

Monkey dander asthma

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We recently cared for two patients who experienced acute asthma with exposure to dander of the cotton top tamarin, a species of New World monkey. Both patients had serologic evidence for an IgE antibody to monkey dander as determined by RAST and a positive immediate skin test response to an extract prepared from monkey dander. Furthermore, by RAST we were able to determine that cotton top tamarin urine and newborn cotton top tamarin dander had antigens that reacted with IgE in the serum of the affected patients. Thus we report two patients with asthma to monkey dander that appears to be mediated by an IgE mechanism. We also found that serum from a subject exposed to another species of New World monkey, the capuchin, yielded a positive RAST to the cotton top tamarin, suggesting that allergenic cross-reactivity may exist between the two species. (J ALLERGY CLIN IMMUNOL 75:268-71, 1985.)

Animal danders are a frequent cause of asthma.¹⁻⁵ This is especially true for the atopic research laboratory worker exposed to animals, particularly rodents.^{6,7} Despite widespread exposure to primates in research and home settings, few patients have documented sensitivity to monkey dander.⁸ We recently cared for a research psychology professor who developed repeated, severe bouts of asthma after exposure to a species of New World monkey, the cotton top tamarin (*Saguinus o. oedipus*), housed in a primate behavior research center. Two other persons in his laboratory had asthma and extensive exposure to New World monkeys. One of these patients experienced acute asthmatic attacks after contact with monkeys, whereas the other asthma patient was not affected.

Case histories

Patient 1, a 40-yr-old psychology professor, had allergic asthma and rhinitis and known sensitivities to pollen, mold, and rat dander. After extensive exposure to the cotton top

tamarin for 6 yr, our patient experienced wheezing and dyspnea on each contact with this primate. The provocative encounter could be minimal or indirect, such as exposure to dander-bearing clothing. These symptoms were initially controlled with the use of inhaled bronchodilators. In February 1982 the patient was hospitalized with severe asthma. His admission FEV₁ was 0.7L with a predicted value of 4.0L. Clinical improvement followed treatment with intravenous bronchodilators and corticosteroids. Although the patient attempted to avoid contact with the monkeys, theophylline (800 mg/day), low doses of prednisone (10 mg/day), and inhaled β -agonists were necessary to control his asthma. Prick-skin testing (50% glycerine, 1:20 w/v, Greer Laboratories, Lenoir, N. C.) produced positive responses to ragweed, a seven-grass mix, *Alternaria*, and rat epithelium. Minimized exposure to monkeys and regular use of inhaled cromolyn has resulted in symptomatic improvement.

Patient 2, a 27-yr-old assistant in the professor's laboratory, had a history of asthma and allergic rhinitis since childhood and was known to be allergic to dust, mold, feathers, and cat dander. She had no respiratory symptoms with monkey exposure during the first 5 yr of work. She then developed asthma and rhinoconjunctivitis after each exposure to cotton top tamarins. The severity of her asthma symptoms was reduced with the use of a face mask and antihistamine treatment.

Patient 3, a 27-yr-old assistant in the same laboratory, developed exercise-related asthma after working in the laboratory for 1 yr. Monkey exposure did not cause the asthma. Immediate hypersensitivity skin tests in this patient were negative to extracts of grass pollen, Bermuda grass, *Alternaria*, *Aspergillus fumigatus*, *Hormodendrum*, *Penicillium*, ragweed, fall pollens, trees, cat, dog, and house dust.

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MATERIAL AND METHODS

Monkey dander extracts

Monkey dander and hair were carefully collected from the animals' housing. Skin test extracts of this monkey material were prepared by soaking in 0.05M Tris-HCl (pH 8.0) at a concentration of 1:20 (w/v). After an overnight agitation at 4° C, the material was centrifuged at $1120 \times g$ for 15 min; the resulting supernatant was filtered through a 0.22 μm filter (Millipore Corp., Bedford, Mass.). Patients were tested with the freshly prepared extracts by the prick skin-test method and examined after 15 min. A 5 mm wheal was considered a positive test.

Preparation of antigen for RAST

Adult cotton top tamarin dander was also extracted in RAST coupling buffer (0.1M NaHCO_3 , 0.5M NaCl, pH 8.3) at a concentration of 1:20 (w/v). After an overnight agitation at 4° C, the mixture was centrifuged at $1000 \times g$ for 20 min at room temperature. The supernatant was re-centrifuged at $12,000 \times g$ for 30 min at room temperature and filtered through a 0.8 μm filter. The protein content⁹ was 2 mg/ml when bovine serum albumin was used as a standard.

Dander from a newborn cotton top tamarin was obtained by shaving the animal within minutes after birth, and extracts were prepared in a similar manner. However, because of the small amount of available dander, the final centrifugation was performed in a Beckman Microfuge (Beckman Instruments, Inc., Palo Alto, Calif.) for 4½ min, and final filtration and protein assay were not possible.

