

Differences in binding affinity of human IgE for receptors in chopped human lung

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Summary. Experiments were performed to ascertain whether IgE in different allergic sera had the same or different sensitizing properties for chopped human lung. When allergic sera were allowed to compete with myeloma IgE for tissue receptors in chopped lung and subsequently challenged with antigen, two groups of sera could be distinguished, one which competed well with myeloma IgE and one which competed poorly. Sera that competed well with myeloma IgE were also able to sensitize for greater histamine release relative to IgE concentration when sensitized lung tissue was challenged with anti-IgE. The converse was true of those sera that competed poorly with myeloma IgE in the antigen assay, in that they sensitized for histamine release only at relatively high IgE concentrations in the anti-IgE assay. The possible significance of these findings is discussed.

INTRODUCTION

Passive sensitization of human lung fragments is a well recognized research technique, used mainly in assessing drugs for anti-allergic activity (reviewed by Becker & Henson, 1973). The technique depends on binding of antibody IgE to surface receptors of

the histamine-containing mast cells in chopped lung fragments. Subsequent challenge of sensitized tissue with appropriate antigen causes rapid histamine release into the supernatant (Sheard, Killingback & Blair, 1967). Assay of the histamine release therefore provides a quantitative assessment of the degree of sensitization achieved on exposure to any given serum and it is an indirect method for estimating the quantity of specific IgE in a serum. Sensitized lung fragments can also be stimulated to release histamine by challenge with anti-IgE antibody (Paul & Weir 1969), usually prepared in goats, sheep or rabbits. This anti-IgE initiates histamine release by binding indiscriminately with IgE molecules bound to surface receptors. It therefore reflects total surfacebound IgE, rather than specific antibody IgE.

We have previously shown that passive sensitization of chopped lung fragments can be prevented by first saturating available IgE binding sites with large amounts of IgE from non-allergic sources (Godfrey & Gradidge, 1976). In the present work this principle has been extended to examine competition between IgE's derived from different sources for the same binding sites.

These competition experiments, together with the other challenge experiments described below, have led us to conclude that different IgE's may have different binding affinities for specific receptors in chopped human lung.

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MATERIALS AND METHODS

Allergic patients

Blood was collected from patients attending for allergic assessment of asthma, hay fever and other related disorders. Skin testing was performed by the modified prick method using standard Bencard antigen solutions. Weals of 3 mm or more were accepted as positive.

Sera

Sera were separated as soon as possible after venepuncture and were stored at -20° without additive. Cord sera were collected, separated and pooled, usually into groups of four or five sera together. Grossly haemolysed specimens were rejected. Pooled cord serum contained negligible amounts of IgE as determined by double antibody radioimmunoassay. A pool of sera from West Africans, containing approximately 6000 iu IgE per ml, was used in some of the experiments.

Human lung fragments

Small pieces from the periphery of human lung lobes, removed at thoracotomy for carcinoma, were finely minced with scissors and divided into 200 mg aliquots. They were washed three times with Krebs solution before sensitization.

Myeloma IgE

Highly purified myeloma protein from patient PS was kindly donated by Dr K. Ishizaka.

Passive sensitization of lung fragments

After thorough washing in Krebs solution, lung fragments were exposed overnight to serum containing IgE at room temperature without mixing. After 18 h, the passive sensitization was completed by incubation at 37° for 1 h. The fragments were then washed three times in Krebs solution before challenge with either antigen or anti-IgE antibodies.

Antigen challenge

Freeze dried extracts of both *Dermatophagoides pteronyssinus* (house dust mite) and Timothy grass pollen were dissolved in distilled water to a final concentration of 1 mg/ml. The sensitized lung fragments were exposed to antigen (0.2 ml) in Krebs solution (1.8 ml) for 15 min at 37° , after which the supernatant was removed and stored at -20° for subsequent histamine assay. Preliminary studies

indicated that these antigen concentrations gave maximal reproducible histamine release from sensitized chopped human lung.

Anti-IgE challenge

Goat anti-human IgE (Miles Laboratories) was used throughout these experiments at a final dilution of 1:1000, and the details parallel closely those shown for 'antigen challenge' (*vide supra*).

