

Changes in challenge tests following hyposensitization with mite extract

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Summary

The changes in a wide variety of *in vivo* and *in vitro* tests following a long course of low antigen dosage hyposensitization therapy in fifteen asthmatic patients with house dust mite allergy are reported.

Evidence of clinical improvement was shown by decreased bronchodilator requirement. Bronchial challenge testing showed a significant increase in bronchial tolerance to mite antigen. Nasal and skin tests with mite extract, however, did not show a significant change.

In contrast to the development of bronchial tolerance and to the clinical improvement, some of the *in vitro* tests seemed to provide evidence of hypersensitization. The leucocyte test, for example, showed increased sensitization to the mite antigen, as well as an increase in histamine release by anti-IgE serum. Total serum IgE was also increased, but this was apparently due to non-allergen specific IgE.

Although the leucocyte test showed increased sensitization to mite antigen, serum reaginic activity as measured by the passive sensitization of human lung did not show any increase. Thus, there seemed to be a difference in the affinity of allergen-specific IgE for leucocytes as compared with lung tissue. It also appeared that leucocyte response to antigen bore no relationship to sensitization of other tissues, particularly the bronchial tree.

The mechanism of clinical hyposensitization by low dosage immunotherapy is discussed. It is suggested that apart from the development of blocking antibodies, other mechanisms seem likely. Among these are a change in the affinity of IgE antibodies for different tissues, and a competition between non-specific IgE (produced in excess as a result of immunotherapy) and allergen-specific IgE.

Introduction

Allergen injection therapy (hyposensitization or immunotherapy) has been practised in the treatment of allergic asthma and rhinitis for many years. Symptomatic improve-

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ment, particularly in pollen allergy, has been reported by numerous authors, but the mechanism by which this is achieved has not yet been established.

In house dust allergy, in spite of the fact that such treatment has been in use for decades, there has been no convincing evidence that hyposensitization with crude house dust extracts is of any value (British Tuberculosis Association, 1968). Recent reports, however, of the efficacy of hyposensitization using specific extracts of *Dermatophagoides* have been more encouraging (McAllen & Assem, 1970; Maunsell, Wraith & Hughes, 1971; Smith, 1971).

We have previously reported the results of a variety of *in vivo* and *in vitro* tests in patients with house dust mite allergy (McAllen, Assem & Maunsell, 1970; Assem & McAllen, 1970). This paper reports the changes in these tests and those in seven additional criteria in a series of fifteen patients treated with a long course of low dosage hyposensitization using an extract of *Dermatophagoides pteronyssinus*.

Patients

Fifteen patients with asthma of at least 2 years duration were studied. All were clinically sensitive to house dust and had positive skin and bronchial challenge responses to *Dermatophagoides pteronyssinus*. Their ages ranged from 16 to 50 years and their asthmatic symptoms were such that they did not remain free of wheezing for longer than a few hours unless they took bronchodilator drugs. The severity of their symptoms was variable, but no patient, even in remission of symptoms, registered a forced expiratory volume in 1 sec (FEV₁) of greater than 80% of the predicted normal. No patient was treated with corticosteroid drugs or with sodium cromoglycate during the period of observation.

Skin tests for immediate-type allergy showed that a single specific sensitivity to house dust and house dust mites was present in nine of the patients. The others had additional positive skin reactions to grass pollen (two patients), animals (three patients) and pollen, animals and mould spores (one patient). The patients with multiple positive skin reactions were not clinically sensitive to antigens other than house dust.

Materials

Allergen extracts

Cultures of *D. pteronyssinus* grown on human skin scales were used for the preparation of the first three batches of extract used for testing and also for the extract which was used for hyposensitization. The method of preparing these extracts was described by McAllen, Assem & Maunsell (1970). Two other batches of *D. pteronyssinus* extract were obtained from the Bencard Division of Beecham Research Laboratories Ltd. These were from cultures grown on animal tissue of non-dermal origin.

Standardization of extracts

The relative potency of each batch of extract was assessed by means of titrated skin tests and by dose-response curves in leucocyte challenge tests, using leucocytes from four patients with known responses to mite antigen.

It was found that neither the dry weight of the mite material in the extract, nor its water soluble protein content, bore any relationship to its ability to elicit a given response in tests.

The most potent of the six batches of extract used in the investigation produced skin weals, on prick testing, of a mean diameter of $9 \text{ mm} \pm 3.5$ in the group of fifteen patients when it was applied in a concentration of 1 mg/ml . This extract was used as the reference antigen and the amount of antigen in the other batches was calculated from their potency relative to the reference material using leucocyte challenge tests as the method of standardization.

It was found that the potency of the extracts was maintained for at least 2 years when they were stored at room temperature.

Methods

The following eleven objective tests have been used to monitor the progress of the patients before, during and after hyposensitization:

In vivo. Skin prick tests; nasal challenge tests; bronchial challenge tests; FEV_1 and FVC.

In vitro. Leucocyte challenge with mite antigen; leucocyte challenge with anti-IgE; serum reaginic activity estimations; serum total IgE estimation; serum haemagglutinin titres; serum precipitin estimations; 'blocking' antibody estimations.

A written record of bronchodilator requirements was also kept.

The tests were carried out at three different points in the period of observation. These were:

Point A. Before treatment.

Point B. On reaching the maximum planned dose of antigen, approximately 6 months after Point A.

