

ANTI-HUMAN IgG CAUSES BASOPHIL HISTAMINE RELEASE BY ACTING ON IgG-IgE COMPLEXES BOUND TO IgE RECEPTORS¹

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We have reexamined the ability of anti-human IgG antibodies to induce histamine release from human basophils. A panel of purified murine mAbs with International Union of Immunological Societies-documented specificity for each of the four subclasses of human IgG was used. Of the 24 allergic subjects studied, the basophils of 75% (18/24) released >10% histamine to one or more anti-IgG1–4 mAb, whereas none of the 13 nonatopic donor's basophils released histamine after stimulation with optimal amounts of anti-IgG mAb. The basophils of 85% (11/13) of the nonatopic donors did respond to anti-IgE challenge, as did 92% (22/24) of the atopic donor cells. Histamine release was induced most frequently by anti-IgG3, and 10/18 anti-IgG responder cells released histamine with mAb specific for two or more different subclass specificities. The rank order for induction of histamine release was anti-IgG3>anti-IgG2>IgG1>anti-IgG4. As in our previous study using polyclonal anti-IgG, 100- to 300- μ g/ml quantities of the anti-IgG mAb were required for maximal histamine release, about 1000-fold higher than those for comparable release with anti-human IgE. Specificity studies using both immunoassays and inhibition studies with IgE myeloma protein indicated that anti-IgG induced histamine release was not caused by cross-reactivity with IgE. Ig receptors were opened by lactic acid treatment so that the cells could be passively sensitized. Neither IgE myeloma nor IgG myeloma (up to 15 mg/ml) proteins could restore the response to anti-IgG mAb. However, sera from individuals with leukocytes that released histamine upon challenge with anti-IgG mAb could passively sensitize acid-treated leukocytes from both anti-IgG responder and nonresponder donors for an anti-IgG response. The only anti-IgG mAb that induced release from these passively sensitized cells were those to which the serum donor was responsive. Sera from non-IgG responders could not restore an anti-IgG response. These data

led to the hypothesis that the IgG specific mAb were binding to IgG-IgE complexes that were attached to the basophil through IgE bound to the IgE receptor. This was shown to be correct because passive sensitization to anti-IgG could be blocked by previous exposure of the basophils to IgE. We conclude that anti-IgG-induced release occurs as a result of binding to IgG anti-IgE antibodies and cross-linking of the IgE receptors on basophils.

The mechanisms and manifestations of allergic or immediate hypersensitivity reactions differ among the various animal species and humans. In mice and guinea pigs, for instance, both IgE and IgG1 antibodies exhibit a capacity to bind with high avidity to receptors on basophils and mast cells and elicit chemical mediators. Although in man there is firm evidence that IgE antibodies have the ability to mediate immediate-type hypersensitivity reactions, the data with respect to IgG antibodies are not clear. During the past several decades, many investigators have attempted to demonstrate the presence of "reaginic human IgG" antibodies that sensitize basophils and mast cells and allow them to respond to Ag. Initial reports in 1970 described a "short-term IgG sensitizing antibody" that caused human skin mast cells to respond to several allergens (1, 2). These and other early reports of human IgG-sensitizing antibody, however, were questioned regarding the possibility that the sensitizing sera contained low levels of contaminating IgE antibody (3–5).

Possibly the best evidence for IgG antibody binding with high avidity to receptors on basophils involved the observation of anti-IgG-induced histamine release from extensively washed peripheral blood leukocytes (6). In 1972, we reported convincing evidence that highly specific polyclonal anti-human IgG caused histamine release from washed basophils (7). This phenomenon has subsequently been confirmed by other investigators (8–12), some using mAb specific for individual human IgG subclasses, in which case only anti-IgG4 was reported to be active. Unfortunately, the question of whether there is an IgG reaginic antibody in man has remained controversial and the mechanisms of anti-IgG-induced basophil histamine release unresolved.

In this study, we expand on our previous work, using basophils from both atopic and nonatopic donors and a panel of purified murine mAb with IUIS³-documented

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³ Abbreviations used in this paper: IUIS, International Union of Immunological Societies; PAG, Pipes buffer containing albumin and glucose; NP, 4-hydroxy-3-nitro-phenylacetyl.

specificity for each of the four subclasses of human IgG. We have reexplored whether IgG avidly attaches to basophils and, if so, what its subclass distribution might be. In contrast to previous studies that demonstrated histamine release from nonatopic donor basophils only with anti-IgG4 (9), we observed that optimal concentrations of mAb with specificity for all four human IgG subclasses could induce mediator release, but only from basophils obtained from atopic donors. Moreover, those mAb directed against the rigid hinge region of human IgG3 were the most active. Our analyses of these responses using passive sensitization and fluid phase Ig inhibition, however, indicate that IgG-specific mAb-mediated histamine release acts through the high affinity IgE receptor on basophils, rather than as a result of IgG antibody bound to Fc γ RII receptors on basophils: specifically, mediator release involves human IgG antibody that is complexed to human IgE and is attached to the surface of basophils through high affinity IgE receptors.

