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Research Article

INVESTIGATION OF THE ALLERGENIC SIGNIFICANCE OF THE AIR BORNE FUNGI

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Abstract

Allergy is one of the oldest maladies of mankind. Allergy which manifested itself in the form of asthma was known in ancient times. For a long time allergy could not be differentiated from other ailments with which it resembled in many respects. In this paper we study of allergenic significance of certain air borne fungi of the Lucknow atmosphere.

Keyword: Allergenic significance, fungal spores, allergy patients.

1. Introduction

Airborne fungal spores have been widely recognized as major allergens capable of causing asthma and allergic rhinitis as well as other allergic diseases (Andrea C. Mallo et al. 2011) .Moreover in the past years an increasing incidence of sensitization to fungi has been reported.

Indoor air quality has become an important health concern and susceptible persons have a high chance of response to these allergens. Considering the fact that an allergic reaction may occur with exposure to minute concentration of an allergen, mold indoor could create health risk for atopic individuals occupying such a building. Although there are several reports on the presence of fungi in the air of some cities, there is not any special focus on home environment for airborne fungi. Regarding the reactive role of fungi in asthmatic patients, this project identified the fungi simultaneously found in the indoor and outdoor asthmatic patients' home(Gerhard Blum et al. 2012).

2. Materials And Methods

Fungal spores

Different strains of fungi in the study were generously supplied by National Collection of Industrial Microorganism,

Poona. Alternaria sp. (CDRI) was kindly provided by Dr. O.P. Shukla, Scientist, Division of Biochemistry, Central Drug Research Institute, Lucknow.

Testing of allergen activity in fungal spore antigens

The allergenicity of fungi spore antigens was elucidated by the skin tests performed on human patients attending the allergy clinic of K.G. Medical College, Lucknow by the procedure described for the assay of CAP allergenic activity.

3. Results

250 patients of the respiratory allergy were screened against six fungal antigenic preparations by intradermal skin tests. Intradermal tests with these extracts were also performed on a group of healthy non-allergic individuals for comparative evaluation. Results of these tests are presented in Table - 1. Skin reactions with 1+ grading were not rare in healthy individuals. On the average with the six antigens studied, the prevalence of 1+ reaction was 7.3% in healthy controls as against 32.4% in allergic individuals attending the allergy clinic. Therefore, in the present study, 1+ skin reactions in patients have not been considered significant. It is clear from the data (Table - 1) that with 1:500 dilution of the antigens, the strong positive reaction of 3+ to 4+ was quite common among patients visiting the allergy clinic, with different antigenic extracts and varied from 4 to 14.7% with an average of 7.4%. The per cent incidence of markedly positive reaction (2+ to 3+) which are clinically important for deciding the allergenicity of fungi varied from 11 to 18.4% with an average of 18.8% with the different antigenic extract from various fungi.

Table-1: Results of skin tests* performed on patients and healthy individuals, with extracts of different fungal spores.

S. No.	Organism tested	Total test	Patients			Healthy person	
			3+ to 4+ (%)	2+ to 3+ (%)	1+ (%)	Total tests (%)	1+ (%)
1.	*Aspergillus niger*	128	10.9	28.0	28.0	26	5
2.	*A. fumigatus*	124	6.5	14.5	29.0	26	10
3.	*A. flavus*	128	4.0	16.0	37.5	20	10
4.	*Alternaria* sp.	128	4.7	11.0	32.0	26	5
5.	*Candida* sp.	88	14.7	28.4	37.5	20	10
6.	*Mucor* sp.	88	5.7	17.0	31.8	29	4
Average		**684**	**7.4**	**18.8**	**32.4**	**147**	**7.3**

* The history of the allergy was carefully established in each patient. Intradermal skin tests were carried out on the patients by injecting 0.2ml of 1:500 (w/v) of various antigens in the dermal layers of the skin. The results of the tests were read at twenty minutes and were graded according to the criteria proposed by Shivpuri (1965).

Heat stability and non-dialyzability of the fungal spore antigens

From the table-2 it is obvious that in most of the cases screened, allergenicity of the fungal extract, after heat treatment and dialysis, remains unchanged or decreased only slightly. The major allergenic principle of all these fungi, therefore, appears to be heat stable and non-dialyzable.

