Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

DOI: 10.1111/all.15377

Mollusk allergy: Not simply cross-reactivity with crustacean allergens

To the Editor,

Mollusk allergy is commonly thought of as clinical cross-reactivity after primary sensitization to shrimps, other crustaceans, or mites.[1,2] Tropomyosin is the major allergen, with primary IgE sensitization in 70% of all shellfish allergies.[3] A high frequency of IgE and basophil reactivity to several mollusk allergens is seen in crustacean and mite-sensitized patients.[4,5] It is still unclear, however, whether mollusks are capable of producing primary allergic sensitization, or whether IgE reactivity is based solely on cross-reactive crustacean-specific antibodies.

Here, we demonstrate for the first time that mollusk tropomyosin can independently elicit a strong primary T-helper Type 2 (Th2)-mediated IgE response in a IP-sensitized mouse model (BALB/c) of food allergy, which was primarily due to tropomyosin, without any prior sensitization to crustacean allergens. Heated protein extracts were prepared from Jade tiger abalone (hAbal; *Haliotis laevigata* x *Haliotis rubra)* and Black tiger shrimp (hBTS; *Penaeus monodon)*. The respective tropomyosins were produced recombinantly (rHal l1 and rPen m1) (Appendix S1). Both proteins share 63% amino acid sequence identity (Figure 1A). Hal l 1 is closely related to oyster and octopus tropomyosin (Figure 1C). In vitro IgE binding was observed to native tropomyosin in hAbal extract (58%) and hBTS extract (75%) as well as for both recombinant tropomyosins, rHal l 1 (62%), and rPen m 1 (75%) as shown by immunoblotting using sera from 12 clinically confirmed shellfish allergic patients (Figure 1B, Tables S1 and S2). Although mono-sensitization to abalone extract or tropomyosin was not observed, subjects 6 and 7 elicited stronger IgE binding to abalone extract and Hal l 1 than to shrimp extracts, implying primary sensitization to abalone. Written informed consent was obtained from all participants, and patient anonymity was preserved. Ethics approval was obtained from the Ethics Committees of James Cook University (H4313), the Alfred Hospital (192/07), and the Monash University (MUHREC CF08/0225).

We established two independent mouse models: (i) shrimp allergen model; intraperitoneal-sensitized either with hBTS or rPen m1, and orally challenged with hBTS (Figure 1D); and (ii) abalone allergen model; sensitized either with hAbal extract or rHal l1, and challenged with hAbal. Mouse IgG antibodies produced against abalone tropomyosin sensitization model elicited cross-binding to tropomyosin in the shrimp extract. Likewise, IgE antibodies produced in shrimp-sensitized models showed binding to tropomyosin in the abalone extract (Figure 1E), demonstrating the cross-reactive nature of Hal l 1 and Pen m 1. A strong IgE response to hBTS was elicited (total and shrimp-specific) with a significant release of mast cell protease (mMCP-1) as compared to the naïve group (Figure 2A,B). A similar response was observed with abalone, however, demonstrating relatively stronger IgE and mMCP-1 responses in ELISA. Mice sensitized to Pen m1 or Hal l1 elicited a much stronger IgG1 antibody response than whole extract-sensitized animals, indicating a more efficient Th2 priming potential with purified allergens (Figure 2B). No significant IFN-γ responses confirmed that no Th1 priming occurred (Figure 2C). Antigen-specific in vitro restimulation of splenocytes revealed a robust IL-5 and IL-13 response, but not IFN-γ, indicating a systemic Th2-polarized immune response (Figure 2D). A significant increase in the frequency of blood- and spleen-based IgE^{+} basophils was observed for abalone- and Hal l1-sensitized mice, similar to shrimp-sensitized mice (Figure 2E).

This study demonstrates that abalone tropomyosin is the major sensitizer in the heated extract. Antibodies generated against abalone tropomyosin is capable of binding to shrimp tropomyosin. We recently showed that T-cell cross-reactivity among tropomyosins is not dependent on amino acid sequence similarity, but instead as a function of structural stability.[6] This supports our findings that tropomyosins from different invertebrate sources may produce cross-reactive antibodies, even when sharing low amino acid sequence similarity[7] Our findings suggest that primary sensitization to tropomyosin from mollusk is possible, and inverse clinical cross-reactivity may occur to crustaceans in mollusk-allergic individuals.

Abbreviations: hBTS, black tiger shrimp heated extract; hAbal, abalone heated extract.

