

## **Increased release of histamine by anti-IgE from leucocytes of asthmatic patients and possible heterogeneity of IgE**

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### **Summary**

A study was made of the significantly increased amounts of histamine released by anti-IgE from the leucocytes of asthmatic and allergic patients, particularly in patients with a low level of serum IgE. By measuring cell-bound IgE and histamine release with anti-IgE, particularly after passive sensitization of human leucocytes, it was possible to show that serum IgE in some allergic patients differed from that in normal subjects in two respects. Firstly, it appeared to have a relatively high leucocyte binding affinity, though this could not be proved conclusively. Secondly, and more importantly, IgE of some patients could more readily mediate histamine release with anti-IgE, that is to say it had a greater efficacy. These properties should be differentiated from another feature of allergic individuals, that is the more ready releasability of histamine from the patients' own leucocytes.

### **Introduction**

In a previous report (Assem, 1974) two correlates of immediate-type allergy were studied, namely total serum IgE and histamine release from isolated human leucocytes induced with anti-IgE. The correlation between these two parameters was generally poor. Allergic patients' serum IgE level was very variable, but their leucocytes showed a consistently high response to anti-IgE. This finding may be explained by three different possibilities. Firstly, allergic patients may have an IgE with high 'affinity' to cell receptors. Secondly, they may have an IgE with high 'efficacy', i.e. an IgE which readily mediates anaphylactic sensitization, perhaps by undergoing the appropriate conformational changes in the Fc region of the molecule, and thirdly, the patients may have increased leucocyte (basophil) reactivity. The latter phenomenon has also been reported by Conroy, Adkinson & Lichtenstein (1977), who termed it 'releasability'.

The importance of these possibilities is their relevance to current diagnostic

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procedures, to our understanding of the mechanisms of anaphylactic reactions, and possibly to the effect of hyposensitization therapy.

We investigated these possibilities, in certain groups of patients with different allergies and different types of asthma, and in a group of patients with house-dust-mite allergy.

## Methods

### *Measurement of serum IgE*

Total serum IgE was estimated by two different techniques, the radioimmunosorbent test (RIST), and the paper radioimmunosorbent test (PRIST). Kits for both tests were obtained from Pharmacia (Great Britain) Ltd. (Johansson, Burglund & Kjellman 1976).

### *Measurement of allergen-specific IgE*

Only one antigen, the house-dust mite *Dermatophagoides pteronyssinus* (Bencard), was tested. Antibodies of the IgE class were detected and assayed by the radioallergo-sorbent test (RAST) (Wide, Bennich & Johansson, 1967), from Pharmacia.

### *Measurement of leucocyte-bound IgE*

Two methods were used: (1) a modification of the RIST procedure, using intact or sonically-disrupted leucocytes (Assem, 1974) and (2) measurement of the uptake of  $^{125}\text{I}$ -labelled anti-IgE by actively- or passively-sensitized leucocytes. The specific activity of the labelled antibody was  $6.8 \mu\text{Ci}/\mu\text{g}$ . This antibody was incubated with mixed leucocytes, i.e. with no enrichment or purification of basophils, for 90 min at  $37^\circ\text{C}$ . Aliquots containing 1–3 million cells used in 3–6 replicates were treated in this way.

### *Histamine release tests*

Leucocytes from normal subjects and allergic patients were isolated, using a modification (Assem *et al.*, 1971) of the method originally described by Lichtenstein & Osler (1964). Histamine release was partly measured by bioassay (Assem & Schild, 1968), and partly by an automated spectrofluorometric technique (Technicon).

The challenge of leucocytes with antigen, and with anti-IgE antibody (sheep anti-IgE serum of Pharmacia) was carried out in a dose-response fashion (Assem & McAllen, 1970).

Apart from the challenge of actively-sensitized cells, similar procedures were carried out on leucocytes from normal donors, which had been passively-sensitized by prior incubation with allergic patients' sera.

