

Allergenic activity of fractions of cocksfoot (*Dactylis glomerata*) pollen

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Summary

A cocksfoot (*Dactylis glomerata*) pollen extract has been fractionated by isoelectric focusing in polyacrylamide gel, the separated components extracted from the gel and assayed by three methods: prick tests in human skin, RAST and PCA tests in monkey skin.

Prick testing in human skin showed that subjects responded differently to the separated components, indicating the presence of more than one antigenic determinant, and a profile of antibody activity against each fraction could be constructed.

In general, good agreement was observed between RAST profiles and skin test profiles, although some patients who gave positive skin tests lacked circulating IgE and consequently did not give a RAST profile or a monkey PCA.

These studies emphasize the current difficulty in attempting to isolate a single pure allergen from cocksfoot which can be used either diagnostically for the detection of pollen allergy, or for the standardization of diagnostic extracts.

Introduction

Recently, much work has been directed towards identifying major allergens from the complex mixture present in pollen extracts. For example, King & Norman (1962) and King, Norman & Connell (1964) isolated two major allergens from ragweed, designated antigens E and K, and subsequently two further allergens of lower molecular weight, Ra3 and Ra5, have also been isolated (Underdown & Goodfriend, 1969; Lapkoff & Goodfriend, 1974). These latter allergens only gave positive skin tests in a small proportion of ragweed-sensitive patients (Marsh *et al.*, 1973).

The importance of defining the allergenic components of pollen extracts is two-fold. Firstly, if a component can be chemically defined and is usually a consistent proportion of the total extract, standardization of extracts could be simplified. Secondly, the isolation of minor determinants, which may only give positive skin

tests in a small proportion of patients allergic to the whole extract, offers scope for investigating the genetics of immune responsiveness in atopy.

We have approached the problem of separation of the active components of complex extracts by isoelectric focusing in polyacrylamide gel, a technique which separates the components on the basis of isoelectric point, which is the resultant of their surface charges. The gels are then sliced into thirty fractions, and the biological activity of the components in each fraction is measured.

Materials and methods

Patients

Subjects suffering from summer hay fever were skin-tested to confirm their allergy. They were then tested with the thirty fractions from the isoelectric focusing gel. There were eight males, mean age 29 years, range 14–46, and four females, mean age 15 years, range 12–19. Seven normal subjects were also tested, mean age 27 years, range 18–42. Skin tests were read at 20 min, the weal outlined and transferred by transparent tape to paper as a permanent record.

Materials and methods

The components in the 1st British Standard extract of cocksfoot pollen (reference No. 75/506, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London) were separated by isoelectric focusing and a series of thirty fractions from gel slices were prepared in sterile Coca's solution containing 50% glycerol, for use in prick tests (Topping *et al.*, 1977). Patients were selected from those attending the Allergy Clinic at the Middlesex Hospital, London. They were prick-tested first with the whole extract, and those that gave a positive result were then prick-tested with the fractions.

The area of the weal size was measured 20 min after testing.

Passive cutaneous anaphylaxis (PCA) tests were performed in the skin of Rhesus monkeys, by the method described by Longbottom *et al.* (1976).

RAST profile analyses were performed as described by Ceska (1972).

Results

A densitometric trace of a stained gel, seen in Fig. 1, shows that the cocksfoot pollen extract contains about twenty peaks, separable by isoelectric focusing in polyacrylamide gel in a pH 3–10 gradient.

The allergenicity of the components in thirty fractions obtained from an isoelectric separation were assayed by three methods: (a) skin prick tests in man; (b) RAST; and (c) PCA tests in monkey skin with human allergenic sera.

(a) Prick tests in man

When the fractions obtained from an isoelectric separation of the cocksfoot pollen were prick-tested in man, it was apparent that the allergenicity of the fractions varied, since the weal sizes obtained in the skin of a single individual varied with the different fractions. The fractions giving the largest weal sizes were titrated out, and it was found that on dilution of each fraction the weal size decreased.

