

[Home](#)[Table of Contents](#)[Past Issue](#)[About the Journal](#)[VPCI](#)[NCCP](#)[Editorial Board](#)[Information for Authors](#)[Subscription Information](#)[Advertisement Tariff](#)[Contact Us](#)

Heterogeneity of Immune Responses to Various *Aspergillus* Species in Patients with Allergic Respiratory Diseases

Sunita Aggarwal, S.K. Chhabra (1), R.K. Saxena (2) and M.K. Agarwal

Departments of Respiratory Allergy and Applied Immunology and Cardio-respiratory Physiology (1), V. P. Chest Institute, University of Delhi, Delhi and Department of Microbiology (2), University of Delhi South Campus, New Delhi

Abstract

In the present study, allergenic significance of thirteen species of *Aspergillus* and their allergenic and antigenic relationship was studied. Of the 3025 ID tests performed with the 13 species of *Aspergillus* on 289 patients suffering with allergic respiratory diseases, 627 (20.7%) were positive (1+ to 4+), 386 (12.8%) being significantly positive (2+ to 4+). Of the 64 patients eliciting a positive cutaneous response to at least one species, 42 (65.6%) were positive to 5 or less number of species while others showed a broad spectrum of positive skin reactivity to different *Aspergillus* extracts. In RAST inhibition assays using pooled sera of patients sensitive to *A. tamarii* dose related inhibition was produced by homologous as well as 5 of the 12 heterologous species. Similarly, in *A. terreus* RAST inhibition was observed with homologous and *A. tamarii* extracts only. Our results suggested the presence of both species specific as well as shared allergenic components among different *Aspergillus* species. In TDIEP experiments using rabbit antisera to *A. tamarii* and *A. terreus* extracts multiple precipitin bands were observed with the homologous extracts. However, only 1–2 bands were produced by 6 heterologous *Aspergillus* species in each system. Collectively, these results gave evidence that there is heterogeneity of immune response in the patients with allergic respiratory diseases to different species of *Aspergillus* and also in rabbits immunized with *Aspergillus* extracts.

Key words : Heterogeneity, Immune response, Allergenic determinants, Homologous extracts, Heterologous extracts.

Introduction

Fungal allergen extracts are usually crude mixtures of a number of biochemical constituents, only some of which may have allergenic and/or antigenic properties. The allergenic and antigenic components may vary with the species and genera of fungi. The patients with allergic respiratory disorders differ in their response to the fungal extracts and show varying degree of response to various allergenic components of a given extract. These biologically active allergenic and antigenic components may be specific to one species¹ or may be shared among different species and genera of fungi (2–5). Cross-reactivity or species specificity of allergens/antigens among different fungi have important clinical and diagnostic implications. Only a limited number of studies on allergens of India are available in literature on this aspect.

Aspergillus species are common air-borne fungi all over the world including Delhi area. More than 16 species of *Aspergillus* were encountered in different aeromycological surveys conducted in Delhi area (6–8). In the present study we have investigated the allergenic significance of 13 common air-borne species of *Aspergillus* for respiratory disease patients.

Material and Methods

Preparation of Allergen Extracts

Allergen extracts from thirteen different species of *Aspergillus*, isolated from Delhi atmosphere, were prepared and their allergenic and antigenic properties were studied and compared employing various clinico-immunologic techniques. For this purpose, each *Aspergillus* species

was mass cultured on synthetic medium (Asparagine Broth) for 3 weeks at 28 ± 2 degrees C. The cultures were checked for purity and harvested by adding ethyl alcohol in each flask. Fungal mats were separated from the culture medium by filtration, washed quickly with distilled water and dried in a lyophilizer. The dried fungal mats were milled to a fine powder and defatted with diethyl ether. Defatted fungal powders were extracted in 0.1 M ammonium bicarbonate at 4 degrees C for 72 hours with intermittent shakings. The extracts were then filtered and dialyzed (Spectrapor membrane tubing No. 3, MW cut off=3500, Spectrum Medical Industries, Los Angeles, USA) against 0.05 M ammonium bicarbonate (3–4 changes) followed by two changes with double distilled water. The dialyzed extracts were clarified by centrifugation at 10,000 RPM for 20 minutes or by filtration and freeze-dried. Allergen extracts were then stored in airtight vials at -20 degrees C.

