

IgE, IgG and patient-self tests during slow hyposensitization to grass pollen

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Summary

Twenty-two patients with severe hay fever unrelieved by antihistamines in previous years were slowly hyposensitized with alum-precipitated pyridine grass pollen extracts. Their responses were objectively assessed as very good (55%), good (18%) or improved (23%). Two mechanisms were detected: (i) an increase in serum blocking activity in 59% and (ii) the prevention in 59% of the serum IgE elevation expected in the pollen season.

Introduction

Hyposensitization for hay fever, with grass pollen extracts, is a reliable means of minimizing symptoms and avoiding secondary complications particularly pollen asthma. It consists of a graduated series of preseasonal injections so arranged for the final dose to be administered 2–3 weeks before the patient symptoms are expected to cause trouble. Prausnitz (1902) discovered that such injections could produce severe constitutional reactions (Brown, 1958).

Noon, from 1909, used aqueous pollen extracts and by 1911 was treating his hay fever patients with weekly injections pre-seasonally; these were weekly—as this was more convenient for both the patient and the administrative staff of the hospital—the number of injections varied from eighteen to twenty-four in the average course (Noon, 1911). With Noon's illness and premature death, Freeman further developed the technique and by 1926 many of his patients were self-administering their injections pre-seasonally (fifty-four injections) (Freeman, 1911, 1950). Various techniques were developed both in the timing of the hyposensitizing dose (Bray, 1936: see Freeman, 1950—the Rush method) and in the nature of the extracts in order to reduce the inconvenience of multiple injections.

Loveless (1940, 1947) used emulsified allergens initially in one and subsequently three injections. Brown (1958) further improved this technique.

Repository injections first became commercially available in the United Kingdom in 1962. In this method necessitating three injections at monthly intervals slow release of the allergen was made possible by using a specially designed syringe, 0.7 ml

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of an inactive shield emulsion being separated by a diaphragm from 0.3 ml of an active allergic emulsion. This was associated occasionally with unpleasant local and constitutional effects and is not recommended for general practitioner use (Blair, 1968). There are also available alum-precipitated pyridine extracts (Fuchs & Strauss, 1959) and extracts adsorbed by a L-tyrosine base (Overell, 1971).

Methods

Selection of patients

The twenty-two patients selected were from the Children's Department of the Prince of Wales's General Hospital, London, N.15 (eight) and from a group practice in East London (fourteen). Nine were female, thirteen were male and their ages ranged from 9–51 years.

The diagnosis was made by a history of seasonal rhinitis and confirmed by prick skin tests and nasal provocation tests with grass pollen extracts. Those patients whose hay fever responded to antihistamines in 1971 were excluded as also were pregnant patients. A history of previous hyposensitization did not automatically exclude the patient providing there had been at least 1 year free from hyposensitization with no response of their hay fever to antihistamines in that year.

The indications of hyposensitization were:

- (1) pollen asthma whether or not this responded to disodium cromoglycate (fifteen patients);
- (2) severe nasal or eye symptoms unresponsive to antihistamines (two patients);
- (3) where the occupation rendered the administration of antihistamines dangerous (bus, lorry, tractor or crane drivers, or those working at heights) (three patients);
- (4) agricultural farm workers (one patient);
- (5) students sitting examinations in the hay fever season (one patient).

Hyposensitization

The hyposensitizing extract used was the commercial preparation Alavac-P made by Bencards Ltd. This is supplied in three multidose vials of allergen in graded strengths, Vial 1 250 noon units/ml, Vial 2 2500 noon units/ml, Vial 3 25,000 noon units/ml and contained twelve grasses (Timothy grass, cocksfoot, Yorkshire fog, bent, broome, dogtail, false oat, wild oat, fescue, foxtail, meadow rye and vernal). It is a pyridine extract of grass pollen precipitated with alum and resuspended in normal saline (0.8%) with 0.4% w/v phenol as preservative.

The course comprises nine injections of increasing strength, planned from the last week of February to finish early in May. After the ninth injection a nasal provocation test with grass pollen was performed to ascertain whether sufficient hyposensitizing extract had been administered. In each case the nasal provocation test was negative—in consequence no additional hyposensitization for that season was required.

