

An Odorant-Binding Protein as a New Allergen from Siberian Hamster (*Phodopus sungorus*)

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Established Facts

- The Siberian hamster or dwarf hamster (*Phodopus sungorus*), while similar in appearance to common hamsters, the European hamster or black-bellied (*Cricetus cricetus*) and golden hamsters (*Mesocricetus auratus*), is a different species.
- Allergens from Siberian hamster are not well known, and although unique allergens are likely to be involved, cross-reactivity with common hamster allergens may also be expected.
- Many of the most important mammalian-derived allergens belong to the family of proteins called lipocalins, which exhibit variable IgE cross-reactivity.

Novel Insights

- A new allergen likely having 3 isoforms and originating in the Siberian hamster (*Phodopus sungorus*) is described.
- No cross-reactivity with other hamster allergens was found.
- The new allergen is an odorant-binding protein from the lipocalin family.

Key Words

Anaphylaxis • Siberian hamster • Bite • Allergens • Lipocalins • Odorant-binding proteins

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Abstract

A case of anaphylaxis following a bite from a Siberian hamster (SH; *Phodopus sungorus*) is described. Skin prick tests with hair, urine and salivary gland extracts from SH were positive, while the tests were negative for hair extracts from other rodents. IgE immunoblotting with the patient serum revealed 3 IgE-binding bands of about 18, 21 and 23 kDa. When the patient's serum was preincubated with rabbit, mouse and gerbil hair extracts, no inhibition of the 3 SH IgE-binding

bands was demonstrated. Proteins extracted from the 3 bands were analyzed by N-terminal sequencing and matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry, and peptides were sequenced. IgE-binding bands were identified as being an odorant-binding protein belonging to the lipocalin family. Analysis of the 3 IgE-binding bands found in the hair, urine and salivary glands of SH showed a new allergenic protein lacking cross-reactivity with allergens from other rodents. The 3 bands likely correspond to isoforms of a single allergen.

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Introduction

The Siberian hamster (SH) or dwarf hamster (*Phodopus sungorus*), while similar in appearance to common hamsters, the European hamster or black-bellied (*Crictus crictus*) and golden hamsters (*Mesocricetus auratus*), is a different species. Two previous reports of anaphylaxis induced by SH bites have been published [1, 2]. In one case, the responsible allergen from the hamster saliva was identified as a 21-kDa protein that showed partial cross-reactivity with *Dermatophagoides pteronyssinus* [2]. Bertó et al. [3] described 2 IgE-binding proteins (molecular weights 18 and 32 kDa) in 5 of the 6 sera samples taken from patients with asthma who were monosensitized to SH. Interestingly, the patients described in these articles did not show specific IgE to extracts from common hamsters. These results demonstrate that allergens from SH are not well known, and although unique allergens are likely to be involved [4], others may be shared with common hamsters (*M. auratus* and *C. crictus*).

Case Report

A 23-year-old woman with no previous history of allergic diseases developed generalized urticaria and facial angioedema a few minutes after she had been bitten on one of her fingers while playing with an SH in her home. The patient's condition later worsened, with general malaise, pharyngeal obstruction, hypotension (90/50 mm Hg), tachypnea, profuse perspiration, shortness of breath and wheezing. A pulse oximetry reading taken in the emergency room showed 79% oxygen saturation, and arterial blood gases revealed hypoxemia and respiratory acidosis. The chest X-ray showed air trapping. The patient received crystalloid fluids, 100% oxygen, subcutaneous epinephrine, intravenous corticosteroid and antihistamine, as well as inhaled salbutamol and antitetanus prophylaxis. She was discharged 1 day after complete remission of her symptoms.

When the patient was treated in our outpatient clinic she was completely asymptomatic, mentioning that she had removed the

hamster from her home. She had bought an SH 3 months before the aforementioned episode. The animal was placed in a cage in the hallway of the house and was given proper care. The patient reported that when she played with the hamster she experienced slight itching and erythema on her fingers. While she made no mention of prior bites, we cannot rule out scratches. Nevertheless, she had never presented rhinoconjunctivitis or asthma symptoms after exposure.

At the time of consultation, the physical examination performed was normal, as was baseline spirometry and exhaled nitric oxide fraction.