Intradermal Tests

To evaluate their allergenic significance, intradermal (ID) tests were performed on a group of patients suffering from respiratory diseases with the extracts of different species of *Aspergillus* (1:500 W/V). These tests were performed and graded as per the method of Agarwal and associates (1990) (9). The criteria for grading the skin reactions are included in table 1.

Table 1. Criteria for grading intradermal reactions (Agarwal et al 1988)

ID test response	Skin test response as compared to histamine control	Pseudopodia
-ve	> 3 mm less	-
1+	Upto 3 mm less	-
2+	Equal	-
3+	Upto 3 mm more	May be present
4+	> 3 mm more	Present

As a control, skin tests were also performed on a group of non-allergic healthy volunteers (NHVs) with no personal or family history of allergy or associated diseases. Prior to performing skin tests, patients were instructed to stop all the short-acting antihistamines for 48–72 hours and long-acting anti-histamines like astemizole for at least 4 weeks. However, as recommended by Agarwal and co-workers (1988) (10) patients were allowed to take various sympathomimetic drugs and bronchodilators upto 2 hours before the performance of the tests.

Radioallergosorbent Tests (RASTs)

Allergen specific-IgE levels in the sera of patients with positive cutaneous reactions to various *Aspergillus* extracts were estimated in RAST assays. For this purpose, 5 ml blood was collected from each patient and healthy volunteer, serum was separated and stored in sterile vials at -20 degrees C. Sodium azide was added as a preservative (0.02%).

Preparation of allergen coupled paper discs : Each *Aspergillus* extract was coupled to cellulose paper discs (6 mm diameter, Whatman No. 50) according to the method of Adolphson and co-workers (1980) (11) with a few modifications. In brief, the discs (1 gm) were washed thoroughly with distilled water and stirred in ice cold cyanogen bromide solution (2 gm in 80 ml dimethyl formamide) for 40–50 minutes at pH 10–11, followed by sequential washings with ice cold 0.1M sodium bicarbonate, distilled water and finally with cold acetone. The activated discs were dried and suspended in 1–2 ml of allergen extract solution in borate buffered saline for 72 hours at 4 degrees C and uncoupled sites on the discs were blocked with ethanolamine solution for 24 hours. The discs were then washed with RAST diluent and stored at -20 degrees C.

Performance of RAST: Rast was performed as per the method of Gleich and Yunginger (1976) (12). Briefly, each allergen coupled disc was incubated with 50ml of patient's serum for 24 hours. Thereafter, the discs were washed thrice with RAST wash and further incubated for 16–20 hours with radiolabelled (I125) antihuman-IgE. The discs were then washed and counts bound were measured in a gamma counter. The percent counts bound per minute (% CPM) to the disc and the RAST ratios were calculated as under. A RAST was considered positive when its ratio with the patient's sera was 2 or more.

$$\% \text{ CPM} = \frac{\text{Total counts bound to the disc}}{\text{Total counts added}} \times 100$$

$$\text{RAST Ratio} = \frac{\% \text{ cpm with patients sera}}{\% \text{ cpm with normal human serum}}$$

RAST Inhibition Assays

Specificity of RAST responses was established by performing inhibition of *A. tamarii* and *A. terreus* RASTs with homologous extracts using the method of Gleich and Yunginger (1976) (12). For these assays, separate pools of patient's sera (PPS) were prepared by mixing equal volumes of sera from a group of patients eliciting significant positive ID reactions and high RAST values to *A. tamarii* and *A. terreus* allergen extracts. For studying the presence of shared allergens among different *Aspergillus* species, inhibition of *A. tamarii* and *A. terreus* RASTs was conducted with varying amounts of homologous and the remaining 12 heterologous *Aspergilli* as inhibitors. Briefly, allergen coupled discs were incubated with PPS in the presence of inhibitors. As a positive control, discs were also incubated in PPS without any inhibitor. After washing, the discs were suspended in radiolabelled antihuman-IgE (1125). Finally, the discs were washed and counted for radioactivity and the inhibition of RAST (%) produced by the inhibitor was calculated as under. Dose response curves with various *Aspergilli* were plotted and the quantity of each extract required for 50% inhibition was calculated.

$$\% \text{ inhibition} = 1 - \frac{\text{CPM (\%)} \text{ with inhibitor}}{\text{CPM (\%)} \text{ without inhibitor}} \times 100$$

Two Dimensional Immunoelectrophoresis (TDIEP)

