

arrowroot, and the patient had consumed several bottles of arrowroot extract without any problems before this episode. He was atopic, had positive responses to house dust mites and weed pollens on a skin prick test, and had been previously diagnosed as having allergic rhinitis and atopic dermatitis. The patient had seafood allergy. To investigate the pathogenic mechanism involved, arrowroot proteins were extracted from finely chopped raw arrowroot by stirring for 2 hours at 4°C in phosphate-buffered saline (PBS). Particles were removed by centrifugation at 10,000g for 30 minutes at 4°C, and the supernatant was dialyzed overnight against PBS. The protein concentration of the extract (2.0 mg/mL) was determined using Bradford analysis.

A skin prick test using arrowroot extract showed a positive response (allergen:histamine ratio of >2+) in the patient, whereas none of the 10 nonatopic, healthy control subjects had a positive response. Serum specific IgE antibodies to arrowroot extract were measured using enzyme-linked immunosorbent assay (ELISA). High levels of specific IgE antibodies were detected in the patient's serum based on a positive cutoff value determined from the mean and standard deviation of absorbance values from 10 nonatopic, healthy controls (Figure 1A). To confirm binding specificity, ELISA IgE inhibition tests were performed by adding arrowroot extract and mugwort, soybean, and house dust mite extracts. Significant inhibition was noted with serial additions of arrowroot extract, whereas inhibition by the other extracts was minimal (Figure 1B).

Traditionally, arrowroot has been a fundamental herb used as a antipyretic, antidiarrhetic, diaphoretic, and antiemetic agent in Chinese medicine.¹ In recent times, it has been used to treat alcohol addiction because it contains many useful isoflavones. Other studies^{3,4} have demonstrated that it effectively inhibits microbial growth. Although it has been used widely, few cases of hypersensitivity reactions induced by arrowroot ingestion have been reported. A case of maculopapular exanthema due to arrowroot ingestion was reported and confirmed by a patch test, suggesting type IV hypersensitivity.² Two cases of toxic hepatitis associated with arrowroot ingestion have been reported in which generalized urticaria was accompanied by hepatitis.⁵ In this case, the patient showed only generalized urticaria without any other organ involvement, suggesting an IgE-mediated reaction. We speculate that arrowroot can cause various clinical manifestations of hypersensitivity reactions involving different pathogenic mechanisms, despite a similar exposure route.

With the increasing interest in healthy foods, food allergies related to herbs are becoming more common. Food allergies caused by herbs such as ginseng and bellflower have already been studied as pollen-food syndromes. Ginseng causes respiratory allergies in patients sensitized to birch pollen, and bellflower root shows cross-reactivity with mugwort and birch pollen.^{6,7} Arrowroot is a plant in the genus *Pueraria*, which is in the pea family *Fabaceae*. We performed IgE inhibition tests with arrowroot extract; mugwort pollen, to which the patient was already sensitized; and soybean, which belongs to the pea family. Significant inhibition was noted with serial additions of arrowroot extract, but inhibition by mugwort pollen and soybean extracts was minimal. The skin prick test using soybean extract revealed an allergen:histamine ratio of 1+. However, this patient never experiences any allergic reactions after eating beans.

In food allergy, both class 1 and class 2 food allergens can cause IgE-mediated allergic reactions. Class 2 food allergens elicit allergic symptoms only after primary sensitization with cross-reactive inhalant allergens. By contrast, class 1 food allergens not only provoke allergic sensitization in the gastrointestinal tract but also cross-link IgE. In this case, considering that the patient had a positive skin prick test response to arrowroot extract, no cross-reactivity between

weed pollens and soybean extracts, and a typical clinical manifestation, we conclude that arrowroot allergen may cause allergic reactions through direct oral sensitization not cross-reactivity with other allergens. However, further studies are needed to reveal the allergenicity of arrowroot.⁸ In conclusion, we report the first case, to our knowledge, of IgE-mediated urticaria to arrowroot extract that was confirmed by a positive skin prick test result and immunologic tests.

