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Immunobiochemical Characterization of *Brassica campestris* Pollen Allergen

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Key Words

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Abstract

Brassica campestris (BC), Eng. Mustard, is an important source of pollen allergen, responsible for type I hypersensitivity disorders. In the present study, BC pollen extract was characterized by TLIEF, SDS-PAGE and immunoprinting. The extract separated into 50 silver stained bands of pI 3-9 on isoelectric focusing whereas it resolved into 14 Coomassie blue stained protein bands of 14-100 kD on SDS-PAGE. Immunoblot analysis with individual patient sera detected four allergenic proteins of 90, 67, 60 and 14 kD. BC separated into 8 peaks (Bras 1-8) on DEAE Sephadex A-50 column. Bras 2 was found to be most potent by IgE specific ELISA, hence further fractionated on Sephadex G-200. A protein of 90 kD (Bras 2a) isolated by gel filtration was found to be most allergenic protein by ELISA inhibition. The findings shall be applicable in standardization of future batches of BC pollen extract to be used for allergy diagnosis and immunotherapy.

Introduction

Allergen extracts are heterogenous mixtures of proteins, glycoproteins, which can bind to human IgE and lead to various allergy manifestations in susceptible individuals [1]. Besides the IgE binding proteins, they also contain non-allergenic components. Since well-standardized antigens are not available, crude extracts are frequently used for management of allergy ailments [2]. The clinical safety and efficacy of the extracts, especially for immunotherapy, depends upon their quality. A general consensus has, therefore, evolved world wide for standardization of allergen extracts. In India, 10 different pollen and fungal extracts have been

characterized by in vivo and in vitro procedures recommended by international societies.

Brassica campestris (BC) pollen has been documented as an important aeroallergen through various aerobiological and clinical studies conducted at many places in the country [3-8]. Based on these reports, its pollen extract is routinely included in allergy diagnosis kits used by allergy practitioners. The present study was undertaken to characterize the allergenic components of BC pollen by isoelectric focusing (IEF), SDS-PAGE, immunoblot and to establish the reference pattern for in-house preparations/standards. BC extract was also fractionated by ion exchange chromatography and gel filtration to isolate the most allergenic protein.