All the *Aspergillus* extracts were subjected to TDIEP analysis using hyper-immune rabbit antisera raised against *A. tamarii* and *A. terreus* extracts according to the method of Axelsen and coworkers (1973) (13). Briefly, 5 ml of molten agarose gel (1%, Low EEO, Pharmacia Diagnostics, Sweden) in tris-calcium lactate-buffer was layered gently on the hydrophilic surface of a gel bond film (5 × 7.5 cm). A well (3–4 mm) was prepared and filled with allergen solution (10–20 ml) and first dimension electrophoresis was carried out at (10 volts/cm) for 25–30 minutes at 8 to 10 degrees C. Second dimension electrophoresis was conducted in antibody containing gel at right angles to the first for 18 hours (2 volts/cm, at 8 to 10 degrees C). The plates were thoroughly washed in normal saline and in distilled water. The plates were then stained with Coomassie brilliant blue R-250 followed by destaining.

Results

Intradermal Tests

Intradermal tests were performed on 289 consecutive patients of respiratory allergic disorders and 26 non-allergic healthy volunteers. Of the total 3025 ID tests performed on patients with the extracts of 13 species of *Aspergillus* 627 (20.7%) were positive (1+ to 4+), 386 (12.8%) being significantly positive (2+ to 4+). Positive skin tests ranged from 13.5 per cent with *A. wentii* to 35.4 per cent with *A. niveus* extracts. The ID results were negative in all the 26 control subjects. Detailed results are presented in table 2.

Table 2. ID test results with different species of *Aspergillus* allergen extracts

Sl. No.	Allergen extracts	Positive ID tests		
		Total tests done	1+ to 4+ No.(%)	2+ to 4+ No.(%)
1.	<i>A.amstelodami</i>	219	63(28.8)	38(17.4)
2.	<i>A.candidus</i>	147	44(29.9)	24(16.3)
3.	<i>A.carneus</i>	275	38(13.8)	23(8.4)
4.	<i>A.flavus</i>	263	45(17.1)	30(11.4)
5.	<i>A.fumigatus</i>	245	62(25.3)	34(13.9)
6.	<i>A.nidulans</i>	206	30(14.6)	21(10.2)
7.	<i>A.niveus</i>	147	52(35.4)	35(23.8)
8.	<i>A.recurvatus</i>	255	60(23.5)	34(13.3)
9.	<i>A.tamarii</i>	288	49(17.0)	33(11.5)
10.	<i>A.terreus</i>	248	62(25.0)	39(15.7)
11.	<i>A.variecolor</i>	266	49(18.4)	35(13.2)
12.	<i>A.versicolor</i>	236	42(17.8)	20(8.5)
13.	<i>A.wentii</i>	230	31(13.5)	20(8.7)

Results of ID tests on 64 patients who were tested with all the 13 extracts and who showed a positive skin response to at least one of them are analyzed in table 3.

Table 3. Distribution of 64 patients according to number of positive skin reactions to the 13 *Aspergillus* species tested

Positive to total no. of <i>Aspergillus</i> species	1+ to 4+		2+ to 4+	
	No.	%	No.	%
1 species	11	17.1	16	33.3
2 species	12	18.8	11	22.9
3 species	7	10.9	3	6.3
4 species	6	9.4	1	2.1
5 species	3	4.7	2	4.2
6 species	5	7.8	1	2.1
7 species	2	3.1	0	0.0
8 species	0	0.0	1	2.1
9 species	2	3.1	1	2.1
10 species	1	1.6	1	2.1
11 species	1	1.6	1	2.1
12 species	5	7.8	5	10.4
13 species	9	14.1	5	10.4
Total	64	100.0	48	100.0

Distribution of these patients has been made according to the number of *Aspergillus* extracts they elicited positive cutaneous response.

Further, ID test results of the 64 patients with *A. tamarii* and *A. terreus* were compared separately with the remaining 12 species of *Aspergillus*. Results are summarized in tables 4 & 5.

