Curr Otorhinolaryngol Rep (2015) 3:186–192
DOI 10.1007/s40136-015-0088-7

Molecular Allergy: A New Paradigm

Guy Tropper[1] · **Habib Chabane**[2]

Published online: 23 June 2015

Abstract Molecular allergy (MA) represents an understanding of allergens down to their molecular, individual protein constituents. Just as allergy entered a new era at the end of 1960s with the discovery of the IgE molecule, the characterization of Der p 1 some 20 years later signaled a new paradigm in allergy. Sitting at the heart the standardization of allergenic extracts, MA opens a new era of allergy diagnosis and treatment that dramatically enhances our ability to advance clinical allergy care. It is not the long sought after Holy Grail of an unequivocal biologic marker for clinical allergy. MA will require a significant educational update as well as a reconsideration of some of our current practices. It may one day allow us to predict the development of clinical allergy in a given patient and might revolutionize our ability to confront the allergy epidemic.

Keywords Molecular allergy · Molecular allergen · Allergen · Allergenic extracts · Respiratory allergy · Food allergy

Introduction

Molecular allergy (MA) represents an understanding of allergens down to their very molecular, individual protein constituents. Just as allergy entered a new era at the end of 1960s with the discovery of the IgE molecule, the characterization of Der p 1 some 20 years later signaled a new paradigm in allergy. Curiously, this has so far unfolded in a somewhat asynchronous fashion, initially in Europe under the leadership of Austrian researchers and more recently in North America.

Various names have been given to this new approach (Table 1) which redefines our clinical understanding of sensitization and clinical patterns of allergy, one sitting at the heart the standardization of allergenic extracts, central to the practice of allergy. This new approach ushers in tremendous advances for clinicians assisting patients affected by respiratory and/or food allergies and even some with venom-related allergies. It has also set the stage for irreversible changes in allergy care.

The Science of Molecular Allergy

MA refines the resolution of our vision of allergens. From an allergen source, such as the hazelnut tree for example, one or more allergenic substances can be produced and extracted (hazelnut tree pollen, hazelnut fruit). Allergy can now go beyond these products to consider the relevance of individual components: molecular allergens, proteins for most of them. Each of these molecular allergens has characteristic epitopes that generate specific IgE recognition.

The WHO/IUIS nomenclature defines molecular allergens by virtue of their sources' Latin family name (first

This article is part of the Topical Collection on *Otolaryngic Allergy*.

✉ Guy Tropper
gtropper@avantgardemedical.ca

Habib Chabane
habib.chabane@free.fr

[1] AVANT GARDE Médical, 550, Boul. de Mortagne, Suite 260, Boucherville, QC J4B 5E4, Canada

[2] 28, Rue de Ponthieu, 75008 Paris, France

three letters of genus and first letter of species) and a number that generally refers to the order of discovery [1]. A prefix specifies their extraction from natural source (*n*) or production via genetic engineering (*r*) (See Fig. 1). In the case of grass allergen components, the relative identity of corresponding proteins of various grasses has led to their designation as a group. Thus, Phl p 1 from *Phleum pratense* is but one member of group 1 grass allergens, belonging to the beta expansin family. Other grasses such as sweet vernal, orchard, and Bermuda grass have group 1 components (respectively, Ant o 1, Dac g 1, and Cyn d 1).

Major allergen components are, by definition, generating IgE recognition in more than 50 % of the patients affected by the corresponding allergy. For instance, Bet v 1 is the major allergen for birch pollen as well over 90 % of patients with birch pollen allergy can be shown to have Bet v 1-specific IgEs. Some major allergens are often used as biologic markers for allergy to an allergen source. The standardization of allergen extracts relies on the dosing of major allergen components.

Minor allergen components, by definition, generate specific IgE recognition in less than 50 % of clinically allergic patients. They often represent a cause of sensitization, generating false positive skin tests and/or traditional IgE serologies in respiratory allergies. Some minor allergens may occasionally have more serious implications in food allergies. This distinction between major and minor allergens is arbitrary and based on statistics relating serologic IgE recognition and clinical expression of allergy for a given population. Increasingly, the clinical relevance of molecular allergens is appreciated beyond an official status of major or minor.