References

- 1 Lernmark A: Immune intervention yes, but for what reason, for whom, when and how? *Diabetologia* 1992;35:1096-1098.
- 2 Bosi E, Bonifacio E, Bottazzo GF: Autoantigens in IDDM. *Diabetes Rev* 1993;1:204-214.
- 3 Thai AC, Ng WY, Lui KF, Cheah JS: Prevalence of islet cell antibodies in Chinese patients with recent-onset insulin-dependent diabetes mellitus: in Min HK, Lee HK, Kaneko T, Baba S, Turtle JR (eds): *Diabetes mellitus in Western Pacific Region. Proceedings of International Diabetes Federation Western Pacific Region Congress 1993*, 12 April 1993, Seoul, South Korea. Seoul, Korea Medical Publishing, 1994, pp 61-64.
- 4 WHO Expert Committee on diabetes mellitus. Geneva, World Health Organisation Technical Series No 727, 1985.
- 5 Ng WY, Thai AC, Lui KF, Yeo PPB, Cheah JS: Non-restricted immunoglobulin-G subclass islet cell antibodies in Chinese. *Autoimmunity* 1993;15:305-309.
- 6 Fujimoto J, Levy S, Levy R: Spontaneous release of the Leu-2(T8) molecule from human T cells. *J Exp Med* 1983;159:752-766.
- 7 Tonkinson BE, Brown MC, Ip SH, Carrabis SH, Sullivan JL: Soluble CD8 during T-cell activation. *J Immunol* 1989;142:2230-2236.
- 8 Symons JA, Wood NC, Di Girolamo FS, Duff GW: Soluble CD8 in patients with rheumatic disease. *Clin Exp Immunol* 1990;80:354-359.
- 9 Nouri Aria KI, Lombard M, Williams R: High serum levels of CD8 antigen in primary biliary cirrhosis: A possible cause of suppressor cell dysfunction? *Clin Exp Immunol* 1991;86:140-144.
- 10 Di Cesare E, Previti M, Ingemi MC, Bagmatou GF, Cucinotta D: High serum levels of soluble CD8 in insulin dependent diabetes. *Clin Exp Immunol* 1994;95:283-286.
- 11 Rubin LA, Kurman CC, Fritz ME, Biddison WE, Boutin B, Yarchoan R, Nelson DL: Soluble interleukin-2 receptors are released from activated lymphoid cells in vitro. *J Immunol* 1985;135:3172-3177.
- 12 Waldmann TA: The IL-2/IL-2 receptor system: A target for rational immune intervention. *Immunol Today* 1993;14:264-270.
- 13 Cavallo MG, Pozzilli P, Bird C, Wadhwa M, Meager A, Visalli N, Gearing AJH, Andreani D, Thorpe R: Cytokines in sera from insulin-dependent diabetic patients at diagnosis. *Clin Exp Immunol* 1991;86:256-259.
- 14 Serjeantson SW, Kohonen-Corish MRJ, Rowley MJ, Mackay IR, Knowles W, Zimmet P: Antibodies to glutamic acid decarboxylase are associated with HLA-DR genotypes in both Australians and Asians with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1992;35:996-1001.
- 15 Omar MAK, Srikantha S, Eisenbarth GS: Human islet cell antibodies. Immunoglobulin class and subclass distribution defined by monoclonal antibodies. *Diabetes Res* 1987;4:155-157.
- 16 Millward A, Hussain MJ, Peakman M, Pyke DA, Leslie PG, Vergani D: Characterization of islet cell antibody in insulin dependent diabetes: Evidence of IgG1 subclass restriction and polyclonality. *Clin Exp Immunol* 1988;71:353-356.
- 17 Lee BW, Chan SH, Tan SH, Wee GB, Yap HK, Wong HB, Tan CL, Tan KW: HLA-system in Chinese children with insulin-dependent diabetes mellitus: A strong association with DR3. *Metabolism* 1984;33:1102-1105.
- 18 Chan SH, Yeo PPB, Lee BW, Tan SH, Cheah JS, Wong HB: HLA and insulin dependent diabetes mellitus in Singaporean Chinese. *Ann Acad Med Singapore* 1985;14:215-218.
- 19 Penny MA, Jenkins D, Mijovic CH, Jacobs KH, Cavan DA, Yeung VTF, Cockram CS, Hawkins BR, Fletcher JA, Barnett AH: Susceptibility to IDDM in a Chinese population. Role of HLA class II alleles. *Diabetes* 1992;41:914-919.
- 20 Szopa TM, Titchener PA, Portwood ND, Taylor KW: Diabetes mellitus due to viruses - Some recent developments. *Diabetologia* 1993;36:687-695.
- 21 Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ: Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* 1992;89:283-292.
- 22 Richter W, Mertens T, Schoel B, Muir P, Ritzkowski A, Scherbaum WA, Boehm BO: Sequence homology of the diabetes-associated autoantigen glutamate decarboxylase with coxsackie B4 2C protein and heat shock protein 60 mediates no molecular mimicry of autoantibodies. *J Exp Med* 1994;180:721-726.
- 23 Ministry of the Environment: Communicable Disease Surveillance in Singapore 1993.

Materials and Methods

Preparation of Extract. Highly pure pollen samples (>98% pollen; <2% flower parts of the same species) were collected and defatted with diethyl ether. BC pollen extract was prepared in three batches using ammonium bicarbonate A.R. buffer (0.05 M, pH 7.8) containing 100 mM phenylmethyl sulphonyl fluoride. The pollen material was subjected to continuous stirring on a magnetic stirrer for 18 h at 4°C. The extract was centrifuged at 10,000 rpm for 30 min and the supernatant brought to 90% saturation with ammonium sulphate. The precipitated proteins were redissolved and dialysed extensively against distilled water using 3,500 molecular weight (MW) cut-off membrane (Spectrum). The extract was distributed in appropriate aliquots, freeze-dried and stored at -20°C until used.