MATERIALS AND METHODS

Donor specimens. Venous blood was collected from 24 atopic individuals defined by a positive history and appropriate positive puncture skin tests and 13 control individuals with no history of atopic disease. Serum and peripheral blood leukocytes were prepared by the methods of Lichtenstein and Osler (13). Briefly, 10 ml of blood were clotted for 1 h at 23°C and centrifuged (10 min, 2000 $\times$ g) to isolate the serum. Venous blood (40 ml) was collected in a 50-ml syringe. The blood was placed in a tube containing a mixture of 5 ml of 0.1 M EDTA, 12.5 ml of 6% Dextran 70 in 0.9% NaCl (Kendall McGaw, Irvine, CA), and 375 mg of dextrose (Fisher Scientific, Fairlawn, NJ). It was then sedimented for 60 min at room temperature and centrifuged at 200 $\times$ g for 8 min at 4°C. The cells were washed twice in a buffer containing 0.003% human serum albumin, 0.1% glucose, 110 mM NaCl, 5 mM KCl, and 25 mM PAG. They were then resuspended in PAG containing 1 mM CaCl₂, 1 mM MgCl₂. These cells are then incubated for 45 min at 37°C with polyclonal anti-human IgE (control) or monoclonal anti-human IgG1-4 and IgG PAN antibodies at final concentrations of 0.004 to 300 μ g/ml. Histamine released from basophils into the final cell medium was measured as described previously (14). The extent of histamine release was computed as a percentage of the total histamine content of the cells as a result of lysis with 1.6% perchloric acid (Fisher, Atlanta, GA). All chemicals were obtained from Sigma Chemical Company, St. Louis, MO unless otherwise stated.

Passive sensitization. In selected experiments, approximately 90% of receptor-bound IgE was removed from basophils using the method of Pruzansky et al. (15), which involved exposing the basophils to 0.01M lactic acid, pH 3.9, containing 140 mM NaCl and 5

mM KCl, for 3.5 min at 23°C. Cells were then diluted in 30 ml PAG, centrifuged, and washed twice in PAG. Passive sensitization was accomplished using the method of Levy and Osler (16). Lactic acid-treated leukocytes were incubated (37°C, 180 min) with IgE or IgG myeloma proteins (0.25 to 24,000 μ g/ml) or sera from atopic and nonatopic donors (10 to 80% original concentration) in PAG containing 4 mM EDTA and 10 μ g/ml heparin. In some experiments, acid-treated leukocytes were preincubated (37°C, 30 min) with IgE myeloma followed by an incubation (37°C, 180 min) with serum from an atopic donor. For other experiments with passive sensitization, a serum from a ragweed-sensitive donor was used and Ag E (gift of Dr. T. P. King) was used as the stimulus.

Myeloma and chimeric proteins. Fifteen IgG subclass 3 paraprotein containing human sera and two purified IgG3 paraproteins from patients with multiple myeloma were kindly provided by Dr. Hans Spiegelberg, La Jolla, CA. IgG1-4 and IgE PS myelomas were chromatographically purified and concentrated to 1 mg/ml for use in immunoassay inhibition experiments for specificity assessment of the mAb. IgE HL myeloma was used as serum in passive sensitization-inhibition experiments. Engineered human IgG1-4 and IgE anti-NP chimeric antibodies with V-region specificity for the hapten NP were produced from clones transfected (17, 18) by the Hybridoma Reagent Laboratory, Baltimore, MD. Specificity analysis of the murine mAb was performed by enzyme immunoassay using IgG1-4 and IgE chimeric antibodies bound to a solid phase NP-human serum albumin conjugate (19).

mAb. IUIS-documented HP series mAb specific for each of the four human IgG subclasses were employed in this study (20). The specificity of each mAb was confirmed by enzyme-immunoassay using a panel of human IgG1, 2, 3, and 4 myeloma and chimeric antibodies (Table I). HP6061 and HP6029 mAb specific for human IgE Fc were prepared from clones produced at the Centers for Disease Control (21). All mAb were purified from ascites obtained from the Hybridoma Reagent Laboratory using a sequential process involving DEAE-anion exchange and hydroxyapatite chromatography (22). Purity was confirmed by agarose line electrophoresis and PAGE and/or IEF immunoblotting (23). The purified antibody concentration was adjusted to 2 mg/ml by OD at 280 nm and both the final immunoreactivity and antibody concentration were confirmed by immunoassay dilutional analysis (24).

Total serum Ig assays. Total IgE in the IgE PS and HL myeloma preparations and for the serum absorption for Table III was measured using a mAb-based immunoenzymetric assay (25). Total serum IgG in the IgG3 myeloma sera and for the serum absorption for Table III was measured by radial immunodiffusion (Kallestad, Chaska, MN).

IgG-anti-IgE antibodies. Because of the central importance of IgG-IgE complexes in this study, all sera from leukocyte donors were assayed for the presence of IgG anti-IgE autoantibodies and preexisting IgG-IgE complexes. The first enzyme immunoassay used was analogous to that of Twena et al. (26) and detected uncomplexed human IgG anti-human IgE by employing a solid phase IgE myeloma or IgE chimeric antibody to bind antibodies specific for IgE followed by an enzyme-conjugated monoclonal anti-human IgG Fc (HP6017, see Table I) to detect bound IgG antibody. The second assay was

