

In vitro basophil histamine-releasing activity of circulating IgG1 and IgG4 autoanti-IgE antibodies from asthma patients and the demonstration that anti-IgE modulates allergen-induced basophil activation

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Summary

In this study we have examined the relationship between the *in vitro* basophil histamine-releasing activity of human IgG anti-IgE, isolated as euglobulin fractions from sera of asthmatic patients, and its IgG1/IgG4 subclass distribution. In particular, we have investigated whether IgG anti-IgE modulates allergen-induced basophil activation. The study has revealed that only a small proportion of IgG anti-IgE samples triggered histamine release from basophils of an asthmatic individual (4/21; 19%), a hay fever sufferer (4/10; 40%) and a healthy person (7/21; 33%). The basophil histamine-releasing activity of IgG anti-IgE did not seem to be determined by the IgG1/IgG4 subclass composition of the IgG anti-IgE preparation used. Furthermore, we have demonstrated that autoanti-IgE antibodies modulate allergen-induced basophil histamine release. The three modulatory effects exerted by IgG anti-IgE antibodies on allergen-triggered basophil activation (i.e. additive, synergistic and blocking) were not dependent on the subclass nature of IgG anti-IgE or the use of histamine-releasing anti-IgE preparations. Our data suggest that IgG anti-IgE antibodies in asthma patients may consist of two functionally distinct subpopulations: those which up-regulate (pro-allergic) and those which down-regulate (anti-allergic) the allergic release of mediators from mast cells and basophils.

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Introduction

Elevated blood levels of IgG anti-IgE are detectable in individuals exhibiting enhanced IgE production, namely those with allergic conditions (e.g. asthma) [1–4] and parasite (hookworm and schistosomes) infections [5]. Our studies of human IgG anti-IgE have mostly focused on allergic asthma [3–7], where IgG anti-IgE is highly prevalent [1–4]. In terms of subclass distribution, the IgG anti-IgE activity is restricted to IgG1 and IgG4, with IgG4 displaying a disproportionately high percentage of the activity [1], particularly in IgE:anti-IgE complexes [7]. The subclass nature of IgG anti-IgE is of potential physiological and pathological relevance because IgG subclasses exhibit considerable variation in their ability to

trigger or bind to effector function molecules (C1q and FcγR).

Cells bearing receptors for IgE (FcεRI and FcεRII), for example, mast cells, basophils and eosinophils, have been shown to be intimately involved in allergic reactions. Given their possession of receptors for IgE, these cells are potential targets for anti-IgE activity. IgG anti-IgE autoantibodies may therefore modulate the activity of these cells either by cross-linking receptor-bound IgE molecules or blocking the binding of IgE to the receptor. *In vitro* experiments have demonstrated that mast cells [8,9] and basophils [9,10] are activated by IgG anti-IgE and that this autoantibody may constitute an important endogenous (as opposed to exogenous) trigger for these cells.

A previous study has demonstrated that only 23% (5/22) of sera with positive titres of autoanti-IgE triggered

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the release of histamine from basophils of healthy individuals [10]. Given that the IgG anti-IgE activity found in asthma patients is represented by IgG1 and IgG4 [1,7], it would be interesting to investigate whether the reported heterogeneity in basophil histamine-releasing activity of IgG anti-IgE is due to differences in the IgG1/IgG4 subclass composition of IgG anti-IgE in the sera tested. In this study we examine the relationship between the basophil histamine-releasing activity of human IgG anti-IgE, isolated as euglobulin fractions from sera of asthmatic patients, and its IgG1/IgG4 subclass distribution. In particular, we investigate whether IgG anti-IgE modulates allergen-induced basophil activation.

Materials and methods

Antibody reagents

Two mouse monoclonal anti-human IgE antibody preparations (E1 and E3) were a kind gift from Dr P. E. C. Brenchley (Department of Immunology, St. Mary's Hospital, Manchester, UK). One further mouse monoclonal anti-human IgE antibody preparation (Le27) was kindly supplied by Professor B. M. Stadler (Institute of Clinical Immunology, Bern, Switzerland).

Alkaline phosphatase-conjugated goat anti-mouse IgG and mouse monoclonal anti-human IgG1 (NL16) and IgG4 (GB7B) antibodies were purchased from Oxoid Ltd. (Basingstoke, UK). Goat polyclonal anti-human IgE ϵ -chain and goat polyclonal anti-human IgG γ -chain antibodies were purchased from Sigma Chemical Company Ltd. (Poole, UK).

