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ORIGINAL ARTICLE

Anaphylactic Shock Caused by Exposure to Sea Anemones

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ABSTRACT

Background: Since the first report of a dog that developed severe systemic symptoms in response to a second injection of sea anemone toxin by Richet and Portier in 1902, no clear human cases of anaphylaxis related to exposure to sea anemones has been reported in the literature.

Methods: A 24-year-old man with an episode of local urticaria on his first contact with a sea anemone (*Stichodactyla haddoni*), developed dyspnea, severe urticaria and hypotension on exposure to water containing the dead bodies of the organism. To study whether this reaction was mediated by antigen-specific IgE, we performed a histamine release test with blood, Western blotting with serum and lymphocyte proliferating test with peripheral blood mononuclear cells of the patient, for the homogenate of sea anemones.

Results: The homogenate of sea anemones induced histamine release from the blood of the patient, but it also induced histamine release from the blood of control subjects. Moreover, it also caused hemolysis of blood of all donors. However, Western-blotting demonstrated the presence of an 86 kd protein-specific IgE in the serum of the patient.

Conclusions: Protein antigen (s) in sea anemones may cause anaphylactic shock under the influence of the cytolytic effects and/or lymphocyte-stimulating activity elicited by the toxin of sea anemones.

KEY WORDS

anaphylactic shock, anaphylaxis, histamine, sea anemone, specific IgE

INTRODUCTION

Maintaining tropical aquariums has become a popular hobby and various kinds of marine organisms, including sea anemones, may be kept at home as well as in aquariums in Japan and other developed countries. However, some anemones have venom in the nematocytes on their tentacles. *Stichodactyla haddoni* , a sea anemone that can be found in the western Pacific Ocean and the Indian Ocean, has a strong venom that causes skin eruptions when humans are exposed to their tentacles. Here we report a case of anaphylactic shock caused by *Stichodactyla haddoni* in a patient who worked in a tropical marine outlet.

METHODS

A 24-year-old man who worked at an aquarium shop arrived at our hospital complaining of dyspnea and systemic urticaria. That morning, the patient had cleaned an aquarium tank in which many sea anemones (*Stichodactyla haddoni*) had died and decomposed. He started his chores at about 10 a.m., and in the process of his work his forehead, arms and fingers were exposed to water from the aquarium. At approximately 12 noon, he experienced a systemic eruption and shortness of breath and came to our hospital.

On admission at 12:30 p.m., the patient was drowsy, with a heart rate of 116/min, and a blood pressure of 60 mmHg (palpation). The patient was in visible distress and had skin wet due to a cold sweat. Marked urticaria was observed on his face, trunk, and extremities. His fingers and palms were severely dry and cracked. He had a slight cough, and a wheeze was apparent in his left lower base. A blood

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gas analysis revealed considerable hypoxia, with pH 7.403; $PaCO_2$ 38.0 torr; PaO_2 53.3 torr; HCO_3 23.2 torr. Blood cell counts showed white-cell at 6500/mm^3, red-cells at 446 × 10^4/mm^3, hemoglobin at 14.8 g/dl, hematocrit of 42.3%, platelets at 18.9 × 10^4/mm^3, neutrophils at 50.4%, lymphocytes at 37.9%, monocytes at 9.2%, eosinophils at 2.5% and basophils at 0.0%. Liver and kidney functions were normal. A chest radiograph and electrocardiogram (ECG) were within normal limits. Total serum IgE was 2100 IU/ml, and antigen-specific IgE detected by CAP-RAST system was 1.06 IU (class 2) for horse-mackerel, 2.06 for tuna (class 1/0), 1.06 IU for salmon (class 1/0), 4.00 IU for flatfish (class 3) and 2.61 for blue mussel (class 2). The ratio of T cell/B cell, the numbers of $CD4^+$ cells and $CD8^+$ cells in peripheral leukocytes were within normal limits.

After injections of 0.2 mg epinephrine subcutaneously and 500 mg methylprednisolone intravenously, we added dopamine chlorate at 15 mg/hr with O_2 inhalation at 1 to 2 L/min. The urticaria disappeared during the procedure.

At 4 p.m., the urticaria recurred, and disappeared shortly after the intravenous administration of 40 mg prednisolone. His condition then improved.