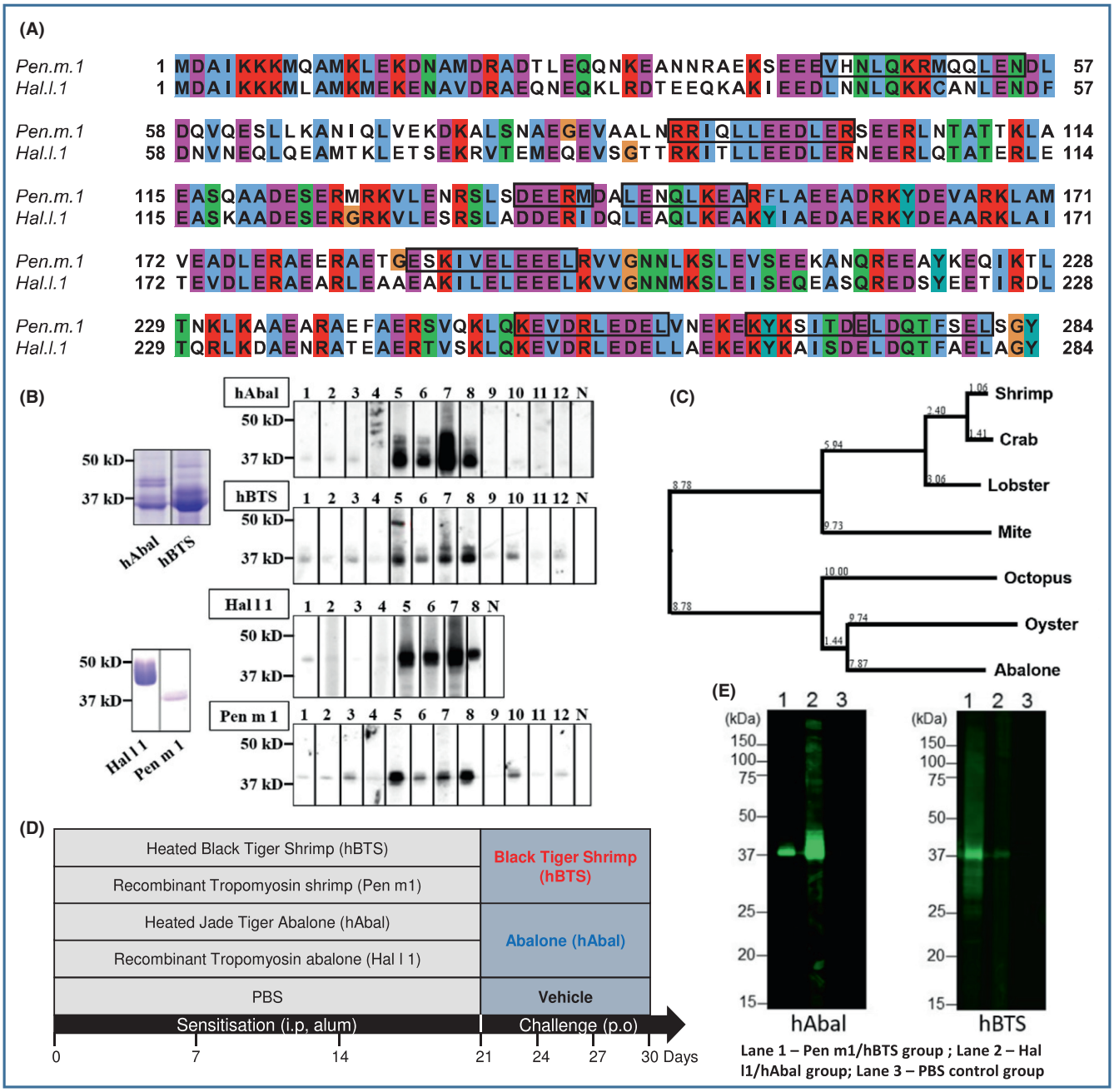

FIGURE 1 Allergenicity of mollusk tropomyosin, Hal l1. (A) Multiple sequence alignment showing amino acid sequence differences and identities in the IgE binding epitopes of shrimp tropomyosin (Pen m 1) and abalone tropomyosin (Hal l 1). (B) Serum IgE binding in whole extracts and recombinant allergens using clinically confirmed shellfish allergic patient sera. (C) Neighbor-joining phylogenetic tree with branch lengths for tropomyosin, highlighting the distance between crustacean, mite, and mollusk species. (D) BALB/c mouse sensitization and challenge regimes used to investigate Th2 response to shrimp or abalone. (E) Mouse IgG immunoblot demonstrating cross-binding of antibodies from abalone tropomyosin-sensitized/abalone extract-challenged mice (Hal l 1/hAbal) to shrimp tropomyosin (~37 kD), and from shrimp tropomyosin-sensitized/shrimp extract-challenged mice (Pen m 1/hBTS) to abalone tropomyosin