The initial dose was 0.1 ml Vial 1 (25 noon units); the patient rested for 30 min after each dose. The subsequent doses were increased regularly provided there were no local or general reactions. If a reaction occurred the subsequent dose was maintained at the same level until no reaction occurred and then the dosage increased.

Patients' assessment of response to hyposensitization

Hay fever is a disease of variable severity in England because of the varying pollen

challenge due to the changeable climate. Subjective assessment is dependent on the patient's memory.

In order to obtain more objective results each patient recorded their eyes, nose and chest symptoms daily on a special score card (no symptoms scored 0, mild symptoms 1, moderate symptoms 2, severe symptoms 3), and also the number of antihistamine and/or bronchodilator tablets necessary to maintain relief from symptoms (Blair & Herbert, 1972, 1973).

Clinician's assessment

Responses were classified as:

- (1) Very good—no hay fever symptoms and no treatment required.
- (2) Good—mild hay fever symptoms, occasionally, for less than 10 days and requiring no treatment.
- (3) Improved—symptoms moderate and relieved by antihistamines.
- (4) Poor—symptoms severe, not relieved by antihistamines for 10 or more days in the season—or time lost from school or work because of hay fever.

Serum immunoglobulins

Sera were collected before and 2 weeks after hyposensitization. Serum IgE was measured by a radioimmunosorbent test (RIST) as previously described (Manners & Ezeoke, 1973). Serum IgG was kindly measured by Miss Linda Slater. The coefficients of variation between batches were for IgE ($\pm 10.2\%$) and for IgG ($\pm 4\%$) (Slater, 1975), so that a change of twice this value would be clearly significant.

Patient-self tests

These were done as described by Maunsell (1945) and Munro-Ashmann, McEwen & Feinberg (1971). Five-fold serial dilutions of sera immediately before and 2 weeks after hyposensitization were incubated with equal parts of aqueous grass pollen extract (200 noon units/ml) overnight at room temperature. (Dilution 1 contained 40 noon units; dilution 5, 8 noon units; dilution 25, 1.6 noon units; dilution 125, 0.32 noon units and dilution 625, 0.06 noon units.)

The two series of serum pollen mixtures were then injected intermittently in parallel vertical columns on the patient's own back, the pre-injection serum dilution on the left (B) and the post injection serum dilution on the right (A) using 0.02 ml of the mixture per injection site. Local diameters were measured and recorded. From the dilution end point in the B and A series a P-S score (equivalent to the increase of 'blocking antibodies') was calculated.

Results

Allergic status of patients

All were grass-pollen prick-test positive, the size of the weals ranging from 8 to 25 mm as measured by plastic gauge. There was a positive family history of 'allergic' disease (hay fever, asthma, perennial rhinitis, infantile eczema) in seven patients each of whom also had pollen asthma. Of the twenty-two patients, fifteen had associated asthma, six had infantile eczema (each of whom also had pollen asthma) and three had perennial rhinitis.

Table 1. Analysis of results of three patients who had required corticosteroids for their hay fever in 1971

No.	Patient			1971 Hay fever season		1972 Hay fever season	
	Initials	Age	Sex	Prednisone dosage	DSCG spincap dosage	Prednisone dosage	DSCG spincap dosage
11	D.F.	10	M	10 mg daily for 2 months	Four spincaps daily throughout the season	10 mg daily for 2 weeks	Four spincaps daily throughout the season
12	N.S.	13	M	10 mg daily for 1 month		5 mg daily for 1 week	
15	B.L.	51	M	15 mg daily for 1 week 10 mg daily for 3 weeks		Nil	Nil

Table 2. Clinical and laboratory assessments of responses to hyposensitization

Patient			Responses		Change in total serum IgE with the pollen season	Patient-self (P-S) test score (increase in dilution)
No.	Sex	Age	Subjective average daily score	Clinical		
1	M	11	0	Very good	→	125
2	M	15	0	Very good	→	1
3	F	13	2	Improved	↑	5
4	F	20	2	Improved	→	1
5	F	48	0	Very good	→	5
6	M	29	2	Improved	→	1
7	M	9	1	Good	→	1
8	M	27	0	Very good	↓	1
9	M	16	0	Very good	↑	1
10	M	30	0	Very good	↑	25
11	M	10	3	Poor	↑	25
12	F	14	1	Good	↑	5
13	M	10	2	Improved	→	1
14	F	10	0	Very good	↑	25
15	M	51	0	Very good	→	125
16	M	10	0	Very good	→	125
17	F	32	1	Good	↓	1
18	F	15	0	Very good	↓	25
19	F	27	1	Good	↑	125
20	M	23	0	Very good	↑	25
21	F	13	0	Very good	↑	125
22	M	13	2	Improved	↓	1

All the patients in this series had severe hay fever unrelieved by antihistamines in previous years.