Protein Estimation. Protein content of each batch of BC extract was determined by Lowry's method using phosphotungstic acid reagent for protein precipitation [9]. BSA was used as reference standard for calibration.

Collection of Human Sera. Intradermal (ID) tests are carried out with BC extract on patients for allergy diagnosis at the Clinical Research Centre, VP Chest Institute, Delhi, India. For skin tests, 0.01 ml of 1:500 v/v antigen diluted with phosphate-buffered saline (PBS) is injected intradermally into the forearm of the patients. Simultaneously, a negative control (PBS) and positive control, i.e. histamine diphosphate 100 µg/ml, are also injected. The ID tests are graded after 20 min, based on the wheal size produced at the injection site in comparison with negative control [10].

We could convince 15 patients showing marked positive ID reactions (2+ to 4+) with BC pollen extract to donate blood (sera) for use in immunochemical experiments. None of the patients were on immunotherapy, when sera were collected. Sera from 10 normal persons with no history of disease and showing negative ID tests to BC extracts were also collected as normal control. The sera collected were screened by ELISA for the presence of specific IgE to BC. Sera showing high antibody titre (optical density, OD, >5 times of the normal control) were used for immunoblot and ELISA inhibition.

IEF. IEF was performed on ready-made gels of pI 3–9, supplied by Pharmacia (Uppsala, Sweden) using the Phast system [11]. An amount of 5 µg protein was loaded per lane for IEF separation. The resolved proteins were stained with silver staining kit obtained from Pharmacia.

SDS-PAGE. SDS-PAGE was performed on 10% gel in 'vertical slab gel' apparatus, LKB Sweden, according to the method of Laemmli, [12]. Electrophoresis was carried out in a discontinuous buffer (0.025 M Tris, 0.2 M glycine and 0.1% SDS) system. Samples (protein) were boiled for 5 min at 100°C in a two fold concentration of sample buffer containing β-mercaptoethanol. Fifty micrograms of BC pollen protein were applied to each lane. Protein standards of 14–100 kD (Sigma) were taken as MW markers.

Immunoblot. Proteins separated by electrophoresis were either stained with Coomassie brilliant blue R-250 (Sigma) or electrophoretically transferred onto nitrocellulose paper using transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3) and 'dry blot apparatus' [13]. Non-specific sites on nitrocellulose paper were blocked with 1% BSA for 2 h at room temperature. After washing with PBS-Tween (0.1%), the blotted strips were incubated overnight in patient serum diluted to 1:10 v/v with PBS-Tween. As a control, a strip blotted with BC pollen proteins was incubated separately with normal human serum in the same dilution. After washing thrice with PBS-Tween, horseradish peroxidase labelled anti-human IgE (1:500, v/v) was added and incubated for 3 h at room temperature. The unbound

antibodies were removed from blots by washing and the bound enzyme was detected by addition of freshly prepared substrate – diaminobenzidine and H₂O₂ in sodium acetate buffer (pH 5.0). The blots were washed after 5 min with distilled water to stop the colour development process. The antigen-antibody binding was recognized by the intensity of the brown colour bands, produced with respect to different protein components.

Fractionation of Proteins. An aliquot of reconstituted BC pollen extract (25 mg protein) was loaded on DEAE Sephadex A-50 column and eluted with phosphate buffer (0.05 M, pH 7.3) using a linear salt gradient of 0.0–0.5 M sodium chloride. The column was washed finally with 1 M sodium chloride. Allergic potency of each fraction was checked by ELISA (IgE specific) using pooled patients sera. All the fractions of each resolved peak were pooled, dialyzed and lyophilized. The fraction showing maximum IgE binding (OD) was further fractionated on gel filtration column, i.e. Sephadex G-200, to isolate the important allergenic proteins in BC pollen extract. To find out the MW of the allergenic components, the column was calibrated with MW markers, viz β-galactosidase 92, bovine albumin 66, β-lactoglobulin 36, pepsin 34, trypsin inhibitor 20 and lysozyme 14.3 kD.