TABLE I
Human Ig-specific mouse mAb used in study^a

Clone No.	Human Isotype Specificity	Mouse Isotype	K _a ^b 10 ⁷	Specificity						
				HuG1	HuG2	HuG3	HuG4	HuE	HuA2	HuM
HP6069	IgG1 Fc	IgG1	4.4-7.5	100	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
HP6070	IgG1 Fc	IgG1	NA ^c	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
HP6002	IgG2 Fc	IgG1	3.3-6.5	<0.01	100	<0.01	<0.01	<0.01	<0.01	<0.01
HP6014	IgG2 Fd	IgG1	1.8-22	<0.005	100	<0.005	<0.005	<0.005	<0.005	<0.005
HP6047	IgG3 hinge	IgG1	18-39	<0.001	<0.001	100	<0.001	<0.001	<0.001	<0.001
HP6050	IgG3 hinge	IgG1	2.4-5	<0.005	<0.005	100	<0.005	<0.005	<0.005	<0.005
HP6023	IgG4 Fc	IgG3	4.1-9.5	<0.001	<0.001	<0.001	100	<0.001	<0.001	<0.001
HP6025	IgG4 Fc	IgG1	4.7-10	<0.005	<0.005	<0.005	100	<0.005	<0.005	<0.005
HP6017	IgG Fc PAN	IgG2a	NA	100	100	100	100	<0.01	<0.01	<0.01
HP6046	IgG Fd PAN	IgG1	NA	100	100	100	100	<0.01	<0.01	<0.01
HP6029	IgE Fc	IgG1	NA	<0.001	<0.001	<0.001	<0.001	100	<0.001	<0.001
HP6061	IgE Fc	IgM	NA	<0.001	<0.001	<0.001	<0.001	100	<0.001	<0.001

^a In the current study, dilution analysis was used to document the specificity of each mAb to purified human IgG1-4 myeloma proteins and human chimeric antibodies (e.g., HuG1), human IgE PS myeloma (HuE), polyclonal human IgM (HuM), and human IgA2 myeloma (HuA2). Specificity is reported as a percentage of the maximal reactivity observed with one of the human Ig (e.g., 100 = 100% reactivity; <0.02 = non-detectable or <0.02% of maximal reactivity as assessed by dilutional analysis).

^b K_a = estimated binding constant of mAb (10⁷ liters/M) for their respective IgG proteins as performed using a sequential saturation immunofluorometric assay (27). The highest and lowest K_a observed with myeloma or human serum as the IgG source are presented.

^c NA, not available.

configured to detect IgG that circulated in blood precomplexed with IgE. One of two anti-human IgE mAb (e.g., HP6061, HP6029) was insolubilized on microtiter plates to quantitatively bind IgE. The same enzyme-conjugated anti-human IgG (HP6017) was then added to detect bound IgG. Both assays were calibrated with an engineered human IgG1 anti-human IgE Fc chimeric antibody to which were added known amounts (1 to 1000 ng/ml) of purified human IgE myeloma. Our attempts to quantitate these assays in weight per volume amounts of IgG-anti-IgE or IgG-IgE complexes were made difficult by the variability of the dose-response standard curve with varying amounts of total IgE in serum. In brief, in both assays were unable to identify a higher frequency of either IgG anti-IgE or preformed IgG-IgE complexes in anti-IgE releaser subjects. Moreover, there were no detectable differences in the semi-quantitative levels of preexisting IgG-IgE complexes or free IgG anti-IgE between the atopic and nonatopic individuals, i.e., all sera from both IgE releasers and nonreleasers contained IgG-IgE complexes (data not shown). Although the preformed IgG-IgE complexes appeared to be relatively higher in all patients' sera than detectable IgG anti-IgE, the quantitative aspects of these assays are complicated by the total serum IgE. This is the subject of a subsequent report. However, important to the present study, performed IgG-IgE complexes were at least detectable in all anti-IgE releaser sera.

RESULTS

Histamine release optimization. Leukocyte histamine release dose-response curves are presented in Figure 1 using 10 to 300 $\mu\text{g/ml}$ of human IgG subclass 1-, 2-, 3-, and 4-specific mAb and is contrasted to the response to 0.004 to 0.1 $\mu\text{g/ml}$ of human IgE-specific polyclonal antibody with cells from two allergic donors. As we previously observed with polyclonal anti-IgG (7), the quantity of human IgG1-4 specific mAb required for maximal histamine release was 100 to 300 $\mu\text{g/ml}$, which was about 1000-fold higher than that required for comparable release with polyclonal anti-human IgE, or with monoclonal anti-IgE (data not shown). The anti-IgG and anti-IgE dose-response curves were similar in shape. We rarely used doses of IgG-specific mAb higher than 300 $\mu\text{g/ml}$, but usually there was less histamine release under these circumstances (data not shown).

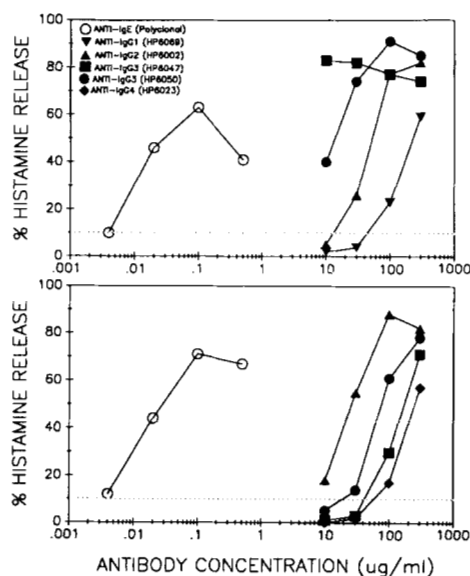


Figure 1. Basophil histamine release from washed cells from two atopic donors (MT, upper panel and JG, lower panel) after incubation with 0.004 to 300 $\mu\text{g/ml}$ of polyclonal anti-IgE or anti-human IgG1-4 mAb. Maximal histamine release with all IgG1-4-specific mAb was achieved with 50 to 300 $\mu\text{g/ml}$ which is 1000-fold higher than the concentration of polyclonal anti-IgE required for optimal release.