Clinical responses to hyposensitization

No local or general reactions occurred with any of the doses of Alavac-P in any of the twenty-two patients. Nasal provocation tests with grass pollen extracts at the end of the course were negative in all the patients. The results are shown in Tables 1 and 2.

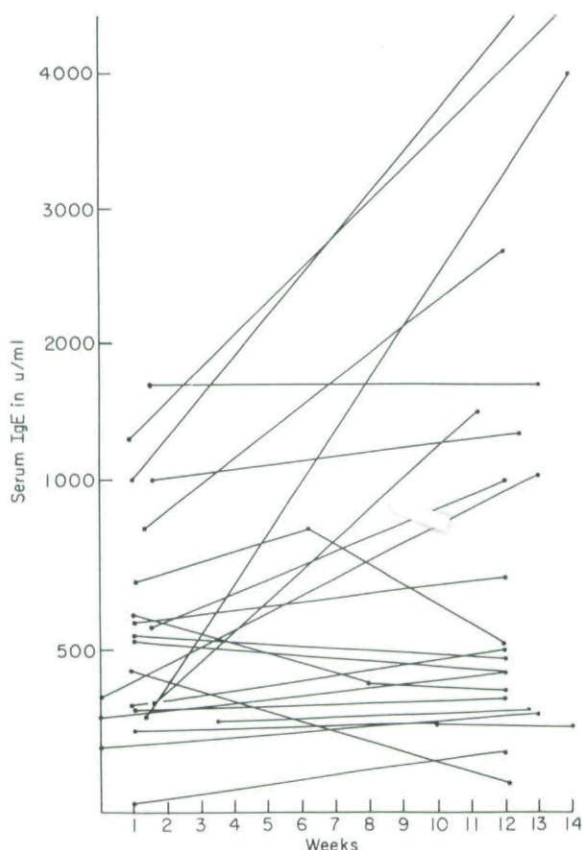


Fig. 1. Changes in serum total IgE levels of twenty-two patients from 'before' and 'during' hyposensitization to 'in' the pollen season.

In twenty patients there was good agreement between the patients' subjective and the clinician's assessments, and only in patients 4 and 6 were these significantly discrepant. Twelve (55%) were completely relieved by a very good clinical response, four (18%) had a good clinical response and five (23%) were improved with only mild symptoms readily controlled by antihistamines. Only one patient (11) was as bad as in the previous year. In the two patients, 4 and 6, the subjective results were poor although clinically it was judged that there had been an improvement as compared with their symptoms in 1971. In 1971 disodium cromoglycate (DSCG) was required for the control of pollen asthma in seven patients (32%), in all of whom it was not required in 1972. Three patients required steroid therapy for the relief of the

hay fever in 1971; in 1972 one was able to do without it and the other two required less, and for shorter periods (see Table 1).

Laboratory responses to hyposensitization

Using patient-self tests, a significant increase in blocking antibodies was inferred by detecting at least a five-fold increase in dilution in thirteen patients, despite the increase in IgE levels in nine of these patients (3, 10, 11, 12, 14, 18, 19, 20, 21). It can be seen in Table 2 and Fig. 1 that the total serum IgE of the patients showed no significant change ($\pm 20\%$ of initial value) in the pollen season in nine, actually fell in four and rose in nine. Total serum IgG (see Fig. 2) showed no significant change ($\pm 8\%$ of initial value) in sixteen patients, rose in four and fell in two.

