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Role of Animal Danders as Inhalant Allergens in Bronchial Asthma in India

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ABSTRACT

The etiological significance of animal danders in Indian patients with nasobronchial disorders has not yet been investigated. In the present study, the role of animal danders in the etiology of bronchial asthma was studied. Extracts of danders from 6 animals along with guinea pig whole pelt were prepared. Intradermal and bronchial provocation tests with these extracts were performed on (i) 68 asthmatics and (ii) 20 nonallergic healthy volunteers. In patients, significant positive skin reactions (2+ to 4+) ranged from 1.4% each with guinea pig whole pelt and ox dander to 8.8% with dog dander extracts. None of the healthy volunteers elicited such a response. On bronchial provocation, 20% and 53.8% of the tests were positive in asthmatics showing 1+ and 2+ skin reactivity, respectively. All the patients as well as controls eliciting negative intradermal responses demonstrated uniformly negative bronchial provocation tests to different dander extracts. Analysis of various clinical features of asthmatics with respect to skin positivity to dander/pelt extract was also carried out. Radioaller-

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gosorbent tests (RASTs) were performed to estimate dander-specific IgE levels in the sera of patients showing different grades of skin response to dander extracts of dog, horse, and goat. Sixty percent of sera from the patients showing 2+ to 4+ skin reactivity to various animal dander extracts showed positive RASTs. RAST positivity as well as RAST ratio increased with increase in the intensity of skin response. All the patients with positive cutaneous as well as positive bronchial responses also showed positive RASTs. Similarly, all the patients with positive skin and positive RASTs showed positive bronchoprovocation tests. These results suggested that animal danders play an important role in the etiology of bronchial asthma. Some of the clinical characteristics of asthmatics, such as (i) early age at onset of asthma, (ii) positive family history, and (iii) asthma with associated allergies, have significant bearing on the cutaneous response to various dander extracts.

INTRODUCTION

We live in close proximity with a number of animals, especially those kept as pets or livestock. Several studies on the role of animal products as causal allergens for bronchial asthma have been reported (1–7). Studies on identification and characterization of such allergens of dog, rabbit, cat, horse, cow, and other animals have also been undertaken (8–11). Recently, Schou (12) has published a review on various major, minor, and yet to be characterized allergens of mammalian origin.

A large number of animals are maintained in dairy farms, stables, slaughterhouses, and other facilities in and around the Delhi area. In addition, there is an abundance of stray animals, including horses, cows, oxen, goats, dogs, sheeps, pigs, and others. Thus, the residents of Delhi are regularly exposed to animal-derived particles, which may play an important role as inhalant allergens. So far, no systematic study on animal danders as inhalant allergens has been reported from India. The present study was undertaken to investigate the etiological significance of animal danders in the causation of bronchial asthma in the Delhi, India, metropolitan area.

MATERIAL AND METHODS

Allergen Extracts

Danders from 6 common animals, cow, dog, horse, goat, ox, and pig, were collected by gentle brushing. Allergen extracts from these dan-

ders were prepared after removal of any visible extraneous material. The method of Agarwal et al. was followed (13). Briefly, the danders were defatted with diethyl ether followed by extraction in 0.1 M ammonium bicarbonate (pH 7.8) at 4°C for 72 hr (2%). These extracts were thoroughly dialyzed using Spectrapor-3 dialysis tubing (mol. wt. cutoff = 3500 daltons; Spectrum Medical Industries, Inc., U.S.A.). The dialyzed extracts were lyophilized and stored in airtight vials at -20°C. Extract from the whole pelt of guinea pig was also prepared. For this purpose, guinea pig whole-pelt powder obtained by lyophilization and pulverization was processed as above. Throughout the study, the same lots of animal dander/pelt extracts were used for various clinical tests.

Protein Estimation

The protein content of each extract was estimated by Lowry's method (14). It ranged from 310 µg/mg of dog dander to 750 µg/mg of horse dander extracts (Table 1).