From these results we assumed that the weal size was a reflection of the relative potency of each fraction, and that plateau skin responses were not being seen.

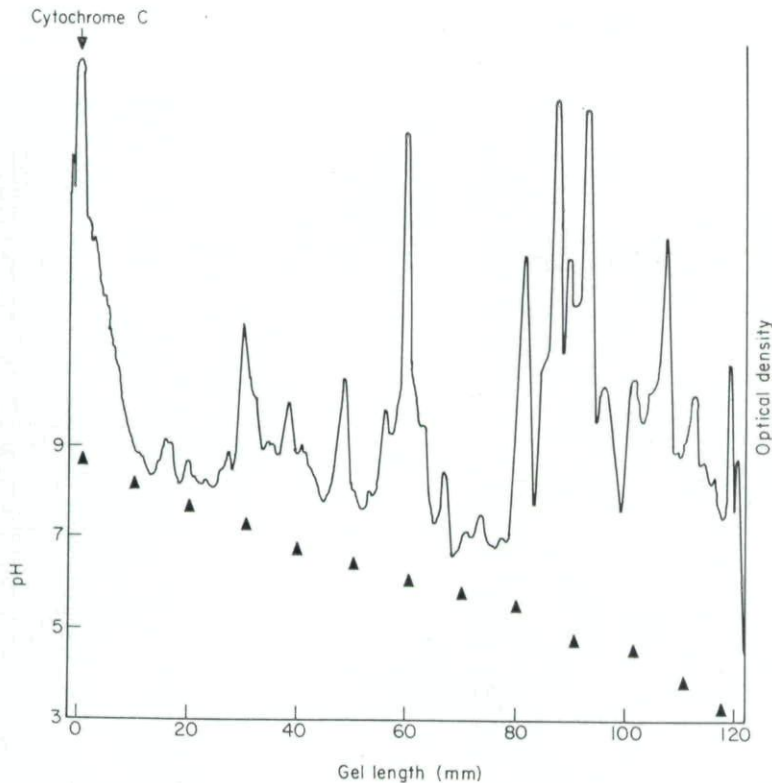


Fig. 1 Densitometric trace of the staining pattern observed after an isoelectric separation of the cocksfoot pollen extract in polyacrylamide gel in a pH 3-10 gradient. (▲) pH.

However, we did not concentrate each fraction further to test if this was indeed the case.

The variation in response in one individual might have been due only to different concentrations of each component, but the same variation in pattern would then have been seen in each patient. This was not seen as, at the time of testing, different patients gave quantitatively different profiles on skin testing, but almost all the patients responded in some degree to all the fractions.

The results obtained in the twelve patients are shown in Fig. 2. Thus the skin test profiles indicate that the components in the fractions have different immunological specificities, especially since one patient (No. 12) only responded to a small number of fractions. This patient, in fact, had perennial vernal conjunctivitis with a seasonal exacerbation.

(b) RAST and passive transfer tests in Rhesus monkey skin

Sera obtained from patients who had been prick-tested with the cocksfoot pollen fractions were used to assess the allergenicity of the fractions by RAST, and also by passive transfer in Rhesus monkey skin.

In patients with circulating specific IgE, a good correlation was obtained between RAST, monkey PCA and prick-testing in human skin, insofar as the peaks of allergenicity occurred in approximately the same fractions (Fig. 3).