Frequency and magnitude of anti-IgG induced histamine release. The data describing the level and frequency of histamine released from the 13 nonatopic and 24 atopic donor basophils by human IgG1-4 mAb and anti-IgE are presented in Figure 2. Although 85% (11/13) of nonatopic donor basophils released histamine after anti-IgE challenge, none released histamine when they were stimulated with optimal amounts of monoclonal anti-IgG. Of the allergic donors studied, 75% (18/24) released >10% histamine to one or more anti-IgG1-4 mAb. The cells of the six atopic patients that did not release histamine to anti-IgG did manifest release to anti-IgE that was quantitatively similar to the release noted with the cells of the other atopics (Fig. 2, A^{nr}, last column). Ninety-two percent (22/24) of atopic donor cells releasing to anti-IgG also showed histamine release to anti-human IgE (Fig. 2, A^r). Interestingly, cells from both of the atopic patients that did not release to anti-IgE did release low but reproducible amounts of histamine when incubated with human IgG3-specific mAb (Table II). Cells from donors releasing histamine responded most frequently to anti-human IgG3 (83%, HP6050; 61%, HP6047). Of 18 anti-IgG responder cells, 10 released histamine with mAb specific for two or more different subclass specificities. The IgG subclass rank order in terms of the induction of histamine release was anti-IgG3 > anti-IgG2 > anti-IgG1 > anti-IgG4 (Table II). It is of interest that there was no correlation between the magnitude and frequency of histamine release induced by mAb of the different subclass specificities.

Diversity of anti-IgG-induced histamine-release patterns. There was no correlation between the amount of histamine release induced by optimal amounts of monoclonal anti-IgG1-4 and polyclonal anti-IgE. Figure 3, A to D displays the wide diversity of histamine-release patterns observed in this study. One reproducible histamine-release pattern, observed in 3 of 18 releasers (DS, JW, and GI in Table II) involved the generation of more histamine release with anti-IgG mAb than was inducible with anti-human IgE. Figure 3A illustrates patient JW's pattern with anti-IgE maximally producing 20% histamine release whereas anti-IgG4 and anti-IgG PAN both produced over 60% histamine release. In our previous studies (7), polyclonal anti-human IgG induced com-

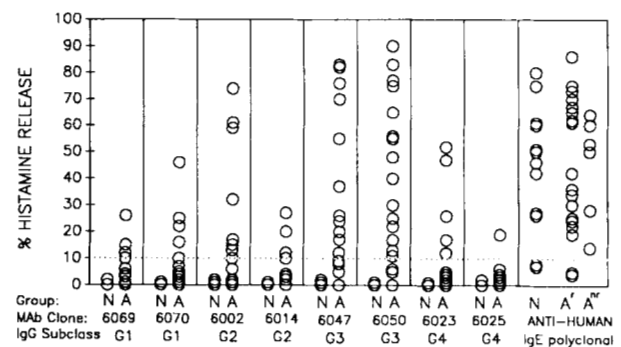


Figure 2. The frequency and magnitude of basophil histamine release from basophils obtained from 12 nonatopic (N) and 25 atopic (A) donors. Atopic donors who released to anti-IgG are denoted by A^r (atopic releaser) whereas those who did not are denoted by A^{nr} (atopic non-releaser). No basophil histamine release to anti-IgG (<10%) was observed with cells from nonatopic donors. All but two nonatopic donor cells released to anti-IgE. Of the 25 atopic donor cells, 18 released histamine to at least one human IgG1-4 mAb. The individual patterns of release to the individual anti-IgG mAb are presented in Table II.

TABLE II
Basophil histamine release patterns induced by monoclonal anti-human IgG1-4^a

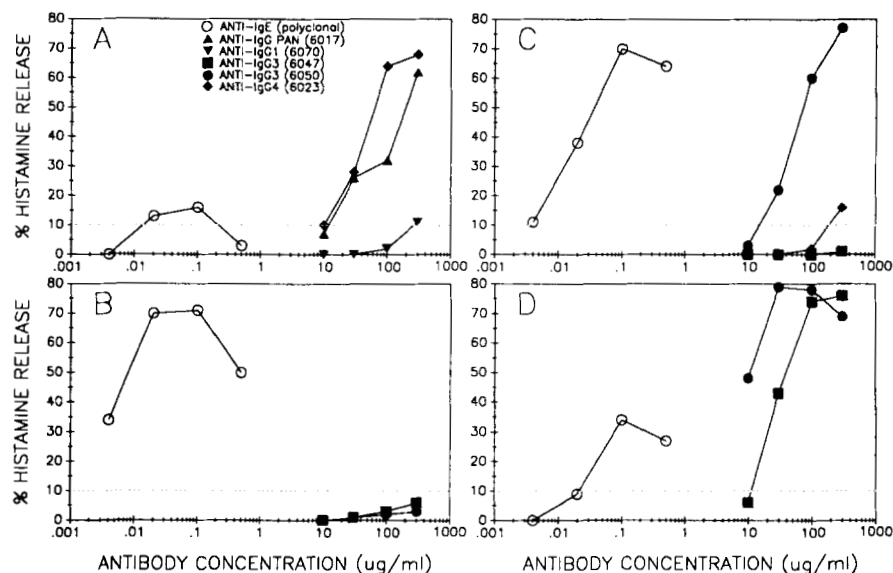
Pt Code ^b	Subclass Reactivity	aG1 69	aG1 70	aG2 02	aG2 14	aG3 47	aG3 50	aG4 23	aG4 25	aGFc 17	aGFd 46	Poly aIgE
TS	G1	0	16	0	0	0	0	0	1	0	1	65 ^c
SD	G12	12	0	59	27	8	5	2	1	0	2	73
BS	G123	11	25	10	20	9	11	1	2	0	11	24
MT	G123	26	1	74	10	76	90	2	0	3	45	65
JG	G1234	6	11	32	12	26	48	52	19	6	8	62
JW	G1234	12	46	13	2	82	83	47	0	16	2	30
EH	G134	3	22	6	4	24	40	12	3	3	5	22
GI	G23	0	0	61	3	8	75	1	2	0	6	34
DO	G23	0	0	15	3	55	30	0	0	1	0	70
CW	G234	1	2	11	2	5	25	13	4	0	3	19
RB	G3	0	0	0	0	5	11	3	0	2	41	42
LL	G3	0	5	0	0	70	77	4	0	3	4	86
TR	G3	0	0	0	0	12	6	5	0	0	0	5
SR	G3	4	3	2	0	37	56	2	3	2	3	75
KR	G3	1	2	0	0	17	23	0	0	0	0	4
DS	G3	0	4	2	0	83	65	1	1	1	2	36
DT	G3	0	0	0	0	0	13	0	0	0	0	25
JE	G34	4	7	1	0	20	55	26	6	0	0	61
No. Positive		4	5	7	3	11	15	5	1	1	3	16
% Positive		22%	28%	39%	17%	61%	83%	28%	6%	6%	17%	88%