Table 1 The different names of molecular allergy

Molecular allergy
Molecular-based allergy diagnostics
Component-resolved diagnosis
Recombinant allergy
Molecular diagnosis
Molecular allergology

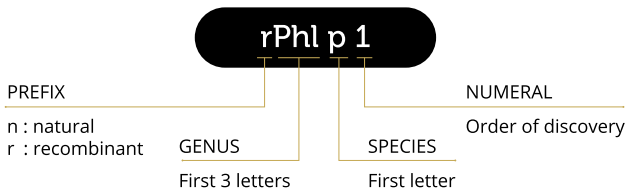

Fig. 1 The WHO/IUIS nomenclature

Molecular Families

Molecular allergens consist, for most of them, of proteins with biologic functions that transcend plant or animal species albeit with some variations. This relative bioidentity (homology) of molecules belonging to a given molecular family allows us to anticipate common allergy features and variable degree of cross-reactivity. Families of molecular (see Table 2) allergens with numerous members across several plants can be referred to as pan-allergens. This underlines their relative ubiquity and the tendency for a patient sensitized to one member of that family to become further sensitized to similar proteins from other plants or fruits. Some molecular allergens are so closely similar that they can, in practice, be clustered in families. For instance, Bet v 1 for birch, Aln g 1 for alder, Cor a 1 for hazel… belong to the PR-10 family (plant Pathogenesis Related proteins), one that extends to several fruits, vegetables, and other plants. The route of sensitization, features of heat and digestion resistance for food allergens, and the severity of eventual allergic reactions are generally consistent within a given molecular family (see Fig. 2).

Such properties of heat and/or digestion resistance are underpinned by the very nature of the molecular allergens' epitopes; the amino acids interacting with the tip of IgE's Fab. *Sequential epitopes*, follow the sequence of amino acids along a particular stretch of the allergenic protein. These tend to resist heat and/or proteolytic acid digestion. Peanut's seed storage proteins and lipid transfer proteins (LTP) are good examples of such epitopes known to cause

Table 2 Main molecular allergen families

Family	Biochemical and clinical characteristics
Polcalcins	Mostly a cause of sensitization, uncertain cause for clinical symptoms of allergy
Profilins	Common panallergen, heat, and digestion labile allergens
PR10 (Bet v 1 –like)	Prominent pollen-food syndrome, usually limited reactions, heat, and digestion labile allergens
Lipid transfer proteins (LTP)	Severe reactions, heat, and digestion resistant allergens. Common in Spain and Southern Europe
Seed storage proteins (SSP)	Several sub-families with inconsistent cross-reactivity, severe reactions as seen with peanut, soy, and nuts
Tropomyosins	Major allergen, heat resistant, marker of crustacean-related severe reactions
Parvalbumins	Major allergen, heat resistant, marker of fish-related severe reactions

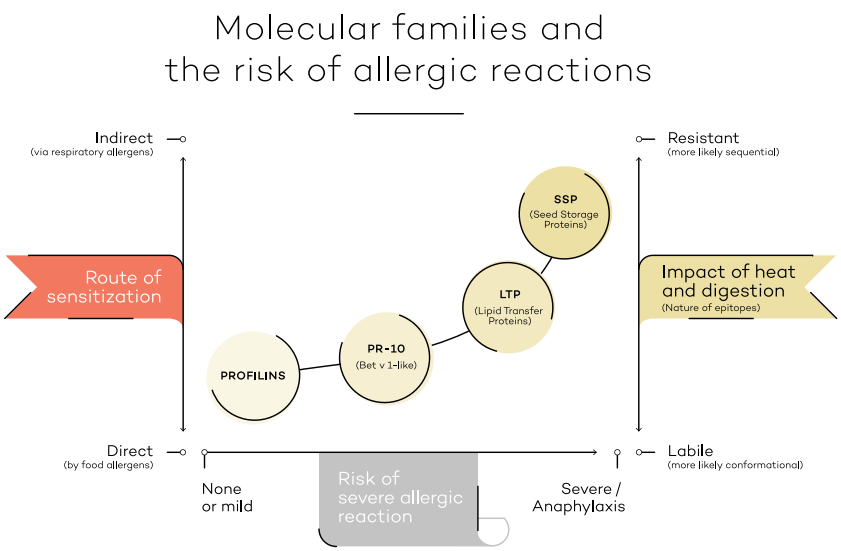

Fig. 2 The route of sensitization, features of heat, and digestion resistance for food allergens and the severity of eventual allergic reactions are generally consistent within a given molecular family (reproduced with permission from Guy Tropper, MD, FRCSC)