Subjects

A group of 68 patients with bronchial asthma with or without allergic rhinitis (aged 12–60 years, mean age = 31 years) attending the medical outpatient department and chest clinics of Lady Hardinge Medical College and associated Sucheta Kriplani Hospital, New Delhi was selected for the study. The patients were diagnosed as having bronchial asthma only when they satisfied the diagnostic criteria of

Table 1. Results of Intradermal Tests with Various Animal Product Allergens on 68 Patients with Bronchial Asthma and 20 Nonallergic Healthy Volunteers

ALLERGEN EXTRACTS	PROTEIN ($\mu\text{g}/\text{mg}$ dry wt)	PATIENTS								HEALTHY VOLUNTEERS			
		Total	1+	2+	3+	4+	1+ to 4+		2+ to 4+		Total	1+	
							<i>n</i>	%	<i>n</i>	%		<i>n</i>	%
Dog dander	310	68	8	5	—	1	14	20.6	6	8.8	20	1	5.0
Horse dander	740	68	10	4	—	1	15	22.1	5	7.4	20	2	10.0
Pig dander	480	68	6	5	—	—	11	16.2	5	7.4	20	0	0.0
Goat dander	ND	68	7	3	—	1	11	16.2	4	5.9	20	1	5.0
Cow dander	650	68	5	2	1	—	8	11.8	3	4.4	20	1	5.0
Ox dander	ND	68	6	—	—	1	7	10.3	1	1.5	20	1	5.0
Guinea pig pelt	438	68	6	1	—	—	7	10.3	1	1.5	20	0	0.0
Total		476	48	19	1	4	73	15.3	25	5.3	140	6	4.3

ND = not done.

Weiss and Segal (15). All the patients were residents of the Delhi metropolitan area and were studied in their asymptomatic phase.

A thorough physical examination was done in each case followed by routine investigations including complete blood count, absolute eosinophil count, stool examination, skiagram of the chest and sinuses, electrocardiogram, and ventilatory function tests. Asthmatics (i) with associated cardiopulmonary disorders, or (ii) in acute exacerbation, or (iii) who were steroid-dependent were not included in the study.

A detailed history was taken in each case and a special note was made of (i) age at onset of asthma, (ii) duration of disease, (iii) precipitating factors, (iv) seasonal pattern and frequency of asthmatic attacks, (v) history of allergic disorders such as eczema/hay fever/atopic dermatitis, (vi) family history of bronchial asthma/rhinitis/other allergic conditions, (vii) development of symptoms on exposure to any particular animal(s), and (viii) presence of pets at home or at work.

As a control, another group of 20 age-and-sex-matched healthy volunteers with no personal or family history of any allergic diseases was also studied. All of them were also from an urban setting.

As recommended by Agarwal et al., the patients were instructed to stop conventional antihistamines 72 hr before and new-generation, long-acting antihistamines at least 45 days before undergoing various in vivo diagnostic tests (16). All other bronchodilators (sym-

pathomimetic amines and xanthines) and steroids were discontinued 12–16 hr prior to skin or bronchial provocation tests.

Intradermal Tests

Intradermal tests with all 7 extracts (reconstituted in 1:500 w/v concentration in phosphate-buffered saline) were performed on all the 68 patients and graded according to the method and criteria of Shivpuri and Agarwal (17). In brief, the tests were performed on the flexor surface of the forearm and/or lateral aspect of the arm. Approximately 0.02 ml of a test solution was injected into the superficial layer of skin using a 1-ml disposable syringe with a 26- or 27-G short beveled needle. The minimum distance between the two injection sites was about 4 cm. Buffered saline and histamine solution (100 $\mu\text{g}/\text{ml}$) were tested as negative and positive controls, respectively (18). The skin test results were read after 15 min of injection and graded as per the following criteria (17).

Grade of reaction	Weal diameter (times that of negative control)	Erythema (mm)
1+	More than twice and >6 mm	15–20
2+	3–3.5 times	>20
3+	3.5–4 times with one or two pseudopodia	>30
4+	4–6 times with several pseudopodia	>35

The tests were performed by the same person (RKB) throughout the course of the study. Positive cutaneous reactions (1+ to 4+) were confirmed by repeating the tests on the same day.