Table 4. Comparison of ID test results of *A. tamarii* with 12 other species of *Aspergillus*

S No.	Name of species	Total +ve pts at least to one sp.	+ve to both sp. (%)			+ve to one species (%)		
			Total	Identl*	Non-identl**	Total	<i>A. tamarii</i>	Other species
1.	<i>A. amstelodami</i>	43	44.2	18.6	25.6	55.8	11.6	44.2
2.	<i>A. candidus</i>	40	47.5	20	27.5	52.5	15	37.5
3.	<i>A. carneus</i>	30	70.0	23.3	46.7	30.0	13.3	16.7
4.	<i>A. flavus</i>	31	67.8	25.8	41.9	32.2	9.7	22.5
5.	<i>A. fumigatus</i>	42	47.6	21.4	26.2	52.4	9.5	42.9
6.	<i>A. nidulans</i>	29	51.7	17.2	34.5	48.3	20.7	27.5
7.	<i>A. niveus</i>	47	44.7	17.0	27.7	55.3	4.2	51.1
8.	<i>A. recurvatus</i>	39	51.3	17.9	33.3	48.7	12.8	35.5
9.	<i>A. terreus</i>	43	44.1	20.9	23.2	55.8	13.9	41.9
10.	<i>A. varicolor</i>	35	62.9	31.4	31.4	32.1	8.6	28.6
11.	<i>A. versicolor</i>	39	51.3	20.5	30.8	48.7	12.8	35.9
12.	<i>A. wentii</i>	36	41.7	13.9	27.8	58.3	30.6	22.8

* Identical cutaneous response.

** Non-identical cutaneous response.

Table 5. Comparison of ID test results of *A. terreus* with 12 other *Aspergillus* species

Sl. No.	Name of species	Total pts. positive	Positive to both (%)			Positive to only one species (%)		
			Total	identl*	Non-identl**	Total	<i>A. terreus</i>	Other species
1.	<i>A. amstelodami</i>	45	64.4	31.1	33.3	35.5	17.8	17.8
2.	<i>A. candidus</i>	50	44.0	28.0	16.0	56.0	30.0	26.0
3.	<i>A. carneus</i>	40	55.0	17.5	37.5	45.0	37.5	7.5
4.	<i>A. flavus</i>	42	50.0	19.0	31.0	50.0	33.3	16.6
5.	<i>A. fumigatus</i>	48	60.4	33.3	27.1	39.6	16.7	22.9
6.	<i>A. nidulans</i>	39	48.7	20.5	28.2	51.2	41.0	10.2
7.	<i>A. niveus</i>	56	46.4	17.8	28.5	53.6	19.6	33.9
8.	<i>A. recurvatus</i>	47	57.4	23.4	34.0	42.6	23.4	19.1
9.	<i>A. tamarii</i>	43	44.2	20.9	23.3	55.8	41.9	13.9
10.	<i>A. varicolor</i>	45	60.0	33.3	26.7	40.0	22.2	17.8
11.	<i>A. versicolor</i>	42	62.3	23.9	40.4	35.7	23.9	11.9
12.	<i>A. wentii</i>	42	42.9	14.3	28.6	57.1	42.9	14.3

* Identical cutaneous response.

** Non-identical cutaneous response.

It is evident from the results that in each comparison, while some patients showed positive skin response to only one of the two extracts tested, there were others who were positive to both. Some patients showed identical skin response to both the extracts, while others varied in their skin reactivity. Of the 64 patients showing positive ID responses to *A. tamarii* and/or to any of the 12 other species tested, positivity to both varied from 41.2% for *A. wentii* to 70% for *A. carneus*. Six of 12 heterologous extracts showed positive ID response simultaneously in 50% or more patients. Similarly, 7 of the 12 heterologous extracts showed simultaneous ID positivity with *A. terreus* extract in 50% or more patients who were skin reactive to at least one of them.

RAST

The specificity of ID test response was evaluated in RAST assays. On an average 76.6% of sera from patients eliciting markedly positive ID response were RAST positive. This positivity varied from 64.5% of *A. amstelodami* to 87.1% of *A. tamarii* RASTs (Table 6).