ELISA. IgE binding capacity of BC fractions obtained by column chromatography was determined by direct ELISA using the method of Voller et al. [14]. A volume of 50 µl of each fraction along with 50 µl of carbonate buffer was coated on each well of the microtitre plate (Corning) and incubated overnight at 4°C. The unbound antigen was washed out with PBS-Tween (0.1%) and the free sites were blocked with 1% BSA in PBS for 1 h at 37°C. This was followed by overnight incubation with 100 µl of 1/10 (v/v) PBS diluted positive serum pool at 4°C. As a control, normal human serum was also checked in the same dilution. After washing, the plate was incubated with 1:500 (v/v) PBS diluted anti-human IgE conjugated with alkaline phosphatase (Sigma) for 18 h at 4°C. After washing the unbound IgE, the substrate *p*-nitrophenyl phosphate was added and incubated at 37°C for 1 h. The reaction was stopped by adding 3 N NaOH. The colour developed was read on a Nunc ELISA reader.

ELISA Inhibition. ELISA inhibitions were performed by mixing 50 µl of pooled patient sera with equal volume of buffer containing various concentrations of inhibitor (10 ng to 5 µg). The mixture was added to antigen-coated wells and incubated overnight at 4°C. IgE antibodies bound to the allergosorbent were detected with alkaline phosphatase labelled anti-human IgE, as described earlier. A similar experiment was carried out with normal human serum. The non-specific binding (OD) was subtracted from the binding value of the samples in each well of the plate and the percent inhibition was calculated as:

$$1 - \frac{\text{OD of the sample with inhibitor}}{\text{OD of the sample without inhibitor}} \times 100.$$

Results

Antigenic extracts from three batches of BC pollen were prepared under similar conditions. Protein content in each batch of extract was quantitated and found to be 12, 14.8 and 15 mg, respectively, per gram dry weight of pollen. All three batches of the extracts were subjected to IEF, SDS-PAGE, immunoblot and other immunochemical experiments. On

IEF, the whole pollen BC extract separated into 50 silver stained bands in pI region of 3–9 (fig. 1). The extract resolved into 14 Coomassie blue protein bands on SDS-PAGE in MW range of 14–100 kD (fig. 2a). Sera of 6 patients showing highly specific IgE levels for BC (>OD 5 times of the normal human serum) were pooled together and also used individually for carrying out immunoblot. Immunoblotting with pooled serum of patients recognized 7 allergenic proteins of MW 14–100 kD in BC extract. The proteins of MW 90, 67, 60 and 14 kD were detected as major allergenic proteins when individual patient sera were analyzed by immunoblot (fig. 2b).

Fractionation of BC pollen extract on DEAE Sephadex A-50 column resulted in eight peaks (fig. 3). Six peaks (Bras 1–6) were eluted with linear salt gradient 0.0–0.5 M sodium chloride in phosphate buffer, whereas the last two peaks, Bras 7 and Bras 8 eluted with 1 M sodium chloride in the same buffer. Compared to other fractions, Bras 2 was found to be more potent by IgE-specific ELISA. It was further fractionated on Sephadex G-200, where Bras 2 separated into two subfractions, viz Bras 2a and Bras 2b (fig. 4). Of these, Bras 2a (90-kD protein) was found to be more allergenic than Bras 2b by ELISA inhibition (fig. 5). Bras 2a required 125 ng protein to generate 50% ELISA inhibition, whereas 500 ng protein of BC pollen extract (WPE) were needed for the same.

Discussion

In the last decade, many allergen extracts of diverse origin were standardized in Europe, USA and Australia using clinical as well as immunobiochemical methods. The majority of the extracts standardized were from tree pollen, weeds and fungal spore allergens. The allergens prevalent in the Indian subcontinent are quite different from that of western countries. In India, few allergen extracts – e.g. pollen, fungi, insects – have been characterized for their allergenic components with standard in vivo and in vitro procedures. The methods followed for labelling the potency of the commercially available extracts in India are W/V (g/ml), PNU and biopotency tests, ID or prick tests. None of these methods are satisfactory, because they do not describe anything about the quality/quantity of the allergenic components present in the extracts. The present study was therefore carried out to characterize the allergenic proteins of BC pollen extract.