Histamine assay

Histamine released into the supernatant was measured by bioassay using strips of guinea-pig ileum bathed at 37° in Krebs's solution containing $60 \mu\text{g/ml}$ of atropine sulphate. Total histamine per 200 mg of lung tissue was determined by boiling the lung fragments for 5 min and then assaying the supernatant.

Total IgE assay

The IgE levels of the various sera were measured by double antibody radioimmunoassay according to the method of Gleich, Averbek & Swedlund (1971), with minor modifications.

Competition experiments

Dilutions of allergic serum were made in Krebs solution. To 0.1 ml of these dilutions was added either 0.1 ml of PS Myeloma IgE (300 iu) in cord serum, or 0.1 ml cord serum alone. These mixtures (0.2 ml) were incubated with 200 mg aliquots of washed human lung tissue contained in 19.8 ml Krebs solution. After subsequent incubation and antigen challenge, histamine released into the supernatant was measured. This method allowed us to compare the amount by which various sera were inhibited in their ability to passively sensitize chopped lung in the presence of a fixed amount of competing myeloma IgE protein.

Consistency of methods

In our hands, passively sensitized lung fragments released comparable amounts of histamine whether challenged by appropriate antigen or by anti-IgE. Unsensitized lung fragments released minimal histamine after anti-IgE challenge, despite the possibility of some sensitization from endogenous donor IgE.

Histamine release from duplicate samples of sensitized chopped lung within the same experiment varied on average by $\pm 4.7\%$. The maximum amount of histamine released from sensitized

chopped lung, either by antigen or by anti-IgE antibody, varied from lung to lung (range of 6–19% of that released by boiling the lung fragments). When dilutions of the same serum were tested on different lung samples, the amount of IgE that caused 50% of maximum histamine release (calculated), varied on average by $\pm 22.4\%$ i.e. there was less than 1 log₂ unit difference in end point between experiments.

RESULTS

Antigen challenge of passively sensitized chopped lung

Figure 1 shows the sensitization achieved by progressive dilutions of two different sera. The dotted lines

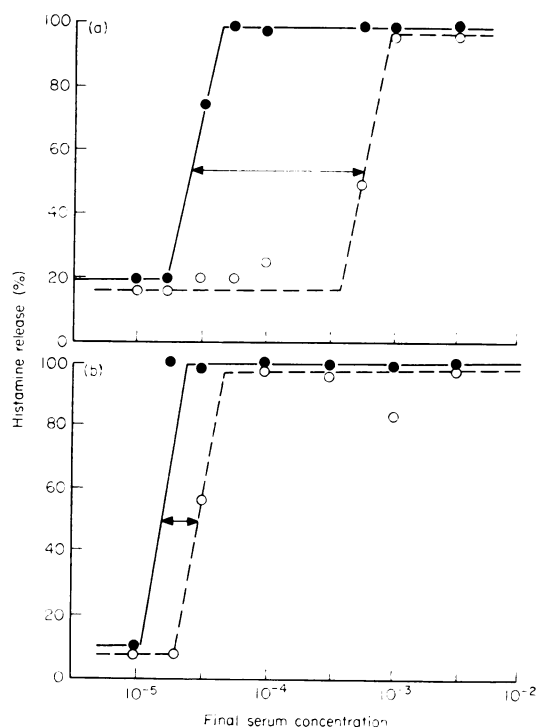


Figure 1. The ability of two different allergic sera to compete unsuccessfully (a) or successfully (b) with a fixed amount of myeloma IgE for binding sites in chopped human lung. The sensitizing ability of serum dilutions was determined in the presence (○) or absence (●) of myeloma IgE, using Timothy grass pollen as antigen for subsequent challenge. 100% histamine release on the vertical axis refers to the amount of histamine released by antigen challenge of lung previously sensitized by serum diluted 1:100 in Krebs solution. The horizontal axis refers to the final serum concentration. All points represent the mean of duplicate samples.