Table 6. RAST results with allergen extracts of different *Aspergillus* species

Sl. No.	Allergen extracts	Skin test grade					
		Negative		1+ to 4+		2+ to 4+	
		TID	+ve (%)	TID	+ve (%)	TID	+ve (%)
1.	<i>A. amstelodami</i>	10	0(0.0)	44	25(56.8)	31	20(64.5)
2.	<i>A. candidus</i>	10	0(0.0)	35	22(62.9)	19	16(84.2)
3.	<i>A. carneus</i>	3	0(0.0)	19	12(63.2)	13	11(84.6)
4.	<i>A. flavus</i>	11	1(9.1)	34	24(70.6)	23	19(82.6)
5.	<i>A. fumigatus</i>	13	0(0.0)	39	21(53.9)	26	20(76.9)
6.	<i>A. nidulans</i>	9	0(0.0)	20	11(55.0)	13	9(69.2)
7.	<i>A. niveus</i>	12	0(0.0)	41	25(61.0)	29	20(69.0)
8.	<i>A. recurvatus</i>	9	0(0.0)	46	22(47.8)	25	18(72.0)
9.	<i>A. tamaritii</i>	18	3(16.7)	42	30(71.4)	31	27(87.1)
10.	<i>A. terreus</i>	11	1(9.1)	45	30(66.7)	30	26(86.7)
11.	<i>A. varicolor</i>	11	2(18.2)	40	24(60.0)	26	19(73.1)
12.	<i>A. versicolor</i>	8	0(0.0)	22	12(54.5)	15	10(66.7)
13.	<i>A. wentii</i>	7	0(0.0)	21	15(71.4)	14	11(78.6)

However, of the 132 tests, performed with the sera from patients with negative skin tests, only 7 (5.3%) were RAST positive. These results gave evidence that skin test results were quite specific.

RAST Inhibition Assays

The results of inhibition of *A. tamaritii* and *A. terreus* RASTs produced by various extracts of different species of *Aspergillus* are shown in figures 1 and 2, respectively.

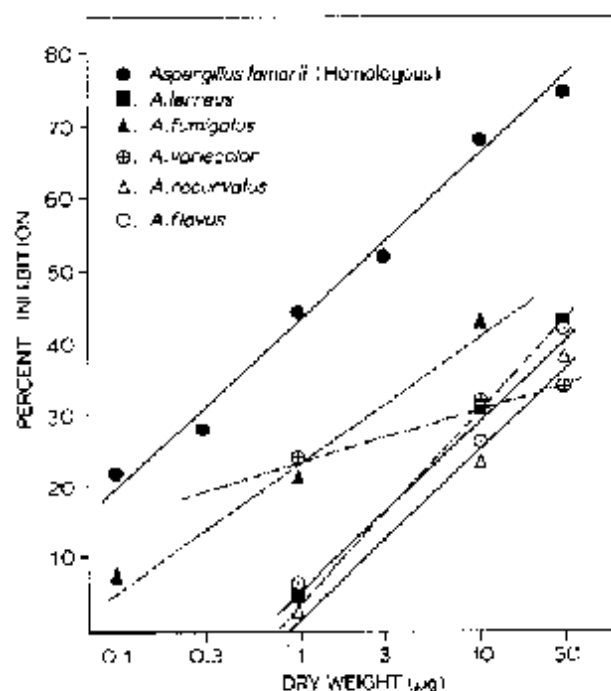


Figure 1. Inhibition of *A. tamaritii* RAST with homologous and five heterologous *Aspergillus* extracts. Estimated amounts required for 50% inhibition : ● *A. tamaritii* 2.1 µg, ▲ *A. fumigatus* 34 µg, ■ *A. terreus* 50 µg, ◻ *A. varicolor* 2.2 µg, △ *A. recurvatus* 120 µg, ○ *A. flavus* 100 µg.

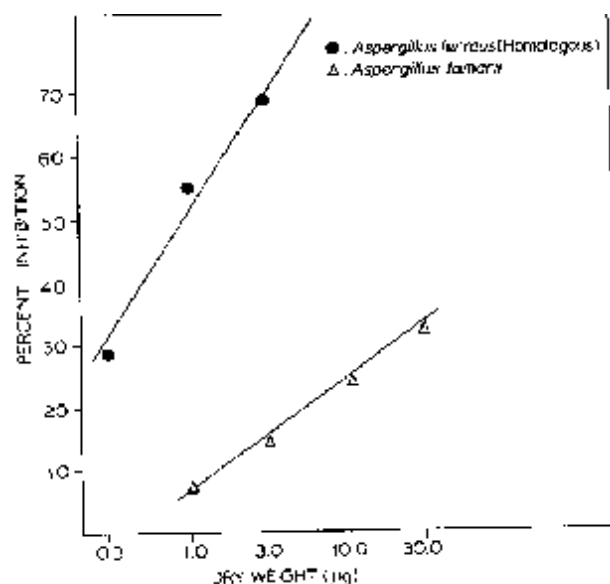


Figure 2. Inhibition of *A. terreus* RAST with the homologous and *A. tamarii* extracts. Estimated amounts required for 50% inhibition : ● *A. terreus* 0.9 µg, Δ *A. tamarii* 300 µg.