Bronchial Provocation Tests

To evaluate the specificity of cutaneous responses and to confirm the etiological significance of animal-derived extracts, a limited number of bronchial provocation tests were performed with the 7 extracts on 25 symptom-free asthmatics showing various grades of skin responses (negative to 4+). As a control, 9 provocation tests with these extracts were also conducted on 5 healthy volunteers. The tests were performed using a spirometer and a nebulizer system. The nebulizer system consisted of a nebulizer containing buffered saline/allergen extract, oxygen cylinder used as a driving gas for nebulization, a T tube to direct the flow of aerosolized solution, and a polymask for inhalation by the patient. When the oxygen passed through the nebulizer, an aerosol of the liquid therein was produced which flows in the T tube. One limb of the T tube was connected to the mask through which the patient inhaled the aerosol while exhaled air passed out through the opposite opening into a corrugated tubing that took it out of the laboratory.

Three basal readings of the patient's forced expiratory volume (FEV) and FEV₁ in the first second (FEV₁) were obtained. Thereafter, the patient was connected to the nebulizer system and allowed to inhale buffered saline aerosol for 1 min while breathing normally. Recordings of FEV and FEV₁ were taken at 2, 10, and 30 min after stopping inhalation of buffered saline. Finally, using the same procedure, the patient was exposed to an aerosol of the test animal dander extract (1:500 w/v) for 1 min and readings of pulmonary functions recorded at 2, 10, and 30 min. The fall in FEV₁, if any, was calculated. The bronchoprovocation tests were graded according to the criterion of Shivpuri and Agarwal (17). A patient showing positive challenge test to an extract was exposed to another dander extract only after 1 week.

Radioallergosorbent Tests (RASTs)

Allergen-specific IgE levels against dog, goat, and horse danders were estimated in the sera of 24 patients eliciting different grades of skin response using disc RASTs. The method of Adolphson, Gleich, and Yunginger (19) was used. The results were graded on the basis of RAST ratio calculated as follows (20):

$$\text{RAST ratio} = \frac{\% \text{ cpm with patient's serum}}{\% \text{ cpm with healthy control serum}}$$

A RAST was considered positive when the RAST ratio was 5 or more.

Statistical Analysis

The chi-square test (with Yates correction for continuity) was applied to test for independence of intradermal positivity and clinical characteristics of the patients (21).

RESULTS

Intradermal Tests

The results of skin tests with various animal allergens are presented in Table 1. The total positive skin responses (1+ to 4+) elicited by patients ($n = 32$; 47.1%) varied from 10.3% each with guinea pig whole pelt and ox dander to 22.1% with horse dander extracts. Similarly, significant positive skin reactions (2+ to 4+) of patients ($n = 14$; 20.6%) ranged from 1.5% each with guinea pig whole pelt and ox dander to 8.8% with dog dander extracts. While none of the healthy volunteers showed any 2+ to 4+ cutaneous response to these extracts, we did obtain a few 1+ reactions with the extracts of dog, cow, ox, goat (5% each), and horse (10%) danders.

Bronchial Provocation Tests

Table 2 shows results of 36 bronchial provocation tests performed on 25 asthmatics showing different grades of skin response to animal dander/pelt extracts. All the asthmatics with a negative intradermal response to animal dander extracts were provocation test negative.

Table 2. Results of 36 BPTs Performed with Animal Product Extracts on Patients with Bronchial Asthma

ID RESPONSE	BPTs PERFORMED	POSITIVE BPTs	
	<i>n</i>	<i>n</i>	%
Healthy volunteers, negative	9	0	0
Patients			
Negative	13	0	0
1+	10	2	20
2+	13	7	53.8

All 9 BPTs performed with animal dander extracts on 5 NHVs were negative. BPTs were performed with dander extracts of dog (*n* = 8), horse (*n* = 8), goat (*n* = 6), pig (*n* = 5), cow (*n* = 2), ox (*n* = 2) and pelt extract of guinea pig (*n* = 5).

Positive responses with these extracts were obtained in 20% and 53.8% of patients with 1+ and 2+ skin responses, respectively. The tests could not be performed on patients showing a 3+ or 4+ cutaneous response as they did not consent to undergo these tests. All the 9 challenges performed with different dander extracts on 5 nonasthmatics were uniformly negative.