^a Results presented as % histamine release. Histamine release results >10% are considered positive.

^b Pt code = initials of the patient.

^c Italicized numbers

Figure 3. Correlation between the magnitude of basophil histamine release induced by optimal amounts of monoclonal anti-IgG1-4 and polyclonal anti-IgE in different donors. Several patterns of histamine release are displayed in A to D. A, anti-human IgG1 and IgG4 produced higher histamine release (80%) than was obtained with anti-IgE (20%). B, anti-human IgG3 produced no histamine release (<10%) whereas anti-human IgE produced 80% histamine release. C, two anti-human IgG3 mAb produced different amounts of histamine release. D, both anti-human IgG3 mAb produced the same amount of histamine release (80%), which was higher than that obtained with anti-human IgE.



parable or less but not more histamine release than was obtainable with anti-IgE. This may simply reflect donor selection bias. A second histamine release pattern is presented in Figure 3B in which anti-human IgE produces detectable histamine release whereas anti-IgG1-4 mAb produced none (<10% histamine release). This was observed with basophils from 6/24 (21%) of atopic donors and 11/13 (85%) of nonatopic donors. Figures 3C and 3D illustrate two very different histamine-release patterns that were produced by the same two mAb with different affinities and specificities for epitopes on the hinge region of human IgG subclass 3 (24). In Figure 3C, anti-IgG3 HP6050 caused more than 80% histamine release whereas HP6047, with a 7.5-fold greater affinity for human IgG3 (27), caused <20% histamine release at the

same concentration. The majority of anti-IgG3-releasing subjects, however, displayed the pattern in Figure 3D in which equivalent amounts of histamine were released when cells were challenged by both IgG3-specific mAb.

Specificity of anti-IgG-induced histamine release. The human IgG subclass 1-4 mAb used in this study were confirmed by immunoassay using purified IgG myelomas and human IgG1-4 chimeric antibodies to be specific for their respective subclasses of human IgG (Table I). We further studied their specificity using the basophil histamine-release assay to ascertain whether the high microgram/milliliter concentrations of anti-IgG required to induce release were causing histamine release because of cross-reactivity with human IgE. As shown in Figure 4, polyclonal anti-IgE and monoclonal anti-IgG3 were

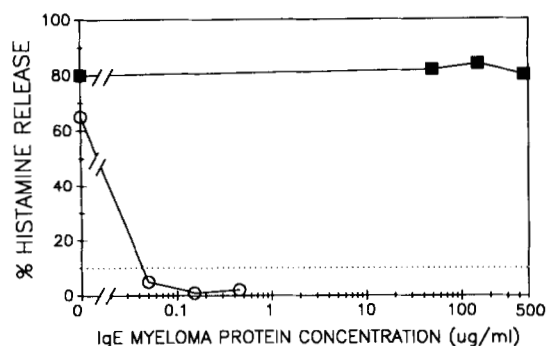


Figure 4. A 10-fold molar excess of fluid phase IgE myeloma did not inhibit anti-IgG induced-histamine release (■), whereas IgE myeloma did inhibit anti-IgE induced histamine release (○). One experiment of three performed with similar results is shown.

adjusted so that approximately equal histamine release was obtained from basophils of a single atopic individual (MT, Table II). Anti-IgE and anti-IgG antibodies were both preincubated with fluid phase IgE HL myeloma in final concentrations ranging from 0 to 450 $\mu\text{g/ml}$ before adding the mixture to basophils. Histamine release induced by 0.1 $\mu\text{g/ml}$ of anti-IgE was completely ablated with 0.05 $\mu\text{g/ml}$ of fluid phase IgE. Histamine release induced by 50 $\mu\text{g/ml}$ of anti-IgG3 (HP6050) was not detectably diminished by up to 450 $\mu\text{g/ml}$ of fluid phase IgE myeloma, indicating that the release was not caused by a small degree of cross-reactivity of the anti-IgG with IgE.