Allergen extracts

A purified house dust mite (*D. pteronyssinus*) extract with a total protein concentration of 1.7 mg/ml (containing 0.9 mg/ml of *Der p* 1) was kindly provided by Dr E. Austin (Cancer Research Campaign Laboratories, University of Nottingham, UK). A grass pollen (*D. glomeratus*) extract with a total protein concentration of 8 mg/ml was kindly supplied by Dr M. R. Walker (Wolfson Research Laboratories, University of Birmingham, UK).

Preparation of euglobulin fractions

The relatively low concentration of IgG anti-IgE found in serum (1/5 being the optimum dilution for detecting anti-IgE) does not allow purification of this autoantibody in sufficiently high concentration for basophil histamine release experiments. However, to avoid complications associated with the use of IgG anti-IgE in serum samples, the basophil histamine release assays were performed with IgG anti-IgE in euglobulin fractions. Anti-IgE-

containing euglobulin fractions were obtained from sera using a 33% saturated solution of ammonium sulphate as described by Catty and Raykundalia [11]. Immunoelectrophoresis and quantitative immunoglobulin determinations carried out on the euglobulin fractions showed considerable depletion of serum non-immunoglobulin proteins (mainly albumin) and selective enrichment of IgG (by up to 30%).

IgG1 and IgG4 anti-IgE assay

IgG1 and IgG4 anti-IgE antibody levels in euglobulin fractions were measured using a previously described [3,5] enzyme linked immunosorbent assay. Briefly, the method involved incubating euglobulin fractions (at an IgG concentration of 3 mg/ml) in IgE-coated microtitre wells and detecting bound IgG1 and IgG4 using mouse monoclonal anti-human IgG1 and IgG4, respectively. The colour reaction was then developed with alkaline phosphatase-conjugated goat anti-mouse IgG and *p*-nitrophenylphosphate as the enzyme substrate. Results were expressed in OD405 units by taking the mean values of duplicate estimations corrected for background (i.e. euglobulin fractions added to wells not coated with IgE).

Basophil histamine release assay

Basophil histamine release experiments were carried out using freshly taken blood samples from an adult asthmatic male with allergy to dust mite (total IgE > 1000 IU/ml; RAST class 4 to mite), an adult female hay fever sufferer (total IgE 67 IU/ml; RAST class 3 to grass pollen) and an adult healthy female with no history of allergic disease (total IgE = 4 IU/ml). The asthmatic individual and the hay fever sufferer were off medication at the time of blood sampling. In the basophil histamine release tests, antibody and allergen preparations were normally used at the following concentrations in the final incubation mixture: anti-IgE-containing euglobulin fractions were used at an IgG concentration of 8 mg/ml, mouse monoclonal anti-human IgE at 0.3 μ g/ml (E1 and E3) or 0.5 μ g/ml (Le27), goat polyclonal anti-human IgE and anti-human IgG at 5 μ g/ml and allergen extracts at 10 μ g/ml.

The ability of antibody and allergen to release histamine from basophils was determined by using a double antibody radioimmunoassay kit (Pharmacia Ltd, Milton Keynes, UK). The method involved incubating 400 μ l aliquots of whole blood, taken by venipuncture using heparin as anti-coagulant, with equal volumes of either antibody or allergen diluted in Pipes buffer. The concentration of histamine liberated into the plasma was then measured by its ability to compete with a fixed amount of ^{125} I-labelled histamine-albumin complex for binding to a

specific monoclonal antibody. Antibody-bound material was separated by the addition of a second antibody immunoadsorbent followed by incubation, centrifugation and decanting of the supernatant. The radioactivity in the pellet, which is proportional to the quantity of histamine released by the basophils in the original blood sample, was then measured. Results of duplicate estimations (which varied by no more than 5%) were corrected for spontaneous release of histamine (usually less than 5 µg/l) and expressed as a percentage of the total histamine content of basophils, determined by lysing cells using repeated rounds of freezing and thawing.