RASTs

Results of dog, goat, and horse dander RASTs using sera from patients eliciting different grades of skin response are presented in

Table 3. While all the RASTs were uniformly negative with the sera of patients with negative skin test response, positivity of RAST varied from 7.7% with the sera from patients showing 1+ intradermal response to 100% with those from 4+ skin reactors to various animal dander extracts. Further, it was observed that the average RAST ratio increased with the increase in the grade of cutaneous response, ranging from 1.34 in patients with negative to 13.18 with 4+ skin test reactions.

All the sera from patients with a positive skin test and a positive bronchial provocation test were RAST positive (Table 4). Similarly, all

Table 3. Results of Animal Dander Allergen RASTs with the Sera from Bronchial Asthma Patients Eliciting Different Grades of Skin Responses

SKIN TEST RESULTS	RADIOALLERGOSORBENT TESTS ^a			
	TOTAL TESTS DONE	POSITIVE		AVERAGE RAST RATIO
	<i>n</i>	<i>n</i>	%	
Healthy volunteers, negative	12	0	0	1.00
Patients				
Negative	43	0	0	1.34
1+	13	1	7.7	1.93
2+	7	3	42.9	6.68
3+	0	0	—	—
4+	3	3	100.0	13.18
1+ to 4+	23	7	30.4	1.93 to 13.18
2+ to 4+	10	6	60.0	6.68 to 13.18

^aRASTs were performed using solid-phase horse (*n* = 24), dog (*n* = 24), and goat dander (*n* = 18) extracts.

Table 4. Results of Intradermal, Bronchial Provocation, and RAST Responses in Some Asthmatics

SERIAL NO.	INTRADERMAL RESPONSES	BRONCHIAL PROVOCATION TEST	RAST	
			RATIO	RESULT
1	Negative	Negative	1.04	Negative
2	Negative	Negative	1.10	Negative
3	Negative	Negative	1.15	Negative
4	Negative	Negative	1.19	Negative
5	Negative	Negative	2.21	Negative
6	1+	Negative	0.56	Negative
7	1+	Negative	0.61	Negative
8	1+	Negative	0.94	Negative
9	1+	Negative	1.06	Negative
10	1+	Positive	7.48	Positive
11	2+	Negative	0.68	Negative
12	2+	Negative	1.23	Negative
13	2+	Positive	10.25	Positive
14	2+	Positive	10.35	Positive
15	2+	Positive	22.38	Positive

the patients with a positive skin test and a positive RAST were bronchoprovocation test positive.

Clinical Characteristics and Intradermal Response

Comparison of clinical characteristics of the patients and their significant intradermal response (2+ to 4+) to animal dander/pelt extracts is presented in Table 5. Patients with an early age at onset of asthma (<20 years) had a significantly higher percentage (10.4%) of positive skin response to the animal dander extracts ($p < 0.001$) as compared to those with late onset of asthma (>20 years, 1.7%).

The duration of disease did not show any correlation with the 2+ to 4+ response to these extracts ($p > 0.01$). Patients with seasonal asthma also did not show any statistically significant difference in cutaneous reactivity to animal dander/pelt extracts as compared to the patients with perennial or irregular asthma ($p > 0.01$). All the patients with pets (dogs) at home had seasonal asthma.

Patients with a positive family history of bronchial asthma and/or any other allergic disorders showed a significantly higher 2+ to 4+ intradermal response (ID) ($p < 0.001$) than those

without a family history, i.e., 12.4% and 1.3%, respectively. Similarly, patients with asthma alone showed only 0.5% ID positivity, while those with an associated allergic disorder showed a significantly higher rate of positive skin response (8.9%). Only 1 of the 68 patients gave a definite history of experiencing symptoms on exposure to the horses. Hence, it was not possible to correlate symptoms of the patients with exposure to the animals. Twelve patients had a pet at their house or place of work. All these patients were seasonal asthmatics and showed a higher percentage of markedly positive cutaneous responses when compared with those without pets (9.2% as against 4.2%). However, statistically the difference was not significant ($p > 0.01$) (Table 5).