Passive sensitization studies. In the passive sensitization experiments, donor basophils were lactic acid treated, which removes approximately 90% of cell-bound IgE and generates open IgE FcR available for binding heterologous IgE antibody (15). The removal of IgE molecules reduced subsequent anti-IgE-inducible histamine release by only $23.8 \pm 9\%$, using basophils from four donors who were known to release histamine to both anti-IgE and anti-IgG (data not shown). In the same experiments, basophil histamine release induced by monoclonal anti-IgG was decreased by $90 \pm 1\%$ as a result of the lactic acid treatment. We had expected that anti-IgE-induced histamine release would not be completely ablated because the few thousand remaining IgE molecules from the original hundreds of thousands before lactic acid treatment are known to be sufficient to induce histamine release with an anti-IgE challenge (28). The more complete effect on anti-IgG release was not anticipated but could be interpreted to mean that the lactic acid treatment also removed IgG molecules from their receptors.

Ig receptors were opened by acid treatment so that we could passively sensitize the cells with various sera and Ig preparations (e.g., IgE and IgG myelomas and sera from anti-IgG responder and nonresponder individuals) to ascertain which restored the response to anti-IgG. The positive results will be presented first. We found that serum from individuals with leukocytes that released histamine upon monoclonal anti-IgG challenge could passively sensitize acid-treated basophils for an anti-IgG response. Moreover, this procedure allowed otherwise anti-IgG unresponsive cells to release histamine with subsequent exposure to the anti-IgG mAb. Importantly, the only anti-IgG mAb that induced histamine release from these passively sensitized cells were those to which the serum donor was responsive. This is demonstrated in Figure 5,

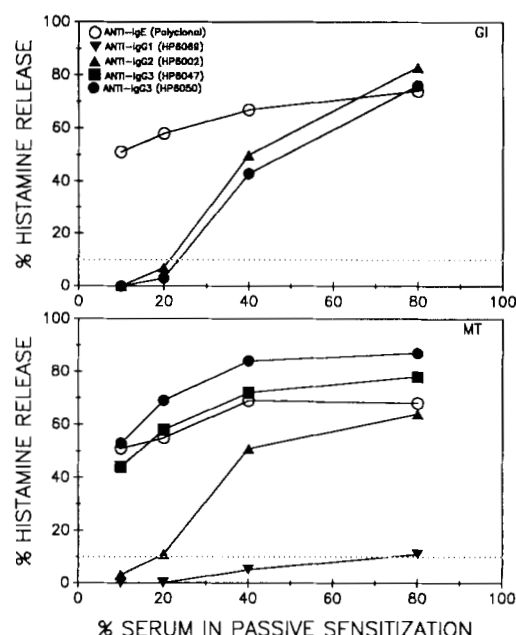


Figure 5. Passive transfer of the monoclonal anti-IgG induced histamine release from lactic acid-treated basophils with sera from two atopic donors (GI, upper panel; MT, lower panel; see histamine release patterns in Table II). Histamine release after passive sensitization was observed with anti-IgG1-4 only at serum concentrations $>1/10$.

in which anti-IgG nonresponder cells were passively sensitized with serum from anti-IgG responder subjects GI (top) and MT (bottom). Serum GI transferred anti-IgG 2- and 3-induced histamine release whereas serum MT transferred anti-IgG1-, 2-, and 3-induced histamine release (see Table II for original specificities). The ability to sensitize nonreleaser basophils for release with IgG-specific mAb diluted out in a variable manner with different sera, and it was usually lost at serum dilutions greater than 1/10 (Fig. 5). Sera from non-IgG responders could not restore histamine release from the same donor cells that had been previously lactic acid-treated (data not shown).

Our attempts to restore anti-IgG-induced histamine release by passively sensitizing acid-treated basophils with polyclonal human IgG or IgE myeloma protein were unsuccessful. Serum from 15 patients with multiple myeloma containing 15 mg/ml of IgG3 were incubated with lactic acid-treated basophils. We failed to achieve passive sensitization with these high concentrations of IgG subclass 3 myeloma protein and also with concentrations of IgE myeloma (PS) up to 3 $\mu\text{g/ml}$ (data not shown). These data together with the successful sensitization of these cells with "anti-IgG releaser" donor sera led us to hypothesize that the human IgG-specific mAb were binding to IgG-IgE complexes, which themselves were attached to the basophil through IgE bound to the IgE receptor. In other words, there were circulating and fixed IgG-IgE immune complexes.

In order to test this latter hypothesis, a specificity study was conducted in which lactic acid treated cells were passively sensitized with a serum from an anti-IgG-releasing individual (serum EH) in the absence or presence of increasing concentrations of fluid phase IgE, washed, and then exposed to anti-IgG. As a positive control (because lactic acid treatment reduced the anti-IgE response by only 20%), we used the same fluid phase IgE prepa-

ration to block passive sensitization with IgE antibody (serum TK) to ragweed Ag E, an Ag to which the basophil donor was not sensitive. The results of this experiment are presented in Figure 6. Fluid phase IgE inhibited passive sensitization with ragweed-specific IgE antibody 50% at a concentration of 0.25 $\mu\text{g/ml}$ and complete suppression was obtained with 1 $\mu\text{g/ml}$ IgE. The dose-response curve for fluid phase IgE inhibition of passive sensitization with the serum of an anti-IgG releasing donor (IgG-IgE complex containing serum) was essentially identical. Exactly similar results were obtained with sera from anti-IgG-responding individuals using basophils from three additional donors.