BC Eng. mustard is cultivated as a major oil yielding crop over a large area of the country. It has a prolonged flowering season starting from November to April releasing

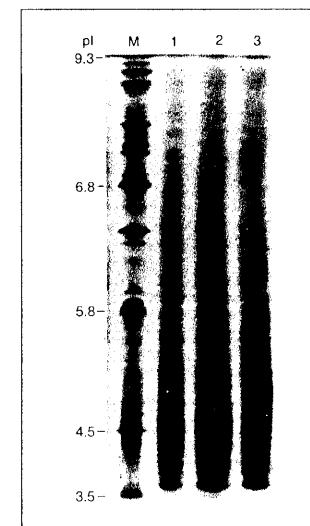


Fig. 1. IEF pattern showing silver stained bands of BC extract (1–3 batches) and pI markers (M).

abundant quantities of pollen into the atmosphere. Aerobiological surveys have quantitated its aerial pollen incidence to be 1.5–6.5% of the total annual pollen catch [3–5]. Clinical studies conducted with BC pollen extract in India have recorded marked positive skin (ID) reactions in 6–9% allergy patients [6–8]. In a random screening of 238 cases (aged 18–43 years) at VP Chest Institute with BC pollen extract marked positive (2+ to 4+) skin reactions were observed in 9.2% allergy patients belonging to different regions of the country. In an earlier study from Europe with *Brassica napus* extract, a low prevalence of allergy (<0.2%) was recorded in a normally exposed population. However, the sensitivity to *B. napus* may be higher in occupationally exposed individuals [15].

The IEF and SDS-PAGE analysis of BC pollen extract followed by staining showed the heterogeneity of the preparation, which contained many components/proteins. All three batches of the extracts showed nearly the same pattern/number of protein bands on IEF and SDS-PAGE. Fifteen patients, positive (ID) to BC extract, showed the presence of specific IgE by ELISA. However, the level of specific IgE in different sera did not correlate exactly with ID test

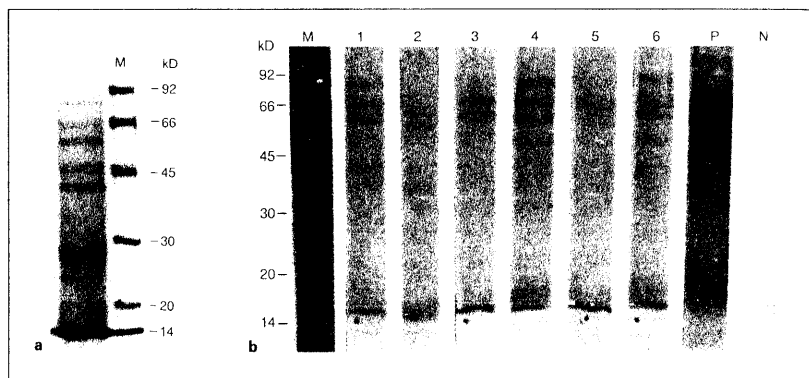


Fig. 2. a) SDS-PAGE pattern showing CBB R-250 stained bands of BC extract and MW markers (M). b) Identification of IgE binding components of BC extract in patient's sera using immunoblot analysis. 1-6 = Individual patient sera; P = pooled patient sera; N = normal human serum (control); M = MW markers.

