment, normal leukocytes were passively sensitized with a serial dilution of a serum from a ragweed-sensitive patient, and sensitized cells were challenged with antigen E. The results showed that maximum histamine release by an optimal concentration of antigen E declined by dilution of the patient's serum for sensitization from 1:12 to 1:24. Thus, we studied the effect of aggregated HGG on passive sensitization with a suboptimal concentration of IgE-antibodies. As shown in Figure 3, two aliquots of the mononuclear cell fraction were passively sensitized with a 12-fold dilution or a 24-fold dilution of the reaginic serum. The third aliquot of the cell suspension was preincubated with 100 µg/ml of aggregated HGG for 30 min, and then

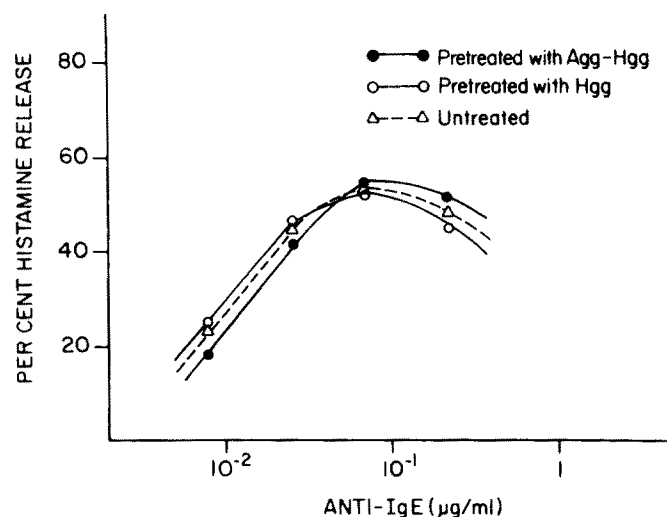


Figure 2. Effect of Agg-HGG to anti-IgE induced histamine release. Aliquots of mononuclear cells were preincubated with 100 µg/ml of Agg-HGG (●—●), 100 µg/ml of monomeric HGG (○—○), or kept in Tris-ACM buffer (△—△), and then challenged with a serial dilution of anti-IgE.

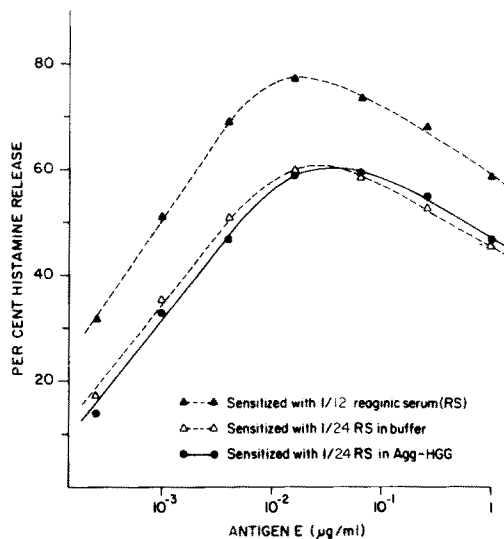


Figure 3. Effect of Agg-HGG on passive sensitization of basophils with IgE antibodies. Mononuclear cells from a normal individual were divided into three parts; one was passively sensitized with a 1:24-fold dilution of a ragweed-sensitive serum after preincubation with 100 μ g/ml of Agg-HGG (●—●), others were sensitized with a 24-fold dilution (Δ — Δ) or 12-fold dilution ($\blacktriangle$ — $\blacktriangle$) of the serum without preincubation with Agg-HGG. After washing, cells were challenged with a serial dilution of antigen E.

sensitized with a 24-fold dilution of ragweed-sensitive serum. Dose-response curves of antigen E-induced histamine release from the three cell suspensions clearly showed that aggregated HGG failed to affect the passive sensitization of basophils with IgE antibodies.

DISCUSSION

Data presented in this paper clearly showed that Fc_γ receptors are present on human basophil granulocytes. The binding of aggregated HGG to basophils was markedly inhibited by monomeric HGG or its Fc fragments but not by the Fab fragments. Similar findings were reported for the Fc_γ receptors on lymphocytes. Rosette formation of murine activated T lymphocytes with IgG-coated ox erythrocytes was inhibited by the presence of monomeric mouse IgG2b myeloma protein (17).