Table 1. Differences between sensitization of chopped human lung by allergic sera in the presence and absence of myeloma IgE

	Serum number	Log ₂ difference in 50 % end point with and without competing myeloma IgE during sensitization
Group A	1	*1.0
	1	1.3
	2	0.85
	2	0.6
	3	1.4
	4 (Fig. 1, b)	0.85
	5	1.2
Group B	6	2.2
	7	4.4
	7	4.9
	8	4.3
	8	4.2
	9	5.4
	10 (Fig. 1, a)	4.4

*Calculated from the dilution of serum that gave 50% histamine release in the absence of myeloma IgE, minus the dilution of serum that gave 50% histamine release in the presence of myeloma IgE. The difference is calculated from the best fitting pair of parallel lines with and without myeloma IgE.

show the results when the sensitization was carried out in the presence of competing myeloma IgE, whilst the continuous lines represent the control experiments in which no competing IgE was present. It can be seen that serum (a) did not sensitize lung in the presence of myeloma IgE so well as in the absence of myeloma IgE, leading to a significant increase in the serum concentration needed to achieve 50% histamine release upon subsequent challenge with Timothy grass pollen. In the case of serum (b) there was no such adverse effect upon sensitization in the presence of competing myeloma IgE, with minimal increase in the concentration needed to achieve 50% histamine release. This experiment was repeated on several occasions with different sera, and it was possible to find other sera showing similar differences in behaviour in the presence of competing myeloma IgE (Table 1). Similar differences were found when *D. pteronyssinus* was used to challenge appropriately sensitized lung.

Next a method was developed to distinguish sera whose passive sensitization capabilities were easily

inhibited by myeloma IgE. It was found that screening of sera at a final dilution of 1 in 10,000 was an effective way to do this. Sera were diluted in Krebs solution to give a final dilution of 1 in 10,000, to which was added 300 iu myeloma IgE in cord serum or cord serum alone. These mixtures were allowed to sensitize chopped lung in the normal way. After incubation, this sensitized chopped lung was challenged with antigen, and the amount of histamine released after sensitization in the presence of competing myeloma IgE compared with that released after sensitization in the absence of myeloma IgE. Figure 2 shows the results from fifty one sera analysed in this way using both Timothy grass pollen and *D. pteronyssinus* as antigens (as appropriate for the various sera). Total IgE in each serum was estimated by double antibody radioimmunoassay. It can be seen that there was no correlation between total serum IgE concentration and the

ability of that IgE to compete well or poorly with myeloma IgE for binding sites in chopped lung.

Anti-IgE challenge of passively sensitized chopped lung

Figure 3 and Table 2 show the results from sensitization of lung with IgE from different sources, followed by anti-IgE challenge. The histamine release, expressed as a percentage of the maximum release, is plotted against the concentration of IgE as determined by double antibody radioimmunoassay. It can be seen that the amount of IgE required for '50% sensitization' varied. Of the controls, a serum from a normal non-allergic donor and PS myeloma IgE both sensitized only at relatively high IgE concentrations, whereas a pool of serum from West Africans gave sensitization at IgE concentrations intermediate between relatively high and relatively low IgE concentrations. It was interesting to find that all sera