Point C. After twelve monthly maintenance injections of the maximum dose as given at Point B.

(a) *In vivo investigations*

Skin tests were carried out by the prick method which introduces about three-millionths of a millilitre of extract into the epidermis (Squire, 1950). The diameter of the weal was read after 15 min and the tests were performed in duplicate. Serial dilutions of extract from $0.1 \text{ } \mu\text{g/ml}$ were used in ten-fold steps, and the lowest concentration which caused a weal of more than 1 mm diameter was recorded as the threshold concentration.

Nasal challenge tests were carried out by spraying the antigen directly onto the nasal septum on one side, using a coarse spray with an output of 0.02 ml per shot. Serial dilutions of the extract were applied on alternate sides until a reaction occurred consisting of sneezing, watering or blocking of the nostril. The threshold dose was defined as the lowest causing a definite response. The net doses of antigen used were from 0.2 to 60 μg .

Bronchial challenge tests were performed by inhalation of a nebulized extract from a closed circuit spirometer using the method of Herxheimer (1951). The dose of antigen was varied by altering the time of the inhalation and the concentration of the extract. The initial challenge was with 0.25 μg of antigen and this was increased by two-fold steps until a positive reaction occurred or a maximum of 400 μg had been inhaled without reaction. The tests were performed at intervals of at least 1 week in order to prevent local hyposensitization of the bronchial tree.

A positive immediate bronchial reaction consists of a reduction in FEV_1 of at least 10% coming on 5–10 min after the end of the inhalation. A positive late reaction

consists of an asthmatic attack of variable severity coming on 4–12 hr after the inhalation. Patients recorded these reactions on peak flow meters taken home.

The smallest amount of antigen which caused an immediate or a late reaction was recorded as the threshold dose.

Clinical observations. FEV₁ and FVC, before and after isoprenaline inhalation, were measured at weekly intervals for 4–8 weeks initially and at intervals afterwards.

A record of bronchodilator requirements was kept by each patient throughout the period of observation.

(b) *In vitro tests*

Leucocyte tests. Blood leucocytes were isolated as described by Assem *et al.* (1971), aliquots of washed leucocytes were challenged with different concentrations of mite extract, ranging from 0.1 to 100 µg/ml, in order to obtain a dose–response curve and to find the concentration which would produce a maximum histamine release. Both maximum histamine release as a fraction of total histamine content and the antigen concentration producing it were taken as parameters of cell sensitivity.

Other leucocyte aliquots were challenged with different dilutions of sheep anti-human IgE serum. This treatment causes histamine release from IgE ‘coated’ leucocytes (mainly basophils). This is an indirect test of the amount of leucocyte-fixed IgE.

Detection of serum reagins. Serum reagins were detected by *in vitro* passive sensitization and histamine release from chopped human lung as described by Assem & Schild (1968). Sera from individual patients, collected on different occasions, were tested in the same experiment in order to compare their relative reaginic activity. This was estimated by the percentage of tissue histamine released on challenge with the mite antigen.

Detection of blocking antibodies. Sera were collected from the patients at different times in order to follow the changes in ‘blocking’ antibody levels. The serum was heated at 56°C for 2 hr and then added to different aliquots of the patient’s own leucocytes to give a final concentration of 10%.

Serum containing ‘blocking’ antibodies to the mite antigen would be expected to shift the antigen dose–response curve to the right (i.e. towards a higher antigen concentration) (Fig. 7a). This is because of the competition between serum ‘blocking’ antibodies and the leucocyte-fixed reagin for binding the antigen. The degree of shift in the antigen dose–response curve correlates with the amount of blocking antibody.

This method, however, does not measure ‘blocking’ antibodies exclusively since the presence of allergen-specific antibodies of any type (whether they are non-sensitizing, ‘true’ blocking antibodies or sensitizing IgE antibodies) can produce this shift. On the other hand, the addition of serum (particularly when antibody-free) to leucocyte suspensions may potentiate their response to antigen, and thus may conceal or underestimate the amount of blocking antibodies (Fig. 7b).

Estimations of total serum IgE. Serum IgE was estimated by the radioimmunosorbent technique (Johansson, 1967), using the assay kits supplied by Pharmacia Limited. Mean IgE levels in the whole group of patients at different stages were calculated, using a logarithmic transformation to base 10 of individual values. Statistical analysis of these results was carried out, using Student’s *t* test.

Test for haemagglutinins. Fresh sheep red cells were preserved with formalin, treated with tannic acid, and then coated with mite antigen. The preparative procedure is a modification of the method described by Fulthorpe *et al.* (1961). Titres of mite

antibody were determined by serial dilution, using Takatsy's microtitrator (Shandon Limited).

Test for precipitins. Precipitins were looked for in neat and five-fold concentrated serum, using a microimmunodiffusion technique (Ouchterlony, 1967).

Control experiments

These were carried out in three groups of subjects:

(1) On the fifteen patients under investigation, in respect of all the direct challenge tests. Both *in vivo* and *in vitro* control experiments were performed using either an extract of the culture medium or 0.5% phenol saline (as was used in preparing the mite extract).

(2) On untreated patients with house dust asthma. Five of these were investigated in respect of their bronchial challenge responses over the same length of time, and three others were similarly followed up in respect of their leucocyte challenge responses to mite antigen and total serum IgE.

