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Sensitization to Different Species of *Aspergillus* in Bakery Workers and General Atopic Population

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SUMMARY Six species of *Aspergillus* predominant in the bakery environment- *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. ochraceous*, *A. sydowi* and *A. versicolor*- were studied for their role in causing Type 1 hypersensitivity among bakery workers and atopic patients from the general population (PGP). Antigenic extracts from the above species were prepared for *in vivo* and *in vitro* studies. The IEF, SDS-PAGE, skin test, ELISA and immunoblot techniques were performed to detect the biochemical- and clinico-immunological characteristics of these species. Among those tested, the important fungal sensitizers among the bakery workers and patients from the general population were *A. sydowi*, *A. fumigatus*, *A. nidulans* and *A. ochraceous*. The protein fractions of different species were in the acidic region (pI 3.0-6.5) and in the molecular weight range of 13.0-91.0 kDa. The protein fraction of 44.0 kDa of *A. flavus* and 20.0 and 70.0 kDa for *A. fumigatus* showed IgE binding in the sera of bakery workers only. Significantly. raised IgG antibodies to dif-ferent species were recorded among the bakery workers as compared to the PGP group. The study showed that different species of *Aspergillus* are of potential allergenic significance in bakery workers and the general atopic population.

Airborne fungal spores are an important component of bioaerosol and their role in respiratory allergy and occupational asthma is well established.[1-3] Several work environments such as bakery, brewery, poultry, food processing facilities, etc. support the growth of certain preferentially selected organisms, which might sensitize individuals, leading to the development of hypersensitivity lung disorders. These disorders are caused by repeated exposure to such organisms in the occupational environments.[4] The main objective of occupational hygiene is the prevention and/or adequate control of exposure of individuals to such fungi arising from the workplaces, as they are hazardous to health.[5] Reliable exposure and effect studies are the major steps towards diagnosis and management of such ailments. In our earlier communication on airborne fungi in a bakery environment we recorded that aspergilli contributed 45% to the total spore load, besides smut and *Cladosporium spp.*[6] A medical questionnaire survey revealed the prevalence of respiratory dysfunctions such as allergic rhinitis, asthma and cough, among 40% of the bakery workers.[6] Liberation of spores in large quantities round the year and their small size make aspergilli an important inhalant allergen.[7] Aspergilli are associated with a spectrum of human diseases such as allergic asthma, allergic broncho pulmonary aspergillosis (ABPA), invasive aspergillosis, and aspergilloma. Although several workers have stressed that different species of aspergilli are of patho-

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genic and allergenic significance,[8] the role of only *A. fumigatus* has been elucidated in detail. In view of the importance of aspergilli in the diagnosis and therapy of respiratory allergic patients, the present study was aimed at determining the allergenicity of dominant airborne *Aspergillus* species among bakery workers and patients from the general population (PGP), and partially characterizing these extracts using biochemical, clinical and immunological techniques.

MATERIALS AND METHODS

Antigens

Six species of *Aspergillus* (*A. flavus*, *A. fumigatus*, *A. nidulans*, *A. ochraceous*, *A. sydowi* and *A. versicolor*) isolated from culture plates exposed to the atmosphere were selected for antigen preparation. Pure isolates of these species were mass cultured in Sabouraud's liquid medium. The defatted fungal powders were then completely dried and stored at 4°C. The defatted fungal powder was extracted in 1:20 w/v in 0.05 M ammonium bicarbonate buffer (pH 8.1) by continuous stirring for 20 hours at 4°C, followed by centrifugation and dialysis against distilled water with visking tubing (cut off 3,500) for 24 hours. Subsequently, centrifugation was repeated and the supernatant passed through millipore filter (0.45 μm), lyophilized in small aliquots and stored at -20°C for further use. The same batch of antigens was used for all the experiments. The protein content of different fungal extracts was estimated using the method of Lowry *et al.*[9]

Isoelectric focusing (IEF)

IEF was conducted on narrow range (pI 4.0-6.5) precasted Ampholine PAG plates (Pharmacia, Sweden) using Pharmacia Multiphor-II system. The resolved protein components were detected by staining with Coomassie Brilliant Blue R-250.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The antigenic extracts were also fractionated using a 10% acrylamide separating gel and 4% stacking gel, prepared using the buffer system of Laemmli.[10] The samples were electrophoresed using Protean II-mini gel electrophoretic apparatus (Bio-Rad, U.S.A).

Skin test

Nineteen bakery workers were selected for intradermal tests, from the group of workers identified by questionnaire as having respiratory disorders. Of the 40% of workers having respiratory problems (unpublished) only 19 volunteered for clinical tests. Skin tests with fungal extracts were also carried out on 53 atopic patients from the general population (not bakery workers) attending the Allergy Clinic of the V.P. Chest Institute, Delhi and 15 healthy volunteers as controls. Along with the extracts, buffered saline and histamine hydrochloride (100 μg/ml) were also injected to act as negative and positive controls, respectively. The wheal diameter and erythema were measured and graded after 15-20 minutes of the test. All the antigens were skin tested simultaneously on each individual. If 2 or more *Aspergillus* antigens gave the same degree of positive reactions in an individual tested, it was called an identical positive reaction, while if a different grade of positive reactions was recorded, it was considered a non-identical positive reaction.