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OCCUPATIONAL ASTHMA CAUSED BY GERBIL: PURIFICATION AND PARTIAL CHARACTERIZATION OF A NEW GERBIL ALLERGEN

Rodents are a well-recognized cause of rhinitis and asthma in occupational and household settings.¹ The gerbil (*Meriones unguiculatus*) is a popular pet that is also used as an animal model for immunology studies.² Cases of allergy to gerbils have rarely been reported, and gerbil allergens have not been characterized to date.^{3,4}

A 33-year-old female biologist and smoker with pollinosis who worked with guinea pigs and gerbils was treated at Fundación Jiménez Díaz. One year after she began working with gerbils, she developed symptoms of nasal and ocular itching, tearing, sneezing, runny nose, and nasal stuffiness while working in the research facility with the gerbils. The symptoms subsided on weekends, and she remained asymptomatic during holidays. She also had no symptoms when working in the laboratory without the animals. In the past 2 months she experienced episodes of bronchospasm, mainly while handling the gerbils. Eosinophilia was present in blood (eosinophil

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count, 830/mm³) and in nasal smears. Spirometry findings were normal, and findings from a methacholine inhalation test performed after a period without working were negative.

Male guinea pig and gerbil epithelium and hair were homogenized and extracted, and the urine was collected and dialyzed. All extracts were sterilized by filtration through a 0.22-μm membrane filter. The epithelium extract protein content was 0.2 mg/mL in guinea pig and 0.15 mg/mL in gerbil. Air samples were collected using an automated sampler (Air Sentinel; Quan-Tec-Air, Rochester, Minnesota) from a 7-m³ chamber where gerbils and guinea pigs were kept separately. Gerbil (0.07 mg/mL) and guinea pig (0.12 mg/mL) extracts were processed by eluting the polytetrafluoroethylene filter membranes.

Skin prick test and specific IgE results are given in Table 1. A nasal challenge to the gerbil epithelium extract (0.008 mg/mL), measured using acoustic rhinometry, caused immediate rhinitis symptoms along with a decrease of 44% and 39% in the minimum cross-sectional area and the nasal volume (2-5 cm), respectively. Specific inhalation challenge with increasing aerosolized allergen concentrations was performed using the tidal breathing method. Guinea pig (0.1 mg/mL) and gerbil (0.15 mg/mL) epithelium extracts elicited isolated early asthmatic responses, with 17% and 19% declines in forced expiratory volume in 1 second (FEV₁), respectively. Twenty-four hours after each specific bronchial challenge, the methacholine inhalation test results changed from negative to positive (provocation concentration that caused a decrease in FEV₁ of 20%, 6.4 mg/mL) for the guinea pig bronchoprovocation and from 6.4 to 2 mg/mL in the case of the gerbil challenge. Moreover, induced sputum samples collected after the bronchial challenges showed intense eosinophilia (30% and 25% eosinophils, respectively).

Proteins from guinea pig and gerbil extracts were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis.⁵ IgE immunoblotting revealed an intense IgE binding band of 23 kDa in the gerbil urine extract that was also present in the epithelium, hair, and air sample filter extracts. Guinea pig IgE immunoblotting revealed protein bands of 17, 19, 21 kDa in the epithelium extract, which may correspond to the Cav p 1 and Cav p 2 guinea pig allergens (Fig. 1A).⁶ Control immunoblot experiments with a serum pool of nonatopic patients did not show bands in the different extracts.

