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Letters

Allergy to hedgehog with carboxypeptidase and chitinase-like and chymotrypsin-like elastase family members as the relevant allergens

Hedgehogs are any of the spiny mammals of the subfamily Erinaceinae, which belongs to the order Erinaceomorpha. They are easily recognized by their spines, which are hollow hairs made with keratin. Unlike the quills of porcupines, hedgehogs' spines are not poisonous, and they only point their spines outward as a defense mechanism and roll into a ball to protect themselves from predators. They live through parts of Europe, Asia, and Africa. The long-eared hedgehog and a hybrid species called African pygmy hedgehog (Fig 1) are very common in captivity, and they are sometimes sold in shops of animals as pets. Only 2 previous cases have reported adverse reactions after exposure to hedgehogs: one case of contact urticaria with hedgehog's spines, with immediate wheal formation after skin prick tests (SPTs), was performed with hedgehog dander collected by stroking the spines with a clean toothbrush[1] and occupational asthma in a doctorate researcher after close contact with the bedding of a hedgehog.[2] Aqueous extracts were prepared in this former patient from hedgehog spines and dander, with a positive SPT result to the dander extract. No additional studies were performed in both cases; thus, no allergen involvement was suggested. We present a case of IgE-mediated allergy to hedgehog that suggests carboxypeptidase A1 and acidic mammalian chitinase-like and chymotrypsin-like elastase family members (CELAs) as the relevant allergens.

We report on 3 women aged 25, 29, and 23 years (patients 1, 2, and 3, respectively) with a history of shortness of breath (patients 1 and 3); ocular and nasal itching, and runny nose (patients 2 and 3); and contact urticaria (patient 3) after exposure to hedgehog. All 3 patients had an African pygmy hedgehog at home as a pet and presented with perennial symptoms coinciding with the introduction of the animals at home. Patients 2 and 3 also reported seasonal rhinoconjunctivitis.

Small, round balls of hedgehog feces with urine were obtained from the bedding material. Although spines and saliva should also be considered as likely sources of allergens, it was not posible to obtain these 2 sources. A protein feces-urine extract was prepared by homogenization in phosphate-buffered saline (2.5% wt/vol), dialyzation, and lyophilization (Bial-Arístegui Laboratory, Bilbao, Spain). Extract protein content (wt/wt) was 10% according to Bradford.[3] SPTs to commercial common aeroallergens (ALK Allergologisk Laboratorium A/S, Horsholm, Denmark), including pollens, dust mites, molds, and danders (cat and dog epithelium), were performed with negative results (wheal <3 mm) to all the allergens tested for patient 1, positive (wheal ≥ 3 mm) to pollen (*Phleum platense*, *Olea europaea*, *Platanus acerifolia*, *Cupresus arizonica*) and cat and dog epithelium for patients 2 and 3, and positive to molds for patient 3. No SPTs were performed with the hedgehog extract for sanitary reasons. The hedgehog extract was analyzed in all the patients by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli,[4] showing protein bands ranging from 97 to 9 kDa (Fig 2). SDS-PAGE IgE immunoblotting assays with the patients' serum were performed and revealed IgE-reactivity with proteins with apparent molecular weights of approximately 40, 34, 32, and 21 kDa in patient 1; 40, 30, 29, and 21 in patient 2; and 42, 40, 32, and 29 kDa in patient 3. The 40-, 34-, 32-, 30-, 29-, and 21-kDa protein bands of the feces-urine extract were manually excised from the gel, digested with trypsin, and analyzed by matrix-assisted laser desorption/ionization time of flight and liquid chromatography coupled to tandem mass spectrometry (MS/MS) following the methods of Pastor et al.[5] The 42-kDa IgE-binding band was not visualized in SDS-PAGE and, thus, could not be analyzed. Protein identification was performed by searching a nonredundant protein sequence database (National Center for Biotechnology Information) using the Mascot program (http://www.matrixscience.com). When compared with the databases, the analysis of the resulting peptides by MS or MS/MS corresponded to the acidic mammalian chitinase-like elastase family member for the 40-kDa band; to carboxypeptidase A1 for the 34-, 32-, and 21-kDa proteins; to CELA3B for the 30-kDa band; and to CELA1 for the 29-kDa band.