Serum samples

Venous blood was collected from all of the individuals, including the 15 healthy volunteers who were skin tested with *Aspergillus* antigens. The blood samples were allowed to clot and the sera separated aseptically and stored at -20°C for future use.

Enzyme-linked immunosorbent assay (ELISA)

Specific IgE and IgG antibodies against different extracts were measured by indirect ELISA according to the method outlined by Sepulveda *et al.*[11] Human sera diluted to 1:5 was used for estimation of IgE, while 1:10 was used for IgG to obtain optimum results. For detecting bound IgE and IgG antibodies, alkaline phosphotase labelled antihuman IgE and IgG antibodies in suitable dilutions were used with *p*-nitrophenyl phosphate as substrate.

Grading of specific IgE and IgG antibodies

To assess the degree of binding, percent binding of the sera with the highest optical density was calculated based on Kauffman *et al,*[12] with slight modifications as outlined below:

$$\frac{A_o - A_y}{A_x - A_y} \times 100$$

where, A_x = mean O.D of 10 highly positive sera

A_y = mean O.D obtained with 10 normals sera

A_o = O.D of individual serum being analysed

Statistical analysis

The results were analysed for significant differences by analysis of variance (ANOVA), wherever required.

Immunoblotting

The method of Towbin *et al.*[13] was followed to electrophoretically transfer the SDS-PAGE fractionated proteins onto nitrocellulose membrane (NCM) using Bio-Rad Mini-Transblot apparatus. The NCM strips were incubated with the pooled sera (1:5) of bakery workers, the PGP group and healthy volunteers. For detecting bound antibodies, both IgE and IgG, strips were incubated in peroxidase-labelled antihuman IgE/IgG antibodies in suitable dilutions. The blot was developed using DAB as substrate.

RESULTS

IEF

The protein components of different species of aspergilli as fractionated on Ampholine-PAG plates (pI range 3.0-6.5) can be seen in Fig. 1. *Aspergillus flavus* and *A. fumigatus* had 12 protein bands each, while *A. nidulans* separated into 13 fractions in the range of pI 3.0 to 4.4. *Aspergillus ochraceous* fractionated into 24 fractions, of which 11 were sharp and thick. *A. sydowi* showed 17 distinct protein components, while *A. versicolor* had only 9 fractions with sharp bands at pI 3.1 and 3.6.

SDS-PAGE

The protein profile of the extracts of aspergilli in SDS-PAGE (Fig. 2) revealed that *A. flavus* had 16 distinct bands with the majority of them below 30.0 kDa. The antigenic extract of *A. fumigatus* showed 18 fractions of which 13.8, 20.0, 33.0 and 70.0 kDa were pro-

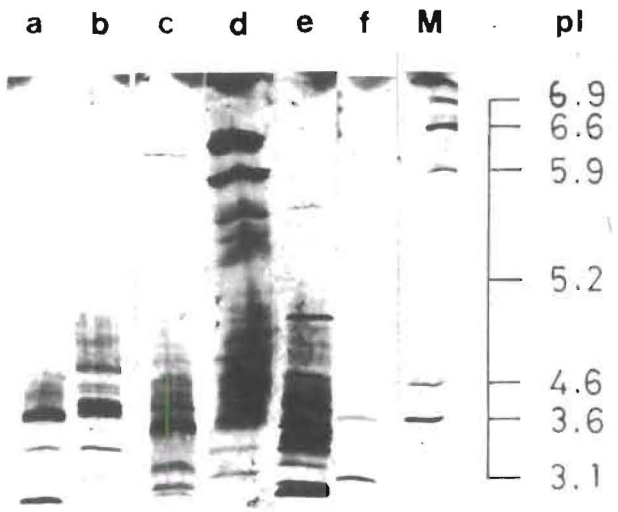

Fig. 1 IEF protein profile of antigenic extracts of different species of *Aspergillus* as electrofocussed on Ampholine-PAG plates (pI 4.0-6.5). Lane a - *A. flavus*, b - *A. fumigatus*, c - *A. nidulans*, d - *A. ochraceous*, e - *A. sydowi*, f - *A. versicolor*, M - pI markers.