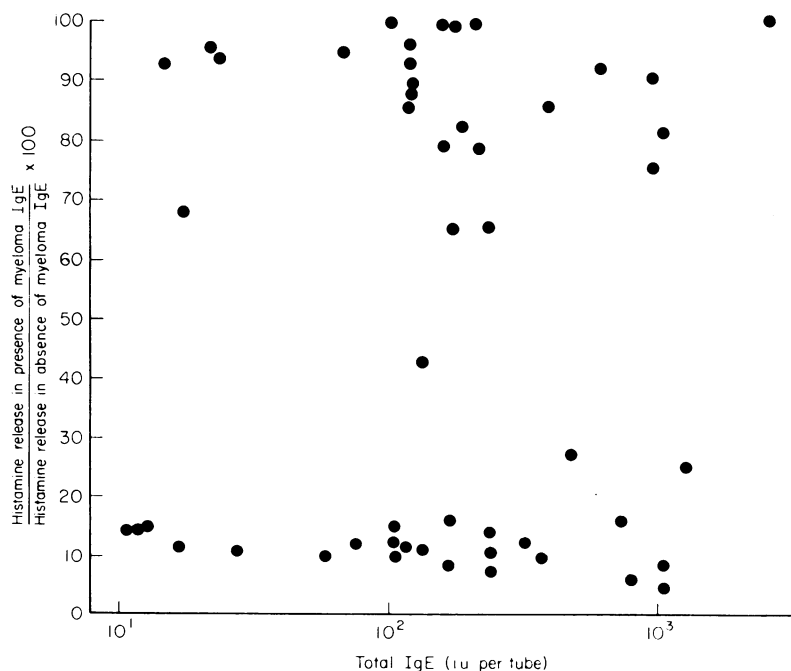


Figure 2. The ability of different patients' IgE to compete with a fixed amount of myeloma IgE for binding sites in chopped human lung tissue. The vertical axis represents the histamine released by antigen challenge of lung previously sensitized in allergic serum (10^{-4} concentration) plus myeloma IgE (300 iu) in cord serum, expressed as a percentage of that released by lung sensitized in allergic serum (10^{-4} concentration) in cord serum alone. The horizontal axis represents the IgE level in the initial serum sample (1 iu $\approx$ approximately 2.4 ng). The antigens used to challenge the sensitized lung fragments were either Timothy grass pollen or *D. pteronyssinus*, depending on the skin test positivity of the patient. All points represent the mean of duplicate samples.

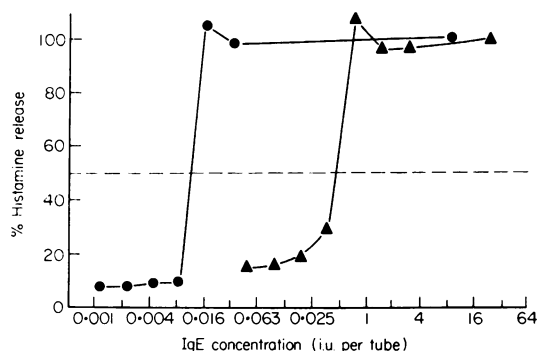


Figure 3. Histamine released by anti-IgE challenge of chopped human lung fragments previously sensitized by IgE from two different allergic subjects. 100% histamine release on the vertical axis refers to the amount of histamine released by anti-IgE challenge of lung previously sensitized by serum diluted 1:100 in Krebs solution. The horizontal axis refers to the concentration of IgE (iu) per tube. All points represent the mean of duplicate samples. (●), Allergic serum with good competing ability. (Serum No. 3); (▲), allergic serum with poor competing ability. (Serum No. 14).

which had shown good ability to compete with myeloma IgE in the previous antigen challenge experiments showed efficient sensitization relative to their total IgE content in the anti-IgE challenge system. The converse was true for those sera which competed poorly with myeloma IgE.

All sera in these experiments failed to sensitize lung after heat treatment at 56° for 1 h.

Figure 4 shows the results of ten allergic sera that were screened in both the antigen challenge assay (see Fig. 2) and the anti-IgE challenge assay (see Fig. 3 and Table 2). It can be seen that the two assays show a negative correlation. Those sera which compete poorly with myeloma IgE in the antigen challenge assay sensitize chopped lung at relatively high IgE concentrations in the anti-IgE challenge assay, whereas those sera which compete well with myeloma IgE in the antigen challenge assay sensitize chopped lung at relatively low IgE concentrations in the anti-IgE challenge assay.

Table 2. Concentration of IgE required for 50% maximum histamine release from chopped lung, following sensitization with various IgE's and challenge with anti-IgE

Experiment number	IgE source	IgE concentration required to sensitize for 50% maximum histamine release (iu per tube)
1	Pooled W. African sera	0.1
2	Pooled W. African Sera	0.15
3	Pooled W. African sera	0.09
4	Pooled W. African sera	0.15
1	IgE myeloma (PS)	0.35
3	Non-allergic serum	1.6
1	Allergic serum No. 3*	0.01
4	Allergic serum No. 4*	0.035
4	Allergic serum No. 5*	0.016
4	Allergic serum No. 11*	0.022
2	Allergic serum No. 12*	0.008
2	Allergic serum No. 8†	0.5
1	Allergic serum No. 10†	1.2
4	Allergic serum No. 13†	4.3
4	Allergic serum No. 14†	0.45
4	Allergic serum No. 15†	5.0

*Sera previously shown to sensitize well in the presence of myeloma IgE.

†Sera previously shown to sensitize poorly in the presence of myeloma IgE.