severe reactions whether in cooked or raw form. *Conformational epitopes*, on the other hand, relate spatially close segments of the allergenic molecule that are nonetheless at fairly distant points along the amino-acid chain. These loops of the protein form the surface of contact, one that is more easily destroyed by heat and/or proteolytic acid digestion as the denatured protein unfolds. This is fairly typical of the molecular allergens of the PR-10 family known generally not to cause reaction in their cooked form. Such conformational epitopes are involved in patients allergic to raw eggs, but tolerant of cooked eggs.

Cross-reactive carbohydrate determinants (CCDs) are sugar-type molecules which are affixed to the proteins of plants and insects by natural biologic processes. Some carbohydrate combinations do not occur in vertebrates. Some patients can generate IgE against these CCDs. Except for a few notable exceptions (alpha-Gal), CCDs rarely cause allergic reactions, but represent a significant source of false positive test results. Recombinant allergens used for in vitro IgE testing carry no such CCD's. For diagnostic purposes, recombinant allergens are thus somewhat more specific than molecular allergens purified from natural sources let alone extracts used for skin tests or traditional IgE serology.

A simplified summary of MA pertinent to the evaluation of respiratory allergies is presented in Fig. 3.

Test Platforms

Singleplex

As is the case for traditional IgE serologic tests, MA serologic tests can be ordered as singleplex, one molecular allergen at a time or, depending on the commercial supplier, via small series geared to the study of a particular food item (peanut, milk, soy etc.). These may combine food-specific IgE to raw food with titrations of one or a few molecular allergens known for their pertinence for that food source. Such tests are usually more sensitive than those available via multiplex units and offer more precise quantification.

For example, the ImmunoCAP Peanut Allergen Component® tests include titrations providing in kU_A/l, the quantification of a patient's IgE specific for Ara h 1, Ara h 2, Ara h 3 (SSPs), Ara h 5 (profilin), Ara h 8 (PR-10: Bet v 1-like), Ara h 9 (LTP), and the traditional raw peanut extract (f13). Many MA tests are now FDA approved.

Multiplex (Micro-Array Technology)

The Immuno-Solid phase Allergen Chip (now called ImmunoCAP ISAC®, Thermo Fisher Scientific®) provides a semi-quantitative assessment of a patient's sensitization across more than 50 different respiratory, food, and venom allergens sources through 112 relevant molecular allergens. It requires one drop of blood (30 µl of serum) and provides results within a range of 0.3–100 ISU (ISAC units), these to be distinguished from the more traditional kU_A/l.

The advantages of a cost-efficient broad inventory must be gauged against the overall cost and the issue of obtaining more information than required by the clinical context at hand. For sure, patients will enquire about "incidental" results! One must be aware that this assay is not as sensitive as singleplex assays for some molecular allergens. Overall, one needs to balance whether a few more expensive tests will provide a pertinent assessment or