The patient had suffered from bronchial asthma during childhood, and had experienced itching around the mouth after eating certain fish. Several months ago, he had also been exposed to the water in an aquarium with sea anemones, and noticed local urticaria. He had no family history of allergy.

HISTAMINE RELEASING TEST

Two milliliters of heparinized peripheral blood of the patient was mixed with 0.2 ml of the homogenate of sea anemones in phosphate buffered saline (PBS) so as to make the final concentrations of the anemones as 0.0, 10.3, 1.03, and 0.103 mg protein/ml, and this was then incubated at 37℃ for 30 minutes. The amount of histamine released to plasma was measured by HPLC.[1] The same test was performed on two normal subjects and two allergy patients (one bronchial asthma, one pollinosis).

LYMPHOCYTE STIMULATION TEST

One million/ml of peripheral blood mononuclear cells (PBMC) was incubated with or without anemone homogenate in the presence of ^{3}H-thymidine at 37℃ for 65.5 hours in 5% CO_2. The amount of ^{3}H-thymidine taken up by the cells stimulated with sea anemone was compared with that of non-stimulated cells.

WESTERN BLOTTING

The homogenate of sea anemones (3.4 μg protein/lane) was separated by 15% SDS- PAGE and blotted to the nylon membrane (Millipore, Billerica, USA). The membrane was blocked with 10% skimmed milk/PBS for 2 hours at room temperature, and incubated with the serum of the patient or control subjects, which was diluted 30 times with 3% bovine serum albumin (BSA), at 4℃ overnight. After washing with 0.05% tween-Tris buffered saline (TTBS, pH7.2), the membrane was then incubated with 1000 times diluted HRP-anti human IgE (Zymed, South San Francisco, USA) with 10% skimmed milk/PBS for 2 hours at room temperature. The binding of IgE to antigens on the membrane was detected by the chemiluminescence of ECL™ (Amersham, Piscataway, USA)

For the experiment of IgE absorption, 100 μl of serum of the patient was pre-incubated with 50 μl of the homogenate of sea anemones at 37℃ for 30 minutes before the incubation with the membrane.

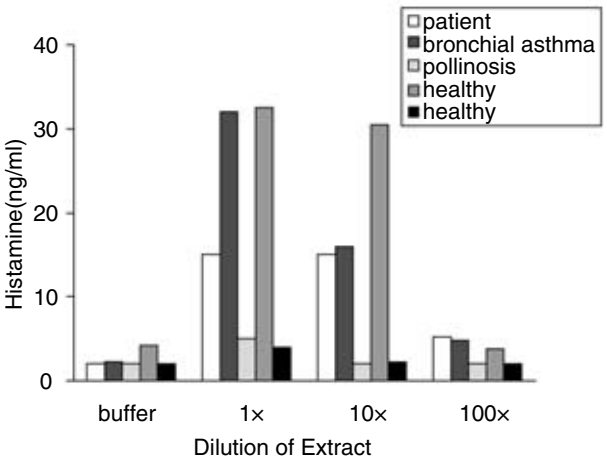

Fig. 1 Histamine releasing test. Heparinized blood obtained from the patient and four control individuals was incubated with or without various concentrations of the homogenate of sea anemones (1×: 10.3 mg protein/ml, 10×: 1.03 mg protein/ml, 100×: 0.103 mg protein/ml).

RESULTS

HISTAMINE RELEASING TEST

The blood of the patient or control subjects released histamine when incubated with the homogenate of sea anemones (10.3, 1.03, 0.103 mg protein/ml) in a dose-dependent manner (Fig. 1). Hemolysis was observed in all samples incubated with sea anemones.

LYMPHOCYTE STIMULATION TEST

^{3}H-thymidine uptake by PBMC of the patient incubated with or without the homogenate of sea anemones (0.136 mg protein/ml) was 703 cpm and 99 cpm, respectively (stimulation index 7.1). Because of the low level of counted radioactivity and the nature of the toxin as cytolysin, there could be the influence of decrease of cell number. However, the cpm of unstimulated lymphocytes itself was low, so the cyto-