To validate the basophil histamine release assay, mouse monoclonal and goat polyclonal anti-human IgE antibodies and dust mite extract were tested for their ability to release histamine from basophils of a dust mite-allergic individual. Goat polyclonal anti-human IgG and grass pollen extract were used in parallel as the control antibody and control allergen, respectively. To further ascertain the specificity of the basophil histamine release assay, aliquots of monoclonal antibodies E1, E3 (0.6 µg/ml) and Le27 (1 µg/ml) were pre-incubated for 1 h with equal volumes of two concentrations of either human IgE or IgG and the histamine release activity measured as before. The percentage inhibition was calculated in each case by comparing the histamine release values with those of E1, E3 and Le27 aliquots pre-incubated with buffer instead of IgE or IgG.

Investigation of the effect of anti-IgE on allergen-induced basophil histamine release

These experiments were carried out by mixing 200 µl aliquots of anti-IgE-containing euglobulin fractions with equal volumes of grass pollen extract and testing the mixture for basophil histamine release using blood from a grass pollen-allergic individual. The amount of histamine released was compared with those obtained using 200 µl of either euglobulin fraction or grass pollen extract mixed with an equal volume of buffer.

Results

Validation of the basophil histamine release assay

The validity of the basophil histamine release assay was assessed by testing the ability of mouse monoclonal and goat polyclonal anti-human IgE and dust mite extract to induce histamine release from basophils of an asthmatic individual allergic to dust mite. This has shown that anti-human IgE preparations and dust mite extract released significant amounts of histamine from basophils of this donor (Table 1). The histamine release process was specific because it did not occur with goat polyclonal anti-

Table 1. Validation of the basophil histamine release assay using a blood sample from an asthmatic individual allergic to dust mite

Antibody/allergen	Histamine release (% total)
E1	89
E3	65
Le27	62
Goat anti-IgE	71
Goat anti-IgG	0
Dust mite extract	94
Grass pollen extract	6

The results are presented as the percentage total histamine content of basophils after correction for spontaneous release. The results show that mouse (E1, E3 and Le27) and goat anti-human IgE and dust mite extract induce histamine release from basophils. No basophil histamine release was demonstrable with goat anti-IgG (control antibody) and pollen extract (control allergen). A histamine release of 10% or more is considered significant.

human IgG (control antibody) and grass pollen extract (control allergen). The specificity of the basophil histamine release assay was further ascertained by demonstrating that the histamine-releasing ability of the mouse monoclonal anti-human IgE antibodies could be inhibited by pre-incubating them with IgE (Table 2). No such inhibition was demonstrable following incubation with IgG.

Basophil histamine-releasing activity of anti-IgE-containing euglobulin fractions

A total of 21 euglobulin fractions with positive levels of IgG1 and/or IgG4 anti-IgE antibodies were tested for basophil histamine release activity (Table 3). Of these fractions, 19% (4/21) released histamine from basophils of an asthmatic individual allergic to dust mite and 33% (7/21) released histamine from basophils of a non-atopic healthy individual. Of the above 21 euglobulin fractions, 10 were randomly selected and tested against blood from a hay fever sufferer, 40% (4/10) triggered basophil histamine release (Table 3).

The histamine-releasing activity of the euglobulin fractions did not seem to be related to the anti-IgE level or to its IgG1/IgG4 subclass composition (Table 3). However, the basophil-histamine releasing activity of the euglobulin fractions was due to anti-IgE because pre-incubation of two representative fractions (TN and DS,

Table 2. Validation of the basophil histamine release assay using a blood sample from an asthmatic individual allergic to dust mite

Treatment	Mouse monoclonal antibody		
	E1	E3	Le27
Unabsorbed	66	58	62
Absorbed with			
IgE 2 µg/ml	54 (18)	46 (21)	26 (57)
IgE 20 µg/ml	2 (97)	15 (75)	0 (100)
IgG 2 µg/ml	57 (14)	50 (13)	65 (0)
IgG 20 µg/ml	62 (6)	54 (7)	65 (0)

The results are presented as the percentage total histamine content of basophils after correction for spontaneous release. The results show that the ability of three mouse monoclonal anti-human IgE antibodies to induce basophil histamine release is inhibited by pre-incubation of the antibodies with human IgE. No such inhibition was demonstrable when the antibodies were pre-incubated with human IgG. The figures in parentheses represent the percentage inhibition. A histamine release of 10% or more is considered significant.

Table 3) with IgE blocked their basophil histamine-releasing activity. Pre-incubation with IgG had no such effect.