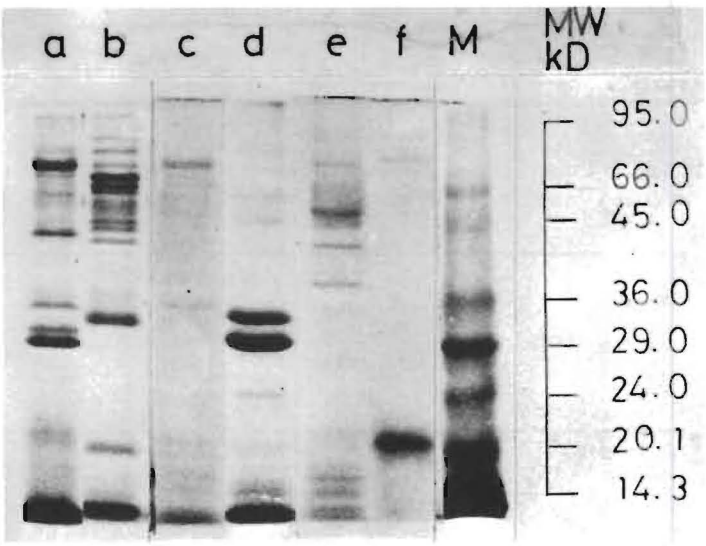

Fig. 2 SDS-PAGE (10%) protein profile of extracts of different aspergilli. Lane a - *A. flavus*, b - *A. fumigatus*, c - *A. nidulans*, d - *A. ochraceous*, e - *A. sydowi*, f - *A. versicolor*, M - M.W markers.

minent. *A. nidulans* showed 10 protein fractions, while *A. ochraceous* had 15 fractions with sharp bands at 13.5, 29.0 and 32.5 kDa. The extract of *A. sydowi* fractionated into 13 conspicuous protein components, whereas *A. versicolor* separated into only four protein fractions with 20.0 kDa being prominent.

Skin test

The results of intradermal testing with different species of *Aspergillus* on 19 bakery workers and 53 PGP (Table 1) revealed workers (15.9%) with marked positivity (2+ to 4+) to *Aspergillus fumigatus* and *A. sydowi*. None of the normals had a marked positivity (2+ or more) to any of the extracts tested, although 1+ reaction was obtained in some individuals, which is not considered to be clinically important. The intradermal test results conducted on the 53 members of the atopic PGP group showed that *Aspergillus fumigatus* elicited marked positivity in 26.4% of cases, followed by *A. versicolor* with 22.6%.

An analysis of identical and non-identical skin reactions to the extracts of different species of *Aspergillus* (performed on the same patients) showed that *A. sydowi* had the maximum identical positive reactions (8/17) (Table2). However, non-identical reactions were observed in more than 60% of cases with different extracts showing limited cross reactivity.

ELISA

According to the formula adopted by us, O.D. values of 0.24 and 0.49 had 15% and 30% binding for IgE, respectively. Similarly , 0.9 and 1.19 O.D. values had 15% and 30% binding, respectively for IgG. Based on the above values, grading of the O.D values of each serum sample was carried out.

Greater than 15% binding in a serum was considered as indicating raised specific IgE/IgG antibodies, while serum with greater than 30% binding had statistically significant ($p < 0.05$) raised antibodies as compared to normals (confirmed by ANOVA).

The percentage of bakery workers and PGP having >15% and 30% binding only in their sera to different *Aspergillus* extracts are shown in Table 3. The maximum number of workers (36.8%) had raised IgE to *A. sydowi*, with 21.0% having significantly raised IgE antibodies (> 30% binding), while in the PGP group, only 11.3% of cases had significant binding. Interestingly, none of the workers had > 30% binding to any other species of *Aspergillus*.

The specific IgG levels among the bakery workers were compared with those of the symptomatic patients from the general population (20/53) to see the effect of bakery environment. Fig.3 shows that almost all the workers had considerably raised IgG antibodies as compared to atopics from PGP. When the mean O.D values of

Table 1. Results of Intradermal skin tests conducted with antigenic extracts of six different species of *Aspergillus* on 19 symptomatic bakery workers and 53 patients from general population

Antigen	Skin Reaction							
	1+ to 4+				2+ to 4+			
	Bakery		PGP		Bakery		PGP	
	No.	%	No.	%	No.	%	No.	%
A. fumigatus	6	31.6	16	30.2	3	15.8	14	26.4
A. sydowi	5	26.3	15	28.3	3	15.8	9	17.0
A. nidulans	6	31.6	12	22.6	2	10.5	6	11.3
A. ochraceous	5	26.3	8	15.1	2	10.5	7	13.8
A. versicolor	5	26.3	15	28.3	2	10.5	12	22.6
A. flavus	4	21.0	12	22.6	1	5.3	9	17.0

None of the healthy volunteers gave a 2+ or more reactions

ELISA from workers and patients from the general population were compared, the workers had significantly ($p< 0.01$) higher O.D values, indicating a higher level of specific IgG in workers, which is important.