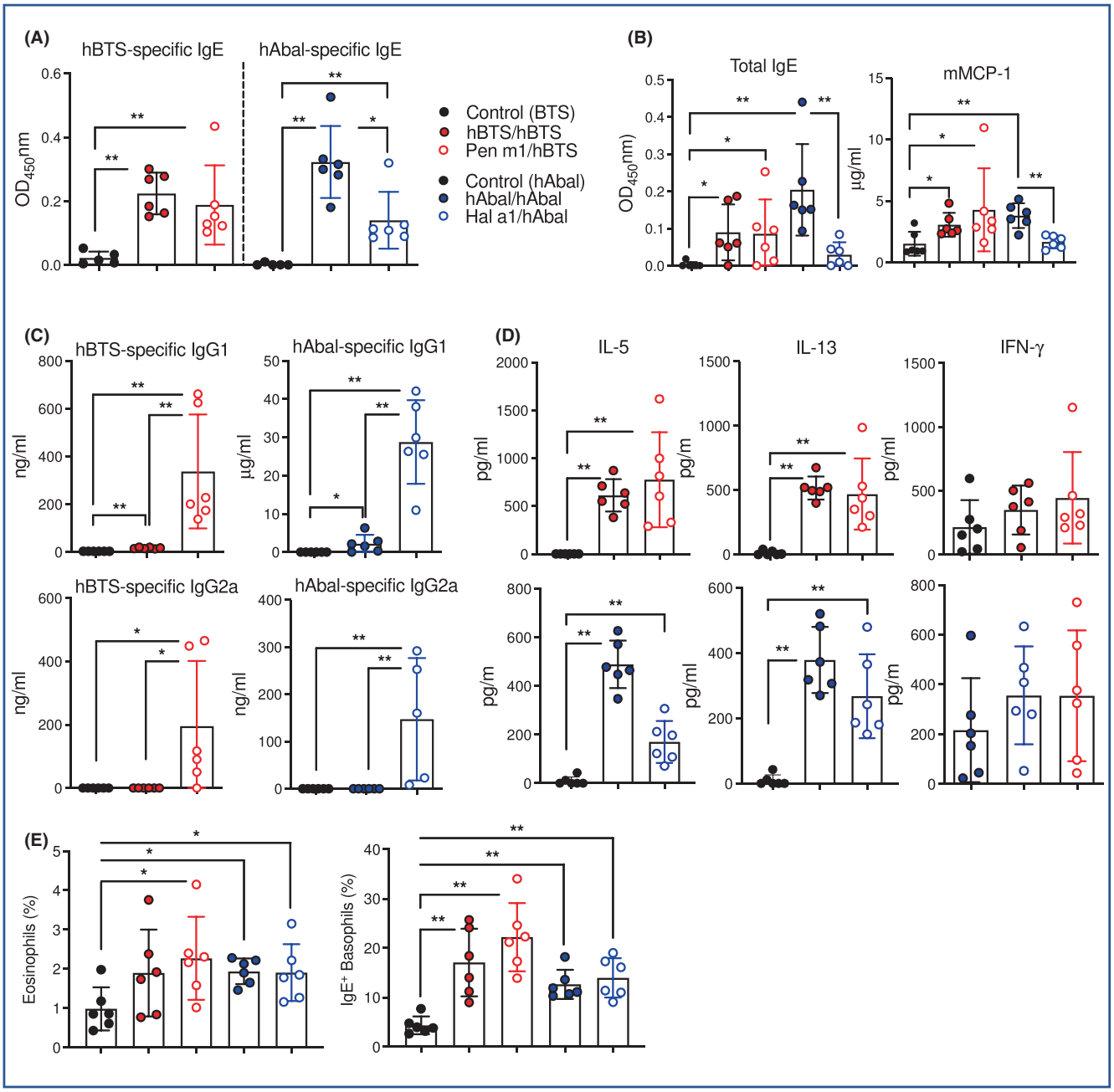

FIGURE 2 Characterization of shrimp (hBTS) and mollusk (hAbal)-induced inflammation, with all challenges performed after 12-h fast. (A–C) Day 31 (24 h post-challenge) allergen-specific serum IgE (A), total IgE and mouse mast cell protease (mMCP) (B), and allergen-specific serum IgG1 and IgG2a antibody quantification (C) were assessed by ELISA. (D) Interleukins (IL)-5 and IL-13, and interferon (IFN) γ were assessed by ELISA in restimulated splenocytes (0.5×10^6 cells/well) with either hBTS or hAbal. (E) Eosinophils and IgE^+ basophils assessed in peripheral blood by flow cytometry with anti-mouse Siglec-F, CD3, IgE, CD11b, CD49b, CD19, and FcεRIa monoclonal antibodies. Statistics: two-tailed Mann–Whitney *U*-tests and mean values with standard deviation. $^*p < .05$; $^{**}p \leq .01$