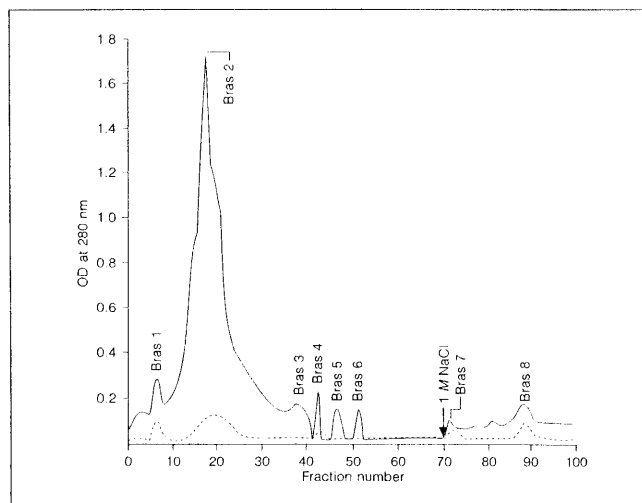


Fig. 3. Elution profile (—) of BC extract on DEAE Sephadex A-50 column (OD at 280 nm). Column size 1.5x40 cm; eluent, 0.05 M phosphate buffer (pH 7.3); linear salt gradient, 0.0–0.5 M NaCl was applied; 1 M NaCl was applied at the point indicated; flow rate, 18 ml/h; sensitivity, 0.5 arbitrary units; chart speed, 0.2 mm/min and fraction size, 5 ml. IgE binding capacity (---) of each eluted fraction as determined by ELISA. Absorbance was read at 405 nm.

gradings. Of the seven allergenic proteins detected with pooled patients (6) sera, four proteins with MW 90, 67, 60 and 14 kD were recognized as major allergenic protein by immunoblotting with individual patient's sera (fig. 2b). Previous studies have shown the presence of a highly allergenic

14 kD-protein in seeds of *Sinapis alba* from the family Brassicaceae. Whereas in case of *Brassica juncea* protein components of 16–16.4 kD were recognized as the major allergen of seeds [16–17].

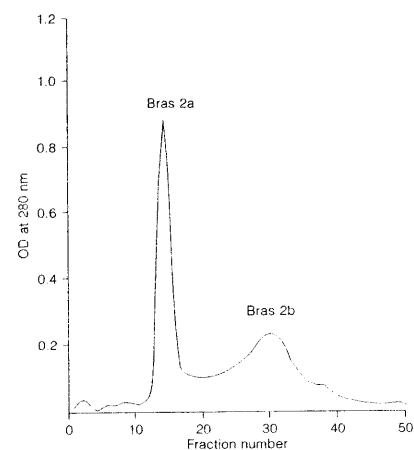


Fig. 4. Elution profile of Bras 2 fraction of BC extract on Sephadex G-200.

An attempt has been made to isolate allergenic components from BC pollen extract by ion exchange and gel filtration chromatography. Elution profile of BC demonstrated 8 peaks on DEAE Sephadex A-50 column. IgE-specific ELISA indicated Bras 2 fraction to be more potent than others. It also accounted for 38% of the total protein recovered. Further fractionation of Bras 2 on Sephadex G-200 resulted in two peaks, viz Bras 2a and Bras 2b. Bras 2a (MW 90 kD) was identified as the most potent/allergenic protein, since it required 125 ng of protein for 50% ELISA inhibition, compared to the crude extract (500 ng) for the same degree of inhibition. Thus, a major allergenic protein, Bras 2a MW 90 kD, has been isolated and identified in BC pollen extract. In India, studies with *Amaranthus spinosus*, *Xanthium strumarium* (weed pollen) and *Cocos nucifera* (tree pollen) have revealed the glycoproteins of higher MW (128, 103, 116–200 kD, respectively) as major allergenic proteins [18–20]. However, with other pollen antigens, such as *Ageratum conyzoides*, *Artemisia scoparia*, *Cynodon dactylon* and *Prosopis juliflora*, low MW proteins of 10–32 kD have been recorded as major allergenic components [21–24].