Immunoblotting

The specific IgE binding pattern to different aspergilli from the sera of bakery workers is given in Fig. 4A. Four SDS-PAGE fractions (13.8, 25.0, 44.0, 47.0) of *A. flavus* showed IgE binding, with greater intensity at 13.8 kDa. *A. fumigatus* had 7 fractions (20.0, 33.0, 40.0, 43.0, 47.0, 66.0 and 70.0 kDa with binding to IgE antiodies of the bakery workers. The fractions of 20.0 and 70.0 kDa had

Table 2. Patients showing identical and non-identical skin reactions to different species of *Aspergillus* conducted on same 53 patients

A. flavus	***A. fumigatus***				***A. ochraceous***				***A. versicolor***				***A. sydowi***				***A. nidulans***			
Skin reactions	-	+	2+	3+	-	+	2+	3+	-	+	2+	3+	-	+	2+	3+	-	+	2+	3+
-	47	4	3	0	49	3	2	0	45	6	3	0	50	4	0	0	46	5	2	0
+	3	1	2	1	3	2	0	2	3	0	3	1	4	2	0	1	1	3	2	1
2+	1	0	3	1	1	2	1	1	1	2	2	0	2	1	2	1	1	2	0	2
3+	0	0	3	2	2	0	1	2	0	1	0	4	0	0	1	4	0	0	3	2
Total positive identical reactions	6/17				5/17				6/17				8/17				5/17			
Total positive non-identical reactions	11/17				12/17				11/17				9/17				12/17			
Total positive reactions	13/17				11/17				13/17				12/17				15/17			

Table 3. Incidence (%) of workers from bakery and general patients population having raised IgE antibodies to different antigenic fractions from different species of *Aspergillus*

Antigen	**Total positivity** (> 15% binding)		**Marked positivity** (> 30% binding)	
	GP	BW	GP	BW
A. sydowi	20.8	36.8	11.3	21.0
A. flavus	11.3	5.3	5.7	0.0
A. ochraceous	7.5	5.3	7.5	0.0
A. fumigatus	5.7	5.3	5.7	0.0
A. nidulans	13.2	0.0	13.2	0.0
A. versicolor	5.7	0.0	3.8	0.0

GP - Atopic patients from general population
BW - Bakery workers

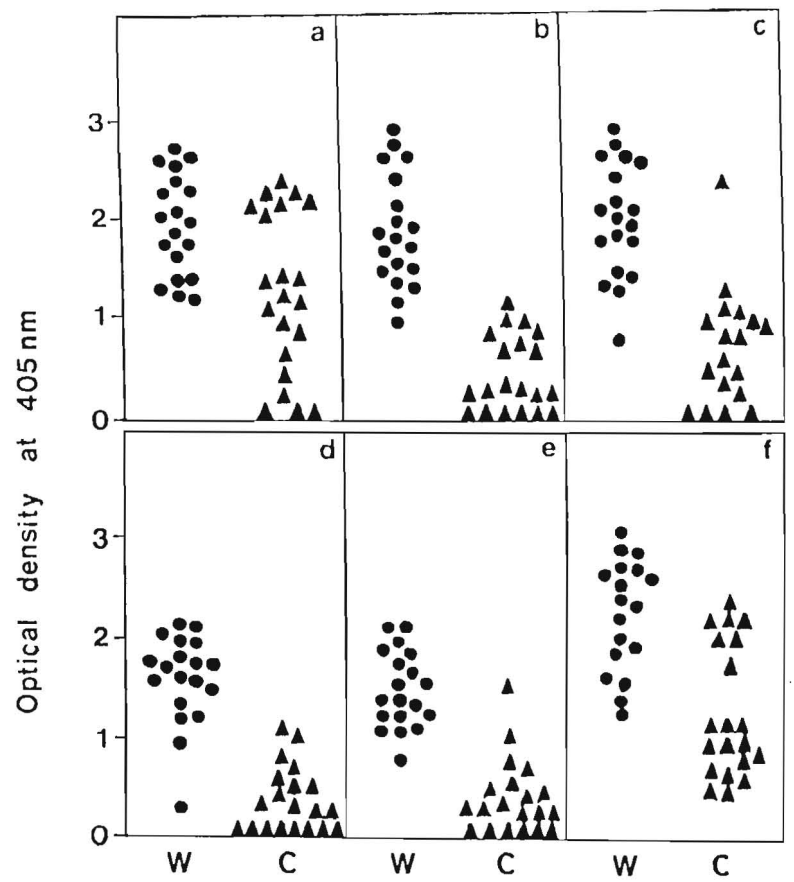

Fig. 3 Optical density (O.D) values of specific IgG antibodies to different extracts of aspergilli in the sera of bakery workers (W) and patients from general population (C), in indirect ELISA assay. a - *A. flavus*, b - *A. fumigatus*, c - *A. nidulans*, d -*A. sydowi*, e - *A. ochraceous*, f - *A. versicolor*.