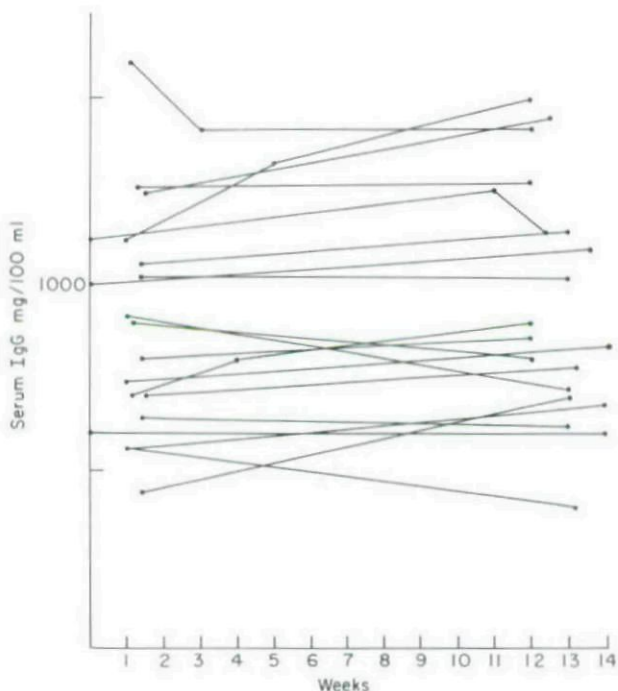


Fig. 2. Changes in serum total IgG levels from 'before' and 'during' hyposensitization to 'in' the pollen season (complete data are only shown for nineteen patients).

Discussion

Of the thirteen patients (59%) showing an increased score in patient-self tests, twelve derived benefit from the presumed increase in blocking antibodies, despite seven of these also showing a rise of IgE. Only patient 11 failed to improve, possibly because his increase in IgE overruled the increase in P-S score, or other factors contributed to the persistence of his attacks of pollen-season asthma.

There were then five patients with very good or good responses who did not show a five-fold increase in P-S score. Patient 9 showed a two-fold increase in his total IgE, possibly overruled by a less than five-fold increase in blocking activity. The other four, however, either showed no increase in IgE (2, 7) or a fall in IgE (8, 17), and these are remarkable findings in the pollen season. Presumably as a result of hyposensitiza-

tion, thirteen patients did not show the high IgE levels expected of them in the pollen season: Manners & Ezeoke (1973) found this occurred in 86% of forty-four subjects with seasonal allergic rhinitis. Only nine of the present patients (41%) showed an increased IgE, with only seven (32%) above 1000 iu. This is obviously a highly significant reduction ($P < 0.01$) in high IgE levels for what was in fact the same pollen season (1972) for the two groups studied.

It is interesting to note that the remaining four patients (4, 6, 13, 22) all showed no increase in IgE even though the P-S score did not increase five-fold. These four were rated clinically as improved, though for patients 4, 6 this was in their own opinion only marginal. Patient 4 had moved to alongside Epping Forest for the season and received a much higher challenge than ever before.

It can therefore be said that a rise in P-S score or the absence of the expected seasonal rise in IgE could account for the satisfactory responses in twenty of the twenty-one patients, patient 9 being the one exception (and patient 11 remaining unimproved). This could indicate that hyposensitization could achieve its effect in two ways.

The first is the obvious production of enough blocking antibodies to show up in P-S tests, and their persistence during the pollen season. The other [as possibly evidenced in thirteen patients (59%), numbers 1, 2, 4, 5, 6, 7, 8, 13, 14, 15, 16, 17, 22] could be by inducing not an obvious titre of antibodies capable of blocking (especially in patients 2, 4, 6, 7, 8, 13, 17, 22), but rather immune response capable of subsequently diverting allergens away from IgE responses in the pollen season. Such immune responses could be 'tagging' antibodies with insufficient avidity to 'block', but resulting in Fc-activation capable of leading to phagocytosis of allergens. Other possibilities are of responses modulating T- and B-lymphocyte interaction, so that IgE cells are not stimulated.

With normal serum total IgG levels so high, and the variance ($\pm 4\%$) of even the best current method for their measurement, it is not surprising that few differences were observed. The four increases were one to each clinical grade, so that no significance can be inferred. It can be concluded that total IgG was not worth doing.

In future studies it might be of advantage to use the RAST approach (Wide, Bennich & Johansson, 1967) to assess not only the changes in pollen-specific IgE but also pollen-specific antibodies in other immunoglobulin classes.

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