

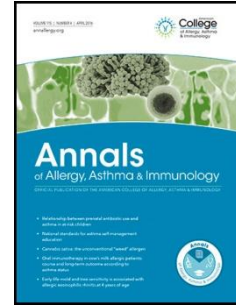
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1 **Manuscript:** JELLYFISH COLLAGEN: A NEW ALLERGEN IN THE BEACH.

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22 **Key words:** jellyfish, collagen, jellyfish allergy.

23 **Abbreviations**

24 BSA (Bovine Serum Albumin)

25 IgE (Immunoglobulin E)

26 LC-MS/MS (Liquid Chromatography–Mass Spectrometry)

27 MALDI (Matrix-Assisted Laser Desorption/Ionization)

28 MS (Mass Spectrometry)

29 SAV-HRP (Horseradish Peroxidase Streptavidin)

30 SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis)

31

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35

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37

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42 Author contributions: C. JA, R-M. JM and R-C. S performed the experiments, analyzed

43 and data interpretation. B. C, F-N. M and del P. V, designed the study, analyzed and

44 interpreted the data and wrote the manuscript.

45

46 Jellyfish stings are a common event in seas worldwide with an estimated 150 million

47 envenomations annually, usually results in acute cutaneous inflammation but some

48 allergic reactions are also documented.¹ Usually the allergy manifests with rashes,

49 erythema, and pruritus and in some cases with even more severe reactions as

50 anaphylaxis. Among jellyfish (considering Scyphozoa, Cubozoa and the siphonophore

51 Portuguese man o'war) there are different species, some of them very dangerous; their
52 sting can lead to severe injuries and even death of the individual, mainly from Cubozoa
53 and Siphonophora. Historically, reactions against jellyfish toxins are one of the
54 landmarks of medical history. In 1902, Richet and Portier coined the term anaphylaxis
55 ("lack of protection") due to experiments conducted in dogs against the *Physalia*
56 *physalis* toxins, where dogs were exposed to sublethal doses of the toxin. They
57 observed that sensitized dogs reacted instantaneously and lethally to subsequent contact
58 with low amounts of the toxin.² This discovery allowed to Richet to win the Nobel Prize
59 for Medicine in 1913.

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

72 A 76-year-old Spanish Caucasian male was accidentally stung by a jellyfish, identified
73 by its morphologic appearance as *P. noctiluca* in the Multidisciplinary Institute for
74 Environmental Studies (University of Alicante, Spain) using photographs from this

75 jellyfish taken by the patient. He was stung on Ibiza (Spain) (38°54'31.79"N
76 1°25'58.66"E) and he felt only one sting. Immediately after, he developed tongue
77 edema, palms itching, dizziness and general discomfort. The patient did not go to
78 emergency room or any doctor office and he treated himself the symptoms with oral
79 methylprednisolone 40 mg, but no topical treatment was applied.

80 He was stung some times before by jellyfishes in the same sea area. He reported that he
81 had itching with crab ingestion in the past. He was evaluated in our outpatient clinic 4
82 weeks after the episode. The patient's tryptase levels were normal. Specific serum IgE
83 results (in kU/l) (Thermo Fisher Scientific, Waltham, MA, USA) were as follows: crab
84 0.02; mussel 0.0; squid 0.0; octopus 0.0; Anisakis 0.58; rPen a 1 tropomyosin 0.0; rPol
85 d 5 Paper wasp *Polistes dominulus* 0.60; *Polistes dominulus* 0.13. The patient reported
86 no reactions to hymenoptera stings.

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

94 In order to confirm these results, immunoblot inhibition assays were performed using as
95 inhibitor purified collagen from *Rhizostoma pulmo* extract. IgE binding was inhibited
96 up to 86% with the highest collagen concentration in both bands. Recently, Suzuki *et al.*
97 described a case of anaphylaxis in a professional diver caused by ingestion of snack

98 made with jellyfish. The authors identified a protein of 250 kDa that it could cause the
99 symptoms and they supposed that this protein was related to collagen.⁸

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

106 Collagen presents the structure of triple helix and its molecular weight is approximately
107 300 kDa; although, specific types of collagen present a lower molecular weight, such as
108 collagen type IV which has approximately 180 kDa,⁹ in mammals. In the UniProtKB
109 database (www.uniprot.org) only one type of collagen IV from jellyfish *Craspedacusta*
110 *sowerbyi* (*Freshwater jellyfish*) is described, which has a molecular weight of 128 kDa
111 (Accession number V9GWB0). Collagen is a very important material in medicine and
112 food industry. Collagen peptides are used as active components for their good
113 properties: bioactivity, biocompatibility, penetrability, reparative ability to skin and hair
114 and no irritation to skin.¹⁰ Allergy to gelatin, a form of collagen, is relevant in food
115 allergy and vaccine allergy so, we also performed a IgE antibody test for gelatin of
116 bovine origin (ImmunoCAP, Phadia SL, Thermo Fisher Scientific, Waltham, MA,
117 USA) and the result was negative <0.35 kUA/L.