(3) On fifteen normal subjects with negative skin tests to mite antigen, grass pollen, animals, and mould spores and with no personal or family history of allergy. These were examined in respect of total serum IgE and reversed leucocyte challenge with anti-IgE. Repeated leucocyte challenge with mite antigen was also carried out on six of these normal subjects.

Desensitization regime

After all the investigations outlined above were complete, the fifteen patients were subjected to the following hyposensitization course:

(A) Subcutaneous injections at weekly intervals: mite extract 0.1 µg/ml concentration, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 ml; mite extract 1 µg/ml concentration, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 ml; mite extract 10 µg/ml concentration, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 ml.

(B) Subcutaneous injections at monthly intervals: mite extract 10 µg/ml, 1 ml for 12 months.

Results

Control experiments

In vivo. The five untreated mite sensitive patients showed no significant changes in their bronchial or skin responses over a period of 18 months.

Control challenge tests with non-antigenic material did not produce any positive reactions at any time in the treated patients.

In vitro. Challenge with mite extract of isolated leucocytes from fifteen clinically non-allergic controls produced a small percentage of histamine release (compared with the substantial release in allergic subjects), usually less than 10%. This small release was found in nearly half of these control subjects. No release was obtained in the rest of them. In untreated mite allergic patients there was no increase in histamine release by either antigen or anti-IgE.

Maximum histamine release by anti-IgE in these control subjects never exceeded 35%. Total serum IgE in normal subjects varied within a wide range (1–1700 ng/ml), with an arithmetic mean of 225 ng, and a standard deviation of 320. Serum IgE estimations in individual normal subjects were repeated during the length of the study. It was found that fluctuations of up to two-fold occurred within the above range, but

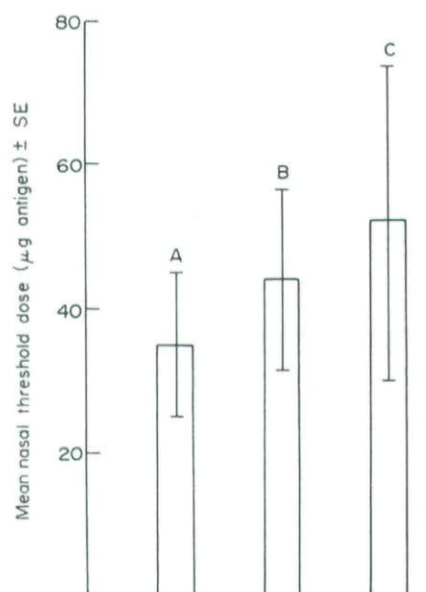
Table 1. Summary of changes in various parameters in the whole group of patients following hypo-sensitization therapy

	No. of patients	Percentage of patients with:			Change in mean value for whole group	Statistical significance in change*
		Increased	Decreased	Unchanged		
<i>In vivo</i>						
Bronchodilator requirement	15	0	87	13	Decreased	$P < 0.005$
Bronchial sensitivity	15	0	67	33	Decreased	$P < 0.01$
Nasal sensitivity	15	47	20	33	Decreased	NS
Skin sensitivity	15	53	13	33	Increased	NS
<i>In vitro</i> tests						
Serum IgE	15	67	0	33	Increased	$P < 0.01$
Serum reaginic activity						
(passive sensitization of lung)	15	20	33	47	Decreased	NS
Serum haemagglutinins	15	93	0	7	Increased	$P < 0.001$
Serum blocking antibody						
activity	9	67	0	33	Increased	$P < 0.001$
Leucocyte response to antigen	15	93	0	7	Increased	$P < 0.001$
Leucocyte response to anti-IgE	7	86	14	0	Increased	$P < 0.002$

NS, Not significant.

* By Student's 't' test.

there was no consistent trend in the individual. Untreated mite allergic patients also showed fluctuations in serum IgE, but mean values for the whole group at various points showed no significant change.

**Fig. 1.** Effect of hyposensitization therapy on nasal sensitivity (fifteen patients). Note that the increase in mean threshold dose after treatment was not significant.

Effects of hyposensitization therapy

Changes in the various tests in the whole group of patients are summarized in Table 1.

Skin tests. When the results of testing before, during and after treatment (Points A, B and C) were compared there was no significant change in the skin responses either in respect of the mean size of the weal produced by the standard extract or in the mean threshold concentrations. There were no delayed skin reactions.

Nasal challenge. The mean threshold response in the nasal test increased slightly but progressively from Point A through Point B to Point C, but these differences were not significant (Fig. 1). There were no delayed nasal responses.

Bronchial challenge. In eight of the fifteen patients immediate-type responses were seen, in the other seven the responses were delayed.

The mean threshold of bronchial response increased markedly during and after the treatment and the differences were significant (Point A v Point C: $P < 0.01$) (Fig. 2).