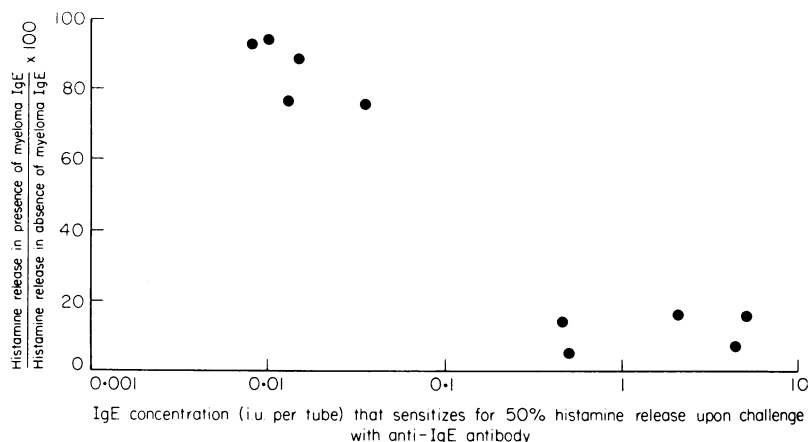


Figure 4. Correlation of the results of ten sera screened in both the antigen assay and the anti-IgE assay. The vertical axis represents the histamine released by antigen challenge of lung previously sensitized in allergic serum (10^{-4} dilution) plus myeloma IgE (300 iu), expressed as a percentage of that released by lung sensitized in allergic serum (10^{-4} dilution) alone. The horizontal axis represents the concentration of IgE (iu per tube) needed to cause 50% of maximum histamine upon challenge with anti-IgE antibody.

DISCUSSION

The experiments reported here demonstrate that IgE's from allergic patients have differing capacities to sensitize chopped human lung for subsequent histamine release by antigen or anti-IgE. Those sera which compete well with myeloma IgE for tissue receptors in the antigen experiments were also able to sensitize tissue receptors at very low IgE concentration in the anti-IgE experiments. The converse was true for those sera which competed less well. This suggests the existence of different types of IgE. The possibility that these differences reflect the activity of IgG tissue sensitizing antibodies (Parish, 1974), has been eliminated by showing complete loss of sensitizing activity in all sera which had been heated at 56° for 1 h. This treatment destroys the ability of IgE to bind to tissue receptors but leaves IgG intact (Ishizaka, Ishizaka & Menzel, 1967).

A further possibility, suggested by a recent publication (Brostoff, Johns & Stanworth, 1977), is that human IgE may exist in complexed form. Preliminary experiments in this laboratory where sera were separated on DEAE cellulose columns (unpublished observations) suggest that the results presented here cannot be explained by IgE in anything other than its monomeric form.

Previous workers have shown that certain allergic sera contain IgM antibodies that have anti-IgE

activity (Williams, Griffiths, Emmons & Field, 1972). The possibility exists that some of the sera used in our study contain these IgM anti-IgE antibodies, which might be expected to interfere with IgE binding to tissue receptors in chopped human lung, or indeed to induce histamine release by crosslinking IgE molecules on the cell surface. That these antibodies do not themselves induce histamine release *per se* is shown by the fact that no sera on their own (without added antigen) would induce histamine release. Whether these IgM anti-IgE antibodies have any relevance to our results has yet to be determined.

The differences shown here between allergic sera in IgE sensitization of chopped human lung may reflect differences in IgE structure. IgE is known to bind to receptors on mast cells and basophils by the Fc portion (Stanworth, Humphrey, Bennich & Johansson, 1968; Ishizaka, Ishizaka & Lee, 1970) and the differences in IgE structure we propose may exist in the Fc portion of the molecule. Assuming that the differences reported here stem from molecular differences amongst IgE molecules, we suggest that these may be due to the presence of IgE subclasses or alternatively of allotypic differences amongst IgE molecules. Between these possibilities allotypic differences may provide the more likely explanation since the pattern of results so far shows at least two separate types of IgE binding. If these

differences were due to the presence of subclasses of IgE, an even scatter of results would be expected, at least in the anti-IgE assay, determined by the proportion of different subclasses in each individual's serum. To establish the existence of IgE allotypes with certainty, anti-allotype antibody will need to be raised. It is interesting to note that IgM antibodies with anti-IgE activity have been found (Williams *et al.*, 1972) recalling the way in which human IgG Gm allotypes were discovered (Grubb & Laurell, 1956). In terms of molecular structure, the differences between IgG allotypes can be small (reviewed by Nisonoff, Hopper & Spring, 1975) but it seems possible that in the case of IgE such small differences may be enough to alter biological activity in the Fc region.

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