In order to perform the allergy study, a group of SHs were bought and kept in good conditions in the Animal Facility of the hospital. The animals were used to obtain samples of urine, hair and salivary glands for the preparation of extracts [5]. The SH hair extract was obtained by means of shaving. The hair (10% w/v) was suspended in a phosphate-buffered saline (PBS) solution for a period of 3 days at 4°C in constant agitation. The extract was centrifuged and the supernatant was dialyzed against PBS and then lyophilized. Urine from SH was obtained by keeping the SH in a metabolic cage for 3 days. The hamster's urine was dialyzed against PBS and then lyophilized. The lyophilized extracts were subsequently reconstituted in 50% glycerine saline solution at a concentration of 2 mg/ml and filtered using a 0.22-µm Millipore filter, and a series of aliquots were prepared.

The salivary glands were obtained by means of surgical dissection performed by a veterinarian on a previously killed SH. After the salivary glands were homogenized, the same procedures as above were followed to obtain the extract.

Total protein content was determined by Coomassie Plus (Bradford) Assay (Pierce, Ill., USA) with the following results: SH hair, 1.3 mg/ml; SH urine, 5 mg/ml; SH salivary glands, 11.6 mg/ml.

Skin prick tests (SPTs) were performed with commercial common inhalants and commercial rodent extracts (1/100 w/v), including rabbit, mouse, gerbil, rat, ferret and common hamster (*C. crictus*). Histamine (10 mg/ml) and glycerol-saline solution were used as controls (all from ALK-Abelló, Madrid, Spain). A skin test was considered positive if the wheal was at least 3 mm greater than the negative control. All SPTs with the extracts used were negative.

SPT results with SH extracts were as follows: SH hair extract, 4 mm in diameter; SH urine extract, 3 mm in diameter, and SH salivary gland extract, 6 mm in diameter with pseudopod formation. The same tests were performed on 5 nonatopic patients used as controls and had negative results.

Total serum IgE (CAP system, Phadia, Uppsala, Sweden) was 117 IU/ml. Specific IgE for dog and cat dander and hair from rat, mouse, rabbit, ferret, gerbil and a mix of common hamsters (*C. crictus* and *M. auratus*) was determined using the CAP system (Phadia). All were negative (<0.35 IU/ml). SDS-PAGE was carried out according to the method of Laemmli [6]. Polyacrylamide concentrations of 14% (w/v) and 5% (w/v) were used for separating and stacking gels, respectively. An amount of 50 µg of protein was applied per lane. A 14% SDS-PAGE gel under reduced conditions was run with 3 different SH extracts. Protein bands separated by SDS-PAGE were transferred by semidry blotting onto nitrocellulose sheets. Immunoblots were carried out as previously described [7]. Briefly, membranes were blocked with PBS containing 0.1% Tween 20 (PBS-T), 3% skimmed milk powder and 3% bovine se-

Fig. 1. SDS-PAGE analyses of SH (*P. sun-gorus*) extracts under reducing conditions and immunoblotting. An amount of 50 µg of protein was applied per lane. **a** SH hair extract. **b** SH salivary gland extract. **c** SH urine extract. Lane A: SDS-PAGE under reducing conditions with Coomassie blue staining; lane B: IgE immunoblotting using patient serum; lane C: IgE immunoblotting using serum from a nonatopic patient as a negative control. **d** IgE immunoblotting inhibition of SH hair extract with other rodent hair extracts. G = Gerbil; M = mouse; R = rabbit; MW = molecular weight in kDa (from BioRad).