Table-2: Results of skin tests* with different antigenic extracts after heat treatment and extensive dialysis against phosphate buffer saline (pH 8.0).

S. No.	Name of the extract	Total tests done	Sin reactivity without any treatment	Skin reactivity after heat treatment	Skin reactivity after dialysis
1.	*Aspergillus niger*	10	+++	++±	++±
2.	*A. fumigatus*	10	+++	+++	++±
3.	*A. flavus*	10	+++	++±	++±
4.	*Alternaria* sp.	10	+++	++±	++±
5.	*Candida* sp.	10	+++	+++	++±
6.	*Mucor* sp.	10	+++	++±	++±

* The history of allergy was carefully established in each patient. Intradermal skin tests were carried out on the patients by injecting 0.02ml of 1:500 (w/v) of various antigens in the dermal layers of the skin. The results of the tests were read at twenty minutes and were graded according to the criteria proposed by Shivpuri (1965).

4. Discussion

Purification of CAP allergens

The results of the present study indicate that the allergenic component of CAP like allergens of most of the pollens (Berrens, 1971) is also non-dialyzable protein which can be precipited from aqueous extract by salts.

The first step of purification involving ammonium sulphate precipitation resulted in 5.2 fold purification of the allrgenic principle, without any significant loss in allergenic activity.

Multiplicity of CAP allergens

Gel filtration effected resolution of two minor allergens of CAP in addition to the main allergenic component. The minor allergens differed from the main component in their molecular size and elicited much lower skin response on human and guinea pig skin. By DEAE-cellulose chromatography also, three minor allergenic fractions were resolved besides the main allergen. All these fractions differed from each other in their net ionic charge. These results suggest that allergenic constituents of CAP occur in multiple forms differing from each other in molecular size and electrical charges. CAP allergens, thus show close resemblance with the allergens of birch, ragweed pollen and ascaris which also display a wide range of molecular size and ionic charge.

Heat Stability

Results of this study indicate the presence of both heat labile and heat stable antigens in CAP as demonstrable by gel diffusion and PCA. The heat labile antigen (precipitinogen) of CAP have higher molecular weight and a lower ionic charge than the stable antigens and could be separated from the later by gel filtration and DEAE-cellulose chromatography. The allergen activity of crude extract of CAP appears to be associated with the heat stable antigens giving precipitin reaction since the allergenic activity of the crude extract remained unaltered after the heat treatment.

The heat stability of the CAP allergenic antigens as discussed above, is in accordance with the observations of Augustin and Belin on grass and birch pollen allergen. The allergenic activity of these pollens has also been shown to be associated with the heat stable precipitinogens (Belin, 1972). According to these authors, the thermolabile pollen antigens are non-allergenic and are found in immunodiffusion analysis to give precipitin lines towards the antigen well, whereas the lines formed by the allergenic thermostable antigens occur near the antiserum well. Similar localization of precipitin lines associated with the allergen activity was observed in the present study and is in agreement with the fact that the molecular weight of the allergens is lower than that of the non-allergenic antigens. The allergenic antigens with a lower molecular size will migrate to a longer distance than the non-allergenic antigens in gel diffusion. In attempting to understand the phenomenon of heat stability, it must be remembered that atopic allergens present themselves as already intrinsically denatured glycoproteins which have survived conditions

potentially deleterious to antigenic structure. As suggested by Berrens and Bleumink (1965), sugar reduces linked to the protein framework of the molecular are presumably, responsible for the stability of the allergen.

Molecular Weight

The purified allergenic principle of CAP has a molecular weight around 24,000. This figure is well within the molecular weight range of 10,000 to 40,000 reported for most of the pollen and other allergens (Ambler et al. 1973; Takao et al. 1974; Strejan, 1975; Malley et al. 1976; Lowenstein et al. 1976). No definite explanation is available as to why an antigen should fit into limited range of low molecular size in order to act as allergen. It is possible that small molecular size of the allergens favours their penetration through mucous membrane. Another logical proposition is that the intramolecular distances between the allergenic determinants would appear to constitute a more critical characteristic for the initiation of the allergic reaction because bridging of the spatial gap between two cell fixed IgE molecules, believed to initiate the type I hypersensitivity reactions would also demand a restricted molecular size.