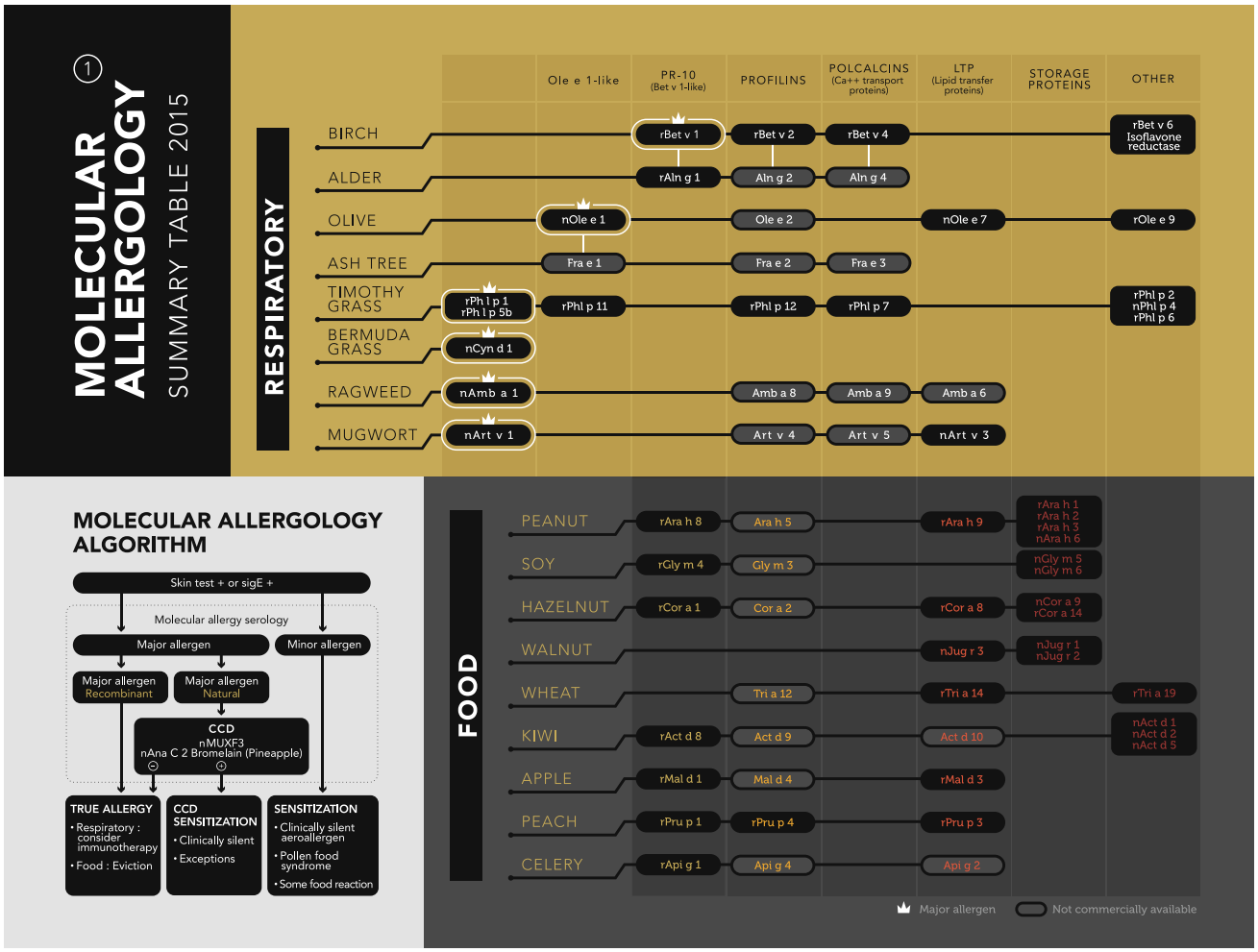

Fig. 3 Simplified summary of molecular allergy pertinent to the evaluation of respiratory allergies (reproduced with permission from Guy Tropper, MD, FRCSC)

whether the number of molecular allergens sought dictates a more cost-efficient investigation.

The MeDALL European survey (Mechanisms of the Development of Allergy) initiative has lead the development of another multiplex unit [2••], not available commercially but used in epidemiological and other clinical research efforts in Europe. This more recent micro-array assesses IgE sensitization to an optimized list of 176 molecular allergens, with enhanced sensitivity.

Molecular Allergy in Clinical Practice

Conceptually, MA provides the clinician with a deeper level of corroboration of a patient's allergy dynamics. Yet, the clinical reality must take precedence. The results of MA serology still need to be gauged against the patient's clinical symptoms. While a diagnosis of allergy can be very reasonably confirmed by skin testing for many clinical situations, others may be more challenging. This is where MA testing can be considered as a second step, one which may eventually replace traditional serologic IgE testing.