strong binding as compared to the others. Only one fraction of *A. nidulans* (35.0 kD) showed IgE binding out of the 10 protein components fractionated on SDS-PAGE. In the extract of *A. ochraceous*, the 32.5 kDa fraction had intense binding, while others were faint but distinct. In the antigenic extract of *A. sydowi*, fractions of 37.0, 45.0 and 70.0 kDa are prominent, as compared to 42.5 and 74.85 kDa fractions which show weak binding. No IgE binding was observed for *A. versicolor* in the pooled sera of the bakery workers. The binding pattern of IgE antibodies of the PGP group to different extracts of aspergilli is documented in Fig. 4B. The 13.8, 25.0. 35.0 and 47.0 kDa fractions of *A. flavus* showed binding to sera of PGP, although the binding was weak as compared to that of bakery workers. The pooled sera showed strong binding to 70.0 kDa fraction of *A. fumigatus*, while moderate to weak binding was observed at 20.0. 43.0, 47.0 and 60.5 kDa. The same fraction (35.0 kDa) of *A. nidulans* showed binding to the pooled sera of both groups studied. The extract of *A. ochraceous* showed additional IgE binding at 25.5, 50.0 and 67.0 kDa, besides the fractions which had binding to IgE in the sera of bakery workers. The 29.0 and 32.5 kDa had a very strong binding. In the extract of *A. sydowi*, strong binding was recorded at 38.0, 42.5 and 74.5 kDa, while those of 20.5, 37.0, 45.0 and 70.0 had a weak binding. As in the case of the bakery workers, none of the fractions of *A. versicolor* showed IgE binding in the PGP also.

The IgE binding patterns of *Aspergillus ochraceous* and *A. sydowi* with individual sera of hypersensitive patients were assessed to identify the major allergenic fractions, as these have been tested for the first time in Indian population (Figs. 5,6). The specific IgE binding pattern to *A. ochraceous* in 19 sera showed that fractions of 29.0 and 32.5 kDa had binding in 100% of sera, while 23.5, 50.0 and 62.0 kDa showed binding in 68.4%. Only 10.5% had binding to 25.0 kDa fraction (Fig. 5). The antigenic extract of *A. sydowi* exhibited IgE binding at 38.0 and 42.5 kDa in 87.5% of cases, while 43.8% of sera bound to 70 and 74.5 kDa fractions, and 31% showed binding to 37.0 kDa protein component. The 20.5 and 45.0 kDa protein fractions recorded IgE binding in only 17.5% and 6.2% of cases, respectively (Fig. 6).

DISCUSSION

The characterization of fungal extracts from various fungi have revealed that protein components are in the acidic region.[14,15] In our study, the extracts of different *Aspergillus* species showed considerable variability in their pIs

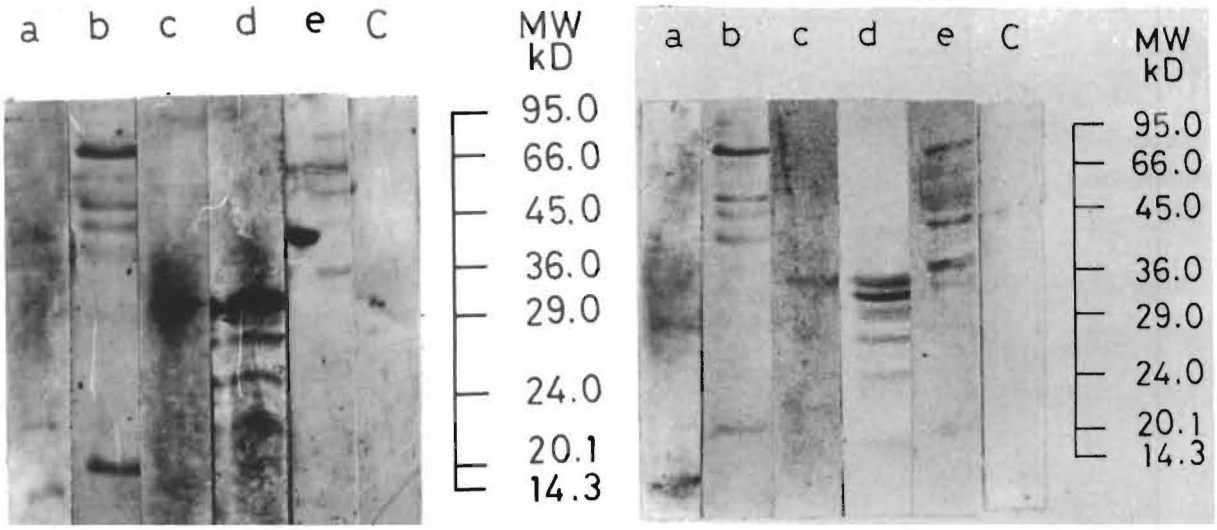

Fig. 4 Immunoblotting of different extracts of *Aspergillus* with the pooled sera of bakery workers (A) and atopics from general population (B). a - *A. flavus*, b - *A. fumigatus*, c - *A. nidulans*, d - *A. ochraceous*, e - *A. sydowi*, C - pooled control sera showing absence of any allergenic fractions against the extract of *A. fumigatus*. Similar control strips were also obtained for other extracts.