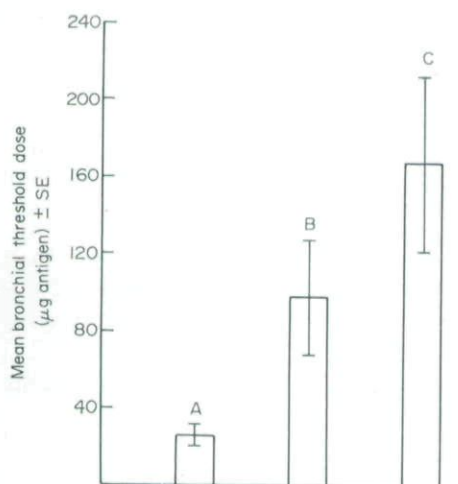


Fig. 2. Effect of hyposensitization therapy on bronchial sensitivity (fifteen patients). Note the significant reduction in bronchial sensitivity (i.e. development of bronchial tolerance to mite antigen) following this therapy (A v C: $0.002 < P < 0.01$).

There were, however, considerable variations between the individual patients. Two patients did not improve at all, but in four patients the increase in antigen tolerance was more than 800%. The remainder were moderately improved.

Clinical response. Improvement in the clinical state as measured by bronchodilator requirements ran parallel to the increase in bronchial tolerance.

Eight of the fifteen patients became symptom-free and stopped taking all bronchodilators. Five others continued to need occasional treatment and two were unchanged.

In the group as a whole, the bronchodilator requirements, in the 2 months following the last injection, were significantly less than before treatment ($P < 0.01$). There was no significant change in the mean levels of FEV₁ and FVC during the period of observation.

In vitro tests

Total serum IgE. Fig. 3 shows the results of serum IgE estimations. They are expressed as a mean of the log values with the standard errors of the mean.

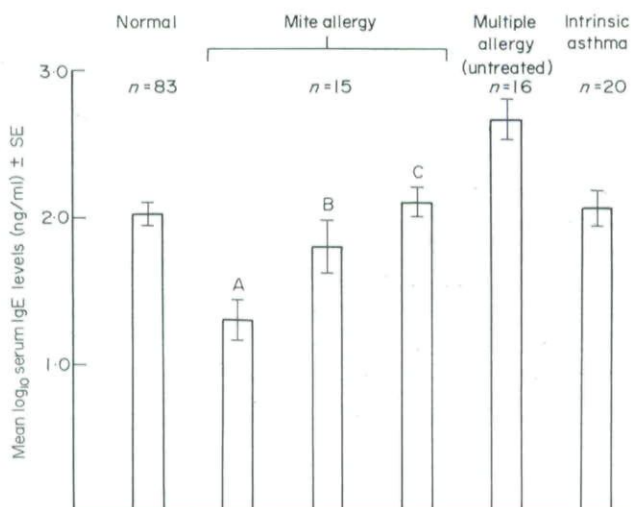


Fig. 3. Total serum IgE in patients with mite allergy: Effect of hyposensitization and comparison with non-allergic subjects, with patients with multiple allergy, and with patients with intrinsic asthma. Note that serum IgE in mite allergy is significantly lower than in normals ($P < 0.001$) and than in patients with intrinsic asthma ($P < 0.001$). Hyposensitization therapy produced a significant increase in total serum IgE (A v C: $0.002 < P < 0.01$).

It was found that the mean serum IgE in mite allergy was significantly lower than normal ($P < 0.001$) at Point A (before treatment). It rose with the progress of desensitization, at Point B, until it reached normal or just above normal level at Point C (for Points A v C: $P < 0.01$).

Fig. 3 also compares the serum IgE of mite allergic patients with that of patients

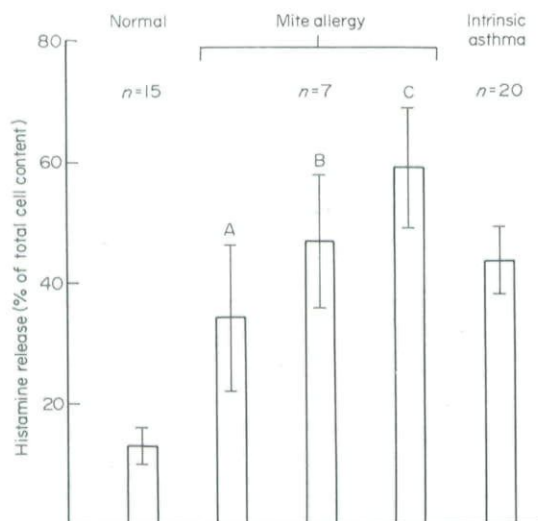


Fig. 4. Maximum histamine release from leucocytes by anti-IgE in patients with mite allergy. Effect of hyposensitization therapy, and comparison with normal subjects and with patients with intrinsic asthma. Note that in mite allergy and in intrinsic asthma the histamine released by anti-IgE is higher than in normals. This contrasts with the low or normal values of serum IgE in the same patients. Hyposensitization therapy produced a measurable, though insignificant, increase in histamine release by anti-IgE.

with extrinsic asthma and multiple positive skin reactions, as well as that of patients with intrinsic asthma. In the former group IgE was significantly higher than normal ($P < 0.05$) while in intrinsic asthma it was similar to that of normal subjects.

Leucocyte response to anti-IgE. The reaction of leucocytes of the mite allergic patients to challenge with anti-IgE at different stages of the hyposensitization course is shown in Fig. 4. Histamine releases are expressed as a percentage of the total leucocyte content of histamine.

An increase in histamine release was observed after hyposensitization, this ran parallel with the increase in serum IgE. However, the fundamental peculiarity which has been noted in these patients is that even before desensitization, when the serum IgE was lower than normal, the reversed leucocyte reaction was higher than normal.