DISCUSSION

Animal products constitute an important group of inhalant allergens (1–7,22,23). These allergens are released from various domestic, stray, and laboratory animals and animals maintained in dairy farms, stables, slaughterhouses, and other facilities. The animal-derived allergens are present in the indoor as well as outdoor environments (24–28). Thus, exposure

Table 5. Analysis of Intradermal Responses (2+ to 4+) to the Extracts of Animal Products According to the Clinical Characteristics of 68 Asthmatics

CLINICAL CHARACTERISTICS	TOTAL TESTS DONE (n)	SKIN TEST POSITIVITY (2+ TO 4+)		p-value
		n	%	
Age at onset ≤20 years	182	19	(10.4)	<0.001
Age at onset >20 years	294	5	(1.7)**	
Duration of disease ≤5 years	273	13	(4.8)	>0.01
Duration of disease >5 years	203	11	(5.4) ^{ns}	
Seasonal asthmatics	154	11	(7.1)	>0.01
Perennial or irregular asthmatics	322	13	(4.0) ^{ns}	
Positive family history of allergies	161	20	(12.4)	<0.001
Negative family history of allergies	315	4	(1.3)**	
Asthma with other allergies	259	23	(8.9)	<0.001
Asthma alone	217	1	(0.5)**	
Pets at home/place of work	98	9	(9.20)	>0.01
Absence of pets at home/place of work	378	16	(4.20) ^{ns}	

**Highly significant; ns = not significant.

to animal allergens is not limited to people who have occupational exposure to animals. The general population is also affected. In our laboratory, experiments conducted to quantify horse dander allergen revealed the presence of the same in the air of the Delhi, India, metropolitan area in sufficient amounts around the year (unpublished data). However, no systematic study on the significance of animal allergens in the etiology of bronchial asthma has been reported from our country, so far.

A major problem in the diagnosis of animal allergy is variability in source of raw material used for allergen preparation. These sources include whole pelt, epithelium, hair, dander, serum, saliva, and urine (4,25,29–32). Even the sex of animals, especially rats, has been found to influence proteins and allergenic components of the extract (33). Thus, it is difficult to compare results of different studies. In addition, low and variable potency of animal dander extracts further makes the comparison difficult. Skin scrapings, which usually contain a large number of serum components, have been reported to be of low potency, while epithelial components dominating in dandruff preparations were found to have better potency (5,34). However, this limitation can be obviated in future studies using specific allergen assays

and assays of biological potency that are now available.

In the present study, extracts were prepared from the danders of goat, dog, horse, cow, ox, and pig. However, in the case of guinea pig the extract was prepared using its pelt as this has been reported to possess significant allergenic activity (26). Further, guinea pig pelt-derived allergens have been detected in small-size particles in the air (26). Larsen et al. compared extracts of dog dander prepared by different extraction procedures (8) and reported that the extract prepared in 0.125 M ammonium bicarbonate (1 g of dander/100 ml) was most suitable. In the present study, the extraction of danders/whole pelt was carried out in 2% w/v concentration and the protein contents of these extracts varied from 310 µg/mg of dog dander to 740 µg/mg of horse dander extracts (Table 1).

To investigate the role of animal allergens in the causation of bronchial asthma, intradermal tests were performed with these extracts on all the 68 patients with bronchial asthma included in this study. Of the 476 ID tests performed on patients, 15.3% were positive (1+ to 4+) as against 4.3% of the 140 tests conducted on 20 healthy volunteers (Table 1). On an average, 5.3% of the skin tests performed on asthmatics

were significantly positive (2+ to 4+). However, absence of such response among controls suggested the specificity of significantly positive skin test reactions. These results gave evidence that animal allergens play an important role in the etiology of bronchial asthma. A few 1+ reactions observed in healthy volunteers may possibly be due to the presence of nonspecific irritants in these allergen extracts or to the presence of latent subclinical allergy in the volunteers to various test animal danders (35). Of the 7 animal allergens tested, the highest percentage of markedly positive cutaneous reactions (2+ to 4+) was obtained with dog (8.8%) and horse and pig (7.4% each) dander extracts (Table 1). In addition to dog (36,37), some other workers have also reported cat allergens as the common sensitizers (10,29,36). We did not include cat extract as cat is not a common household pet in our country.