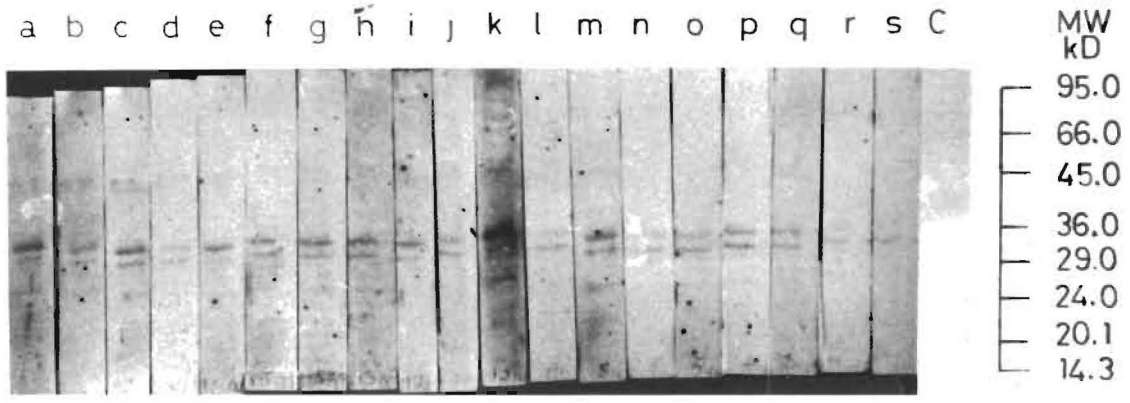

Fig. 5 Immunoblotting showing heterogeneity in the binding of IgE of patients from general population to the protein components of *Aspergillus ochraceous* as separated by SDS-PAGE. Lanes a - s represent individual serum and C - pooled sera of healthy volunteers.

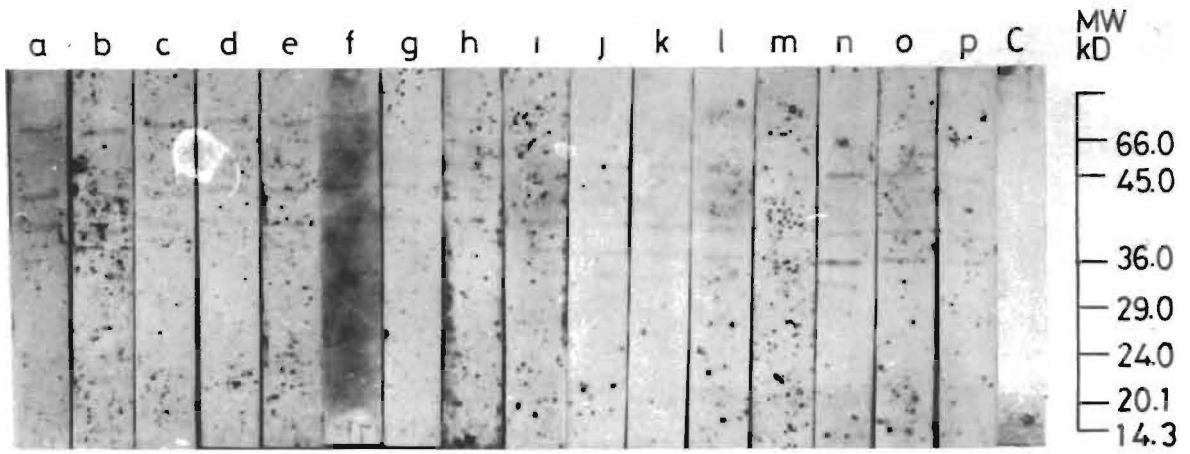

Fig. 6 Heterogeneity in allergen recognition by the serum IgE of patients (PGP group) sensitive to *Aspergillus sydowi*. Lanes a - p represent individual serum and C - pooled sera healthy volunteers.

and molecular weights. The antigenic extracts when fractionated on a broad pI range Ampholine-PAG plate (pI 3.5-9.5) were clustered together in the acidic region only, and therefore the antigens were also fractionated on narrow range Ampholine-PAG plates. All the 6 extracts had protein fractions in the acidic region (pI 3.0-6.5) and the number of fractions varied from 9 to 24 for different species. *A. ochraceous* had a few common fractions of pI 5.8, 6.0, 6.2 and 6.3 with *A. flavus,* while, pI 4.4 was shared with *A. sydowi.*