### *Patients*

For the comparison between serum IgE and histamine release with anti-IgE, samples were obtained from 105 patients. Fifty-four of these had extrinsic (allergic) asthma, twenty had intrinsic asthma, and thirty-one had drug allergy, mainly to penicillin. Skin tests were made on all of these patients, and were the main criterion for defining intrinsic asthma. The controls were eighteen normal (non-allergic) subjects. Fifteen of the allergic asthmatics had repeated leucocyte challenge tests. These fifteen patients comprised a group with house-dust-mite allergy, who had been included in a

prospective study on the effect of long-term allergen hyposensitization therapy (Assem & McAllen, 1973).

## Results

### Total serum IgE

Total serum IgE varied within an extremely wide range, both in normal and allergic subjects (Table 1). It also showed fluctuations of up to three-fold in any one individual. There were problems of great discrepancies between RIST and PRIST assay procedures, and much variability in results obtained in different experiments. The coefficient of correlation between RIST and PRIST was relatively poor, being only 0.356. The coefficient of variation for repeated assay of the same samples using different RIST kits was 25.6% on average. Figures obtained by PRIST kits were generally lower than by RIST, and more reproducible.

In eighty-five normal subjects total serum IgE (estimated by RIST) ranged from 0.5 to 850 i.u./ml (i.u. = 2 ng), with an arithmetic mean of 113 and a standard deviation of 160. In PRIST the arithmetic mean for the same group was 62 i.u./ml. Because of the skew distribution, geometric means were calculated. The results are summarized in Table 1, where the patients whose leucocytes were tested for histamine release with anti-IgE are divided into groups according to their predominant allergy. In all groups the 95% confidence limits overlapped considerably.

### Histamine release with anti-IgE

*Actively sensitized leucocytes.* Maximum histamine release with anti-IgE was elicited in all subjects by using a wide range of anti-IgE concentrations. The majority of allergic patients released more histamine than that of normal persons. Some patients with serum IgE levels at the lower end of the normal range, i.e. hardly detectable serum

**Table 1.** Serum total IgE (estimated by RIST and PRIST) and maximum leucocyte histamine release with anti-IgE in normal and allergic subjects

|                                                       | n  | Serum IgE (i.u./ml) |       |                       |       | Histamine release by anti-IgE |                       |
|-------------------------------------------------------|----|---------------------|-------|-----------------------|-------|-------------------------------|-----------------------|
|                                                       |    | Geometric mean      |       | 95% Confidence limits |       | Mean                          | 95% Confidence limits |
|                                                       |    | RIST                | PRIST | RIST                  | PRIST |                               |                       |
| Normal                                                | 18 | 53                  | 21    | 6-501                 | 2-423 | 15.7                          | 4.5-26.9              |
| House-dust allergy (mainly <i>D. pteronyssinus</i> )  | 18 | 50                  | 57    | 6-601                 | 7-488 | 46.2                          | 32.0-60.4             |
| Mould allergy* (mainly <i>Aspergillus fumigatus</i> ) | 20 | 569                 | 49    | 31-11, 090            | 2-731 | 48.7                          | 36.1-61.3             |
| Pollen allergy (grass pollens)                        | 16 | 225                 |       | 112-2, 344            |       | 52.6                          | 30.4-74.8             |
| Drug allergy (mainly penicillin)                      | 31 | 229                 |       | 11-457                |       | 34.6                          | 20.8-48.4             |
| 'Intrinsic' asthma†                                   | 20 | 110                 |       | 6-347                 |       | 46.0                          | 29.6-62.4             |

\* Patients with bronchopulmonary aspergillosis; IgG2 was also involved in 'sensitization' (Assem & Turner-Warwick, 1976).

† Assem *et al.* (1971).

IgE, also released significantly more than normal amounts of histamine. This difference was seen in all groups of patients (Table 1). The mean maximum histamine release in normal subjects was 15.7%, while in allergic patients it ranged from 34.6 to 52.6% in the different groups. In the intrinsic asthmatic patients it was 46%. The increase in all groups was significant at the 1% level.

There was no positive correlation between the release of histamine with anti-IgE and serum IgE levels.