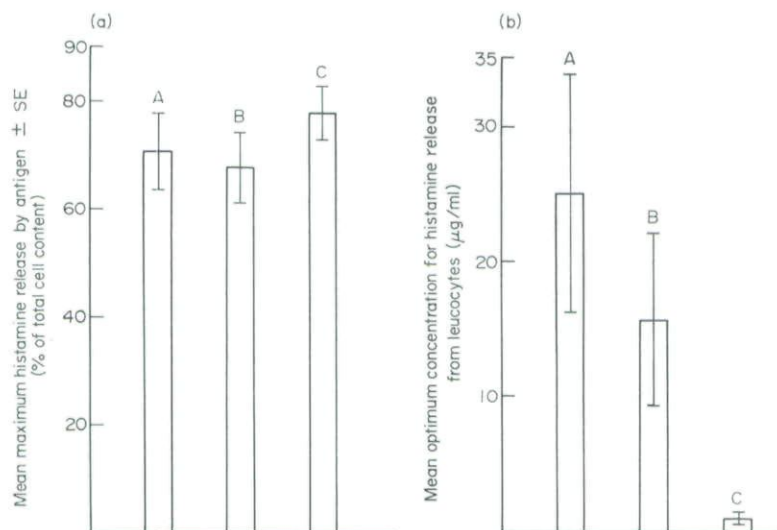


Fig. 5. Effect of hyposensitization therapy on the response of patients' leucocytes to challenge with mite antigen. Note that although maximum histamine release by antigen did not change following hyposensitization therapy (a), the optimum concentration of antigen required for maximal histamine release showed a marked drop (b), i.e. the leucocytes have become more sensitized (A v C: $P < 0.001$).

The difference between the non-allergic subjects and the allergic patients after treatment was significant ($P < 0.02$) in respect of histamine release by anti-IgE. This contrasts with the serum IgE levels in the same two groups of subjects where there was no difference, i.e. the previously abnormally low serum IgE level in the mite allergic subjects rose to a normal level after hyposensitization, whereas the leucocyte response to anti-IgE was greatly increased above normal. In intrinsic asthma (where the serum IgE is normal), leucocytes challenge with anti-IgE released significantly more ($0.01 < P < 0.02$) histamine than in normal subjects. Thus, in respect of this reaction, patients with intrinsic asthma resemble those with mite allergy.

Leucocyte response to antigen. Figs. 5(a) and 5(b) show the 'direct' leucocyte challenge with mite antigen. In Fig. 5(a) the results are expressed in terms of the maximum percentage histamine release from leucocytes, while Fig. 5(b) shows the optimum concentration of antigen which produced this release. In normal subjects histamine release with mite extract, even in high concentrations, never exceeded 16%. In the mite allergic patients, the maximum histamine release was around 70% before

desensitization and it did not show any significant change as treatment proceeded. However, the optimum concentration required for the release of histamine dropped significantly with the progress of desensitization in all patients. This suggests that the leucocyte sensitivity to antigen is *increased* by the treatment.

Serum reagins. Serum reaginic activity was examined in all the patients at Points A and B. This was carried out by passive sensitization of chopped normal human lung tissue followed by challenge with mite antigen. Histamine release was measured, thus providing a quantitative, though indirect, assay of serum reagins. No significant change between Point A and Point B was observed in the group as a whole. However serum reaginic activity showed a marked drop in two patients, and a moderate reduction in three others. We have also examined the sera of three patients by this test at Point C. The reaginic activity remained unchanged in these patients.

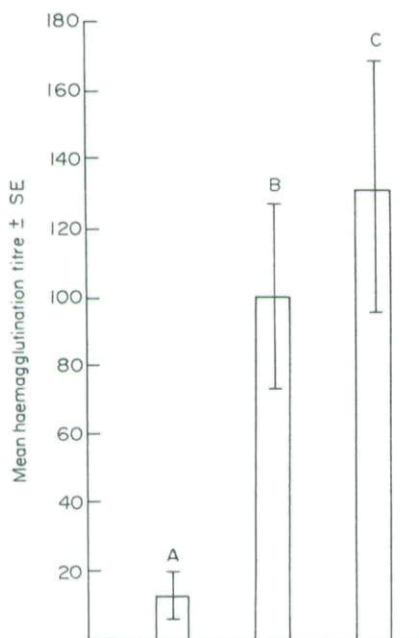


Fig. 6. The increase in serum haemagglutinins following hyposensitization therapy (A v B: $0.002 < P < 0.01$).

Initially (at Point A) we found correlation by ranking order between the serum reaginic activity and the leucocyte response to antigen (Assem & McAllen, 1970). Following hyposensitization treatment, this correlation disappeared.

Serum haemagglutinin tests. Fig. 6 shows the titres of haemagglutinating antibodies.

Before desensitization, haemagglutinins were found infrequently and the titres were low. As expected, when hyposensitization was carried out, there was a marked rise in the number of patients with positive results and also a marked increase in the titres.

There was no correlation between haemagglutinin titres and clinical response to treatment (Table 1). For example, there were three patients with very low haemagglutinin titres after treatment; two of these responded well to treatment and also showed increased bronchial tolerance to antigen.

Serum precipitins. All the sera were examined for precipitins to the mite antigen in unconcentrated serum and also in serum concentrated five-fold. We did not find any precipitins in any patients at any stage of the treatment.