118 Approximately, 15 years ago collagen was identified as an allergen. However, few
119 reports involving collagen in allergic reactions have been published.¹¹ Proteins from our
120 *P. noctiluca* extract were separated by SDS-PAGE. The bands recognized by IgE were

121 sliced and isolated by anion exchange chromatography and later analyzed using Liquid
122 Chromatography–Mass Spectrometry (LC-MS/MS). Peptides obtained from 100 and
123 150 kDa bands were characterized as collagen type alpha-IV (GPIGVPGEKGR,
124 GPIGVPGEGAGR, GPIGPVGEGAGR and Acetyl-NGEKGYLGLR). They showed
125 70-91% sequence homology with others collagens from different organisms. This study
126 revealed that jellyfish collagen presents a conserved sequence with other
127 phylogenetically distant species. IgE from our patient's serum is able to recognize
128 collagen from both *P. noctiluca* and *R. pulmo*, which belong to the same class
129 (Scyphozoa), but belong to different orders (Semaestomeae and Rhizostomeae
130 respectively). So this individual could show allergic reactions after sting of other
131 cnidarians.

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

136

137 **References**

- 138 1. Frazão B, Campos A, Osório H, et al. Analysis of *Pelagia noctiluca*
139 proteome reveals a red fluorescent protein, a zinc metalloproteinase and a
140 peroxiredoxin. *Protein J* 2017;36:77–97.
- 141 2. Portier P, Richet C. De l'action anaphylactique de certains venins. *C R Soc*
142 *Biol (Paris)* 1902;54:170-2.
- 143 3. Tibballs J, Yanagihara AA, Turner HC, Winkel K. Immunological and
144 toxinological responses to jellyfish stings. *Inflamm Allergy Drug Targets*
145 2011;10:438–46.
- 146 4. Mariottini GL, Giacco E, Pane L. The mauve stinger *Pelagia noctiluca*
147 (Forsskål, 1775). Distribution, ecology, toxicity and epidemiology of stings.
148 A review. *Mar Drugs* 2008;6:496–513.
- 149 5. Burnett JW. Treatment of Atlantic cnidarian envenomations. *Toxicon*
150 2009;54:1201–5.
- 151 6. Morabito R, Costa R, Rizzo V, et al. Crude venom from nematocysts of
152 *Pelagia noctiluca* (Cnidaria: Scyphozoa) elicits a sodium conductance in the
153 plasma membrane of mammalian cells. *Sci Rep* 2017;7:41065.
- 154 7. Horiike T, Nagai H, Kitani S, Identification of Allergens in the Box Jellyfish
155 *Chironex yamaguchii* That Cause Sting Dermatitis. *Int Arch Allergy*
156 *Immunol* 2015;167:73-82
- 157 8. Suzuki S, Miyata Y, Jinno M, et al. An adult case of anaphylaxis caused by
158 allergy to jellyfish. *Arerugi* 2017;66:804–8.
- 159 9. Timpl R, Wiedemann H, Delden V, Furthmayr H, Kuhn K. A network
160 model for the organization of type IV collagen molecules in basement
161 membranes. *Eur J Biochem* 1981;120:203–11.
- 162 10. Hoyer B, Bernhardt A, Lode A, et al. Jellyfish collagen scaffolds for
163 cartilage tissue engineering. *Acta Biomater* 2014;10:883–92.
- 164 11. Kobayashi Y, Kuriyama T, Nakagawara R, Aihara M, Hamada-Sato N.
165 Allergy to fish collagen: Thermostability of collagen and IgE reactivity of
166 patients' sera with extracts of 11 species of bony and cartilaginous fish.
167 *Allergol Int* 2016;65:450–8.

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169 **Figure Legend**

170 Figure 1: IgE-immunoblot of *P. noctiluca* protein extract and collagen purified; P:

171 serum from patient , C-: serum from non-allergic donor and NET buffer.

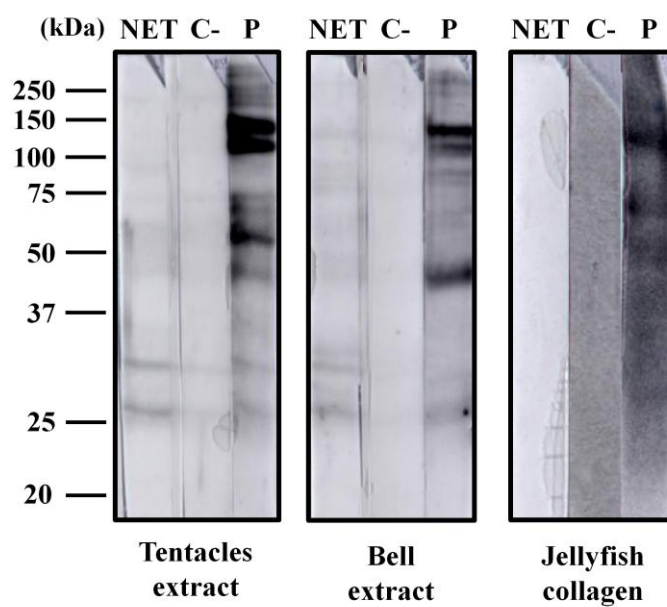
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