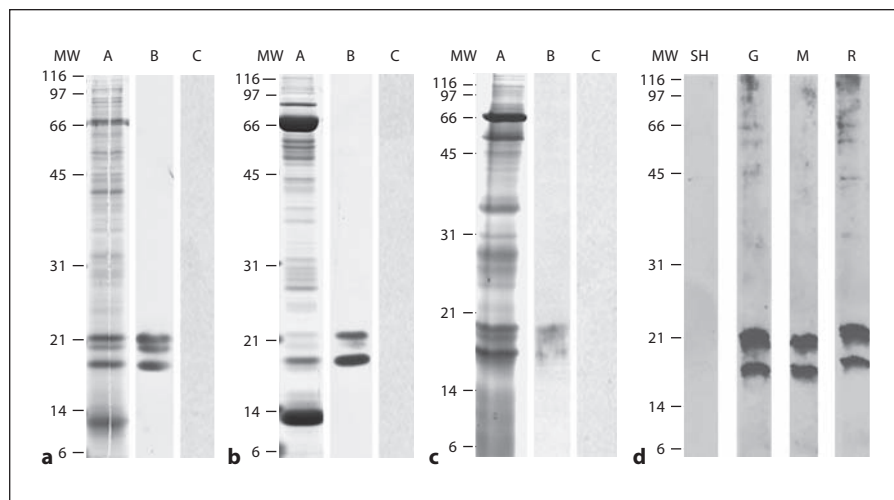


Table 1. Amino acid sequences identified in IgE-binding bands from SH

		Identity, %	Positives, %
N-terminal sequencing			
Sequenced peptide	NDYAELEG		
Odorant-binding protein 3 (<i>Myodes glareolus</i>)	HA-----	75	75
Mass spectrometry			
Sequenced peptide	FKPVYATPENIVFTNK		
Odorant-binding protein 3 (<i>M. glareolus</i>)	----H---D---ISQ	69	75
Sequenced peptide	AGQETNEMYVV_GK		
Aphrodisin (lipocalin family) (<i>Cricetus cricetus</i>)	-----_-I--A--	85	85

rum albumin for 2 h at room temperature. These were incubated overnight at 4°C with the patient serum diluted 1:10 in 20 mM Tris-buffered saline (pH 7.5) containing 0.1% Tween 20 and 3% bovine serum albumin. After washing 5 times with PBS-T, the blots were incubated for 1 h at room temperature with goat anti-human IgE (Serotec) diluted 1:2,500 in PBS containing 0.05% Tween 20 and 1.5% skimmed milk powder. Finally, they were washed 5 times with PBS-T, and the blots were incubated for 1 h at room temperature with rabbit anti-goat IgG peroxidase (Nordic, Culti) diluted 1:5,000 in PBS containing 0.05% Tween 20 and 1.5% skimmed milk powder. After 5 washes in PBS-T, the protein bands were visualized by chemiluminescence with ECL (GE Healthcare), following the instructions given by the manufacturer. IgE immunoblotting with the patient serum revealed 3 IgE-binding bands of 18, 21 and 23 kDa. No IgE-binding bands were found in control serum obtained from a nonatopic patient (fig. 1). An IgE immunoblotting inhibition assay showed that the 3 different IgE-binding bands (18, 21 and 23 kDa) were strongly (100%) inhibited with the SH hair, urine and salivary gland extracts used as inhibitors. When the patient's serum was preincubated with rabbit, mouse and gerbil hair extracts, no inhibition of the 3 SH IgE-binding bands was demonstrated (fig. 1).

Proteins extracted from the 3 bands observed from the 3 sources (hair, urine, saliva) were analyzed by N-terminal sequencing and matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry [7], and peptides were sequenced. The results showed the following N-terminal sequences: for the 23-kDa protein, NDYAELEG; for the 21-kDa protein, NDYAELEG, and for the 18-kDa protein, no sequence. To verify the protein identification, we performed tandem mass spectrometry and obtained the sequences of 2 internal peptides: AGQETNEMYVVVGK and FKPVYATPENIVFTNK (table 1). Identical sequences were observed in the 3 bands of the same origin and among the 3 investigated sources for each band. These data indicate that those IgE-binding bands correspond to a unique protein or its isoforms. Research conducted with protein databases using the Blast program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and the nonredundant protein sequences database identified the IgE-binding bands as odorant-binding proteins [8]. The peptides shared significant homology with odorant-binding proteins from *Myodes glareolus* (bank vole) and with aphrodisin from *C. cricetus* (black-bellied hamster), all of which belong to the lipocalin superfamily.

Discussion

We describe a new case of IgE-mediated anaphylaxis induced by a bite from an SH in a patient monosensitized to the animal. Analysis of 3 IgE-binding bands found in the hair, urine and salivary glands of the SH showed a new allergenic protein belonging to the lipocalin family and lacking cross-reactivity with allergens from other rodents. Amino acid sequences from the allergen are de-

scribed. We suggest that these allergens be provisionally named Pho s 18 kDa, Pho s 21 kDa and Pho s 23 kDa. Allergens from various sources have been shown to comprise several isoforms.

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