

Shrimp Serology: We Need Tests with More and Less Cross-reactivity

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Cross-reactivity between “unrelated” allergen source materials has been a diagnostic challenge for many years. Invertebrates are a particularly rich source of unexpected cross-reactivities, with all possible modes of exposure to our immune system: in swimming water (chironomids^{1,2}) and in the air (seafood processing³), in floor dust (mites, roaches, silverfish^{4,5}), as stinging or biting bugs,^{6,7} exo- or endoparasites,^{8,9} and, increasingly, as items on our menu.¹⁰ Should we be happy or worried by all this cross-reactivity? Should we search for more specific allergens or should we perhaps look for even more cross-reactive allergens?

The current status is well illustrated by the comprehensive serological investigation on shrimp sensitization described in the paper by Pascal et al in this issue.¹¹ They studied 86 individuals with a positive skin test to shrimp extract, of whom 58 were established to be allergic. IgE to a panel of 10 recombinant crustacean allergens (mostly muscle proteins) was tested in a dot-blot assay. As expected, tropomyosin was the dominating component being positive not only in 83% of the 58 allergic subjects but also in 44% of the 16 tolerant subjects. Its false-negativity rate of 17% is disappointing and intriguing. Of the other components tested, arginine kinase had the highest sensitivity (48%), followed by myosin light chain (38%) and sarcoplasmic calcium-binding protein (34%). However, even with the complete panel the false-negativity rate was still 12%. This deficiency of the panel could be due to technical problems with the expression of the recombinant proteins, but could also reflect species differences. Lured by the high cross-reactivity of tropomyosin, we might be tempted to assume erroneously that our immune system is unable to distinguish invertebrates. An illustrative example to the contrary is the limited cross-reactivity of the major mite allergens.

THE 4 DIMENSIONS OF ALLERGY DIAGNOSIS

The first dimension is diagnostic sensitivity: the ability to identify more than 95% of the patients with allergy to the source in question. Cross-reactivity is convenient for shrimp allergy diagnosis, because of the many different shrimp species that

patients may be exposed to. Without cross-reactivity, we would need to test for reactivity to all these species. From this perspective, it is a positive feature that the cross-reactivity amongst invertebrate tropomyosins is very high. On the other hand, more than 10% of the shrimp-allergic patients were found to be negative for tropomyosin. Because positivity to shrimp extract was the inclusion criterion, the potential exclusion of some patients may even have resulted in a too optimistic estimate of test sensitivities. It might be worthwhile to look for other cross-reactive shrimp allergens that might be used alone or in combination with tropomyosin to achieve a higher diagnostic sensitivity.

The other 3 dimensions are all related to specificity. The second dimension is the ability to distinguish between sensitized subjects with allergy and those without allergy. Exposure to a cross-reactive substance is often (but not always, see below) better tolerated than exposure to the sensitizing substance. In the present study population¹¹ (as in other studies⁴), almost all shrimp-allergic patients were sensitized to mites. It would be interesting to know how many of these mite-sensitized patients were mite-tolerant and negative for the major group 1 and group 2 mite allergens.

The third dimension is the ability to distinguish between allergen source materials that do or do not induce symptoms. In the case of shellfish allergy, it is generally assumed that consumption of all species of invertebrates should be avoided. In view of the promotion of invertebrate meat sources as an alternative for mammalian meat, the general validity of this approach is under scrutiny. In a fish allergy study, consumption of cross-reactive fish species was in some cases well tolerated.¹²