The protein profile by SDS-PAGE of the aspergilli under study varied from species-to-species, except for *A. fumigatus* and *A. flavus* which had three common fractions of 13.8, 47.0 and 70.0 kDa. *A. sydowi* also had a common protein fraction of 70 kDa, but was not conspicuous. Cross reactivity between *A. fumigatus* and *A. flavus* has also been shown by Bardana *et al.*[16] by inhibition studies. Antigenic extracts of different *Aspergillus* species had most of the protein components below 50.0 kDa, except *A. fumigatus* which had 9 out of 18 bands above 50.0 kDa, The prominent fractions of 70.0 kDa in the extract of *Aspergillus fumigatus* have also been reported by Leung *et al.*[17] *A. nidulans, A. ochraceous* and *A.versicolor* had 4 to 15 fractions ranging from 13.5 to 89 kDa. However, the results cannot be compared with that of other workers since literature is scanty on the characterization of different species of *Aspergillus.*

Several techniques have been used for *in vivo* and *in vitro* valuation of the allergenicity of fungal antigens. Assay using biological and immunological systems was limited in the case of fungal extract until the recent past, and such studies are important for the identification and characterization of allergenic fungal extracts. The reason for less extensive studies was in the high variability of antigenic content,[18] and the presence of shared allergenic components among related and even unrelated fungal taxa.[19]

Variability in the degree of sensitization to different species of aspergilli was recorded among the patients. *A. fumigatus* is the most important sensitizer for Type I hypersensitivity among both groups as investigated by skin test, followed by *A. sydowi, A. ochraceous* and *A. nidulans,* which are tested for the first time in Indian population. Studies based on ELISA showed statistically significant raised IgE antibodies to *A. sydowi* among the bakery workers only, while in the PGP group, significantly raised IgE antibodies were recorded against *A. nidulans, A. sydowi* and *A. ochraceous.* A variable degree of sensitization has also been observed with different species of *Aspergillus* by Novey

and Wells.[20] They recorded raised specific IgE to *A. fumigatus, A. niger, A. flavus, A. terreus* and *A. fishceri* in 15% of sera studied, while only 0.8% to *A. ochraceous.*

The reason for the disparity between skin test and ELISA could be that the wheal and flare reaction involves several biological variables which vary from individual-to-individual. It could also be because they were not well proven cases. Such observation has also been made by other workers.[21] Thus, the need for skin test in conjunction with *in vitro* tests for specific IgE is required for the diagnosis of allergy, as also emphasised by Nelson *et al.*[22]

Type I hypersensitivity to 6 species of aspergilli, except *A. sydowi*, as assessed by skin test and ELISA was found to be higher in the atopic PGP group as compared to bakery workers. The low skin reactivity and specific IgE level to different aspergilli among the bakery workers, could be due to the lower number of workers investigated; Or it could also be interpreted that atopy was due to some other environmental materials, such as flour, storage mites, enzymes, etc. which are known causative agents for it in the bakery. *A. flavus,* although it was recorded in high concentration in the outdoor and indoor environments,[6] was not observed to elicit markedly positive skin reactions or raised IgE antibodies in most of the bakery workers and PGP. This indicates a low level of sensitization to *Aspergillus flavus,* so rendering it of low importance for Type I hypersensitivity.

The antigenic extracts of different species of *Aspergillus* tested on the patients indicate that none of the extracts showed high identical positive skin reactions, as more than 60.0% of patients elicited non-identical reactions. This points towards a low degree of shared antigenicity among different species of aspergilli. Kim and Chaparas[23] made a similar observation while studying *A. fumigatus, A. flavus* and *A. niger* with rocket immunoelectrophoresis using the sera of skin test positive cases.

In our previous report we recorded a high concentration of aspergilli in the bakery environment, and thus bakery workers are regularly exposed to them.[6] A prolonged and heavy exposure to spores of aspergilli is known to cause several IgG mediated respiratory disorders, thus it was imperative to estimate the specific IgG antibodies against different aspergilli among the bakery workers. The fact that all the bakery workers had significantly raised ($p < 0.01$) specific IgG to different aspergilli as compared to even the symptomatic PGP, shows that they experience a continuous exposure to high concentrations of these species. In the closed environment of the bakery, the concentration is maintained for a prolonged time due to the lack of an escape route, while in the outside air the concentration is diluted. Kaukonen *et al.*[24] have observed that, *A. umbrosus* specific IgG antibodies were significantly higher ($p < 0.001$) among farmers with or without Farmer's Lung disorder, as compared to healthy controls. Thus, it is necessary to have fixed standard upper limits for biological particles in closed environments for the proper management of susceptible individuals/workers, it is necessary to monitor the trend over time of respiratory diseases in occupational environments.

We observed that the protein fraction of 44.0 kDa of *A. flavus* was unique to the sera of bakery workers, while 35.0 kDa had binding to IgE in the sera of the PGP group. The 20.0 and 70.0 kDa fractions of *A. fumigatus* antigen are important, as they showed binding in sera of both the groups studied, while 33.0 and 40.0 kDa fractions are unique for bakery workers only. The 70.0 kDa protein fraction of *A. fumigatus* has also been found to be allergenically important by Leung *et al.*[17] Topping *et al.*[3] made an interesting observation that the commercial extract of *A. niger* did not produce a wheal and flare reaction among workers of citric acid manufacturing unit, but the one isolated from the unit gave a positive reaction. Thus, identification of allergenic fractions of different aspergilli, inducing raised IgE in bakery workers, will be useful in the diagnosis of occupational allergies.