There are three main clinical indications for MA testing identified by the WAO-ARIA-GA^{2}LEN consensus document on MA diagnostics [3]:

1. *Increase accuracy and resolve cross-reactivity.* Differentiate true allergy from sensitization (false positives) from minor allergen components or CCDs.
2. *Optimize the prescription of immunotherapy.* As a consequence of item 1 above.
3. *Assess the risk and type of reaction.* Identify/confirm the possibility of pollen-food syndrome and/or clarify the risk of food-related anaphylaxis.

To these, we should add the following:

4. Monitoring of specific molecular allergen levels to assist a clinical decision for the reintroduction of some

food items (milk, egg, peanut) into patient's diet or maintain the advice for the wear of an epinephrine auto-injector and continued food eviction.

5. Epidemiologic surveys, such as some that have revealed important regional differences in patterns of sensitization. From this, insights as to the mechanism of allergy development and the associated risk of asthma, in particular, have emerged.

Respiratory Allergies

For clinicians and clinical researchers alike, distinguishing between true allergy and mere sensitization makes all the difference. While no allergy test is perfect, MA titrations based on major respiratory allergens offer improved accuracy, or at least more specificity over traditional skin testing and serologic IgE titrations. More common respiratory allergens and their major allergen counterpart are listed in Table 3. Some investigators are now choosing to use MA testing to optimize the selection of patients included into their clinical studies. For these, the appropriate documentation of symptoms and corresponding sensitization to the pertinent major allergen(s) optimizes the selection of truly allergic patients. In clinical practice, there is no indication for immunotherapy when the sensitization documented relates to minor allergens [4]. Increasingly, poly-sensitized patients considered for immunotherapy may represent an appropriate indication for MA testing [5].

Pollen-Food Syndromes

Molecular allergy testing can provide helpful insights to the clinician confronted to an individual's food-related symptoms in the context of pollen sensitization with or without the corresponding respiratory allergy. MA can readily distinguish a PR-10 (Bet v 1-like, birch related) pollen-food syndrome sensitization pattern from that associated to a SSP allergy issue. The potential for severe reactions may be limited in most patients with a PR-10 pattern of sensitization and negative LTP or SSP results. The case of peanut-related symptoms in a birch pollen sensitized patient may benefit from the guidance of MA testing before deciding on whether or not the patient needs to wear an epinephrine auto-injector. One must keep in mind that, while pollen-food syndromes usually involve more limited, mostly oral symptomatology (Lessof syndrome), more severe reactions can nevertheless occur. Food-related molecular allergens commonly associated to birch pollen allergy include apple (Mal d 1), hazelnut (Cor a 1), peach (Pru p 1), celery (Api g 1), kiwi (Act d 8), peanut (Ara h 8), soy (Gly m 4), etc.

For some patients, a past history of positive skin test to peanuts, soy, or hazelnut with equivocal clinical reactions may seriously hinder career choices such as culinary or military functions. MA can provide with a solid basis for an adjudication that differentiates a severe, SSP (seed storage protein)-related allergy from a usually more benign PR-10 pollen-food syndrome.

Table 3 Common respiratory allergens and related major allergen components

Allergen	Major molecular allergen
Birch	Bet v 1
Grass	Phl p 1 and Phl p 5
Ragweed	Amb a 1
House dust mites (HDM)	Der p 1, Der p 2
	Der f 1, Der f 2
Cat	Fel d 1

Food Allergy

Some molecular markers are associated with a high risk of severe reactions. Depending on the clinical context, the identification of such markers in a given patient may confirm the need for strict food eviction and the wear of an epinephrine auto-injector. For nuts, peanuts, soy, wheat, and some fruits, the identification of specific IgE to SSP and LTP families signals a risk of severe reactions. With all due reserve, a patient's molecular allergen-specific IgE titers may sometimes provide guidance as to prognosis and the consideration for food challenge tests and/or oral desensitization protocols.

When exploring the possibility of food allergy in a given patient, the limited library of currently available molecular allergens for testing must be considered. In this regard, the provision of molecular allergens Cor a 9 and Cor a 14 (hazelnut SSP) was a welcome addition to, Cor a 8 (LTP), the only marker of serious hazelnut allergy risk previously available. One should beware the implications of a negative test for a major molecular allergen in food allergy situations. Although a positive result for parvalbumin, a major allergen of fish (Gad c 1, cod), is strongly associated with severe reactions to white fish meat, a negative result by no means rules out this risk. Other fish proteins (minor molecular food allergens) can elicit anaphylaxis. The same goes for crustaceans where, in spite of negative IgE to tropomysin (major allergen Pen a 1, shrimp), some patients will react to other allergens beyond the currently available repertory of molecular allergens.