*Passively sensitized leucocytes.* In order to find an explanation for the high histamine release with anti-IgE in allergic patients, and particularly to test for the possibility of a 'high-efficacy' IgE, the sera from two patients were used for the passive sensitization of leucocytes of a normal donor. This donor's cells did not release histamine (< 10%) on challenge with anti-IgE and his serum IgE was higher than that of the two allergic patients. Table 2 summarizes the results of one experiment. It shows that the donor's cells released more histamine (55%) after incubation in the patient's serum (containing 56 i.u. IgE/ml, before desensitization) but it released only 6.5% after incubation with the donor's own serum (though containing 72 i.u. IgE/ml). Furthermore, the same allergic patient showed a slight increase in his serum IgE following hyposensitization therapy but when this serum was used for passive sensitization it did not give increased histamine release; on the contrary, histamine release was significantly reduced to 20% (Table 2). Histamine release with antigen (*Dermatophagoides pteronyssinus*) was also significantly reduced from 48 to 9.8%.

Studies of the uptake of labelled anti-IgE (Table 2) described in detail below showed that the IgE of the normal donor was capable of 'fixation' to his own cells though it did not mediate histamine release. They also helped to show reduced IgE

**Table 2.** Single experiment showing the passive sensitization of normal leucocytes with 'high-efficacy' IgE in the serum of an asthmatic patient with house-dust-mite allergy

| Serum used                                      | IgE level<br>(i.u./ml) | Uptake of labelled anti-IgE after incubation with serum (ct/min) |           | Maximum histamine release with anti-IgE after incubation with serum (%) | Maximum histamine release with antigen |
|-------------------------------------------------|------------------------|------------------------------------------------------------------|-----------|-------------------------------------------------------------------------|----------------------------------------|
|                                                 |                        | mean<br>(n = 3-6)                                                | s.e. mean |                                                                         |                                        |
| Autologous, normal serum                        | 72                     | 1843                                                             | 120       | 6.5                                                                     | 3.5                                    |
| Patient's serum before hyposensitization        | 56                     | 191*                                                             | 133       | 55†                                                                     | 48                                     |
| Patient's serum during hyposensitization course | 70                     | 1899                                                             | 156       | 23†                                                                     | 16‡                                    |
| Patient's serum after hyposensitization course  | 75                     | 1834                                                             | 135       | 20†                                                                     | 9.8‡                                   |

Total  $^{125}\text{I}$ -labelled anti-IgE incubated with each aliquot gave nearly 50 000 ct/min (about 34 i.u.)

\* In subsequent experiments the uptake of labelled anti-IgE was not significantly different from the other sera.

† Similar rank order confirmed in two other experiments.

‡ Histamine release reduced though allergen (mite)-specific IgE (RAST) was unchanged.

**Table 3.** Comparison between mean values of serum-IgE, leucocyte-bound IgE and leucocyte histamine release with Anti-IgE in normals and in allergic asthmatics

| Subjects                   | Serum IgE<br>i.u./ml<br>(RIST) | Leucocytes-<br>bound IgE<br>(i.u./million<br>cells, RIST<br>modification) | Maximal<br>histamine<br>release by<br>anti-IgE (%) |
|----------------------------|--------------------------------|---------------------------------------------------------------------------|----------------------------------------------------|
| Allergic<br>asthma<br>(14) |                                |                                                                           |                                                    |
| Mean                       | 306                            | 5.8                                                                       | 49.7                                               |
| s.e.mean                   | 124                            | 3.3                                                                       | 6.9                                                |
| Normal<br>(12)             |                                |                                                                           |                                                    |
| Mean                       | 165                            | 3.4                                                                       | 15.7                                               |
| s.e.mean                   | 43                             | 1.9                                                                       | 3.0                                                |

efficacy following hyposensitization therapy, i.e. reduction in antigen-induced histamine release for the same amount of antibody.

#### *Leucocyte-bound IgE*

*Actively sensitized leucocytes.* The method first attempted for the 'direct' measurement of cell-bound IgE was a modification of RIST (Assem, 1974). Briefly, the results (Table 3) showed that leucocyte-bound IgE was greater in the allergic subjects than in the normal controls, but the increase did not appear to be disproportionate to the serum level in the group of patients as a whole. However, individual differences in this respect were clear, i.e., high-affinity IgE was apparently present in some patients (Fig. 1).