Content Of Allergen In Pollens

The allergenic principle of CAP comprised 0.08% of the total dried and defatted pollen. The concentration of allergenic material in CAP is comparable to that present on ragweed pollen (0.03%), Kapok (0.04%), Fish (0.05%) and Cow's milk (0.1%) (Berrens, 1971). Wide variations have however been observed in the percentage of allergenic constituents of other sources. The values range from 0.0025% as reported in the case of tomato allergen to 10% demonstrated for horse dandruff. In addition to the variation of allergenic contents observed among different sources, variation in percentage of allergenic principle has also been demonstrated in different batches of the same allergen preparation. The later variations are attributable to variation in heating and drying conditions during the isolation process of the different batches. Furthermore, the proportion of allergen in the dried source is governed to some extent by certain external factors specially those regulating generation, destruction or inactivation of allergen (Berrens, 1970a).

Chemical Composition of Purified CAP allergen

Protein and Carbohydrate Contents

Chemical analysis revealed the purified allergenic principle of CAP to be a glycoprotein, composed of about 86% protein and 14% carbohydrate. Exact value of protein contents of the allergenic component was established by multiplication of the Kjeldahl nitrogen value with the conversion factor of 6.25 because Lowry method for protein estimation yielded exaggarated protein contents, probably due to the interference of carbohydrate residues when estimated by Folin-Oiocalteu phenol reagent (Lowry et al. 1951). The proportion of sugars found in purified allergen (14.2%) was very close to that reported in horse dandruff (13.9%) and hay allergen (14.6) but was higher than that reported in ragweed pollen allergens E (0.5%), K (0.6%), Ra3 (12.4%) and rye grass pollen allergen (5.4%) (Berrens, 1971). Considerably higher proportion of carbohydrate residues have been reported in house dust (28-32%) (Berrens, 1970b), green pea (30%) (Malley et al. 1976), liquorice (34.4%) (Berrens, 1964), ascaris (76.5%) and tichophytin (83%). The sugar residues, mainly, sialic acid, hexosamine, glucose, galactose and arabinose, found in the allergenic component of CAP, have also been shown to constitute the carbohydrate moiety of allergens from various other sources (Berrens, 1971). Presence of sialic acid and hexosamine with certain other sugars has been demonstrated in horse and human dandruff allergens (Berrens, 1971). Carbohydrate moiety of ragweed allergen has however been found to be exclusively composed of arabinose.

From the data discussed above it appears that the chemical composition of individual allergens with respect to the protein and carbohydrate contents varies considerably, both qualitatively as well as quantitatively. Several allergen of plant origin are mainly built up of complex heteropolysaccharide chains combined with a small proportion of peptide as in liquorice and tichophytin allergens (Barker et al. 1967), on the other hand, some protein allergens display barely detectable carbohydrate residues e.g. cotton beans and ragweed allergen Ra5 (Lapkoff and Goodfriend, 1974). For simplicity it is safe to state that atopic allergens fall into a category of glycoproteins of widely varying overall composition.

Amino Acid Composition

As revealed by amino acid analysis, the protein moiety of the purified CAP allergen is constituted by most of the essential and non-essential amino acids. The allergen molecule was, however, found deficient in arginine, proline and cysteine residues. The allergen was also deficient in tryptophan and methionine but deficiency of these amino acids is most likely to be due to their destruction under the conditions of hydrolysis employed. The overall amino acid recovery was poor and accounted for 13-14 per cent, of the protein subjected to hydrolysis. This figure however compares well with the amino acid recoveries reported in case of house dust (11.06%) (Kraewinkels-Versine, 1969) and feathers (11.86%) allergen protein. Poor amino acid recoveries in house dust and feather allergens were ascribed to the hard nature of their proteins. Furthermore, acid hydrolysis in the presence of large amounts of sugars, as present in these allergens, entails heavy losses of amino acids resulting in poor recoveries.

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