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Manuscript: JELLYFISH COLLAGEN: A NEW ALLERGEN IN THE BEACH.

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Abbreviations

BSA (Bovine Serum Albumin)
IgE (Immunoglobulin E)
LC-MS/MS (Liquid Chromatography–Mass Spectrometry)
MALDI (Matrix-Assisted Laser Desorption/Ionization)
Jellyfish stings are a common event in seas worldwide with an estimated 150 million envenomations annually, usually results in acute cutaneous inflammation but some allergic reactions are also documented.\textsuperscript{1} Usually the allergy manifests with rashes, erythema, and pruritus and in some cases with even more severe reactions as anaphylaxis. Among jellyfish (considering Scyphozoa, Cubozoa and the siphonophore...
Portuguese man o’war) there are different species, some of them very dangerous; their sting can lead to severe injuries and even death of the individual, mainly from Cubozoa and Siphonophora. Historically, reactions against jellyfish toxins are one of the landmarks of medical history. In 1902, Richet and Portier coined the term anaphylaxis (“lack of protection”) due to experiments conducted in dogs against the Physalia physalis toxins, where dogs were exposed to sublethal doses of the toxin. They observed that sensitized dogs reacted instantaneously and lethally to subsequent contact with low amounts of the toxin.\(^2\) This discovery allowed to Richet to win the Nobel Prize for Medicine in 1913.

Here, we describe the sting of *Pelagia noctiluca*, which is a bell-shaped pelagic jellyfish (open waters).\(^3\) *P. noctiluca* is ubiquitous worldwide, especially in warm and temperate waters, and it is common in Mediterranean Sea, moreover it is known to be one of the most abundant and venomous jellyfish in this area.\(^4\) The sting of the *P. noctiluca* is poisonous, but in humans typically does not cause more than a local cutaneous reaction. However in some cases humans can develop allergic reactions, including anaphylactic shock.\(^5\) Several studies have described the cytolytic and hemolytic properties of crude venom of *P. noctiluca*\(^6\) like so the complete proteome of this jellyfish;\(^1\) however, none of them have described specific allergens responsible for their allergic effects. Only, a study conducted with proteins belonging to *Chironex yamaguchii* nematocysts, have identified several proteins as allergens: a protein toxin (CqTX-A) and a N-linked glycoprotein.\(^7\)

A 76-year-old Spanish Caucasian male was accidentally stung by a jellyfish, identified by its morphologic appearance as *P. noctiluca* in the Multidisciplinary Institute for Environmental Studies (University of Alicante, Spain) using photographs from this
jellyfish taken by the patient. He was stung on Ibiza (Spain) (38°54'31.79"N 1°25'58.66"E) and he felt only one sting. Immediately after, he developed tongue edema, palms itching, dizziness and general discomfort. The patient did not go to emergency room or any doctor office and he treated himself the symptoms with oral methylprednisolone 40 mg, but no topical treatment was applied.

He was stung some times before by jellyfishes in the same sea area. He reported that he had itching with crab ingestion in the past. He was evaluated in our outpatient clinic 4 weeks after the episode. The patient’s tryptase levels were normal. Specific serum IgE results (in kU/l) (Thermo Fisher Scientific, Waltham, MA, USA) were as follows: crab 0.02; mussel 0.0; squid 0.0; octopus 0.0; Anisakis 0.58; rPen a 1 tropomyosin 0.0; rPol d 5 Paper wasp Polistes dominulus 0.60; Polistes dominulus 0.13. The patient reported no reactions to hymenoptera stings.

An in vitro study including SDS-PAGE analysis and immunoblot was performed with both nematocysts extracts from body or bell and tentacles of P. noctiluca. Two prominent bands of approximately 130 kDa were recognized by serum Immunoglobulin E (IgE) of the patient (Figure 1). These bands were excised, digested and analyzed by mass spectrometry (MS). De novo sequencing was carried out and several peptides were obtained that present homology with different collagens in a non-redundant protein sequence database (NCBI).

In order to confirm these results, immunoblot inhibition assays were performed using as inhibitor purified collagen from Rhizostoma pulmo extract. IgE binding was inhibited up to 86% with the highest collagen concentration in both bands. Recently, Suzuki et al. described a case of anaphylaxis in a professional diver caused by ingestion of snack
made with jellyfish. The authors identified a protein of 250 kDa that it could cause the symptoms and they supposed that this protein was related to collagen.\(^8\)

Collagen is the main structural protein in the extracellular matrix of several connective tissues in animal bodies and, it is distributed in the skin, bone and cartilage. It is phylogenetically conserved along evolution and between organisms. Specifically, in jellyfish this protein forms the structure of nematocysts. A nematocyst is a type of subcellular organelle produced by cells called cnidocytes (also called nematocytes), that is used for the injection of toxins for the capture of prey and the defense of the animal.

Collagen presents the structure of triple helix and its molecular weight is approximately 300 kDa; although, specific types of collagen present a lower molecular weight, such as collagen type IV which has approximately 180 kDa,\(^9\) in mammalians. In the UniProtKB database (www.uniprot.org) only one type of collagen IV from jellyfish *Craspedacusta sowerbyi* (*Freshwater jellyfish*) is described, which has a molecular weight of 128 kDa (Accession number V9GWB0). Collagen is a very important material in medicine and food industry. Collagen peptides are used as active components for their good properties: bioactivity, biocompatibility, penetrability, reparative ability to skin and hair and no irritation to skin.\(^10\) Allergy to gelatin, a form of collagen, is relevant in food allergy and vaccine allergy so, we also performed a IgE antibody test for gelatin of bovine origin (ImmunoCAP, Phadia SL, Thermo Fisher Scientific, Waltham, MA, USA) and the result was negative <0.35 kUA/L.

Approximately, 15 years ago collagen was identified as an allergen. However, few reports involving collagen in allergic reactions have been published.\(^11\) Proteins from our *P. noctiluca* extract were separated by SDS-PAGE. The bands recognized by IgE were
sliced and isolated by anion exchange chromatography and later analyzed using Liquid Chromatography–Mass Spectrometry (LC-MS/MS). Peptides obtained from 100 and 150 kDa bands were characterized as collagen type alpha-IV (GPIGPVPGEGAGR, GPIGVPGEGAGR, GPIGVPGEGAGR and Acetyl-NGEKYLGLR). They showed 70-91% sequence homology with others collagens from different organisms. This study revealed that jellyfish collagen presents a conserved sequence with other phylogenetically distant species. IgE from our patient’s serum is able to recognize collagen from both *P. noctiluca* and *R. pulmo*, which belong to the same class (Scyphozoa), but belong to different orders (Semaeostomeae and Rhizostomeae respectively). So this individual could show allergic reactions after sting of other cnidarians.

In conclusion, we report one case of anaphylaxis after jellyfish sting that resulted from sensitization to an allergen found in nematocysts from scaffolds and tentacles that has been characterized by MS as collagen type alpha-IV. This is the first report of the presence of collagen type alpha-IV in *P. noctiluca* as an allergen.
References


Figure Legend

Figure 1: IgE-immunoblot of *P. noctiluca* protein extract and collagen purified; P: serum from patient, C-: serum from non-allergic donor and NET buffer.
Figure 1.tif