Adult cotton top tamarin dander extract was coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Piscataway, N. J.) at a concentration of 2 mg of protein per milliliter of swollen gel as previously described.¹⁰ Briefly, gel swollen in 1 mM HCl was mixed with monkey dander-extract protein that was dissolved in RAST coupling buffer at a gel:buffer ratio of 1:2. This mixture was agitated for 2 hr at room temperature and allowed to stand overnight at 4° C. After filtration, the suspension was washed twice with deionized water (water:gel, 2:1), and the protein content in each filtrate was determined by the method of Lowry.⁹ The remaining active groups were blocked with 1.0M ethanolamine, pH 8.0 (4 ml ethanolamine to 1 ml of swollen gel), at room temperature for 2 hr with occasional stirring. Excess uncoupled protein was removed with four washes, alternating high-pH buffer (0.1M Na borate, 1.0M NaCl, pH 8.5) with low-pH buffer (0.1M Na acetate, 1.0M NaCl, pH 4.0). After a wash in RAST buffer (0.05M Na_2HPO_4 , 0.2% bovine serum albumin, 0.05% w/v NaN_3 , 0.5M NaCl, 0.5% v/v Tween, pH 7.5), the gel was dried to a moist cake and stored in 50% RAST buffer (v/v) at 4° C. The gel bound 34% to 47% of the protein that was added.

Direct RAST measurements

Direct RAST was performed as previously described.¹¹ Briefly, 0.5 ml of 3% sorbent was incubated with 0.1 ml of serum that was diluted 1:5 in RAST buffer with constant

rotation at room temperature for 4 hr. After three washes with RAST buffer, Phadebas ^{125}I -labeled anti-human IgE (Pharmacia) (67,000 to 85,000 cpm per tube) was added, followed by overnight rotation at room temperature. After three additional washes, the bound radioactivity was counted on a Micromedic 2/200 automatic gamma counter (Rohm and Haas, Philadelphia, Pa.). All assays were performed in duplicate. The average background cpm bound was subtracted from each result before data analysis. The percentage cpm bound was determined as follows:

$$\% \text{ cpm bound} = \frac{\text{cpm}_s}{\text{cpm}_{\text{total}}} \times 100$$

where cpm_s = the cpm bound by the subject's serum and $\text{cpm}_{\text{total}}$ = the cpm added to each sample. Determination of the ratio of radioactive binding in the sample serum to the negative control serum was calculated as follows:

$$\frac{\text{cpm}_s}{\text{cpm}_{\text{normal}}}$$

where $\text{cpm}_{\text{normal}}$ = the cpm bound by a negative control serum.

In addition to the sera of the three research workers described above, we obtained serum from three members of a family who owned a capuchin monkey (*Cebus capucinus*) as a pet. One member of this family (subject 4) had asthma during contact with the pet monkey. The other two family members (subjects 5 and 6) remained asymptomatic with exposure to this primate. We were interested in determining if sera from these family members had IgE antibody to an extract from the cotton top tamarin monkey, a species related to the capuchin in the New World monkey group.

RAST-inhibition measurements

RAST inhibition was conducted as described by Gleich et al.¹² Equal volumes of sera from subjects 1 and 2 were pooled and diluted 1:5 in RAST buffer. Diluted sera (0.1 ml), 0.5 ml 3% sorbent, and varying quantities of the following inhibitors were allowed to react with constant rotation at room temperature for 18 hr: (1) adult cotton top tamarin dander extract, 1:20 w/v in coupling buffer; (2) newborn cotton top tamarin dander extract, 1:20 w/v in coupling buffer, (3) undiluted male cotton top tamarin urine, (4) *Alternaria* (70% $[\text{NH}_4]_2\text{SO}_4$ precipitate of a 1:20 extract of locally isolated organism), 50 mg/ml in RAST coupling buffer, (5) *Dermatophagoides farinae*, 1:100 w/v 50% glycerine, and (6) house dust extract, 10,000 PNU (Greer Laboratories). The remainder of the procedure was the same as that for the direct RAST. The amount of radioactivity bound to the Sepharose particles was compared to that of a control to which inhibitor had not been added. In all tests the amount of nonspecific binding of radioactivity was taken to be the amount bound by a nonallergic subject. Results of duplicate tests varied <16%. Percentage inhibition was calculated as follows¹³:

TABLE I. Results of monkey dander prick-skin tests

	Wheal diameter (mm)		
	Subject 1	Subject 2	Subject 3
Histamine (2.75 mg/ml)	6	7	7
Cotton top tamarin monkey	15	11	0

TABLE II. Direct RAST to cotton top tamarin monkey dander

Subject	Skin test results	Presence of symptoms with exposure to New World monkeys	Type of monkey subject exposed to	% Total cpm bound	cpm _x cpm _{normal}
1	+	+	Cotton top tamarin	4.03	23.7
2	+	+	Cotton top tamarin	20.5	120.6
3	—	—	Cotton top tamarin	0.15	0.88
4	ND*	+	Capuchin	3.83	9.0
5	ND	—	Capuchin	0.26	0.62
6	ND	—	Capuchin	0.31	0.72
Atopic-unexposed	ND	—	—	0.15	1.12
(n = 6)				(0.13 to 0.17)†	(0.96 to 1.21)
Non-atopic	ND	—	—	0.18	1.38
(n = 5)				(0.12 to 0.23)	(0.94 to 1.74)

*Not done.