The fourth type is the ability to distinguish between routes of allergen exposure (particularly oral vs airway mucosa), both for sensitization and for symptom elicitation. The cross-reactivity between shrimp and mites is largely due to tropomyosin. It is not fully established yet whether IgE to *Dermatophagoides pteromyssinus* 10 (Der p 10; for the nomenclature of the 4 major allergens mentioned in this paper, see Table 1) is relevant for mite allergy, but the allergen is presumably too big (and the allergen-carrying particle in dust too heavy) to be an important airborne allergen. It is of interest to contrast this with the reversed situation of birch-apple cross-reactivity related to allergens of the pathogenesis-related protein 10 (PR-10) family, in which the airborne allergen *Betula verrucosa* 1 (Bet v 1) is the sensitizer and the food allergen *Malus domestica* 1 (Mal d 1) is the nonsensitizing cross-reactive allergen. Some, but not all, antibodies to Bet v 1 react with Mal d 1. The interaction with Mal d 1 is weaker. Eating apple often causes only oral symptoms, because the allergenic activity is destroyed on gastrointestinal digestion.

THE CROSS-REACTIVE HIERARCHY

Two allergens are cross-reactive if antibodies exist that recognize both allergens. In many instances, the antibody will

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TABLE I. Official nomenclature for the 4 major allergens mentioned in this paper

IUIS allergen nomenclature	Species	Species	Protein name
Bet v 1	<i>Betula verrucosa</i>	Birch	Pathogenesis-related protein 10 (PR-10)
Der p 10	<i>Dermatophagoides pteronyssinus</i>	House dust mite	Tropomyosin
Lit v 1	<i>Penaeus (Litopenaeus) vannamei</i>	Pacific white shrimp	Tropomyosin
Mal d 1	<i>Malus domestica</i>	Apple	Pathogenesis-related protein 10 (PR-10)

IUIS, International Union of Immunological Societies.

have a preference for one allergen over the other. This preferential recognition provides a clue that points to the more relevant of the 2 allergens. Many different epitopes on each allergen can induce IgE antibodies. In the case of the induction of IgE antibodies to tropomyosin induced by eating shrimp (*Litopenaeus vannamei* 1 [Lit v 1] in the case of Pacific white shrimp), 2 types of IgE antibodies can often be distinguished based on their reactivity with Der p 10, the tropomyosin of *D. pteronyssinus*. Some IgE antibodies will not react with Der p 10, because they are directed to a nonconserved part of Lit v 1. Other IgE antibodies will react not only with Lit v 1 but also with Der p 10. The latter reaction will usually be of lower affinity.

Using IgE serology it is possible for each type of allergen (Lit v 1, Lit v 2, etc. for shrimp) to rank allergen source materials (shrimp, mite, and other invertebrates) in a cross-reactive hierarchy. This is most reliably done by using a quantitative bidirectional cross-inhibition protocol,¹³ but less demanding protocols may also be informative. The neutralization or removal of IgE to shrimp tropomyosin from the serum of mite and/or shrimp double-sensitized subjects is likely to allow some discrimination between mite-allergic and mite-tolerant subjects. A risk of such a simpler protocol might be that quantitative differences might mask qualitative differences: a source material with a very low concentration of the allergen in question could be underestimated.

CONCLUSION

In many cases, the clinical history of a patient offers sufficient information, without the need for much further testing. However, the situation might occasionally be less clear-cut. The number of invertebrate species that a patient may have become sensitized to might exceed the number of tests that can be performed. One option would be to combine whole-body extracts of many different species into a single ill-defined test reagent. A more scientific approach would be to use a few well-defined broadly cross-reactive allergens. However, we do not yet have a suitable panel. Finding components for such a test might be facilitated by an in-depth analysis of the specificity of the shrimp-allergic patients who are negative for tropomyosin. The aim would be to combine a small number of recombinant allergens into a single high-sensitivity screening test. The specificity of such a test will be low. To increase sensitivity, we might fall back

on the use of optimal cutoff levels, which would require quantitative assays. Additionally, inhibition assays may be performed to establish a hierarchy of allergen source materials that the patients should avoid. However, a better approach to increase the specificity would be to identify species-specific reagents, such as allergen-derived peptides selected for low cross-reactivity with clinically irrelevant homologous allergens. The most promising perspective would be offered by a discovery of species-specific allergens that would also be able to reveal the most likely route of a patient's allergen exposure.

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