With respect to the relationship between IgE-receptors and Fc_γ receptors on mast cells, Halper and Metzger (6) have shown that insoluble antigen-IgG2a antibody complexes inhibited the binding of IgE to mast cells, and suggested the possibility that IgG2a may have a weak affinity for IgE-receptors. Indeed, Bach *et al.* (18) reported that IgG2a could block passive sensitization of rat mast cells by IgE. The present experiments, however, clearly showed that IgE-receptors on human basophils are distinct from Fc_γ receptors on the same cells. It was found that aggregated HGG bound to basophils that had been saturated with IgE; neither the percentage of basophils labeled with aggregated HGG nor the number of grains per basophil was affected by saturation of IgE-receptors. Considering the low dissociation rate of IgE from IgE-receptors (19), it is quite unlikely that cell-bound IgE was replaced by aggregated HGG. On the other hand, pretreatment of basophils with aggregated HGG failed to affect the binding of ^{125} I-IgE with basophils. It was also found that aggregated HGG failed to inhibit passive sensitization of human basophils with a suboptimal dose of IgE antibodies. More definitive evidence for the difference between IgE-receptor and Fc_γ receptors was obtained by cap formation of IgE-receptors. Our previous studies have shown that IgE-

receptors redistribute together with cell-bound IgE when IgE molecules are capped by the treatment with anti-IgE (20). If IgE-receptors were saturated by IgE before the treatment with anti-IgE, cap formation of IgE induced redistribution of virtually all the receptors into one pole, and left the rest of the cell surface free of IgE-receptors. When such "capped" cells were treated with aggregated HGG followed by ^{125} I-anti-HGG, radioactive grains distributed all over the cells. The results indicated that Fc_γ receptors failed to co-cap with IgE-receptors.

It has been shown that nonspecifically aggregated IgE induced histamine release from basophils, but aggregated HGG failed to do so (10). The present experiments confirmed the failure of aggregated HGG to induce histamine release from basophils even in the presence of D_2O . It was also found that the reaction of anti-HGG with cell-bound aggregated HGG failed to induce histamine release. Furthermore, the binding of aggregated IgG to Fc_γ receptors did not increase histamine release by anti-IgE. Recent studies on the mechanisms of IgE-mediated histamine release from rat mast cells indicated that bridging of IgE-receptors rather than polymerization of cell-bound IgE is responsible for triggering (21). A series of experiments with anti-receptor antibodies suggested that some local changes in membrane structures or possible interaction between adjacent receptor molecules may induce activation of the biochemical pathway for mediator release (22). Following this concept, induction of histamine release by aggregated IgE would be due to the binding of polymerized IgE with adjacent receptors. Since aggregated IgG should have bound to multiple Fc_γ receptors already, it is not surprising that further reaction of aggregated HGG to anti-HGG failed to induce histamine release.

A question remains why multiple binding of aggregated IgG to Fc_γ receptors failed to trigger histamine release. Previous studies on rat mast cells have shown that antigen-antibody reaction on mast cells does not necessarily induce histamine release and suggested that the participation of certain membrane components is required for triggering (22). It is possible that antigen-antibody reaction on Fc_γ receptors may not induce a sufficient signal for histamine release. Another possibility to be considered is that the binding of aggregated IgG to Fc_γ receptors is not strong enough for triggering. Although aggregated IgG firmly bind with basophils because of multipoint attachment, one may visualize that the strength of the individual bond between IgG and each Fc_γ receptor is not sufficient to cause disturbance in membrane structure, or to bring adjacent receptors into close proximity. Under certain circumstances, however, Fc_γ receptors may be involved in histamine release. Däron *et al.* (23) reported that IgG1 alloantibody against histocompatibility antigen can induce degranulation of mouse mast cells, whereas both IgG2 antibody and the $F(ab')_2$ fragments of IgG1 antibody failed to do so. More recently, Mossman *et al.* (24) reported that alloantibodies against rat histocompatibility antigen (H-1), but not the $F(ab')_2$ fragment of the antibody, induced histamine release from normal rat mast cells. From these findings, they suggested that participation of Fc receptors is essential for the alloantibodies to induce degranulation or histamine release.

The presence of Fc_γ receptors on basophils may suggest the possibility that the binding of monomeric IgG with basophils may go through the Fc_γ receptors. As reported in our previous article (24) and confirmed in the present experiments, a small number of molecules of IgG could be demonstrated on human basophils by autoradiography. It is also known that incubation of mononuclear cell fraction from some individuals with anti- γ

chain antibody resulted in histamine release (25), particularly in the presence of D₂O (26). Parish (27) has detected IgG-type skin-sensitizing antibody against β -lactoglobulin, tetanus toxoid, and horse γ -globulin in the serum of some patients, and showed that sensitization of normal human leukocytes with the IgG fraction of these sera enhanced anti-IgG-induced histamine release. More recently, Assem and Turner-Wabrich (28) demonstrated that serum of a patient with allergic bronchopulmonary aspergillosis could sensitize normal leukocytes for histamine release when challenged with anti-IgG2, and claimed that IgG-type sensitizing antibody may belong to the IgG2 subclass. It is possible that the Fc_γ receptors described in this paper are involved in sensitization by a minor IgG subclass that may have higher affinity for the receptors than the major subclasses. A problem to be considered, however, is the heterogeneity of Fc_γ receptors. Anderson and Grey (29) reported the presence of two types of Fc_γ receptors on rat macrophages: one type binds aggregated IgG of any subclass, whereas another type is specific for monomeric IgG2b. A possibility remains that human basophils may also have two types of Fc_γ receptors, one of which is specific for monomeric IgG of a minor subclass and is responsible for sensitization with IgG-type sensitizing antibody.

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