

Species specific grass pollen sensitivity: diagnosis and treatment with single grass species Allpyral vaccines

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Summary

Skin test titrations and nasal provocation tests in sixty patients with hay fever showed specific reactions to extracts of individual grass species. There was, however, no correlation between skin and nasal sensitivity. Repeat testing after treatment with Allpyral vaccines consisting of only the grass species to which the nasal reaction was most severe, or only one of several pollens to which reactions were equally severe, showed marked diminution of skin and nasal sensitivity not only to the single pollen used for immuno-therapy but to all five common pollens used in the Allpyral grass mix. Clinical results seemed much improved as compared with results in the same year for Allpyral five grass mix vaccines, especially in the case of patients treated with Timothy, rye, or cocksfoot. It was concluded that these three grasses were to be preferred for treatment in England, and that these grasses contain common allergens.

Introduction

Previous investigations at this clinic (Brown, 1965) revealed that species specific nasal test responses to challenge with grass pollen extracts do occur, and that the frequency of responses to the common grasses is greater than to uncommon species. It was for this reason that Allpyral five grass mix was preferred for treatment, as described by Brown & McIntyre (1977) but the results of treatment using conventional dosage schedules were poor.

It was postulated that if only one species of grass pollen, to which the patient was shown to be sensitive by nasal challenge, was used in treatment, then the clinical result, using conventional dosages only, might be improved.

Materials and Methods

Case selection

An appeal for volunteers resulted in the selection of sixty previously untreated cases of

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hay fever, sensitive only to grass pollen, and aged from 16–70 years, twenty-one being female and thirty-nine male.

Methods of investigation

Using species grass pollen extracts, made by our rapid method (Brown & Thantrey, 1976), the sensitivity of each patient to each of the five main pollens, *Phleum pratense* (Timothy), *Lolium perenne* (rye), *Dactylis glomerata* (cocksfoot or orchard), *Holcus lanatus* (Yorkshire fog), and *Festuca pratensis* (meadow) was determined by nasal and skin tests. For skin test titration each of the five grass pollen extracts was diluted to represent that obtained from 400, 80, 40, 20, 10, 2, and 1 μ g of pollen per ml (Noon units) respectively. Each patient was titrated to these pollens on the skin of the back, using the standardised prick testing needle developed in Derby (Brown, Su & Thantrey, in press), and serum samples taken.

Nasal provocation tests were carried out using 0.05 ml of pollen extract containing 10 or 25 Noon weight/volume units of each grass, sealed in a thin plastic tube. The patient carried out these tests at home by cutting off both ends of the tube, sniffing the extract up one nostril, and recording the results on a special form. The results were recorded as $\pm$ for sneezing for a few minutes only, + for sneezing lasting an hour or more, and ++ when they wheezed as well.

Treatment

The choice of grass pollen species for treatment was made by selecting the one to which the nasal test reaction was the strongest. If several pollens gave strongly positive reactions, then a random choice of only one of these was made. The patients then received nine weekly injections of the selected single grass pollen Allpyral vaccine, reaching a top dose of 10,000 PNU, using the makers' recommended dosages for mildly sensitive patients, after which skin and provocation tests were repeated and a serum sample taken.

All patients kept a Symptoms & Treatment Chart during the season. Excellent correlations with grass pollen counts can be obtained using this chart. Daily pollen counts were carried out, as is usual at this centre.

All patients were interviewed for a third time after the season, nasal tests and skin test titrations repeated, and a third serum sample obtained for immunological studies.

Fifty patients who had been routinely treated with the standard Allpyral five grass mix on the same dosage schedule in the same year also kept charts, and provided a control group.

Results

The results of pre-treatment skin test titrations disclosed marked differences between different pollens on the same individual, but against the 40 and 20 μ g/ml strengths almost all patients had a positive reaction. Post-treatment the skin reactions were diminished to *all* the grass pollens even though the patient had been treated with only one. After the season further non specific diminution of the skin reactions was found.