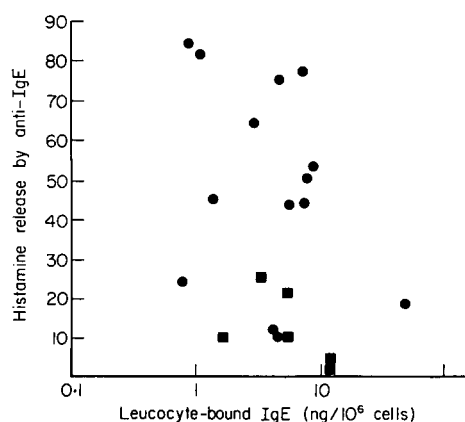


**Fig. 1.** Leucocyte-bound IgE as estimated by a modification of RIST procedure, and maximal histamine release by anti-IgE serum. Note the wide individual differences and the lack of correlation between these two parameters (see text). ■, Normal subjects; ●, allergic patients.

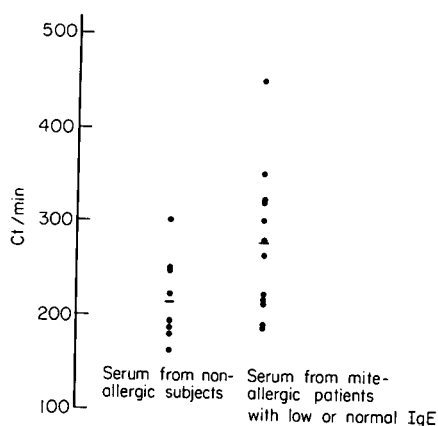


Fig. 2. Measurement of cell-bound IgE in passively sensitized leucocytes by the uptake of  $^{125}\text{I}$ -labelled anti-IgE. All the eleven allergic patients suffered from house-dust allergy, and had a total serum IgE (RIST)  $< 100$  i.u./ml (four of them had an IgE  $< 10$  i.u./ml).

There was no positive correlation between cell-bound IgE and histamine release with anti-IgE as shown in Fig. 1 ( $r = -0.3349$  in patients).

*Passively sensitized leucocytes.* Since some technical difficulties in the direct RIST assay of cell-bound IgE were encountered, an alternative test procedure was used for confirmation. The uptake of  $^{125}\text{I}$ -labelled anti-IgE was measured.

Figure 2 shows that in allergic patients with normal or low total serum IgE, the uptake of labelled anti-IgE by donor cells pre-incubated (passively sensitized) in these patients' sera was greater than by cells pre-incubated in normal sera containing comparable IgE levels. However, the difference was not statistically significant. Thus, the results of this test agree in this respect with the direct RIST measurement. Individual differences in IgE affinity were also noted, but they were not as widely different as suggested by the results of the RIST technique.

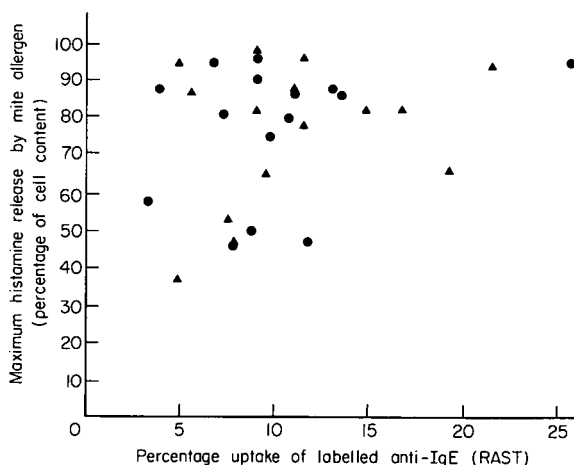


Fig. 3. Comparison between allergen-induced histamine release from the leucocytes of patients with house-dust mite allergy, and allergen-specific IgE estimated by RAST. This comparison was held in fifteen patients, before and after a course of hyposensitization (injection) therapy.  $\blacktriangle$ , Before desensitization;  $\bullet$ , after desensitization.