Modulation of allergen-induced basophil histamine release by anti-IgE

Ten euglobulin fractions, randomly selected from those tested for basophil histamine release activity (Table 3), were employed to investigate their modulatory effect on basophil histamine release by allergen. This involved incubating blood samples from a grass pollen-allergic individual with an optimal concentration of pollen extract (see Fig. 1) in the presence and absence of anti-IgE-containing euglobulin fractions. Of these fractions, 50% showed either a synergistic (3/10) or an additive (2/10) effect on pollen extract-induced basophil histamine release, the other 50% showing either a blocking effect (2/10) or no effect (3/10) (Table 4). None of these modulatory effects seemed to be related to the anti-IgE level or to its IgG1/IgG4 subclass composition. Furthermore, the synergistic effects of the anti-IgE antibodies did not seem to be dependent on whether they induced histamine release from basophils in the absence of allergen.

Discussion

In this paper we have demonstrated that only a small proportion of IgG anti-IgE samples from asthma patients

Table 3. Basophil histamine release results (expressed as the percentage total histamine content after correction for spontaneous release) obtained with 21 IgG1 and/or IgG4 anti-IgE-containing euglobulin fractions using blood samples from an asthmatic individual (FS), a healthy person (SS) and a hay fever sufferer (SJ)

Euglobulin fraction	IgG1 anti-IgE (OD405)	IgG4 anti-IgE (OD405)	Blood donor		
			FS	SS	SJ
CW	0.190	0.313	2	29	ND
TG	0.307	0.098	33	50	ND
JM	0.558	0.452	7	11	6
LM	0.337	0.239	13	46	19
TN	0.388	0.105	12	32	13
BW	0.065	0.256	6	0	14
TY	0.047	0.115	4	10	ND
DS	0.077	0.193	20	8	9
MN	0.087	0.119	8	17	11
CS	0.384	0.182	4	0	ND
BG	0.246	0.421	4	0	2
JB	0.445	0.117	5	5	9
GM	0.379	0.453	6	0	ND
JD	0.173	0.248	4	0	ND
AM	1.031	0.274	1	0	ND
CG	0.431	0.125	1	0	ND
JP	0.100	0.077	4	0	1
SP	0.194	0.054	8	1	ND
RN	0.055	0.117	4	0	ND
EO	0.070	0.243	5	3	9
BB	0.014	0.292	8	0	ND

A histamine release of 10% or more is considered significant. IgG1 and IgG4 anti-IgE levels of 0.1 OD405 units or more are considered positive. Euglobulin fractions CW to MN induced basophil histamine release from one or more blood donor. Fractions CS to BB did not.

trigger *in vitro* histamine release from basophils of an asthmatic individual (4/21; 19%), a hay fever sufferer (4/10; 40%) and a healthy person (7/21; 33%). These data are very similar to those (5/22; 23%) reported by Gruber *et al.* [10] using leucocytes from healthy individuals. We have also shown here that the basophil histamine-releasing activity of IgG autoanti-IgE is not restricted to either IgG1 or IgG4.

We have recently demonstrated, using a series of overlapping recombinant human ϵ -chain peptides, that IgG anti-IgE antibodies in asthma patients recognize two autoantigenic epitopes located within the C ϵ 2-C ϵ 3 inter-domain region (aa301-339) and the C ϵ 4 domain, respectively [6]. Such heterogeneity in the epitope specificity of autoanti-IgE antibodies may, therefore, be responsible

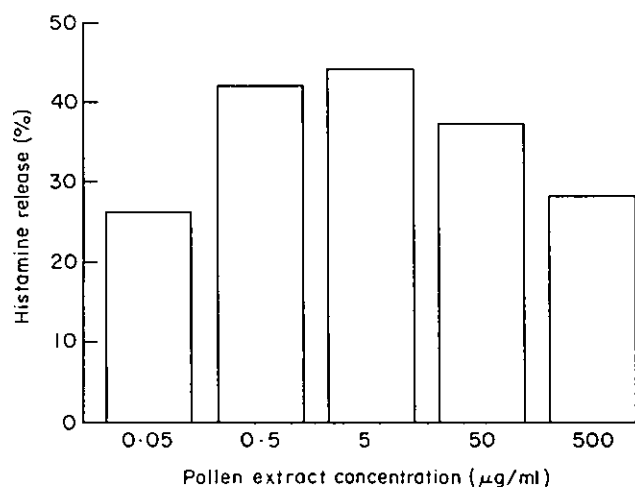


Fig. 1. Pollen extract-induced basophil histamine release results obtained using blood from a hay fever sufferer. Data show the bell-shaped concentration-response curve of the pollen extract.