Results for two individual patients are featured in Table 1 showing examples of the type of result observed. In the case of H.M., skin sensitivity was shown to all five grasses before treatment, but the skin responded in a non specific way after treatment and after the season. The nasal tests were positive for all pollens until after the season,

Table 1. Results for two individual patients (H.M. and C.B.) treated with Phleum

	Pre-treatment concentrations					Post-treatment concentrations					Post-season concentrations				
	40	20	10	2		40	20	10	2		40	20	10	2	
Skin tests															
Phleum	+	+	+	-		±	-	-	-		-	-	-	-	-
Lolium	+	+	±	-		+	+	+	-		+	-	-	-	-
Dactylis	+	+	±	-		+	±	-	-		±	±	-	-	-
Holcus	+	+	±	-		+	+	-	-		-	-	-	-	-
Fescue	+	+	+	±		±	-	-	-		-	-	-	-	-
			10					25					25		
Nasal tests															
Phleum			+					+					-		-
Lolium			+					+					-		-
Dactylis			+					+					-		-
Holcus			-					±					-		-
Fescue			-					+					-		-

H.M. was treated with *Phleum* Allpyral only. Both skin and nasal sensitivity decreased to all five pollens after treatment and after the season, showing lack of specificity.

In the case of C.B. both skin and nasal challenge suggested that *Phleum* was the best pollen to use. The nasal tests show remarkable specificity.

yet the use of Timothy (Phleum), alone for treatment clearly had a non specific effect, and the clinical result was excellent.

In the case of patient C.B. skin sensitivity was again not specific but the nasal sensitivity was highly specific. After treatment and after season skin reactions were markedly less, nasal tests were negative, and the clinical result was excellent.

The case of D.A. (Table 2) is particularly interesting as he came into the trial rather late, so that when the skin tests were done a quick decision had to be made regarding which pollen to choose. As rye (Lolium) showed the greatest skin sensitivity this grass was chosen, but later the nasal tests were found positive for Dactylis, Holcus and Festuca only. Instead of being a failure of treatment this case was one of the most successful ones, nasal tests being all negative after treatment and also skin sensitivity was non-specifically reduced.

Clinical results

After the season the results were assessed and classified using the symptoms and treatment charts which had been kept by all patients. We recorded 'excellent' results for patients who had no symptoms or only a sniffle at the peaks of the pollen count, and who had taken no anti-histamine or other treatment. 'Moderate improvement' was defined as definite hay fever symptoms, sometimes requiring drug treatment, but improved on previous years. The rest were classed as failures, especially those who had required oral steroids for a short period. We felt that it was important to assess results strictly, as we had done in our previous work (Brown & McIntyre, 1977).

The results for each pollen are shown separately in Fig. 1 where it is obvious that really good results were obtained mainly from patients who had received Phleum,

Table 2. Results for an individual patient (D.A.) treated with Lolium

	Pre-treatment concentration				Post-treatment concentration				Post-season concentration			
	40	20	10	2	40	20	10	2	40	20	10	2
Skin tests												
Phleum	—	—	—	—	—	—	—	—	—	—	—	—
Lolium	+	+	+	±	+	—	—	—	—	—	—	—
Dactylis	+	+	+	—	±	—	—	—	—	—	—	—
Holcus	+	+	+	—	+	—	—	—	—	—	—	—
Fescue	+	±	—	—	±	—	—	—	—	—	—	—
				10				25				25
Nasal tests												
Phleum				—				—				—
Lolium				—				—				—
Dactylis				++				—				—
Holcus				++				—				—
Fescue				+				—				—

The decision to use Lolium Allpyral was made on the results of skin testing, this case being a late entry into the trial. Challenge tests were negative for Lolium and Phleum only, yet treatment with Lolium alone produced demonstrable desensitisation to all five pollens.