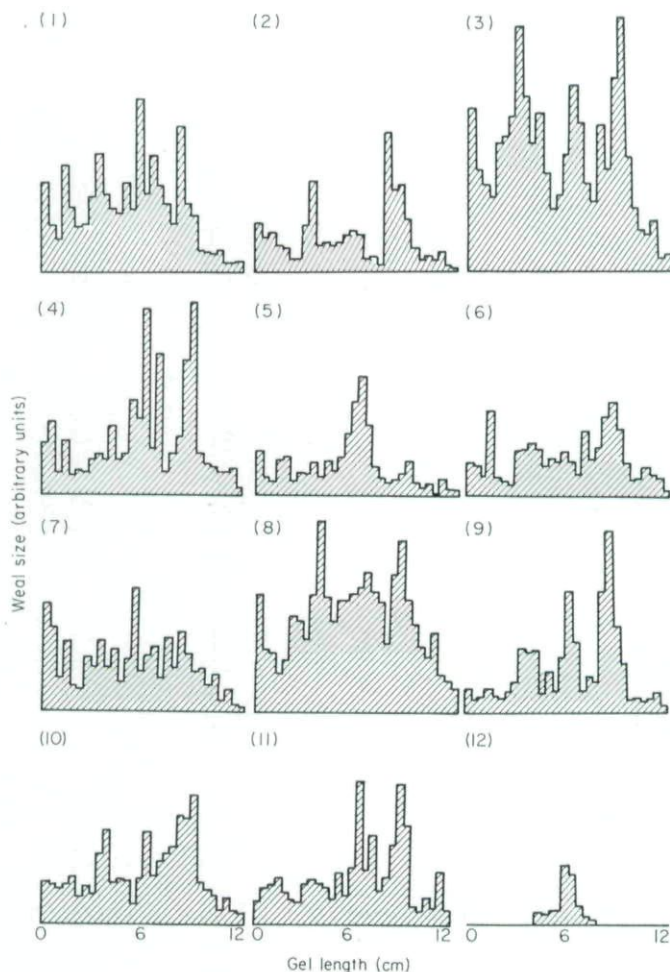


Fig. 2. Weal sizes observed on prick testing the thirty fractions in twelve patients.

Interestingly, in one patient (K.), negative RAST and monkey PCA were seen in contrast to the positive skin tests. This suggests that although the skin mast cells were sensitized, specific circulating IgE was lacking.

The serum from patient J. showed a different phenomenon. The RAST did not mirror the *in vivo* skin test results, in general showing rather low specific IgE-binding. However, on monkey PCA a peak did show up that was neither present on skin testing the patient nor was it seen in the RAST.

Discussion

We have previously reported on the separation of a cocksfoot pollen extract by isoelectric focusing (Topping *et al.*, 1977), and we now describe an investigation into the allergenicity of the fractions. The thirty fractions gave different weal sizes on skin-testing, and this could be due to the fact that the fractions contained different allergens or different concentrations of the same allergen. We favour the former interpretation, especially as on skin testing patients responded to different fractions,