Even though a positive intradermal test is an important indicator of hypersensitivity to an allergen, the etiological significance of an allergen may be confirmed by performing a bronchial provocation test. In our study, a total of 36 bronchoprovocation tests were conducted with animal dander extracts on 25 of the 68 patients. Of the 23 tests on patients showing different grades of positive cutaneous reactions (1+ and 2+), 39.1% were positive. The positivity increased to 53.8% in 2+ skin reactors ($n = 13$). All the challenge tests ($n = 9$) conducted on nonasthmatics were found to be negative. Similarly, all the 13 bronchial provocation tests on patients with negative skin reactions to different animal dander extracts were also negative.

RASTs ($n = 66$) performed with the sera of 24 patients revealed the presence of circulating animal dander-specific IgE antibodies. Of the 23 RASTs performed with the sera from patients showing a positive skin response to dander extracts, 30.4% were found to be positive. The RAST positivity increased to 60% with the sera from patients with markedly positive cutaneous responses. However, all the sera from healthy volunteers as well as from patients showing a negative intradermal response to different animal dander extracts gave uniformly negative RASTs (Table 3). Some other workers have also reported a varying degree of RAST positivity (20–85%), with the sera from

patients showing a positive skin response to animal allergens (38–41).

The lower degree of RAST positivity in patients with a positive cutaneous response has been attributed to various factors, such as use of different allergen preparations for RASTs and skin testing (39,42). This factor was eliminated in our study, as we used the same lot of extracts for both skin testing and RASTs. It has also been proposed that activation of a nonspecific mediator system may result in a false positive intradermal response leading to discordance between the skin test and RAST results (43). On the other hand, the number of multiple allergenic components present in an extract which get coupled to the paper disc is also an important factor affecting RAST results.

In the present study, RAST positivity increased with an increase in the grade of skin reactions, varying from 7.7% with the sera from 1+ skin reactors to 100% with the sera of patients eliciting 4+ intradermal reactions. Similarly, the RAST ratio increased from 1.34 in negative to 13.18 in 4+ skin reactors to various animal dander extracts (Table 3). It was also observed that RASTs were always positive with the sera of patients eliciting 4+ intradermal reactions (Table 3). Further, all the sera from patients showing a positive skin as well as a positive bronchial response to various animal dander extracts were invariably RAST positive.

Only a limited number (8 patients) consented to undergo all 3 tests (intradermal and bronchoprovocation tests and RAST). On critical analysis of the results of 15 such comparisons, some important observations may be made (Table 4): (i) all 5 patients with negative cutaneous responses to the dander extracts showed uniformly negative bronchial provocation tests and RASTs, (ii) all 11 patients with negative bronchial provocation tests had negative RASTs, and (iii) all 4 patients with positive bronchial responses showed positive RASTs as well as positive skin reactions. These results suggested the specificity of both RAST and the bronchoprovocation test.

Our results of intradermal tests, bronchial challenge tests, and RASTs gave evidence that animal danders play an important role in the etiology of bronchial asthma. Investigations on the relationship of the presence of hypersensi-

tivity to animal allergens (as demonstrated by positive skin responses) and clinical characteristics of the patients revealed some interesting findings (Table 5). Patients with (i) younger age at the onset of symptoms, (ii) a positive family history of allergic disorders, or (iii) associated allergic disorders elicited significantly higher skin positivity to animal dander/pelt extracts as compared to those without these features. These results probably reflect the presence of atopy in our patients as IgE-mediated asthma is more common in children and runs in families.

The duration of disease and the presence of pets at home or at work did not seem to correlate with intradermal positivity. All the patients with pets (dogs) at home had seasonal asthma. It may be mentioned here that asthmatics with high serum levels of IgE are usually atopics. In the absence of IgE estimation in our study, we cannot say definitely how many of our perennial patients were having IgE-mediated and how many non-IgE-mediated disease. Since we did not find any significant difference in skin reactivity of the two groups (seasonal and perennial) it seems that the majority of our perennial cases were suffering with IgE-mediated asthma. It was not possible to correlate clinical symptoms of the patients with exposure to the animals as only 1 of them had a definite history of developing symptoms on exposure to horses.