A dose related inhibition of *A. tamarii* RAST was obtained with homologous as well as five of the twelve heterologous *Aspergillus* extracts, namely, *A. flavus*, *A. fumigatus*, *A. recurvatus*, *A. terreus* and *A. variegatus*. However, in *A. terreus* RAST only homologous and one heterologous *Aspergillus* extract viz. *A. tamarii*, produced dose related inhibition.

Two Dimensional Immunoelectrophoresis (TDIEP) Experiments

In TDIEP experiments with homologous as well as heterologous *Aspergillus* extracts using rabbit hyperimmune sera to *A. tamarii* and *A. terreus* extracts multiple precipitin bands were obtained with both the allergen extracts with homologous hyperimmune antisera. However, six of the twelve heterologous fungal extracts namely, *A. candidus*, *A. fumigatus*, *A. niveus*, *A. recurvatus*, *A. terreus* and *A. variegatus* produced only one to two precipitin bands in TDIEP experiments using rabbit *A. tamarii* antibodies. Similarly, only six heterologous *Aspergilli* (viz. *A. fumigatus*, *A. niveus*, *A. recurvatus*, *A. tamarii*, *A. variegatus* and *A. versicolor*) showed 1 to 2 precipitin bands with rabbit *A. terreus* antibodies.

Discussion

Patients with respiratory allergic diseases, sensitive to fungi, generally show simultaneous skin and RAST positivity to multiple fungal extracts. This co-existing hypersensitivity in patients may be either due to the presence of shared allergenic components in different fungi or chance occurrence of co-existing hypersensitivity to multiple heterologous allergens. Using RAST inhibition assays Hoffman and Kozak (1979) 14 observed cross reactivity among *Curvularia*, *Alternaria*, *Dreschlera*, *Spondylocadium* and *Stemphylium* extracts. Tee and co-workers (1987) 15 also reported significant cross-reactivity between *Alternaria* and *Cladosporium* extracts. Contrary to this, Bonilla-Soto and associates (1961) (4) did not find any cross reactivity in these two fungi. Karr and co-workers (1981) (16) also reported the absence of shared allergenic components between *Alternaria* and three species of *Aspergillus* i.e. *A. flavus*, *A. fumigatus* and *A. glaucus*. Presence of both shared and/or specific allergenic and antigenic components has also been demonstrated among different species of basidiomycetes (17). Agarwal and associates (1982) (3) reported the presence of shared allergenic and antigenic components in *Alternaria* and *Stemphylium* extracts.

In the present study, ID tests were performed on 289 patients with allergen extracts of 13 species of *Aspergillus* (Table 2). ID test positivity to individual *Aspergillus* species varied from 13.5 per cent with *A. wentii* to 35.4 per cent with *A. niveus*, illustrating the heterogeneity of immune response to these extracts. Analysis of RAST and skin test results revealed the specificity of skin test responses. While 5.3 per cent RASTs were positive with the sera of patients with negative ID response, RAST positivity with the sera of patients with markedly positive skin responses was quite high ranging from 64.5% in *A. amstelodami* to 87.1% in *A. tamarii* RASTs (Table 6). Distribution of 64 patients, who were tested with all the 13 allergen

extracts and who elicited cutaneous reactivity to at least one species, was made according to the number of *Aspergillus* species they were positive to. The results showed that while 23 patients (35.9%) were positive to only one or two species, only 14 (21.8%) were sensitive to 12–13 species (Table 3). These results suggested the presence of both shared as well as species specific components in different species of *Aspergillus*.

On performing various clinico-immunologic tests with the extracts of 4–5 species of *Aspergillus*, Agarwal and co-workers found *A. tamarii* to be the most important allergenic species (6,18). However, on prick testing with *A. fumigatus* and *A. terreus* extracts, Eaton *et al* (1980) (19) found *A. terreus* to be an important allergenic species. Thus, in the present study the presence of shared and/or specific allergens among different *Aspergillus* species was investigated by comparing the results of ID tests performed with *A. tamarii* and *A. terreus* extracts, individually with each of the other twelve species of *Aspergillus* (Tables 4 & 5). It was observed that more than 50 per cent of the patients showed positivity to both *A. tamarii* and each of the 6 other species, namely, *A. flavus*, *A. carneus*, *A. versicolor*, *A. recurvatus*, *A. nidulans* and *A. variegatus*. Similarly, *A. terreus* also showed co-existing skin reactivity in patients (50% or more) with 7 different *Aspergillus* sp. viz. *A. recurvatus*, *A. variegatus*, *A. fumigatus*, *A. versicolor*, *A. amstelodami*, *A. flavus*, and *A. carneus*. These results suggested the presence of some shared allergenic components among these Aspergilli. However, simultaneous positivity to more than one allergen extract in these patients may also be due to the presence of co-existing, independent hyperreactivity to their heterologous allergenic components.