Blocking antibodies. The presence of 'blocking antibodies' was also looked for in nine patients by leucocyte challenge with antigen at point B. A 'blocking' effect of serum antibodies on the histamine release could be demonstrated clearly in five patients. In others it was not seen; this was probably due to a non-specific action of the serum potentiating the response to antigen, the net result being a balance between

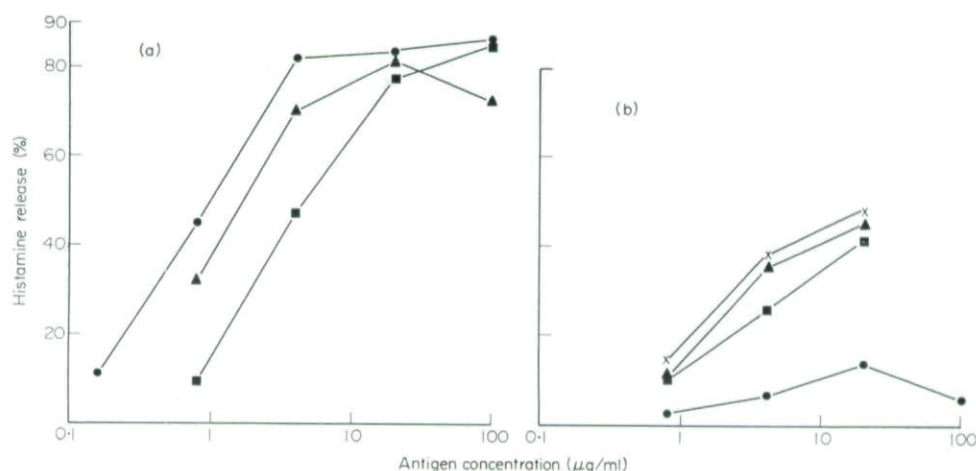


Fig. 7. Effect of serum (10%) on the antigen-leucocyte challenge test. (a) In this patient a 'blocking' antibody effect is seen. The presence of the patient's own serum, particularly after hyposensitization, has produced a decrease in leucocyte histamine release; the antigen dose-response curve is shifted to the right. (b) In this patient a potentiation of leucocyte response is seen. The presence of normal serum and of the patient's own serum, both before and after hyposensitization, has in each case produced an increase in leucocyte histamine release; the antigen dose-response curve is shifted to the left. ●, Antigen alone; ▲, serum before hyposensitization (with antigen); ■, serum after hyposensitization (with antigen); ×, normal serum (with antigen).

two different effects in opposite directions; the blocking effect shifting the antigen dose-response curve to the right, i.e. towards a higher concentration of antigen (Fig. 7a) and the non-specific effect shifting the curve to the left (towards a lower concentration of antigen). In one patient with weak leucocyte response to antigen alone, serum produced marked potentiation (Fig. 7b).

Serum IgG. The total serum IgG was estimated at Points A and C. There was no significant difference in the levels, all being within the normal range at both times (9–17, mean 13 mg/ml).

Discussion

This investigation has shown that hyposensitization therapy has produced significant changes both in respect of the clinical status as well as in the various tests applied. These findings are summarized in Table 1. The improvement in the clinical state was indicated by symptomatic improvement and a reduction of the need for bronchodilator drugs. More than half of the patients became symptom-free and thus stopped taking bronchodilators.

The bronchial challenge test showed a marked increase in the mean threshold dose of the mite extract, i.e. an increased tolerance. Since this test appears to be the most relevant parameter in allergic asthma, the finding of an increased tolerance to antigen provides objective evidence in support of the symptomatic improvement.

By contrast, both skin and nasal tests did not show evidence of hyposensitization. These differences between the various *in vivo* tests are difficult to explain. Among the various possibilities, two seem likely:

(1) Hyposensitization therapy may produce changes in the affinity of tissue-sensitizing antibodies for the various target tissues (lung, skin and nasal mucosa).

(2) Repeated bronchial challenge testing may produce local desensitization. However, precautions were taken to avoid this happening. According to our previous experience, bronchial desensitization does not occur provided that the tests are carried out at intervals of not less than 7 days and that the challenge dose is increased by not less than 100% at each session.

Confirmation of this conclusion is provided in the series by the five control mite sensitive patients who were subjected to a similar procedure of bronchial challenge as the treated patients and who did not show any evidence of local desensitization.

In contrast to the development of bronchial tolerance and to the clinical improvement, some of the *in vitro* tests seem to provide evidence of hypersensitization. The leucocyte test, for example, clearly showed increased sensitization to the mite antigen (Fig. 5b). Both total serum IgE and the leucocyte 'reversed' reaction to anti-IgE showed an increase following therapy and this also suggests increased sensitization (Figs. 3 and 4). However, the latter two parameters, which are non-specific, may not necessarily be associated with an increase in allergen-specific IgE. The increase in total serum IgE could be almost totally due to non-allergen specific molecules. Had there been no evidence of increased leucocyte sensitization to allergen, one might have been tempted to speculate that the increase in non-specific IgE may increase the competition with allergen-specific IgE for binding to basophil leucocytes and to tissue mast cells, thus raising the possibility of yet another mechanism for desensitization.