Particular Entities

- *The midnight urticaria syndrome, also called red meat allergy* was traced down to a CCD (galactose alpha 1,3-galactose, alpha-Gal) allergy. This particular syndrome is characterized by urticaria and eventual full blown anaphylaxis generally starting within 8 h after a meal involving red meat (beef, pork, lamb). The sensitization to this CCD arises from bites of tick *Amblyoma americanum* in the south-eastern US, the same responsible for excessive prevalence of anaphylaxis to cetuximab in the same region. Other ticks in the US and abroad represent vectors of this condition.
- *Exercise-induced anaphylaxis* could be due to wheat protein Tri a 19, an ω-5 gliadin, in a large number of cases [6•]. Avoidance of physical exertion in the 4 h before or after consumption of wheat products averts anaphylaxis. Other foods have been associated also such as soy, hazelnut, celery, milk, etc.

Discussion

For clinicians, insights gained from the clinical use of MA will quickly renew a healthy dose of skepticism regarding the results of skin tests (SPT, IDT, MQT) or traditional IgE serology. Especially as allergen immunotherapy becomes a first line option in respiratory allergies, the concept of MA provides a finer grain definition of a patient's sensitization. Differentiating between allergy and mere sensitization, MA is generally more specific than allergy skin testing and traditional serology. Whether the patient's symptoms are equivocal, or take place during overlapping pollen seasons, MA can help determine more pertinent allergen immunotherapy. Some molecular allergens can serve as biologic markers of severity; since Phl p 5 for grass and Ole e 7 for olive tree pollen for example have been shown to be associated to an increased risk of asthma in pollen allergic patients.

The identification of major allergens has improved the process of standardization of allergenic extracts and from there, enhanced our ability to confront therapeutic dosage issues. Increasingly, MA will be relied upon for the selection of patients in the trials of new immunotherapy products. While MA assists mostly the dosage of allergenic extracts developed from traditional harvesting methods, newer strategies will increasingly involve allergenic molecules developed via DNA technology. The day may come where either skin testing will rely on purified MA products or a strictly serologic approach might become recommended for specific clinical situations.

Yet, for all its scientific appeal, the clinical usefulness of MA is dependent on its judicious use by the clinician.

MA tests, like more traditional IgE serology, provide results to be gauged against statistics of sensitivity and specificity that are far from absolute. Whether for respiratory or for food allergy, the patient's clinical symptoms remain the most reliable guide to the clinician. A given patient's symptoms may relate to a molecular allergen beyond those currently available for testing. As things progress however, more and more pertinent molecular allergens assays will become available.

In food allergy, traditional skin testing and/or serologic IgE titrations may be more sensitive. Some patients may have serious reactions due to molecular allergens that are not present in the extract used for testing, complicating the diagnosis process. This can be overcome by spiking the total extract with some important molecular allergens that are naturally underrepresented as was done for hazelnut (Cor a 8-LTP) and latex (Hev b 5) extracts.

For many situations, MA results may be more specific and clinically relevant. However, just as is the case for traditional IgE serology, the patient's sensitization to a specific molecular allergen cannot be diagnostic by itself. There are, for example, some rare patients quite tolerant to peanuts in spite of significant levels of IgE to Ara h 2, currently our best marker of the risk for serious reactions.

Cost is a significant issue. For each clinical situation, the diagnostic need for the IgE titration of selected molecular allergens must be weighed against its cost. Beyond a few individual tests, micro-assays may represent, in spite of their limitations, a preferable option. Issues of cost regarding immunotherapy may eventually lead third party payers to request the confirmation of a patient's respiratory allergic status via documentation of sensitization to pertinent major allergen(s).

MA shines in recognizing pan-sensitization across molecular families and corresponding pollen-food syndromes. MA helps us understand the mechanism of the development of more severe reactions in some patients, but not why the oral allergy syndrome sometimes arises before the corresponding clinical pollen allergy.