Table 4. Modulation of pollen extract-induced basophil histamine release by 10 randomly selected IgG1 and/or IgG4 anti-IgE-containing euglobulin fractions using blood from a hay fever sufferer

Euglobulin fraction	Histamine release (% total)		Change (%)	Enhancement/inhibition
	Euglobulin	Euglobulin + pollen		
		37*		
EO	9	71	92	++
JM	6	62	68	++
JP	1	51	38	++
TN	13	55	33	+
BW	14	42	14	+
MN	11	40	8	NC
JB	9	35	5	NC
LM	19	35	5	NC
BG	2	27	-27	—
DS	9	19	-49	—

A histamine release of 10% or more is considered significant. The effects of mixing euglobulin fractions with pollen extract are also expressed as the percentage change in basophil histamine release from the value (*) obtained using just pollen extract. The pollen extract was used at the optimum concentration of 5 µg/ml (see Fig. 1) and the euglobulin fractions at 8 mg/ml. ++, Synergistic effect. +, Additive effect. NC, No change. —, Blocking effect.

for differences in the basophil histamine-releasing ability of different anti-IgE antibody preparations, as shown here and elsewhere [10].

The novelty of this study is the demonstration that autoanti-IgE antibodies modulate *in vitro* allergen-induced basophil histamine release. The three modulatory effects exerted by IgG anti-IgE antibodies on allergen-triggered basophil activation (i.e. additive, synergistic and blocking) were not dependent on the subclass nature of IgG anti-IgE or on using histamine-releasing anti-IgE preparations. In theory, the above modulatory effects of IgG anti-IgE, which may well have been exhibited by distinct epitope-specific subpopulations of anti-IgE, can be explained as follows.

- (i) The additive effect is due to cross-linking of receptor-bound IgE molecules which are not already engaged by allergen.
- (ii) The synergistic effect is due to the additive mechanism described above and to allergen and anti-IgE having favourable effects on IgE cross-linking by each other (i.e. cross-linking by one ligand brings the two IgE molecules in close proximity for efficient engagement by the other).
- (ii) The blocking effect is the result of the binding of IgE by a non-triggering anti-IgE, thereby sterically hindering cross-linking by allergen.

It is now appropriate to expand on the synergistic (3/10), additive (2/10) and blocking (2/10) effects demonstrated by the anti-IgE-containing euglobulin fractions. The concentration-response curve for pollen extract-induced basophil histamine release was shown to be bell-shaped, increasing or decreasing the optimal dose of pollen (5 µg/ml) resulted in a progressive decrease in the amount of histamine released (Fig. 1). It is, therefore, significant that the synergistic effect of anti-IgE-containing euglobulin fractions increased allergen-induced basophil histamine release by up to 92% and the blocking effect decreased allergen-induced basophil histamine release by up to 49%. In other words, the presence of some anti-IgE preparations (3/10) in the pollen extract-cell mixture resulted in a significant increase in basophil histamine release, which could not be achieved by increasing the concentration of pollen extract by up to 100-fold (i.e. from 5 to 500 µg/ml). The presence of other anti-IgE preparations (2/10) in the pollen extract-cell mixture resulted in a significant decrease in basophil histamine release, which exceeded that achieved by decreasing the concentration of pollen extract by up to 100-fold (i.e. from 5 to 0.05 µg/ml).

If we were to extrapolate from these observations to the *in vivo* situation, it may be reasonable to regard IgG anti-IgE antibodies in asthma patients as consisting of two functionally distinct subpopulations: those which up-

regulate (pro-allergic) and those which down-regulate (anti-allergic) the allergic release of mediators from mast cells and basophils. As indicated earlier, we have already demonstrated two epitope-specific subpopulations of IgG anti-IgE in asthma patients [6]. It would, therefore, be interesting to investigate whether the functional heterogeneity of anti-IgE preparations demonstrated here is due to differences in the epitope specificity exhibited by these antibodies.

In an attempt to answer this question, we are currently constructing human monoclonal IgG anti-IgE antibodies and phage-encoded Fab fragments representative of *in vivo* specificities. The availability of such novel reagents and their experimental use will greatly enhance our understanding of the biological roles of epitope-specific human anti-IgE antibodies in IgE-mediated events, particularly those resulting from exposure to allergens.

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