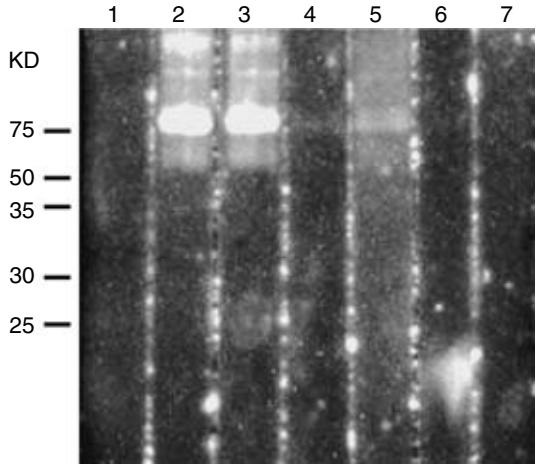

Fig. 2 Western blotting. The homogenate of a sea anemone (3.4μg/lane) separated by SDS-PAGE was blotted to the nylon membrane and incubated with diluted serum of the patient or control subjects. IgE bound to the membrane was visualized by chemiluminescence.

lytic effect was thought to have a minimal contribution.

WESTERN BLOTTING

Western blotting showed the presence of the patient's IgE that was bound to a 86 kd protein in the homogenate of sea anemones. This IgE was completely absorbed by pre-incubation of sera with the homogenate of sea anemones. Sera of two normal controls did not show any binding of IgE, however, the sera of one patient with atopic dermatitis showed a weak reaction of IgE to the same band of protein (Fig. 2).

DISCUSSION

More than thirty kinds of sea anemones are known to have toxic proteins. Most of the toxins of sea anemones make pores in membranes.[2,3] Some of these proteins are cardiostimulants, neurotoxins, or ion channel blockers and may induce papules, linear lesions, bullae and vesicles when these tentacle-laden toxins make contact with the human skin.[3,4] Other symptoms, including somnolence, dizziness, nausea, vomiting, muscle pain, edema of the eye lids and acute renal failure due to acute tubular necrosis have also been reported.[5,6]

The phenomenon of anaphylactic shock caused by anemone toxin has been widely known, since it was first reported by Richet and Portier in 1902, and became the very origin of the term of anaphylaxis. Nevertheless, such a clear case of anaphylaxis in humans, as we have reported here, has not been reported before.

Our patient developed only local urticaria on his first contact with water from the aquarium contaminated with sea anemones. However, upon the second exposure, he developed severe anaphylactic symptoms, suggesting that his lymphocytes had been sensitized or their immunological memory had been boosted by the anemone antigen upon the first contact. The skin of his forehead and arms were apparently intact, but the skin on his fingers 'cracked' in such a fashion as to allow the antigen to pass into the body through the skin. Likewise, hypothetically, contaminated water could have entered his gloves and invaded the body through the finger legion. This may be the reason why it required almost two hours for the patient to develop symptoms after his second exposure to the sea anemones.

Dose-dependent histamine release from the blood of the patient supports the involvement of immediate-type hypersensitivity. However, similar or even larger amounts of histamine release were induced by the homogenate of sea anemones from the blood of control subjects. Moreover, they accompanied hemolysis, suggesting that histamine release may be due to toxicity rather than an IgE-mediated mechanism. On the other hand, Western blotting with the homogenate of sea anemones demonstrated the presence of IgE specific to a 86 kd protein of this creature. The IgE was completely absorbed by preincubation with the homogenate of sea anemones, indicating that this IgE recognized the 86 kd protein in the homogenate.

The serum of a control subject with atopic dermatitis, who had never previously been exposed to sea anemones, showed a weak reactivity against the same antigen as the sera of the patient. The serum of the patient also showed IgE reactivity to several types of fish and shellfish. Although sea anemones and these marine organisms are not closely related zoologically, they may share some antigenicity.

A small but significant increase of the stimulation index of PBMC incubated with the anemone homogenate suggests the activation of lymphocytes and/or monocytes, as well as hemolysis, may also be involved in the anaphylactic symptoms.

In conclusion, the symptoms of this patient might have developed via specific IgE against a 86 kd protein under the influence of a direct cytolytic and/or lymphocyte/monocyte stimulating effect of the toxins of sea anemones. As home aquariums become increasingly popular with the public, we must pay great

attention to the fact that this will bring individuals into contact with various types of marine life that they have never encountered before, leading to the risk of provoking anaphylactic symptoms.

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