The serum reaginic activity as measured by passive sensitization of human lung did not show any change in the group as a whole following hyposensitization therapy. Thus, the result of this test did not correlate with the change in leucocyte sensitization, again suggesting a difference in affinity of sensitizing antibodies for these tissues.

Since there was no change in serum reaginic activity in the *in vitro* lung test despite an increase in total serum IgE, we were interested to know if this increase could nevertheless produce a greater release of histamine when passively sensitized lung was challenged with anti-IgE. Sera from two patients were tested in this way and an increase in histamine release with anti-IgE was in fact obtained in the sample taken after hyposensitization treatment. The increase in both the serum IgE and in the histamine release by anti-IgE in the absence of any increase in histamine release by antigen provides strong evidence of an increase in non-specific IgE.

On the other hand, in the few patients where the serum reaginic activity was decreased when the leucocyte sensitivity was increased, this paradox suggests an alteration in the relative affinity of reagins for these two tissues.

The development or increase in serum haemagglutinins after hyposensitization therapy is usually taken as an indication of development of non-sensitizing allergen specific antibodies, which is probably not absolutely true. It has also been assumed that these antibodies compete with cell fixed reagins (mainly IgE) for binding the

allergen, thus producing a 'blocking' effect. A blocking effect was seen in our assay of these antibodies in the leucocyte test, though this was partly concealed by the non-specific potentiating effect of serum. The shift to the right of the antigen dose-response curve in this test (Fig. 7), which was taken as an indication of 'blocking' antibodies, could, however, be produced by antibodies of any class, including IgE antibodies which have not been 'fixed' to basophil leucocytes.

Changes in various *in vitro* tests after hyposensitization have been reported by several authors. It has been shown that repeated injection of pollen allergen may produce one or more of the following effects:

(1) Stimulation of the production of blocking antibodies (Loveless, 1940; Lichtenstein *et al.*, 1966).

(2) Reduction in serum reaginic activity as detected either by the Prausnitz-Küstner reaction (Sherman, Stull & Cooke, 1940; Connell & Sherman, 1964, 1969; Melam *et al.*, 1971), or by the passive sensitization of human leucocytes (Levy *et al.*, 1971).

(3) Reduction in the histamine release from leucocytes induced either by antigen or by anti-IgE (Van Arsdel & Middleton, 1961; Pruzansky & Patterson, 1968; Sadan *et al.*, 1969; Levy *et al.*, 1971).

(4) Reduction in lymphoblast transformation by allergen (Brostoff & Roitt, 1970).

In our investigation we employed a different antigen, and though we achieved similar clinical results as in these previous reports, we did not find evidence of reduction in sensitization in the *in vitro* tests. On the contrary, leucocyte response to antigen and anti-IgE was increased in nearly all patients. This, however, was not associated with an increase in the ability of serum to sensitize human lung passively. In fact, two patients showed marked reduction in serum reaginic activity, and three others showed slight reduction. This reduction in serum reaginic activity in some patients may suggest that following therapy newly formed reagins may have a lower affinity for lung tissue, and may possibly explain the increased bronchial tolerance to antigen. The increase in leucocyte sensitization may thus have no bearing on bronchial sensitivity. This apparent paradox is not different from the frequent occurrence of bronchial desensitization following immunotherapy in the absence of any changes in skin sensitivity.

The reduction in leucocyte sensitization which has previously been reported by several authors was obtained by injections of relatively large amounts of antigen, and, as pointed out by May, Schumacher & Williams (1972), may simply reflect a gradual occupation by the allergen of the antigen-binding sites of the basophil-fixed IgE. The same authors have shown that the reduction in leucocyte responsiveness to allergen may also produce a concordant reduction in leucocyte sensitivity to an unrelated allergen.

The present study and previous investigations suggest that apart from the effect of blocking antibodies three other possible mechanisms for the effect of hyposensitization may exist.

(1) Reduction in cell reactivity (mast cell and basophil leucocytes) to allergen induced by the occupation of allergen-binding sites of cell-fixed reagins. This effect is more likely with immunotherapy using large amounts of antigen.

(2) Alteration in affinity of reaginic antibodies for various target tissues.

(3) It is also possible that a substantial increase in non-specific IgE, induced by

hyposensitization therapy, may, at least in some tissues, interfere with sensitization by allergen-specific IgE.

In the majority of reports which have been published on the clinical effects of hyposensitization therapy, objective clinical criteria have been lacking. It has been shown, however, that in pollen allergic asthmatics such treatment can produce significant increases in bronchial tolerance (McAllen, 1961; McAllen, Heaf & McInroy, 1967), but similar increases were not found after hyposensitization with a crude house dust extract (McAllen, 1961).

The present study shows an increase in bronchial tolerance and a significant clinical improvement following hyposensitization with an extract of *D. pteronyssinus* in patients with house dust mite asthma. It also shows changes on five of the other ten criteria which have been examined. It is difficult to interpret these changes in the light of the clinical results, but our findings suggest some hitherto unrecognized factors which may be concerned in the mechanism of hyposensitization.