The family Brassicaceae includes broccoli, cabbage, turnip and mustard species. Most of them are known to cause different types of allergic reactions, based on the route of contact [15–17, 25]. The seeds and oil of *B. napus* and *Sina-*

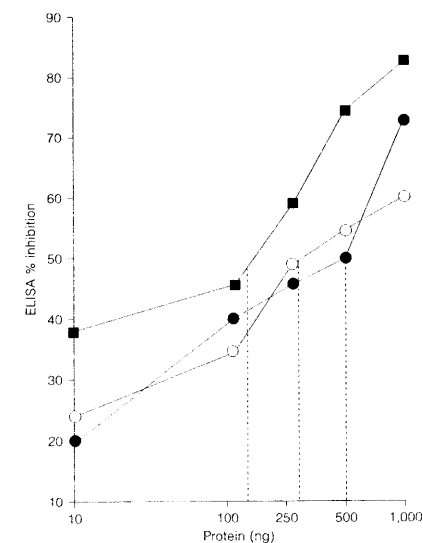


Fig. 5. ELISA inhibition, dose response curves of subfractions of Bras 2, purified on Sephadex G-200. ● = Whole pollen BC extract; ■ = Bras 2a; ○ = Bras 2b.

pis alba have also been reported as important allergens from other countries. The oil from seeds of BC is used extensively for cooking in the Indian subcontinent. Therefore, it would be interesting to study the presence of cross-reacting allergens if any in BC pollen and in a variety of mustard seeds.

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References

- Suphioglu C, Singh MB, Knox RB: Peptide mapping analysis of group I allergens of grass pollen. *Int Arch Allergy Immunol* 1993;102:144-151.
- Gangal SV, Jaggi KS, Sridhara S: Standardization of Allergens in India. *Arbeiten aus dem Paul-Ehrlich-Institut*. Stuttgart, Fischer, 1991.
- Gaur RD: Aeropalynology of Meerut I - Pollen grains. *J Indian Bot Soc* 1978;57:353-365.
- Singh AB: Pollen production in common allergenic plants of Delhi and their atmospheric prevalence. *Sci Cult* 1984;50:65-67.
- Singh AB, Babu CR: Survey of atmospheric pollen allergens in Delhi: Seasonal periodicity. *Ann Allergy* 1982;48:115-122.
- Singh BP, Singh AB, Prakash D: Skin reactivity to airborne pollen and fungal antigens in patients of nasobronchial allergy: in Chandra N (ed): *Atmospheric Biopollution*. Maharashtra, Environmental Publications, 1987, pp 125-137.
- Acharya PJ: Skin test response to some inhalant allergens in patients of nasobronchial allergy from Andhra Pradesh. *Aspects Allergy Appl Immunol* 1980;13:14-18.
- Shivpuri DN: Clinically important pollen, fungal and insect allergens for nasobronchial allergy patients in India. *Aspects Allergy Appl Immunol* 1980;13:19-23.
- Singh BP, Sridhara S, Arora N, Gangal SV: Evaluation of protein assay methods for pollen and fungal spore extracts. *Biochem* 1992;27:477-484.
- Shivpuri DN, Agarwal MK: Studies on the allergenic fungal spores of Delhi, India, metropolitan area - Clinical aspects. *J Allergy* 1969;44:204-213.
- Phast System Owner's Manual, Pharmacia AB, Uppsala, 1986.
- Laemmli UK: Cleavage of structural proteins during assembly of the head of the bacteriophage T₄. *Nature* 1970;227:680-685.
- Towbin H, Gordon J: Immunoblotting and dot immunobinding: Current status and outlook. *J Immunol Methods* 1984;72:313-340.
- Voller A, Bidwell D, Barlett A: Enzyme linked immunosorbent assay; in Rose NR, Friedman H, (eds): *Manual of Clinical Immunology*. Washington, American Society for Microbiology, 1980.
- Fell PJ, Soulsby S, Blight MM, Brostoff J: Oil-seed rape: A new allergen? *Clin Exp Allergy* 1992;22:501-505.
- Gonzalez de la Pena MA, Menendez-Arias L, Monsalve RI, Rodriguez R: Isolation and characterization of a major allergen from oriental mustard seeds. *Bra J*. *Int Arch Appl Immunol* 1991;96:263-270.
- Dominguez J, Cuevas M, Urena V, Munoz T, Monco I: Purification and characterization of an allergen of mustard seed. *Ann Allergy* 1990;64:352-357.
- Choudhry A, Saksena RP, Tandon A, Shukla OP, Zamil Z, Saksena KC: Purification and partial characterization of a major allergenic component of *Amaranthus spinosus* pollen. *Indian J Allergy Appl Immunol* 1990;4:9-16.
- Jaggi KS, Gangal SV: Purification and characterization of allergens from *Xanthium strumarium* pollen. *Mol Cell Biochem* 1987;78:177-180.
- Jaggi KS, Arora N, Nipadkar PV, Gangal SV: Immunochemical characterization of *Cocos nucifera* pollen. *J Allergy Clin Immunol* 1989;84:378-385.
- Jaggi KS, Gangal SV: Isolation and identification of allergens and characterization of *Ageratum conyzoides* whole pollen extract. *Indian J Allergy Appl Immunol* 1987;1:39-48.
- Jaggi KS, Gangal SV: Isolation and identification of pollen allergens of *Artemisia scoparia*. *J Allergy Clin Immunol* 1987;80:562-572.
- Matthiesen F, Schumacher MJ, Lowenstein H: Characterization of major allergen of *Cynodon dactylon* pollen *Cyn. dt.* *J Allergy Clin Immunol* 1991;88:763-774.
- Sridhara S: Purification and standardization of allergens; PhD thesis, University of Delhi, 1985.
- Blaiss MS, McCants ML, Lehrer SB: Anaphylaxis to cabbage: Detection of allergens. *Ann Allergy* 1987;58:248-250.

