

Occupational asthma due to frogs

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INTRODUCTION

Many animal species have been implicated as causes of human allergy. In most instances, the allergens emanating from these animals are airborne and produce respiratory reactions, such as asthma and rhinoconjunctivitis.¹ These antigens are receiving increased recognition as important etiologic agents of occupational disorders in laboratory animal handlers, veterinarians, farmers, and zookeepers. The animals most commonly involved include guinea pigs, rats, mice, rabbits, horses, cows, lions, tigers, and insects.

To the best of our knowledge, there have been only three reports of similar disease in relation to handling frogs.²⁻⁴ We describe a case of occupational asthma that was probably due to an allergic reaction to frog venom components.

CASE REPORT

A 58-year-old man, a professional frog catcher, was admitted to our hospital for asthma. He had a 10-year history of seasonal asthma. Two years after he began handling frogs, he developed rhinitis and asthma. He reported that his symptoms only appeared on the days he handled frogs. These symptoms consisted of conjunctivitis and rhinitis that progressed to asthma and were directly related to inhalation of the emanations of a fluid expelled by the frogs when caught. He was symptom-free on the days he was not occupied with this activity.

Eight years later, his asthma worsened and he was admitted to our hospital. The patient had no per-

sonal or family history of atopy. He lived in a rural area and had a dog.

LABORATORY STUDIES

Blood counts, biochemical profile, urinalysis, and immunoglobulin levels (total IgE was 60 IU/mL) were normal.

Routine skin testing (prick-test) with a battery of commercially available allergens was negative. The control was 1/100 wt/vol histamine acid phosphate, and our criteria for considering the skin test positive was production of a larger wheal than the control substance.

A frog venom extract was employed for prick, intradermal, and passive transfer (Prausnitz-Küstner) tests. This venom was collected by manual expression of the skin glands of frogs of the species (*Rana esculenta*) caught by the patient. One milliliter of this fluid was dialyzed against phosphate buffered saline (PBS), pH 7.2, for 24 hours, then centrifuged at 48,000 g and passed through a 0.45- μ m Millipore filter (Millipore Corp, Bedford, MA). This was used as the "stock solution." Protein levels (Bio-Rad protein assay) of the preparation were adjusted to 420 μ g protein/mL. Prick testing was performed with a 1/10 dilution in PBS. Dilutions to a final concentration of 1/1000 were made for the intracutaneous test.

The prick test with this antigen, diluted to 1/10, was positive in the patient, producing a 10 $\times$ 10-mm wheal with respect to the histamine control. None of four nonatopic control subjects had positive skin test results. In a healthy volunteer with no reaction to the frog antigen, 0.1 mL of the patient's serum was injected. The volunteer was challenged intracutaneously with 0.4 μ g of venom antigen 48 hours later, which resulted in marked wheal and

flare response at the injection site 15 minutes later. No reaction occurred when serum from normal subjects was used for passive sensitization or when the patient's serum was previously heated at 56 °C for two hours.

Other *in vivo* tests, such as the provocative inhalation test, were not performed because patient and family consent could not be obtained.

To determine venom IgE antibodies, we applied reverse enzyme immunoassay (REIA), using a technique previously described by our group.⁵ Briefly, 0.130 mL of "stock solution" was conjugated with 0.5 mg peroxidase (type VI, Sigma). This antigen was labeled using the periodate oxidation method described by Nakane et al.⁶ The method followed in the rest of the technique was that described in previous publications.⁵

Reverse enzyme immunoassay disclosed specific serum IgE antibodies to venom antigen with 3,480 optical density (OD) units. Specific IgE antibodies to this venom were not detected in normal serum or in serum from ten other asthmatic atopic individuals studied. RAST test using commercial paper discs coupled with other allergens (ie, house dust, mites, molds, and pollens) scored 0 with the patient's serum and normal sera.

Precipitins to the frog antigen and serially diluted patient's serum (Ouchterlony's agar gel double diffusion) were not found.

DISCUSSION

Allergy to frogs has only been communicated in three reports. Nakazawa et al.³ demonstrated the presence of IgE specific for frog antigen in a case of occupational allergy in a laboratory technician who had

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asthma-like symptoms and dermatitis due to an allergic reaction to antigens of brain and bull frog secretions (*Rana catesbeiana*).³ Earlier, Charpin et al² in 1953 described a patient who developed asthmatic attacks when he touched *Rana esculenta* (the same species as in our case), but they did not demonstrate the presence of IgE antibody specific for frog antigens. Recently, Von Steurich and E. Barnstedt⁴ reported a case of a cook who presented anaphylactic reactions after ingesting frog legs.

We describe a new case of this rare occupational allergy: asthma due to inhalation of a venom expelled by frog skin glands. This venom is normally used by the frog to protect its skin against bacteria and fungi and not to repel attack. Very low concentrations (10^{-3} to 10^{-5} moles/L) of venom inhibit growth of bacteria and fungi.⁷ It is composed of a mixture of simple amines, peptides, steroids, and alkaloids. The LD50 of the pure venom is 12 mg/kg (determined in rabbit).

The frog species that affected this patient was *Rana esculenta*, family Ranidae, the most common frog in western Europe.⁸

Exposure to frog venom can be considered as a possible etiologic cause of occupational allergy be-

cause numerous professionals handle these amphibians, eg, laboratory workers, cooks, workers in restaurants and food factories, biologists, pharmacologists, and frog catchers.

Our patient developed allergic symptoms that could be attributed to the venom expelled by the frogs because his symptoms developed 2 years after he began handling the frogs and disappeared soon after he changed his occupation. Specific IgE antibody to frog venom was demonstrated by REIA and Prausnitz-Küstner testing. In addition, IgG antibodies to frog antigen were not detected by agar gel diffusion.

We think that the patient's symptoms were due to development of Type I allergic reactivity and were not produced by irritative or toxic mechanisms because skin tests and detection of IgE specific antibody were negative in our controls.

Our group is now attempting to fraction and separate the frog venom compounds responsible for this patient's pathology.

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