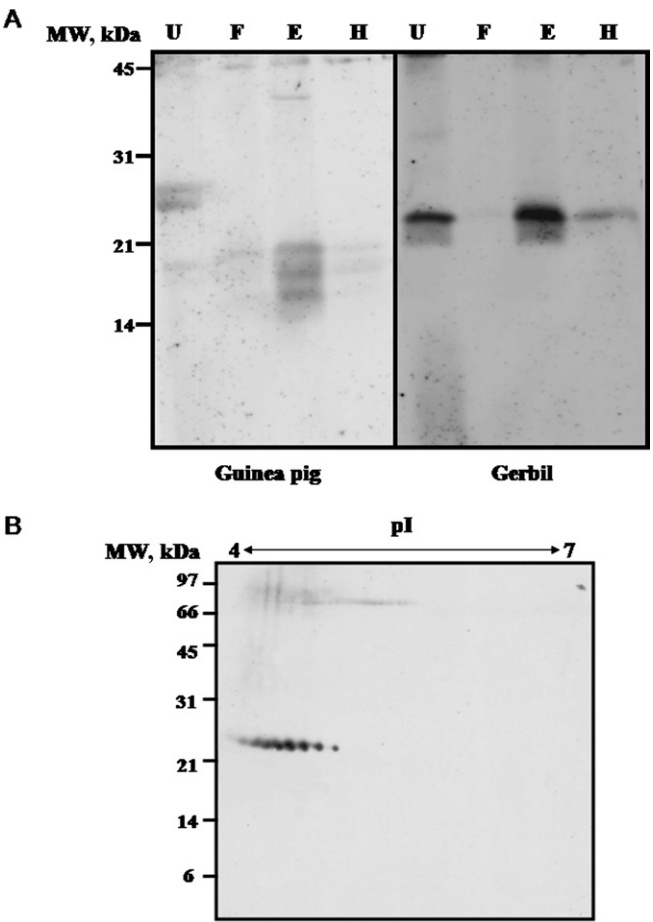


Figure. A, IgE-binding profile of the patient's serum to guinea pig and gerbil extracts by immunoblotting. Molecular weight (MW) markers are shown on the left. Lanes U show urine extracts; lanes F, membrane filter extracts; lanes E, epithelium extracts; and lanes H, hair extracts. B, IgE reactivity of the patient's serum to gerbil epithelium extract by 2-dimensional immunoblotting.

Table 1. Results of Skin Prick Tests and Specific IgE Measurement

Aeroallergens ^a	Histamine, mm	Glycerosaline, mm	Extracts, mm ^b		Total IgE, kU/mL	CAP results, kU/L
			Guinea pig	Gerbil		
Positive ^c to grass pollens, dog, cat, and mouse	4	<1	Epithelium, 5	Epithelium, 7	78	Cat dander, 0.66
Negative to mites, molds, cockroach, and other animal danders			Urine, 5	Urine, 5 Filter-membrane extract, 4		Guinea pig, 1.47 Gerbil, 1.07 Rat urine, 1.28 Mouse epithelium, 1.27 Mouse urine, 1.26 < 0.35 to rabbit, hamster, dog, horse, and cow epithelium

^a From ALK-Abello, Madrid, Spain.

^b Five atopic controls yielded negative test results.

^c Positive refers to a wheal diameter greater than 3 mm.

Sera preabsorbed with gerbil and guinea pig epithelium extracts (50 µg/mL) lost significant IgE antibody binding activity to both guinea pig and gerbil allergens, which suggests the existence of IgE-binding cross-reactivity epitopes. Two-dimensional electrophoresis and immunoblotting assays showed several allergens located around 23 kDa with acidic isoelectric point values of 4 to 4.7, possibly indicating isoforms (Fig.1B).

The 23-kDa gerbil protein was purified in an fast protein liquid chromatography system.⁵ First, ion exchange chromatography was performed using a DEAE Sepharose column, followed by gel filtration chromatography in a Superdex 75 column (GE Healthcare, Piscataway, New Jersey). A prick test performed with this purified protein elicited a 7-mm wheal in the patient and was negative in 5 atopic individuals.

The 23-kDa purified allergen was studied by analyzing the amino-terminal sequence and by means of mass spectrometry using MALDI-TOF-TOF.⁷ The 14 amino-terminal amino acid residues of the 23-kDa gerbil band was S-P-F-D-P-K-E-L-E-G-E-W-H-T-L-R. A computer database search found no matching results with known peptide sequences, indicating that this might represent a novel protein or proteins. However, this contains the GXW triplet in the 10–12 position, which is the most highly conserved motif present in lipocalins.⁸

We report the first case, to our knowledge, of occupational rhinitis and asthma due to gerbil, as demonstrated using specific nasal and bronchial challenge. Few other cases of suspected gerbil allergy have been reported to date, and the implicated allergens were not studied.^{3,4} From the present analysis, it seems that the 23-kDa binding band is the major sensitizing allergen and is present in different gerbil sources and in air sentinel samples, which suggests an ability to act as an aeroallergen. Sequencing and mass spectrometry did not identify the specific protein(s) corresponding to this

band, thus indicating that this is potentially a novel antigen that may correspond to a lipocalin.

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