Elastase is an enzyme from the class of serine proteases that break down elastine, an elastic fiber that determines the mechanical properties of connective tissue, and also plays a key role in breaking down some of the virulence factors of bacteria. Several human genes exist for elastase, and 6 of them belong to CELA: 1, 2, 2A, 2B, 3A, and 3B. CELA1, unlikely other elastases, is not expressed in the pancreas. In the other hand, CELA3B is secreted in the pancreas as a zymogen and has a digestive function in the intestine and little elastolytic activity.[6] Proteases (serine, cysteine, aspartic) have been widely described as allergens.[7] Serine proteases, which include CELAs, have been widely reported as allergens and were first described as allergens from *Aspergillus fumigatus* and later also identified as allergens from house dust mites and Hymenoptera.[8]

Chitin is a polysaccharide composed of *N*-acetylglucosamine repeats. It functions as a major structural polymer in many lower life forms, including the cell walls, and it is also an important nutritional source for many organisms. Chitinases are a family of evolutionarily conserved hydrolases characterized by their ability to cleave chitin. Evidence in animals and humans suggests that both chitinases and chitinase-like proteins are potent up- or down-regulators of the innate immune response by interacting with and

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Figure 1. Picture of a hedgehog.[13]

degrading chitin and independent of chitin by modulating immune responses.[9] The mammalian chitinases have been implicated in the pathogenesis of asthma and atopy,[10] mainly, chitotriosidase and acidic mammalian chitinase, both true chitinases, and a chitinase-like-protein that has the ability to bind chitin but not to degrade it.[9]

A carboxypeptidase is a protease enzyme that hydrolyzes a peptide bond at the carboxy-terminal end of a protein, in contrast with an aminopeptidase. Humans, animals, and plants contain several types of caboxypeptidase that have diverse functions, ranging from catabolism to protein maturation. The first caboxypeptidases studied were those involved in food digestion (pancreatic carboxypeptidases A1, A2, and B). Most of the remaining known carboxypeptidases are involved in regulation biological processes not in catabolism. In the case of pancreatic carboxypeptidase A, the inactive zymogen form (procarboxypeptidase A) is converted to its active form (carboxypeptidase A) by the enzyme trypsin. This mechanism ensures that the cells in which procarboxypeptidase is produced are not themselves digested.[11] Carboxypeptidase has been reported as a relevant Hymenopetera allergen (mainly *apis*).[8]

The number of households with pets is progressively increasing, and this increase is even bigger within the group of exotic or uncommon animals.[12] As it happens with traditional pets, such as dogs and cats, sustained contact with exotic animals can sometimes lead to the development of allergic symptoms. To our knowledge, we present the first demonstrated case of IgE-mediated allergy to hedgehog that suggests carboxypeptidase A1, acidic mammalian chitinase-like family members, CELA1, and CELA3B as the relevant allergens.

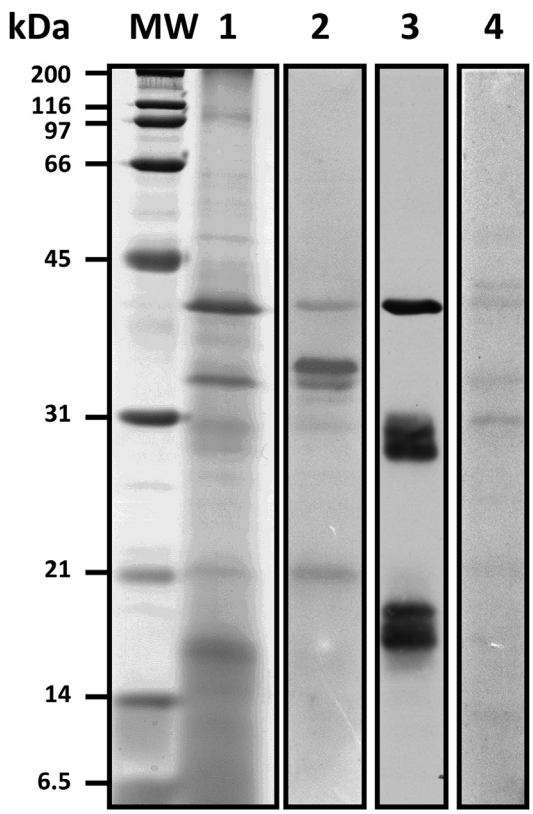

Figure 2. Identification of proteins in the hedgehog feces-urine extracts. 1, SDS-PAGE after Coommassie Blue staining with the hedgehog feces-urine extract. 2, SDS-PAGE IgE immunoblotting with patient's 1 serum. 3, SDS-PAGE IgE immunoblotting with patient's 2 serum. 4, SDS-PAGE IgE immunoblotting with patient's 3 serum. MW = molecular weight marker; kDA = kilo Dalton.

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