MA may eventually help us ascertain the dynamics of certain cross-sensitization such as that to tropomyosins. A significant cause of shellfish allergy, tropomyosin, a muscular protein, is also present in house dust mites (HDM), cockroaches, and some worms. More recent studies have alleviated fears that HDM immunotherapy might increase the occurrence of shellfish allergy. Epidemiological studies will help discern possible subgroups of patients sensitized to HDM for whom the risks and benefits of immunotherapy should be reconsidered.

The significance of certain patterns of sensitization is gradually being recognized via epidemiological studies emerging from Europe. In Spain for example, sensitization to olive tree pollen (Ole e 1) has been associated to a higher

prevalence of LTP-related food allergy (often peach-Pru p 3), possibly as a consequence of patients' sensitization with the olive pollen's LTP (Ole e 7). This sensitization to Ole e 7 also carries a sevenfold increase in asthma risk. Clinicians can use this information to optimize the management of affected patients.

Can MA predict the future for an allergic patient? Hatzler's brilliant work [7•] describes how children develop increasing levels of IgE specific for various grass molecular allergens before their becoming allergic to grass. The rise of group 5 specific IgE serum levels could precede the onset of clinical symptoms of grass allergy by some 2–3 years. This opens the door on the eventual understanding of the sequence of events leading to clinical allergy for various inhalants and possibly allergenic foods as well. The day may come when we might accurately predict the advent and specifics of a patient's allergies. The question might then arise as to whether a preemptive immunotherapeutic intervention should be considered.

MA is at the heart of novel strategies aiming to effect immunotherapy in a few injections [8]. Equally exciting is Europe's FAST program [9] where food allergen molecules are being targeted for modifications that would enhance immunogenicity while limiting allergenicity. This could revolutionize our ability to confront the food allergy epidemic.

Conclusion

MA marks a turning point for the world of allergy. MA testing could be viewed as somewhat of an HD version of traditional extract-based evaluations. MA brings us closer to the level where our immune system interacts with proteins from the environment. Even more importantly, this science opens a new era of allergy diagnosis and treatment that dramatically enhances our ability to help our patients perhaps one day even before the appearance of clinical symptomatology.

MA is still defining its precise clinical role. It is not the long sought after Holy Grail of an unequivocal biologic marker for clinical allergy. It nevertheless represents a major advance in clinical allergy care, improving the management of allergy. MA will require a significant educational update as well as a reconsideration of some of our current practices.

Compliance with Ethics Guidelines

Conflict of Interest Guy Tropper and Habib Chabane declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Radauer C, Nandy A, Ferreira F, et al. Update of the WHO/IUIS Allergen Nomenclature Database based on analysis of allergen sequences. Allergy. 2014;69:413–9.
2. •• Lupinek C, Wollmann E, Baar A, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. Methods. 2013;66:106–19. *Clear summary of fundamental notions of molecular allergy.*
3. Canonica G, Ansotegui I, Pawankar R, et al. A WAO-ARIA-GA2LEN consensus document on molecular-based allergy diagnostics. World Allergy Organ J. 2013;6:17.
4. Valenta R, Twaroch T, Swoboda I. Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. J Investig Allergol Clin Immunol. 2007;17(Supplement 1):88–92.
5. Sastre J, Landivar M, Ruiz-García M, et al. How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. Allergy. 2012;67:709–11.
6. • Palosuo K, Varjonen E, Kekki O, et al. Wheat omega-5 gliadin is a major allergen in children with immediate allergy to ingested wheat. J Allergy Clin Immunol. 2001;108:634–8. *Fascinating insight into the development of grass pollen sensitization and allergy over time.*
7. • Hatzler L, Panetta V, Lau S, et al. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. J Allergy Clin Immunol. 2012;130:894–901.e5. *Molecular allergy as it opens new therapeutic avenues.*
8. Valenta R, Campana R, Marth K, et al. Allergen-specific immunotherapy: from therapeutic vaccines to prophylactic approaches. J Intern Med. 2012;272:144–57.
9. http://www.allergome.org/fast/index.jsp Accessed 23 May 2015.