Original Paper

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Characterization of the Allergen Group VI in Timothy Grass Pollen (Phl p 6) I. Immunological and Biochemical Studies

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Key Words

Epitopes
Grass pollen allergen
IgE binding
Immunoblotting
Microsequencing
Two-dimensional electrophoresis

Abstract

For the identification and characterization of allergen grass group VI we performed two-dimensional immunoblotting of timothy grass pollen (*Phleum pratense*). Two intense 13-kD protein spots of pI 5.2 and 5.5 were found to be IgE reactive. By N-terminal microsequencing and amino acid analysis we identified them as Phl p 6 isoallergens. An antiserum was raised against Phl p 6 by immunizing BALB/c mice with allergen bearing nitrocellulose particles of one isoform. The antiserum revealed an intense reactivity to Phl p 6 isoforms, but also showed a weak cross-reactivity with Phl p 5 allergens. After immunoabsorption of patients' serum to Phl p 6 spots on the blotting membrane, we were able to demonstrate that the eluted human IgE antibodies cross-react with the grass group V allergens as well. Therefore, Phl p 5 and Phl p 6 possess one or more common IgE binding epitopes.

Introduction

Grass pollens are one of the main causes of IgE-mediated allergy. Of all the temperate grasses the major allergens belong to groups I and V. Timothy grass (*Phleum pratense*) possesses an additional major allergen which was not detected in other grasses [1]. This was classified as group VI (Phl p 6), formerly termed Ag 19 [2]. The skin test and CRIE (cross radioimmuno-electrophoresis) showed an IgE prevalence of 75% [3]. Monitoring of IgE antibodies to Ag 19 before and during hyposensitization therapy followed typical courses: before immunotherapy starts the titer of IgE antibodies against Phl p 6 is usually low, during initial hyposensitization it reaches a high titer or remains unchanged.

This high IgE response was thought to be a suitable marker for prospective evaluation of clinical effect [4].

Lowenstein [5] studied Phl p 6 components in depth and determined a molecular mass of 11 kD by gel filtration and a pI of 4.9. Due to the difficulty in separating the allergen from Phl p 5 by gel filtration, ion exchange chromatography and copper chelate affinity chromatography [5, 6], it was suspected that it would be similar to grass group V components. Furthermore, Matthiesen et al. [1] observed a high degree of similarity between the N-terminus of group VI and an internal part of the Phl p 5. They did not detect any cross-reactivity between the two grass groups after raising an antiserum against Phl p 6 by immunizing rabbits.