†Range.

% inhibition =

$$100 - \frac{\left(\text{cpm bound in the presence of inhibitor} \right) - \left(\text{cpm bound nonspecifically} \right)}{\left(\text{cpm bound in the absence of inhibitor} \right) - \left(\text{cpm bound nonspecifically} \right)} \times 100$$

RESULTS

Skin tests

Results are recorded as millimeters of wheal observed 15 min after the prick-skin test (Table I). The two individuals with asthma symptoms on monkey exposure (subjects 1 and 2) had positive skin tests to the extract from the cotton top tamarin. Subject 3 did not have asthma when she was exposed to the monkey, and her skin tests were negative.

Direct RAST

An excellent correlation between RAST results and symptoms was found (Table II). Subjects 1 and 2, who were symptomatic with cotton top tamarin monkey exposure, had radioactive binding that was 23.7 and 120.6 times that of a negative control serum, respectively. The radioactive binding of subject 3, who remained asymptomatic with exposure to this primate, did not differ from that of the control.

The radioactive binding to cotton top tamarin mon-

key-dander sorbent in subject 4 was 9.0 times that in the control. Although this subject had no known contact with the cotton top tamarin, he did experience asthma with contact to the capuchin monkey. The asymptomatic family members (subjects 5 and 6) did not demonstrate increased radioactive binding.

RAST inhibition

The results of the RAST inhibition are illustrated in Fig. 1. The highest inhibition of the RAST occurred with adult monkey dander. By comparison, dander obtained from the newborn monkey and the monkey urine were much weaker inhibitors. No inhibition was observed with *Alternaria*, house dust, or *Dermatophagoides farinae*.

DISCUSSION

Animal dander sensitivity is a major cause of allergic symptoms for many people.^{6,7} There are several reasons why monkeys have not, heretofore, been reported to cause allergic symptoms. Monkeys are not a common pet or laboratory research animal. Thus human exposure to monkeys is infrequent. Moreover, because the monkey is a primate, human sensitivity may be less likely. We have identified two individuals who developed allergic symptoms after exposure to monkeys and have both positive skin tests to a monkey

dander extract and IgE antibodies to this material by RAST.

Only sera from the two patients who were symptomatic after exposure to monkeys had specific IgE antibody to the monkey dander extract by RAST. Interestingly, the IgE antibody to cotton top tamarin monkey dander was also found in serum from the symptomatic owner of the capuchin monkey. This suggests that an antigenic cross-reactivity may exist between these two species of New World monkeys. Bronchial challenges would have confirmed monkey dander as a new cause of occupational asthma. Unfortunately, we were not able to conduct this aspect of the evaluation because one subject moved out of the study area and the other subject declined to participate because severe asthma had followed each monkey exposure. Nevertheless, we believe that the association of exposure-induced symptoms, RAST, and skin test results offer strong presumptive evidence that monkey dander can sensitize certain individuals and induce asthma.

Antigens in animal dander may arise from several sources, including the epithelium, albumin, urine, and saliva.^{6, 13-16} Although the origin and biochemistry of allergen(s) in monkey dander are not presently known, some characteristics of monkey dander have been established. RAST-inhibition results found adult monkey dander to be considerably more allergenic than newborn monkey dander, whereas monkey urine was the weakest allergen tested. Newborn monkey dander differed from adult monkey dander only in that it was obtained before the mother had a chance to clean the infant. It was therefore devoid of salivary proteins. Whether or not this means that saliva is an important allergen remains to be observed.

We have identified three patients in whom monkey dander apparently causes asthma. In two of these patients, we were able to demonstrate an immediate skin test response to monkey dander. RAST identified specific-IgE antibodies to the monkey dander in the sera of the three symptomatic individuals. The source of the allergen is the dander, with an unknown contribution from the urine and possibly the saliva.

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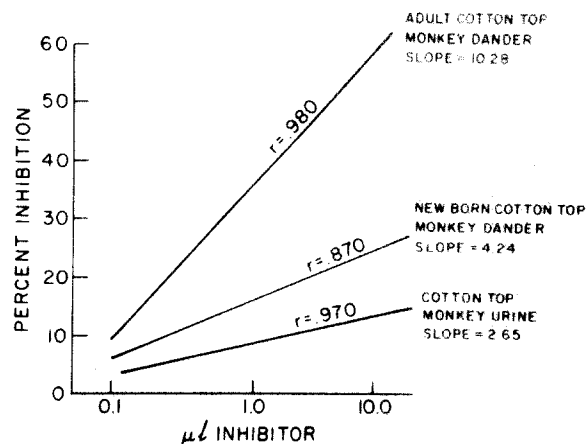


FIG. 1. RAST-inhibition results with use of cotton top tamarin solid-phase polymer; r = correlation coefficient.

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