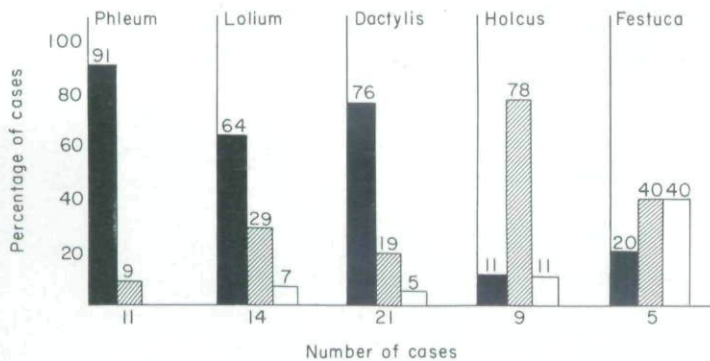


Fig. 1. Success rate of treatment with single grass Allpyral. ■, No or slight symptoms; ▨, symptoms, but improved; □, no better or worse. The results, using strict criteria as above, suggest that only the use of *Phleum*, *Lolium* and *Dactylis* seems to be worthwhile.

Lolium and *Dactylis*. Only fourteen received *Holcus* or *Festuca*, with poor results. The combined results of all cases give 52% excellent, 35% moderate, and 13% failures, but if *Holcus* and *Festuca* are excluded the figures become 77%, 19% and 4% respectively. The results of our previous trial of Allpyral five grass mix (Brown & McIntyre, 1977) provides an interesting contrast.

In this previous trial the results of using the same dosage schedules had been poor, but were greatly improved by administering from four to ten extra weekly doses of 10,000 PNU until the nasal test with dry pollen became negative. In the present trial, however, no extra injections were given to either group, yet the results are much better, especially if only the three main pollens are considered.

The patients received the same treatment for a second year in 1977. 44% had even lower symptom scores than in 1976, 31% were the same, and 8% were higher.

Discussion

This work has confirmed our earlier observations (Brown, 1965) that many patients are sensitive only to certain species of grass pollen, as shown by direct nasal challenge. In this previous investigation the dose of pollen used for nasal testing had been excessively large, thus making a negative response more significant. On this occasion the doses may have again been excessive, because the clinical results were still good in spite of the persistence of a positive nasal reaction after treatment in many cases.

A possible explanation of the good results could have been a low pollen count, but it so happened that in 1976, the first year of this trial, we experienced our worst pollen season since pollen and spore counts were begun here in 1967. (Fig. 2). The fifty patients who had been treated with the usual Five grass mix had a bad season in 1976. (Table 3)

In most patients nasal provocation tests were still positive post-treatment and also post-season, but the test dose may have been excessive. The nasal tests after treatment and after the season were two-and-a-half times the dose used before treatment, showing that the patients had become less sensitive if the reaction was no worse than before. Some patients did become nasal test negative to some of the grasses after treatment, and more became negative after the season. This was non specific and bore no relationship to the grass type with which they had been treated.

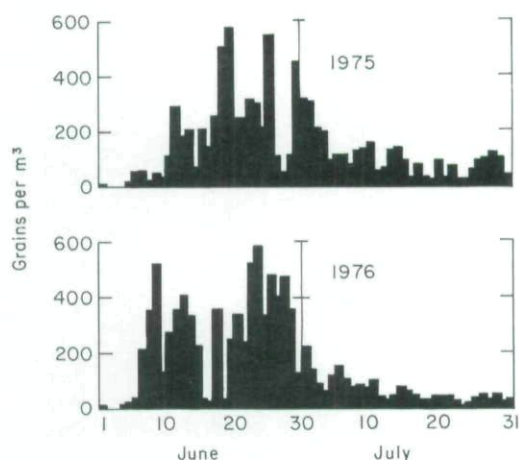


Fig. 2. The daily grass pollen counts in Derby for 1975 and 1976. The 1976 season was the worst for many years, and was also the first year of our trial. A low count would not account for the good results.

Table 3. Allpyral in treatment of hay fever

Mild dosage schedule (top dose 10,000 PNU)	Results			
	No.	Excellent (%)	Moderate (%)	Failure (%)
Five grass mix (Brown & McIntyre 1977)	188	27	33	40
Five grass mix 1976	50	20	26	54
Specific single pollens 1976	60	52	35	13
Specific single pollens 1977	50	60	28	12

Assessment: Excellent—no or slight symptoms; Moderate—symptoms but improved; Failure—no better or worse.

Skin test titrations using our standard needle have now shown that the skin also shows an individual pattern of species specific grass pollen sensitivity. It was also found that the skin did not correlate reliably with the nasal sensitivity to individual grass species, so the skin tests alone could not indicate the correct pollen species to use.