KEYWORDS

allergy, crustacean, mollusk, shellfish, tropomyosin

FUNDING INFORMATION

SK was supported by a National Health and Medical Research Council early career research fellowship (GNT1124143). SN is supported by a Children's Hospital Foundation grant (RCP10317). ALL was supported by a National Health and Medical Research Council project grant (GNT1086656).

CONFLICT OF INTEREST

The authors have no conflict of interest in relation to this study.

ACKNOWLEDGEMENT

The authors thank Prof. Robyn O'Hehir for providing the allergic patient sera. Open access publishing facilitated by James Cook University, as part of the Wiley - James Cook University agreement via the Council of Australian University Librarians.

Sandip D. Kamath[1,2,3]
Tiange Liu[4]
Paul Giacomin[1,3]
Alex Loukas[1,3]
Severine Navarro[5,6]
Andreas L. Lopata[1,2,7]

[1]*Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, QLD, Australia*
[2]*Molecular Allergy Research Laboratory, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, QLD, Australia*
[3]*Centre for Molecular Therapeutics, James Cook University, Cairns, QLD, Australia*
[4]*National University of Singapore (Suzhou) Research Institute, Suzhou, China*
[5]*QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia*
[6]*Woolworths Centre for Childhood Nutrition Research, Faculty of Health, Queensland University of Technology, Brisbane, QLD, Australia*
[7]*Tropical Futures Institute, James Cook University, Singapore, Singapore*

Correspondence
Andreas L. Lopata, James Cook University, 1 James Cook Drive, DB47, Pharmacy and Medical Research, 4811 Douglas, QLD, Australia.
Email: andreas.lopata@jcu.edu.au

Severine Navarro, QIMR Berghofer Medical Research Institute, 300 Herston Road, 4006 Brisbane, QLD, Australia.
Email: severine.navarro@qimrberghofer.edu.au

Sandip D. Kamath and Tiange Liu shared equal authors.

ORCID

Sandip D. Kamath https://orcid.org/0000-0002-5956-8552
Severine Navarro https://orcid.org/0000-0003-4568-9721
Andreas L. Lopata https://orcid.org/0000-0002-2940-9235

REFERENCES

1. Taylor SL. Molluscan shellfish allergy. *Adv Food Nutr Res*. 2008;54:139-177.
2. Azofra J, Lombardero M. Limpet anaphylaxis: cross-reactivity between limpet and house-dust mite dermatophagoides pteronyssinus. *Allergy*. 2003;58:146-149.
3. Davis CM, Gupta RS, Aktas ON, Diaz V, Kamath SD, Lopata AL. Clinical management of seafood allergy. *J Allergy Clin Immunol Pract*. 2020;8:37-44.
4. Azofra J, Echechipía S, Irazábal B, et al. Heterogeneity in allergy to mollusks: a clinical-immunological study in a population from the north of Spain. *J Investig Allergol Clin Immunol*. 2017;27:252-260.
5. Nugraha R, Kamath SD, Johnston E, Karnaneedi S, Ruethers T, Lopata AL. Conservation analysis of B-cell allergen epitopes to predict clinical cross-reactivity between shellfish and inhalant invertebrate allergens. *Front Immunol*. 2019;10:2676.
6. Kamath SD, Scheiblhofer S, Johnson CM, et al. Effect of structural stability on endolysosomal degradation and T-cell reactivity of major shrimp allergen tropomyosin. *Allergy*. 2020;75:2909-2919.
7. Cox AL, Eigenmann PA, Sicherer SH. Clinical relevance of cross-reactivity in food allergy. *J Allergy Clin Immunol Pract*. 2021;9:82-99.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

DOI: 10.1111/all.15395

Delta or Omicron BA.1/2-neutralizing antibody levels and T-cell reactivity after triple-vaccination or infection

To the Editor,

In Germany, SARS-CoV-2 infections in fall 2021 were caused by the Delta (B.1.617.2) variant of concern (VOC), which was completely replaced by the Omicron (BA.1, B.1.529.1/BA.2, B.1.529.2) VOC in winter. Meanwhile, the BA.2 sublineage dominates, apparently having a selection advantage.[1]

We studied the kinetics of anti-spike (S) protein IgG, Delta neutralizing antibodies (NA), and the release of interferon-gamma