The presence of cross-reactivity and/or species specificity was further investigated in RAST inhibition assays. While allergen extracts from five species of *Aspergillus* viz. *A. flavus*, *A. fumigatus*, *A. recurvatus*, *A. terreus* and *A. variegatus* produced dose related inhibition in *A. tamarii* RAST, only one heterologous fungus namely *A. tamarii* produced inhibition of *A. terreus* RAST. The results of inhibition assays of these two RASTs gave evidence for the presence of some shared allergenic components among these species. The fact that the slopes of inhibition curves with different species were not parallel to the standard curve suggested qualitative and quantitative variations in the shared allergenic components in these extracts. Thus, only an estimate of the amount required for 50% inhibition could be made for comparison purposes. The dose required for 50 per cent inhibition of *A. tamarii* and *A. terreus* RASTs with the homologous extracts were 2.1 and 0.9 mg, respectively. However, the extrapolated amounts of other heterologous extracts required for 50 per cent inhibition in these RASTs were very high and even 30 mg of various heterologous extracts failed to produce such inhibition (Figures 1 & 2). These results suggested that only a few allergenic components of *A. tamarii* and *A. terreus* extracts may be shared with other *Aspergillus* species and most of them seem to be species specific. In crossed-RAST inhibition assays, Karr and associates (1981) (16) also observed the existence of both shared as well as specific allergenic components among three species of *Aspergillus* viz. *A. flavus*, *A. fumigatus* and *A. glaucus*. Likewise, in fused rocket radio-immuno-electrophoresis experiments, Kim and Chaparas (1979) (20) observed 19 to 35 per cent cross reactivity among *A. flavus*, *A. fumigatus* and *A. niger*.

In TDIEP experiments, multiple precipitin bands were observed with homologous *A. tamarii* and *A. terreus* allergen extracts, suggesting the presence of multiple antigenic components in these extracts. On the other hand, only one to two bands were observed with 6 of the 12 heterologous *Aspergillus* extracts viz. *A. candidus*, *A. fumigatus*, *A. niveus*, *A. recurvatus*, *A. terreus* and *A. variegatus* in TDIEP experiments with rabbit anti-*A. tamarii* antibodies. These results suggested the presence of some shared antigenic components between *A. tamarii* and these six *Aspergillus* species. Similarly, one to two shared antigenic components were observed between *A. terreus* and six heterologous *Aspergillus* species, viz. *A. fumigatus*, *A. niveus*, *A. recurvatus*, *A. tamarii*, *A. variegatus* and *A. versicolor*. These results gave evidence that though a large number of antigenic components present in these two fungi are species specific, only a limited number of them are shared with other *Aspergillus* species. The presence of some cross reacting antigens among different fungi has also been reported by other investigators. Schumacher and co-workers (1975) (21) observed a few shared antigenic components between *Alternaria* and three other genera viz. *A. fumigatus*, *Curvularia* and *Stemphylium*. Similarly, Longbottom and Pepys (1964) (22) re-reported the presence of some antigenic components of *A. fumigatus* in other species of *Aspergillus*, namely, *A. flavus*, *A. nidulans*, *A. niger* and *A. terreus*. Interestingly, in the present study while six heterologous species produced precipitin bands in TDIEP experiments with rabbit anti-*A. terreus* antibodies, only one species *A. tamarii* showed inhibition in *A. terreus* RAST. These results indicate that the allergenic and antigenic components in fungal extracts may be different. Collectively, our findings provide evidence that majority of the allergenic and antigenic components in different species of *Aspergillus* seem to be species specific and only a few of them may be shared.