Treatment with single pollen Allpyral instead of a mixture, giving the pollen type to which there was the most intense nasal response, or one selected at random in the event of several equally positive reactions, appears to have achieved much improved clinical results as predicted. However, the surprise has been that the effects on both skin and nasal sensitivity of the use of a single species vaccine have been so remarkably non-specific.

Lolium (rye) grass is now the most popular hay grass in England and our findings suggest that it has become of great importance to hay fever subjects. On the results presented here it would seem logical to propose that a three grass mix should be used in this country, consisting of rye, Timothy, and cocksfoot only, and that the inclusion of less common grasses may dilute the mixture with irrelevant allergens. On the other hand our results could equally well suggest that a mixture is unnecessary, and that any one of the three major common pollens would be adequate for therapy.

The results of this investigation suggest that some of the major allergens of grass pollen are common to all species. Amongst clinical allergists there has always been argument regarding whether species specific allergens are important in hay fever, and serological studies as long ago as 1952 (Gosselin, Mynors & Coombs, 1953; Hoet, Mynors & Coombs, 1952), demonstrated common allergens in different pollens, and also an antigen mosaic characteristic for each pollen. In 1964 a survey (Milner & Tees, 1972) of skin test reactions to twelve grass pollens identified a group of patients (19%) who had from one to nine negative skin reactions using strong testing extracts. Follow up of these patients revealed good results from treatment with a mixture of equal parts of all twelve pollen extracts.

With the advent of the RAST technique extensive cross-reactivity has been demonstrated (Bernstein *et al.*, 1976). Four major allergens of Timothy pollen extracts have been isolated (Løwenstein, 1978a) and two of them shown to contain about 90% of the allergenic activity. Recently this work has been extended (Løwenstein, 1978b) to show that immunological partial identity exists between the two most important allergens of Timothy, and those of cocksfoot, rye and false oat (*Avena elatior*) grass pollen. Thus both protein analysis and immunological data support our clinical findings and our suggestion that these three pollens alone or together might be most suitable for treatment purposes.

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References

- BERNSTEIN, I.L., PERERA, M., GALLAGHER, J., MICHAEL, J. G. & JOHANSSON, S.G.O. (1976) *In vitro* cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *Journal of Allergy & Clinical Immunology*, **57**, 141.
- BROWN, H.M. (1965) Nasal provocation testing in asthma and rhinitis. *Proceedings of the World Asthma Conference, Eastbourne*. Chest & Heart Association, London.
- BROWN, H.M., SU, S. & THANTREY, N. A standardised prick testing needle. *Allergy*, in press.
- BROWN, H.M. & MCINTYRE, D. (1977) Immunotherapy for grass pollenosis with Allpyral allergen extracts: improved results by objective control of treatment requirements. *Acta allergologica*, **32**, 321.
- BROWN, H.M. & THANTREY, N. (1976) Rapid extraction of grass pollen allergens and separation of their active fractions. *Acta allergologica*, **31**, 22.
- gosSELIN, M.L., MYNORS, L.S. & COOMBS, R.R.A. (1953) Serological studies on pollen extracts. II. Demonstration by a serological method of the specificity of the antigen-mosaic of the extracts of six grass pollens. *Acta Allergologica*, **6**, 96.
- HOET, J.J., MYNORS, L.S. & COOMBS, R.R.A. (1952) Serological studies on pollen extracts. Demonstration of the specificity of the antigenic mosaic of pollen extracts and the estimation of the relative strengths of the serologically reactive components in extracts by means of inhibition tests. *Acta Allergologica*, **5**, 239.

- LØWENSTEIN, H. (1978a) Isolation and partial characterisation of four allergens of Timothy pollen. *Allergy*, **33**, 30.
- LØWENSTEIN, H. (1978b) Immunological partial identity and *in vitro* inhibitory effect of two major Timothy pollen allergens to whole pollen extract of four grasses. *International Archives of Allergy & Applied Immunology* **57**, 379.
- MILNER, F.H. & TEES, E.C. (1972) Specific sensitivity to individual pollens in some hay fever patients. *Clinical Allergy*, **2**, 83.

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