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References

- ASSEM, E.S.K. & McALLEN, M.K. (1970) Serum reagins and leucocyte response in patients with house-dust mite allergy. *British Medical Journal*, **ii**, 504.
- ASSEM, E.S.K. & SCHILD, H.O. (1968) Detection of allergy to penicillin and other antigens by *in vitro* passive sensitization and histamine release from human and monkey lung. *British Medical Journal*, **iii**, 272.
- ASSEM, E.S.K., TURNER WARWICK, M., COLE, P. & SHAW, K.M. (1971) Reversed anaphylactic reaction of leucocytes in intrinsic asthma. *Clinical Allergy*, **1**, 352.
- BRITISH TUBERCULOSIS ASSOCIATION (1968) Report of Research Committee on 'Treatment of house dust allergy'. *British Medical Journal*, **iii**, 774.
- BROSTOFF, J. & ROITT, I.M. (1970) Induction of tolerance to grass pollen in patients with summer hay fever. *Excerpta Medica International Congress Series*, **211**, 136.
- CONNELL, J.T. & SHERMAN, W.B. (1964) Skin-sensitizing antibody titer. III. Relationship of skin-sensitizing antibody titer to the intracutaneous skin test, to the tolerance of injections of antigens, and to the effects of prolonged treatment with antigen. *Journal of Allergy*, **35**, 169.
- CONNELL, J.T. & SHERMAN, W.B. (1969) Changes in skin sensitizing antibody titer after injections of aqueous pollen extract. *Journal of Allergy*, **43**, 22.
- FULTHORPE, A.J., ROITT, I.M., DONIACH, D. & COUCHMAN, K.G. (1961) A stable sheep cell preparation for detecting thyroglobulin auto-antibodies and its clinical applications. *Journal of Clinical Pathology*, **14**, 654.
- HERXHEIMER, H. (1951) Induced asthma in man. *Lancet*, **i**, 1337.
- JOHANSSON, S.G.O. (1967) Raised levels of a new immunoglobulin class (IgND) in asthma. *Lancet*, **ii**, 951.
- LEVY, D.A., LICHTENSTEIN, L.M., GOLDSTEIN, E.O. & ISHIZAKA, K. (1971) Immunologic and cellular changes accompanying the therapy of pollen allergy. *Journal of Clinical Investigation*, **50**, 360.
- LICHTENSTEIN, L.M., NORMAN, P.S., WINKENWERDER, W.L. & OSLER, A.G. (1966) *In vitro* studies of human ragweed allergy: Changes in cellular and humoral activity associated with specific desensitization. *Journal of Clinical Investigation*, **45**, 1126.
- LOVELESS, M.H. (1940) Immunological studies of pollinosis. I. The presence of two antibodies related

- to the same pollen antigen in the serum of treated hay fever patients. *Journal of Immunology*, **38**, 25.
- MAUNSELL, K., WRAITH, D.G. & HUGHES, A.M. (1971) Hyposensitization in mite asthma. *Lancet*, **i**, 967.
- MAY, C.D., SCHUMACHER, M.J. & WILLIAMS, C.S. (1972) Significance of concordant fluctuation and loss of leucocyte sensitivity to two allergens during injection therapy with one non-specific desensitization. *Journal of Allergy*, **50**, 99.
- MCALLEN, M.K. (1961) Bronchial sensitivity testing in asthma. *Thorax*, **16**, 30.
- MCALLEN, M.K. & ASSEM, E.S.K. (1970) Observations on challenge testing and hyposensitization in asthma due to house dust mite allergy. *Excerpta Medica International Congress Series*, **211**, 4.
- MCALLEN, M.K., ASSEM, E.S.K. & MAUNSELL, K. (1970) House dust mite asthma: Results of challenge tests on five criteria with *Dermatophagoides pteronyssinus*. *British Medical Journal*, **ii**, 501.
- MCALLEN, M., HEAF, P.J.D. & MCINROY, P. (1967) Depot grass pollen injections in asthma: Effect of repeated treatment on clinical response and measured bronchial sensitivity. *British Medical Journal*, **i**, 22.
- MELAM, H., PRUZANSKY, J., PATTERSON, R. & SINGER, S. (1971) Clinical and immunologic studies of ragweed immunotherapy. *Journal of Allergy*, **47**, 262.
- OUCHTERLONY, O. (1967) Immunodiffusion and immunoelectrophoresis. *Handbook of Experimental Immunology* (Ed. by D. M. Weir), p. 655. Blackwell Scientific Publications, Oxford.
- PRUZANSKY, J.J. & PATTERSON, R. (1968) Immunologic changes during hyposensitization therapy. *Journal of the American Medical Association*, **203**, 805.
- SADAN, N., RHYNE, M.B., MELLITS, E.D., GOLDSTEIN, E.O., LEVY, D.A. & LICHTENSTEIN, L.M. (1969) Immunotherapy of pollinosis in children: Investigation of immunologic basis of clinical improvement. *New England Journal of Medicine*, **280**, 623.
- SHERMAN, W.B., STULL, A. & COOKE, R.A. (1940) Serologic changes in hay fever cases treated over a period of years. *Journal of Allergy*, **11**, 225.
- SMITH, A.P. (1971) Hyposensitization with *Dermatophagoides pteronyssinus* antigen: Trial in asthma induced by house dust. *British Medical Journal*, **iv**, 204.
- SQUIRE, J.R. (1950) The relationship between horse dandruff and horse serum antigens in asthma. *Clinical Science*, **9**, 127.
- VAN ARSDEL, P.P., JR & MIDDLETON, E., JR (1961) The effect of hyposensitization on the *in vitro* histamine release by specific antigen. *Journal of Allergy*, **32**, 348.

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