To more directly confirm that it was IgE-IgE immune complexes in the sera from anti-IgG responders that were responsible for the passive transfer of response to anti-IgG, the following experiments were performed. As shown in Table III, first the IgE was removed from a serum that was known to passively sensitize lactic acid-treated basophils for anti-IgG-induced histamine release. Although >99% of the IgE was absorbed using anti-IgE-coupled Sepharose, the total IgG level was essentially unaffected (1.3% removal) compared with sham-absorbed serum. Next, the anti-IgE and sham-absorbed sera were used for the two experiments shown in Table IV. As seen in column A, the cell donors were not anti-IgG responders. The buffer blanks in columns B, C, and D indicate that lactic acid treatment did not increase spontaneous histamine release. The crucial data are in column C showing that if IgE (and therefore IgG-IgE complexes) is removed from the serum of an anti-IgG responder, it is no longer capable of transferring sensitivity

to anti-IgG. This contrasts with the sham-absorbed serum (D), which transferred responses to two anti-IgG3 mAb. Thus, our hypothesis appears to be correct; histamine release by anti-IgG mAb is caused by an interaction with IgG-IgE complexes on the surface of basophils.

DISCUSSION

Since our description of histamine release with anti-IgE and shortly thereafter with anti-IgG, other investigators have studied the low but detectable levels of histamine release that are inducible from basophils after *in vitro* incubation with polyclonal and monoclonal anti-human IgG (6-11). There has also been long interest in the autoantibodies specific for human IgE (29) and the relevance of naturally occurring human IgG anti-IgE to a variety of IgE-related disorders (26, 29, 30). Our study merges these two lines of investigation and provides evidence that anti-IgG-induced histamine release from basophils is a result of human IgG antibody that is complexed to human IgE and attached to the surface of basophils through high affinity IgE receptors. Moreover, these data provide an explanation for the previously reported low (i.e., 1% of IgE density) but detectable level of IgG on human basophils (31) and the ability of high amounts of human IgG-specific antibodies (i.e., 1000-fold > anti-IgE) to induce histamine release *in vitro* (7). They also explain the ability of anti-IgG to activate well washed basophils even though the IgG receptor on basophils has been shown to be the low affinity Fc γ RII (32, 33). These data are also consistent with the concept of IgG "reagins."

The present experiments, however, show that our previous observations and those of others utilizing anti-IgG were misinterpreted. The work indicates that the responsiveness of basophils to anti-IgG is mediated via an interaction with the IgE receptor. Thus, passive sensitization with sera that render basophils responsive to monoclonal anti-IgG1-4 can be completely blocked by prior exposure of the cells to nonspecific IgE (Fig. 6), whereas the response is unmodified after cells have been exposed to much higher concentrations of IgG (data not shown). This can be explained by one of two possible IgG-IgE complexes. IgG exists as an antibody to IgE and these complexes are binding tightly to basophils via the interaction of the IgE portion of the complex with the basophil IgE receptor. Alternatively, IgG may be linked to IgE on the basophil surface through concurrent binding to the same allergen molecule although this is much less likely inasmuch as we can detect in serum both free and complexed IgG anti-IgE. In either case, our results argue strongly that previous reports of anti-IgG-induced release were

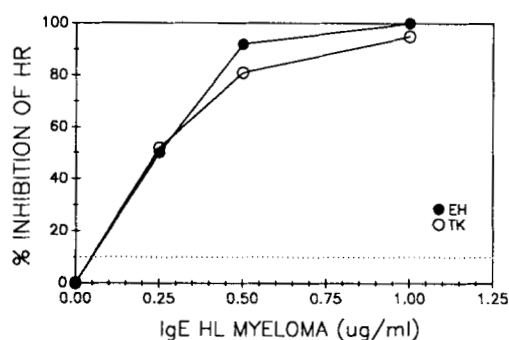


Figure 6. Inhibition by IgE myeloma of passive sensitization of lactic acid-treated basophils using a serum from a ragweed-sensitive donor (TK) and a serum from an IgG-responding donor (EH). The cells were incubated for 90 min at 37°C in serum without or with increasing concentrations of IgE myeloma, washed, and then exposed to ragweed Ag E or anti-IgG. Control histamine release to ragweed Ag E was 21% and that to mAb anti-IgG3 (HP6050) was 26%. Similar results were obtained in three additional experiments.

TABLE III
Ig levels in absorbed serum

Specimen	Absorption Condition	Total IgE (ng/ml)	Total IgG (mg/ml)
MT-1	Anti-HuIgE Sepharose	<1	13.79
MT-1	Anti-HuIgE Sepharose	8	13.71
	Average	4 99.8% removal	13.75 1.3% removal
MT-2	Uncoupled Sepharose	1984	14.06
MT-2	Uncoupled Sepharose	2104	13.79
	Average	2044	13.93

* Experimental conditions: 1 ml of serum (in duplicate) from an anti-IgG responder was incubated with either uncoupled Sepharose CL4B or with Fc-specific monoclonal IgE (HB6061 and HB6029)-coupled Sepharose CL4B. The mixtures were rotated end-over-end for 4 h at 25°C and then overnight at 4°C. The serum was separated from the Sepharose by centrifugation and stored at -20°C until assayed.