Although, *A. nidulans* separated into 10 fractions, only one allergenic fraction of 35.0 kDa was observed in the antigenic extract by bakery workers and PGP sera, thus making it allergenically important. *A. ochraceous* has been studied mostly for toxicity, which is due to ochratoxin, but not for allergenicity. Most of the important allergenic fractions were of low molecular weight (18-32.5 kDa). *A. sydowi* gave strong IgE binding at 37.0, 45.0 and 70.0 kDa fractions with the sera of bakery workers. Except for the 70.0 kDa, the rest of the important allergenic fractions for all the other species of *Aspergillus* studied were less than 45.0

kDa, which conflicts with the observation of Kauffman *et al.*,[15] that the IgE binding is poor with low molecular weight fractions, but conforms with the observation of Leung *et al.*[17] that the most common IgE reacting band were above 30.0 kDa in *Aspergillus fumigatus*.

A. fumigatus had one common allergenic fraction (70.0 kDa) with *A. sydowi* and another of 47.0 kDa with *A. flavus*, Thus showing that shared allergenicity does exist but is poor among the *Aspergillus* species. The 70 kDa fraction of both species has shown binding to IgG antibodies in the pooled sera of bakery workers and the PGP group (Unpublished). We observed that in the same individual, *A. sydowi* elicited a Type III reaction, while *A. fumigatus* did not, as confirmed by raised IgG levels in the serum (ELISA) (Unpublished). Thus, further work is necessary to determine whether the 70 kDa fraction of *A. sydowi* corresponds to that of *A. fumigatus*. Since the patients showing ABPA symptoms but with low IgG to *A. fumigatus*, may be sensitized by *A. sydowi*. Shen *et al.*[26] recorded only one common fraction of 67.0 kDa between *A. fumigatus, A. niger, A. terreus* and *A. flavus*. We recorded a 67.0 kDa fraction only in the extract of *A. fumigatus* when fractionated on SDS-PAGE, but it had no IgE binding.

All the sera showed binding of specific IgE antibodies to 29.0 and 32.5 kDa fraction of *A. ochraceous*, and thus are the major allergenic fractions. The 38.0 and 42.5 kDa fraction of *A. sydowi* had binding in almost 88% of cases and can therefore be considered as major components of this fungus. Thus, homogeneity was observed in the IgE binding pattern of *A. ochraceous* and *A. sydowi* antigenic extracts with individual sera from the PGP group as most of the bands were recognized by the majority of the sera. These major allergens require further purification and characterization.

The sera from both the groups did not show binding to any of the fractions of *A. versicolor*, although it showed a positive skin test and ELISA. This can be attributed to cross-reactive substances. Another reason could be that the important epitopes responsible for IgE binding would have become denatured due to heat or the SDS-PAGE system. Brouwer[27] made a similar observation with the *A. fumigatus* extract, where 48% ELISA positive sera gave negative or normal blots for IgG antibodies.

The work presented here, in conjunction with the aerobiological investigations reported elsewhere,[6] demonstrated that different species of aspergilli are important sensitizers in the bakery environment, and a potential cause of IgG mediated allergic diseases, besides the PGP group. Recently, Zock *et al.*[28] observed significantly raised IgG antibodies against airborne dust due to occupational exposure, among workers in the potato processing industry. Thus, care should be taken in limiting environmental exposure, besides diagnosis and therapy of susceptible individuals. The result of this study will provide the basis for long-term follow-up studies on *Aspergillus* and other important fungal species, such as *Neurospora sitophila, Penicillium, Paecilomyces,* for their role as occupational sensitizers.

Finally we conclude that, i) the extracts of different species of *Aspergillus* should be used for effective diagnosis and treatment of respiratory diseases as they are species specific in nature, ii) *A. fumigatus, A. sydowi* and *A. ochraceous* are important sensitizers in Type I respiratory disorders in bakery workers and the general atopic population alike, iii) bakery workers revealed a higher level of IgG to different aspergilli as compared to the general atopic population, and thus are likely to develop Type III hypersensitivity, iv) most of the allergenic fractions of aspergilli were below 45.0 kDa, except for 70.0 kDa fraction of *A. fumigatus* and *A. sydowi*, v) shared allergenicity was limited and recorded with one fraction each of *A. fumigatus* with *A. flavus* and *A. sydowi*, and vi) the important allergenic fractions for *A. ochraceous* were 29.0 and 32.5 kDa, while, 38.0 and 42.5 kDa for *A. sydowi*.

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