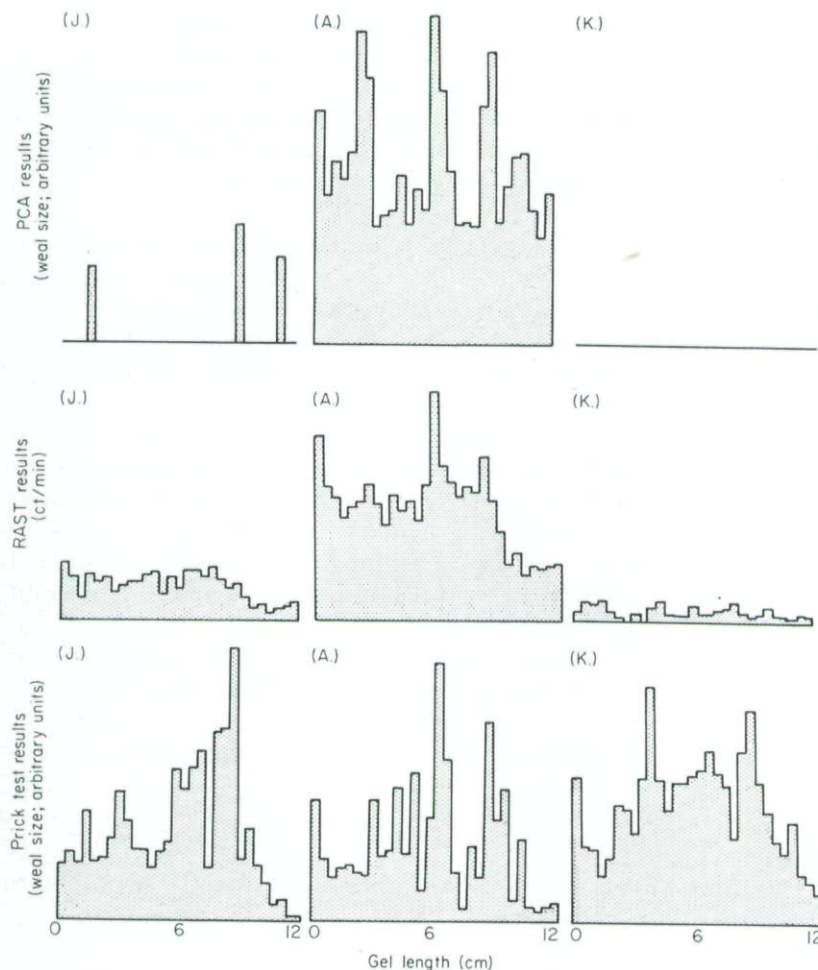


Fig. 3. Comparison of the profiles obtained by three methods: prick testing, RAST and PCA tests. Bottom row, skin test profiles from three patients. Centre row, RAST profiles with sera from the same patients. Top row, profiles obtained by PCA tests with the same sera.

indicating that the separated components could have distinct immunological specificities. Further support for this point of view comes from our recent studies, where several antigenic determinants are shown in the various pollen fractions using the technique of RAST double-inhibition (Topping *et al.*, in preparation).

We do not know whether these profiles in particular patients are constant, or whether they change as a result of allergen challenge.

The reaction of some patients, e.g. No. 12, Fig. 2, to a single peak of the cocksfoot extract is analogous to that observed for Ra5 sensitivity in patients with ragweed sensitivity. There, a small proportion of ragweed-allergic individuals respond to Ra5 only, most of whom have the histocompatibility antigen HLA-A7 creg (Marsh *et al.*, 1973). The isolation of minor determinants which may only give positive skin tests in a small proportion of patients with hay fever offers scope for genetic studies.

If the skin test response is considered as an 'all or none' reaction, then fourteen out of the fifteen patients (93%) responded to all the thirty fractions. There are two

points of view from which this can be considered. Firstly, from the purely diagnostic point of view, will a pollen extract lacking a major component give a positive skin test in a particular hay fever patient? From our results it is likely that even this 'bad' extract will be satisfactory diagnostically, i.e. in giving a positive skin test in subjects with grass pollen allergy. However, from the therapeutic point of view, it is possible that the so-called 'bad extract' which is lacking major bands may be inferior to one with a full complement.

As in our previous paper (Topping *et al.*, 1977), the similarity between the results obtained by skin testing, RAST and monkey PCA show the reproducibility of the techniques used, and also that the circulating IgE has equivalent specificities to that bound to the skin mast cells. Some patients, however, lacked circulating IgE antibody and consequently gave neither a RAST profile nor positive monkey PCA, although they showed strong skin reactivity.

Huggins & Brostoff (1975) have reported similar findings in another group of patients where skin tests and RAST were negative, but the allergen provocation test was positive. In these patients, the IgE antibody was produced locally. Caution must therefore be placed on the use of RAST alone in diagnosing allergy.

These studies also emphasize the current difficulty in attempting to isolate a single pure allergen, which could be used either diagnostically for the detection of cocksfoot pollen allergy, or for the standardization of diagnostic extracts.

Isoelectric focusing enables allergen extracts to be separated into many different components. In addition, the biological assays reveal that the fractions can also show different immunological specificities. The method can presumably be applied to many allergen extracts, and is currently being used to study the components of house dust and mite extracts.

Acknowledgments

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