

Standardization of pollen allergens of *Parthenium hysterophorus* and selection of an in-house reference extract

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Summary

A standardized in-house reference extract from the pollen of *Parthenium hysterophorus*, which is responsible for the high incidence of allergic rhinitis in India, was generated and examined by skin test, radio-allergosorbent test inhibition and isoelectric focusing. *Parthenium* reference allergen discs and positive reference serum were also generated. These reference reagents could not only be used for the quantitation of *Parthenium*-specific IgE in the sera of rhinitis patients but also for the evaluation of allergenic activity (relative potency and lot-to-lot variation) of different batches of *Parthenium* pollen.

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Introduction

It is increasingly realized that the airborne pollen of *Parthenium hysterophorus* (American feverfew) is a potent atopic allergen. Cases of rhinitis due to exposure to *Parthenium* pollen have been reported from the U.S.A. [1], India [2,3] and Australia [J. A. Stewart, personal communication]. In a recent clinical survey [4], we have found that 7.1% of the study population ($n=2035$) residing in the South Indian city of Bangalore are suffering from rhinitis due to exposure to the airborne *Parthenium* pollen, the concentration of which goes up to 48% during monsoon months (July–October) [5]. A high prevalence of rhinitis was also recorded from several parts of India where the alien weed *P. hysterophorus* is growing wild. Positive skin test reactions and radio-allergosorbent test (RAST) for *Parthenium* pollen allergens were reported, respectively from the residents of Brisbane (Australia) [J. A. Stewart, personal communication] and the Texas gulf coast (U.S.A.) [6]. To our knowledge, such a high incidence of allergic rhinitis due to a single pollen

has never previously been reported elsewhere in the world.

Although preparations of *P. hysterophorus* pollen extracts are available in India from different commercial sources for their use in diagnosis and immunotherapy, no national or international standard has yet been set up. In the present study, allergen extracts prepared from *Parthenium* pollen have been standardized by skin tests, isoelectric focusing (IEF) and RAST inhibition. For reasons of practicality and reproducibility, RAST inhibition has been selected as the method of choice for the generation of an internal reference standard. The results are presented in this paper.

Materials and methods

Materials

Bovine serum albumin (BSA), acrylamide, Coomassie Brilliant Blue R-250, Fast Blue B, TEMED, diethanolamine, ammonium persulphate, β -galactosidase anti-human IgE conjugate and 2-naphthyl β -D-galactopyranoside were purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.). Pharmalyte (pH 3.5–10), ^{125}I -labelled anti-human IgE (Phadebas RAST tracer), IEF marker proteins (pI 3.5–10), CNBr-activated filter paper discs and the Phadebas reference diagnostic kit (serum and allergen discs) were obtained from Pharmacia Diagnostics (Upp-

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ml were stabilized by the addition of 0.05% azide and stored at 4°C, while aliquots of the stock serum without azide were stored at -90°C. The serum samples stored at 4°C when determined by RAST were found to be stable for at least 2 months (not tested beyond this period). The coefficient of variation of specific IgE titres for each of the five dilutions of *Parthenium* reference serum obtained from six independent assays varied from 0 to 8.8% (Table 2). Comparison of the levels of *Parthenium*-specific IgE measured in the sera of 10 *Parthenium*-sensitive rhinitis patients (skin prick-test positive) by RAST using standard curves generated with the in-house *Parthenium* reference and Phadebas reference sera are shown in Table 3.

Discussion

The number of standardized allergen extracts available as reference reagents for diagnosis and immunotherapy of atopic allergy is steadily increasing. The list includes pollens of birch, short ragweed, grasses (cocksfoot, timothy, bermuda, kentucky and rye), mites of *Dermatophagoides* and *Acarus siro*, danders of cat, dog and humans, moulds, honey bee venom, etc. [17]. Among these, extracts of *Ambrosia* (ragweed) [18], timothy [19], birch [20], *Dermatophagoides pteronyssinus* [21] and dog dander [22] are already available as international standards. The extract of rye is pending with the WHO/IUIS for its approval as an international standard [23]. The first international standard of *D. pteronyssinus* has been used to compare and evaluate allergen extracts prepared from two different source materials [24].

Although extracts of *Parthenium* pollen are commercially available from three different manufacturers in India, properly standardized extracts are not yet available for *in vivo* and *in vitro* diagnostic tests as well as for hyposensitization. Preliminary studies suggested that the commercial extracts may not be effective in accurate diagnosis and immunotherapy of *Parthenium* rhinitis. In the absence of stringent regulations for the production of standardized allergen extracts in India, and in view of the high incidence of *Parthenium* rhinitis in this country and probably in Australia and southern parts of the U.S.A. and other countries where *Parthenium* is growing wild [25], it was found necessary to standardize and generate an in-house reference preparation of *Parthenium* pollen extract, serum and allergen discs with a view to controlling lot-to-lot variations in composition and activity of allergen extracts for their use in diagnosis and immunotherapy.

Various *in vitro* and *in vivo* procedures have been employed for the standardization of allergen extracts [9,26-32]. In the present investigation, however, the

standardized extract of *Parthenium* pollen was generated and examined by RAST inhibition, IEF and skin-prick test. A reference serum was generated by pooling the sera of 10 patients with regression lines for RAST that were parallel to each other (Fig. 2) and having similar titres. Generation of a reference serum pool is essential for the standardization of the RAST inhibition assay. The criteria for the selection of appropriate dilutions of serum samples for RAST inhibition, however, have received attention only rather recently [33]. An in-house reference allergen preparation of *Parthenium* pollen was generated by pooling different batches of the pollen with comparable composition and activity, as established by IEF and RAST inhibition (Figs 3 and 5). Although it may be valid to include pollen batches with relatively high potency in a pool if the composition remains the same, it was decided to exclude lots 1D1 and 2W2 so that the overall potency of the reference extract was not altered. The stability of the allergens was not significantly influenced when the pollen was stored dry at -20°C for different periods of time.

The methods described in this paper for the generation of a standardized in-house reference extract will be useful in the production of standardized allergen extracts for the pollen of *P. hysterophorus*, which is now recognized as a worldwide allergen, for diagnosis and immunotherapy. Further studies on the immunochemical characterization of the major 31 kD *Parthenium* pollen allergen (*Par h I*), which are underway, could lead to the development of better preparations for the treatment of *Parthenium* rhinitis.

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