References

1. Nemergut RA, Leathers CR, Northey WT. A search for species specific antigens in the genus *Penicillium*. *Ann Allergy* 1977; 38 : 219–221.
2. Agarwal MK, Shivpuri DN, Mukerjee KG, Shali PL. Species specific antigens in fungi. *Aspects Allergy Applied Immunology* 1969; 2 : 69–80.
3. Agarwal MK, Jones RT, Yunginger JW. Shared allergenic and antigenic determinants in *Alternaria* and *Stemphylium* extracts. *J Allergy Clin Immunol* 1982; 70 : 437–444.
4. Bonilla Soto O, Rose NR, Arbesman CE. Allergenic molds. Antigenic and allergenic properties of *Alternaria tenuis*. *J Allergy* 1961; 32 : 246–270.
5. Vijay HM, Huang H, Young NM, Bernstein IL. Studies on *Alternaria* allergens I. Isolation of allergens from *Alternaria tenuis* and *A. solani*. *Int Arch Allergy Appl Immunol* 1979; 60:229–237.
6. Agarwal MK, Shivpuri ND. Fungal spores—Their role in respiratory allergy. *Adv Pollen Spore Res* 1974; 1 : 78–128.
7. Aggarwal S. Clinico-immunologic studies on yet unknown allergenic fungal spores of Delhi metropolitan area. Ph. D. Thesis, University of Delhi, 1992; pp, xv.
8. Sandhu DK, Shivpuri DN, Sandhu RS. Studies on the airborne fungal spores in Delhi. Their role in respiratory allergy. *Ann Allergy* 1964; 22 : 374–384.
9. Agarwal MK, Bansal S, Chaudhry S, Jhamb S. A clinico-immunologic study on allergen preparation, skin tests and RAST. *Indian J Allergy Appl Immunol* 1990; 4 : 48 (Ab).
10. Agarwal MK, Gaur SN, Chaudhry S. Effect of prior medication on intradermal test response in the patients with respiratory allergies. *J Asthma* 1988; 25 : 275–279.
11. Adolphson CR, Yunginger JW, Gleich GJ. Standardization of allergens. In: Rose NR, Friedman H, ed. *Manual of Clinical Immunology*; 2nd edn. Washington DC: American Society for Microbiology; 1980: pp 778–788.
12. Gleich GJ, Yunginger JW. Standardization of allergen. In: *Manual of Clinical Immunology*. Washington DC: American Society for Microbiology; 1976 : 575–584.
13. Axelsen NH, Kroll J, Weeke B. A manual of quantitative immunoelectrophoresis/methods and application. *Scand J Immunol* 1973; 2 : 1.
14. Hoffman DR, Kozak PP. Shared and specific allergens in mold extracts. *J Allergy Clin Immunol* 1979; 63 : 213 [Ab].
15. Tee RD, Gordon DJ, Newman Taylor AJ. Cross reactivity between antigens of fungal extracts studied by RAST inhibition and immunoblot technique. *J Allergy Clin Immunol* 1987; 79 : 627–633.
16. Karr RM, Wilson MR, Anicetti VR, Lehrer SB, Butcher BT, Salvaggio JE. An approach to fungal antigen relationships by radioallergosorbent test inhibition. *J Allergy Clin Immunol* 1981; 67 : 194–198.
17. O'Neil CE, Hughes JM, Butcher BT, Salvaggio JE, Lehrer SB. Basidiospore extracts: Evidence for common antigenic/allergenic determinants. *Int Arch Allergy Appl Immunol* 1988; 85 : 161–166.
18. Shivpuri DN, Agarwal MK. Studies on the allergenic fungal spores of Delhi, India, metropolitan area (Clinical Aspects). *J Allergy* 1969; 44 : 204–213.
19. Eaton KK, Auld RM, McNulty DW. Prick testing for *Aspergillus* (letter to the Editor). *Clin Allergy* 1980; 10 : 639–641.
20. Kim SJ, Chaparas SD. Characterization of antigens from *Aspergillus fumigatus*. Comparison

of antigenic relationships of clinically important Aspergilli. *Am Rev Respir Dis* 1979; 120 : 1297–1303.

21. Schumacher MJ, McClatchy JK, Farr RS, Minden P. Primary interaction between antibody and components of *Alternaria*. I. Immunological and chemical characteristics of labelled antigens. *J Allergy Clin Immunol* 1975; 56 : 39–53.

22. Longbottom JL, Pepys J. Pulmonary aspergillosis: Diagnostic and immunological significance of antigens and C substance in *Aspergillus fumigatus*. *J Path Bact* 1964; 88 : 141–151.

Correspondence :

Dr M.K. Agarwal,
Professor and Head,
Department of Respiratory Allergy and Applied Immunology,
V.P. Chest Institute,
University of Delhi,
Delhi–110 007