Studies of the uptake of labelled anti-IgE by passively sensitized leucocytes also helped to establish the existence of IgE with different efficacies in mediating histamine release. Thus, the normal donor whose cells did not respond to anti-IgE was shown to have IgE molecules on the surface of his leucocytes, as detected by an uptake of labelled anti-IgE of comparable level to that of cell aliquots passively sensitized with the allergic patient's serum (Table 2).

#### *Comparison between RAST and allergen-induced histamine release*

A correlation made between allergen-specific IgE, as estimated by the RAST procedure, and allergen-induced histamine release from allergic patients' leucocytes was statistically significant. The results are shown in Fig. 2. Statistical significance was obtained both with respect to maximal histamine release (Fig. 3,  $r = +0.6055$  before hyposensitization) and to the concentration of antigen required to achieve this maximum ( $r = -0.6191$ ,  $0.01 < P < 0.05$ ). However, in the same group of patients after hyposensitization the correlation was poorer ( $r = 0.4368$ ) for maximum histamine release.

#### **Discussion**

Tests for histamine release from blood leucocytes (basophils) by antigen or by anti-IgE antibodies are *in vitro* correlates of immediate-type allergy, corresponding to RAST and RIST tests, respectively. These histamine-release tests have certain advantages. When made on passively sensitized cells, they provide greater opportunity for studying the properties of IgE, e.g. its affinity to cell receptors or its ability to mediate anaphylaxis (i.e. its efficacy). 'Cell-reactivity', i.e. the property of the cell itself to react to challenge with antigen or anti-IgE with histamine release, is a different property. In fact, it could prove difficult to distinguish between cell-reactivity and IgE efficacy, but the tests most likely to show the latter are those involving passive sensitization coupled with the measurement of cell-bound IgE. The experiment summarized in Table 2 illustrates a method of differentiating between the two.

Cell-reactivity may be of much importance in individual variability of response to stimuli leading to histamine release, and in intrinsic asthma where allergy and IgE may play a relatively small role, but the other two parameters, IgE efficacy and affinity to cell receptors, are also important. The studies by Ishizaka & Ishizaka (1973) showed wide individual variations in IgE affinity, which are compatible with the earlier findings of one of us (Assem, 1974). However, the work of Conroy *et al.* (1977), and the passive sensitization experiments of this study (Fig. 1) indicate that the individual differences in IgE affinity, in general, are not great. In the normal subjects, IgE appeared to 'fix' to cells in the same way and in the same relative quantities as IgE in allergic patients, as detected by the uptake of labelled anti-IgE, while the earlier findings (Assem, 1974, Table 2) were based on measurement by cell-bound IgE in sonicated cells by RIST procedures. The reason for the discrepancy between those two methods is unclear.

The evidence presented here suggests that human IgE is heterogeneous, and that the heterogeneity is expressed most clearly by the 'efficacy' of IgE. Thus IgE(s) of somewhat similar affinities for cell-receptors may differ in their relative efficacies. This situation is similar to drug receptor agonists, some of which behave as 'full' or 'complete' agonists, while others act only as 'partial' agonists. Allergic patients may have IgE of the former type, while normal subjects have the latter.

The differences shown between allergic patients and normal subjects in IgE efficacy were also detected to some extent between allergic patients' IgE before, and after hyposensitization therapy. Although the level of allergen-specific IgE did not change (it even increased in some patients) the sensitizing ability of IgE (i.e. its efficacy) was reduced.

Three other research groups have investigated the heterogeneity of IgE, using different methods for measuring IgE cell-binding affinity. Ishizaka & Ishizaka (1973) measured leucocyte-bound IgE by the direct Cl-fixation transfer test, and found great individual variability in IgE cell-binding affinity, both in atopic patients and in normal subjects. Conroy *et al.* (1977) eluted leucocyte-bound IgE at pH 3.7 and measured it by RIST. They concluded that there were small individual differences in IgE binding affinity and that there was no significant difference between allergic and normal individuals. Godfrey *et al.* (1978), using myeloma IgE as a 'competitive antagonist' of the passive sensitization of human lung by the serum of allergic subjects, could distinguish two types of IgE which may be described as 'high affinity-high efficacy', and 'low affinity low efficacy' types, according to the definitions outlined previously in this paper. Our findings suggest that IgE in normal subjects belongs to the low-efficacy type.

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