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REFERENCES

- Berrens L: Epidermal allergens. *Rev Allergy* 24:917-925 (1970).
- Frankland AW: Allergy to animals. *Acta Allergol* 22:175 (1967) (abstract).
- Foucard T: A follow-up study of children with asthmatoïd bronchitis. *Acta Paediatr Scand* 62:633-644 (1973).
- Lowenstein H, Markussen B, Weeke B: Identification of allergens in extract of horse hair and dandruff by means of crossed radioimmuno-electrophoresis. *Int Arch Allergy Appl Immunol* 51:38-47 (1976).
- Vanto T, Viander M, Koivikko A: Skin prick tests in the diagnosis of dog dander allergy. *Clin Allergy* 10:121-132 (1980).
- Vanto T, Koivikko A: Dog hypersensitivity in asthmatic children. *Acta Paediatr Scand* 72:571-575 (1983).
- Venables KM, Tee RD, Hawkins ER, Gordon DJ, et al: Laboratory animal allergy in pharmaceutical company. *Br J Indust Med* 45:660-666 (1988).
- Larsen JN, Ford A, Gjesing B, Levy D, et al: The collaborative study of the international standard of dog, *Canis domesticus*, hair/dander extract. *J Allergy Clin Immunol* 82:318-330 (1988).
- Warner JA, Longbottom JL: Allergy to rabbits. III. Further identification and characterization of rabbit allergens. *Allergy* 46:481-491 (1991).
- Azofra J: Detection of allergens in a cat pelt extract. *Allergy* 47:110-114 (1992).
- Spitzaner S, Schweiger C, Anrather J, Ebner C, et al: Characterization of dog allergens by means of immunoblotting. *Int Arch Allergy Appl Immunol* 100:60-67 (1993).
- Schou C: Defining allergens of mammalian origin. *Clin Exp Allergy* 23:7-14 (1993).
- Agarwal MK, Sethi S, Jhamb S, Bansal S: Current concepts in the etiologic significance of insects in respiratory allergy. *Proc. CME International Symposium on Recent Advances in Allergy and Immunology*, Chandigarh, Nov. 4-6, 1988, 30-36.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265-275 (1951).
- Weiss EB, Segal MS: *Bronchial Asthma: Mechanism and Therapeutics*. Little, Brown, Boston, 1985.
- Agarwal MK, Jhamb S, Bansal S: The inhibitory effect of Astemizole on allergen and histamine induced skin wheal response in type 1 respiratory allergic disorders. *Indian J Allergy Appl Immunol* 5:32 (1991) (abstract).
- Shivpuri DN, Agarwal MK: Studies on the allergenic fungal spores of Delhi, India, metropolitan area: Clinical aspects. *J Allergy* 44:204-213 (1969).
- Agarwal MK: Skin testing and respiratory allergic disorders. *Indian J Chest Dis Allied Sci* 28:179-182 (1986).
- Adolphson CR, Gleich GJ, Yunginger JW: Standardization of allergens. In *Manual of Clinical Immunology* (Rose NR, Friedman H, Fahey JL, editors) American Society for Microbiology, Washington, DC: 1986, 652-659.
- Agarwal MK, Chaudhry S, Jhamb S, Gaur SN, et al: Etiologic significance of mosquito (*Anopheles stephensi*) in respiratory allergy in India. *Ann Allergy* 67:598-602 (1991).
- Snedecor GW, Cochran W: *Statistical Methods*, 6th Indian ed. Oxford and IBH Publishing Company, New Delhi, 1969, 217-218.
- Lutsky IT, Neuman I: Laboratory animal dander allergy: An occupational disease. *Ann Allergy* 35:201-205 (1975).
- Price JA, Longbottom JL: Allergy to rabbits. I. *Allergy* 41:603-612 (1986).
- Twiggs JT, Agarwal MK, Dahlberg MJE, Yunginger JW: Immunochemical measurement of airborne mouse allergens in a laboratory animal facility. *J Allergy Clin Immunol* 69:522-526 (1982).