TABLE IV
Passive sensitization of leukocytes with absorbed serum

Stimulus	% Histamine Release			
	A ^a	B	C	D
None	7/6 ^b	6/7	7/8	8/5
mAb 6050 (anti-IgG3 100 µg/ml)	0/0	0/0	0/1	35/44
mAb 6047 (anti-IgG3 100 µg/ml)	0/0	0/1	0/1	25/57

^a Experimental conditions: A, not acid-treated, not sensitized; B, acid-treated, not sensitized; C, acid-treated, sensitized, IgE removed; D, acid-treated, sensitized, IgE not removed.

^b Results for two leukocyte donors.

based on IgG-IgE immune complex-basophil interaction.

Our study differs from the previous reports of anti-IgG-induced basophil histamine release in several important areas (7-11). First, we performed our study with basophils from a cross-section of atopic and nonatopic donors (Fig. 2). Previous studies used almost exclusively nonatopic donor cells. In fact, no histamine release (<10%) was detected in our study from any of the 13 nonatopic donor cells that were incubated with the entire panel of anti-IgG mAb, whereas 85% of these same cells released to anti-IgE. This contrasts with the 75% of atopic donor cells that released >10% histamine to one or more human IgG-specific mAb. Interestingly, basophils from the two atopic donors that failed to release histamine to anti-IgE both released histamine after incubation with an IgG3-specific mAb. This indicates a possible role for basophil-resident IgG-IgE complexes in the enhancement of histamine release from cells that are otherwise more resistant to the induction of histamine release by standard stimuli such as allergen.

Second, all anti-IgG mAb used in our study were selected because their specificity and performance had been extensively documented by an IUIS collaborative study; they were chromatographically purified from ascites fluid and quantitated in known weight per volume units of antibody. With these antibodies, we confirmed that 50 to 100 µg/ml concentrations of anti-IgG were required to obtain optimal histamine release from human basophils as was shown with polyclonal antibodies directed against total IgG (Fig. 1) in our previous study (7). This anti-IgG concentration is approximately 1000-fold higher than that required for comparable monoclonal and polyclonal anti-IgE release. Other investigators have used only unpurified mouse mAb that were not quantitated (8-12).

Third, fluid phase Ag (IgE) inhibition, lactic acid treatment, and passive sensitization experiments were performed in the current study to confirm the specificity of the in vitro histamine-release reactions. Fluid phase IgE was able to inhibit anti-IgE-, but not anti-IgG-, induced histamine release (Fig. 4). Lactic acid treatment ablated anti-IgG-induced histamine release. Purified IgG myeloma proteins could not restore histamine release by passive sensitization, indicating that IgG interactions with low affinity IgG FcR on basophils are not the major mechanism by which anti-IgG induces histamine release. These results support the presence of IgG antibodies that are complexed to IgE and resident on the basophil surface by means of IgE FcR.

Finally, experiments were performed that we feel conclusively show that it is IgG-anti-IgE-IgE complexes in the sera and, therefore, on the cells of donors who respond to anti-IgG mAb that confer sensitivity to anti-IgG.

When essentially all of the IgE (and, therefore, any IgG-IgE complexes) was removed from the serum of an anti-IgG responder (Table III), this absorbed serum was no longer able to passively sensitize basophils for anti-IgG-induced release, whereas sham-absorbed serum sensitized the cells for release by two anti-IgG mAb (Table IV).

Interestingly, serum from "anti-IgG releaser" donors did resensitize the basophils for anti-IgG-induced histamine release with the same IgG subclass pattern as seen with the donor cells (Fig. 5). Histamine release was most commonly seen with the two human IgG3-specific mAb (HP6050 and HP6047), both of which bind to unique hinge region epitopes on all allotypic forms of IgG3. The frequency data in Table II (IgG3 > IgG2 > IgG1 > IgG4) suggest that either IgG3 antibodies are most often bound to IgE on the basophil surface and/or they are most readily accessible for cross-linking by the mAb used in the study. The latter possibility is most likely because IgG3 molecules possess the most structurally rigid and extended hinge region of the four human IgG subclasses. This would, therefore, allow the monoclonal anti-IgG3 to have increased access to their hinge region-binding sites compared with the other mAb specific for Fc and Fd regions of IgG1, IgG2, and IgG4.

As noted (see *Materials and Methods*), we were able to detect circulating IgG anti-IgE autoantibodies and IgG anti-IgE complexes in the serum of all donors. Despite the presence of these antibodies and complexes in all sera, only those from donors whose basophils could be directly stimulated by anti-IgG could transfer sensitivity to anti-IgG. Whether this is caused by lower amounts of complexes (see below) in nonresponder sera, to the subclass specificity distribution of the complexes in responder vs non-responder sera, or to other factors is not known. It is not, however, caused by an intrinsic difference in the donor cells because the basophils of non-IgG responders could both bind the IgG-IgE complexes and subsequently release histamine to anti-IgG. Importantly, we were unable to quantitate either the free IgG anti-IgE or preformed IgG-IgE complexes, and our understanding of the literature suggests that other investigators have overlooked the difficulties that we appreciate. To document this, however, is a major *arbeit* and will be the subject of another manuscript.

The data presented here have led to a reinterpretation of the findings of Ig-induced mediator release and, in the process, greatly weakened the argument for the existence of human IgG reagins. We conclude that only atopic individuals have basophil IgE receptor-bound IgG anti-IgE-IgE complexes or that only cells from these individuals can be directly triggered by anti-IgG antibodies that cross-link the IgE receptors through this complex. The clinical significance of these antibodies remains to be elucidated and investigation of their pathophysiologic role will require quantitative assays for both the free and the complexed Ig.

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