25. Vanto T, Viander M, Schwartz B: Dog serum albumin as an allergen: IgE, IgG and lymphocyte responses in dander sensitive asthmatic children. *Int Arch Allergy Appl Immunol* 69:311–315 (1982).
26. Swanson MC, Agarwal MK, Yunginger JW, Reed CE: Guinea-pig-derived allergens: Clinico-immunologic studies, characterization, airborne quantitation, and size distribution. *Am Rev Respir Dis* 129:844–849 (1984).
27. Swanson MC, Agarwal MK, Reed CE: An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air-sampler: Studies with mite, cockroach, cat, mouse and guinea-pig antigens. *J Allergy Clin Immunol* 76:724–729 (1985).
28. Virtanen T, Eskelinen T, Hosman K, Mantiyarvi R: Long term and short term variability of airborne bovine epithelial antigen concentration in cow sheds. *Int Arch Allergy Appl Immunol* 98:252–255 (1992).
29. Anderson MC, Baer H, Ohman JL: A comparative study of the allergens of cat urine, serum, saliva and pelt. *J Allergy Clin Immunol* 76:563–569 (1985).
30. Lindgren S, Belin D, Dreborg S, Einarsson R, Pahlman I: Breed-specific dog-dander allergens. *J Allergy Clin Immunol* 82:196–204 (1988).
31. Franke D, Maasch HJ, Wahl R, Schultze-Werninghaus G, Bretting H: Allergens of horse epithelium. I. Physicochemical and immunochemical characterization of five different horse epithelium raw materials used for allergen extract preparation. *Int Arch Allergy Appl Immunol* 92:309–317 (1990).
32. Gordon S, Tee RD, Taylor AJN: Comparison of proteins and allergens in rat hair and urine. *Clin Exp Allergy* 23(Suppl 1):56 (1993) (abstract).
33. Gordon S, Tee RD, Taylor AJN: Influence of age and sex on the protein and allergen composition of rat urine. *Clin Exp Allergy* 23(Suppl 1):56 (1993) (abstract).
34. Varga JM, Ceska M: Characterization of allergen extracts by polyacrylamide gel isoelectrofocusing and radioimmunosorbent allergen assay. II. Dog and cat allergens. *Int Arch Allergy* 42:438–453 (1972).
35. Gupta S, Jain S, Chaudhry S, Agarwal MK: Role of insects as inhalant allergens in bronchial asthma with special reference to the clinical characteristics of patients. *Clin Exp Allergy* 20:519–524 (1990).
36. Brandt R, Yman L: Dog dander allergens: Specificity studies based on the radioallergosorbent technique. *Int Arch Allergy Appl Immunol* 61:361–370 (1980).
37. Rohatgi N, Dunn K, Chai H: Cat or dog-induced immediate and late asthmatic responses before and after immunotherapy. *J Allergy Clin Immunol* 82:389–397 (1988).
38. Fagerberg E, Wide L: Diagnosis of hypersensitivity to dog epithelium in patients with asthma bronchiale. *Int Arch Allergy Appl Immunol* 39:301–309 (1970).
39. Apold J, Havnen J, Hvatum M, Oseid S, Aas K: The radioallergosorbent test (RAST) in the diagnosis of reagenic allergy: A comparison between provocation tests, skin tests and RAST employing allergosorbents which were arbitrarily prepared with commercial extracts. *Clin Allergy* 4:401–409 (1974).
40. Havnen J, Apold J, Hvatum M, Oseid S, Aas K: The radioallergosorbent test in the in vitro diagnosis of multiple reagenic allergy: Comparison of two different extract preparations from timothy pollen, birch pollen and dog's hair respectively, as used for tests in vivo and in vitro. *Clin Allergy* 4:411–420 (1974).
41. Eriksson NE: Diagnostic methods in reagenic allergy. A clinical evaluation in patients with bronchial asthma and allergic rhinitis. M.D. thesis, University of Goteberg, Goteberg, Sweden, 1977.
42. Aas K, Ludkvist U: The radioallergosorbent test with purified allergen from codfish. *Clin Allergy* 3:255–261 (1973).
43. Berrens L, Henocq E, Koers WJ: The specificity of animal dander allergens: A semiblind clinical and experimental study. *Acta Allergol* 29:10–24 (1974).