

Component Resolved Testing for Allergic Sensitization

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Abstract Component resolved diagnostics introduces new possibilities regarding diagnosis of allergic diseases and individualized, allergen-specific treatment. Furthermore, refinement of IgE-based testing may help elucidate the correlation or lack of correlation between allergenic sensitization and allergic disease. Novel tools to predict severe outcomes and to plan for allergen-specific treatment are necessary, and because only a small amount of blood is needed to test for a multitude of allergens and allergenic components, component resolved diagnostics is promising. A drawback is the risk of overdiagnosis and misinterpretation of the complex results of such tests. Also, the practical use and selection of allergenic components need to be evaluated in large studies including well-characterized patients and healthy, sensitized controls and with representation of different geographical regions.

Keywords Allergen · Allergenic component · Cross-reactivity · CRD · Diagnostics · Individualized treatment · Pollen-related food allergy · Sensitization pattern

Introduction

Since the discovery of IgE in the late-1960s, the relationship between allergic disease and the presence of IgE in serum has

been intensively investigated. Antigen-specific IgE plays a key role in the immediate-type hypersensitivity reaction, also referred to as the *type I allergic reaction*, and the importance of the IgE-mediated immune response in the development of the most severe allergic manifestation, anaphylaxis, is undisputed.

The technical performance of the IgE assays has improved significantly over the years, with the sensitivity thereby also enhanced. No IgE assay will be better than the quality of the employed allergens, however, and one of the barriers for further improvement of the assays is linked to the inclusion of all relevant allergens. In the absence of detectable sensitization evaluated by a highly sensitive assay, IgE-mediated allergy can be ruled out with a high level of certainty [1, 2]. However, the clinical efficacy—that is, the ability of an IgE test to predict allergic symptoms and severity of disease—is nonetheless mediocre.

This review deals with the different aspects of component resolved IgE diagnostics (CRD) and allergic sensitization, but the real challenge is to establish the connection between sensitization and clinical allergic disease. Even though this aspect may be equally important for all allergen sources and symptoms from a theoretical point of view, it seems particularly important when dealing with potential severe allergic reactions and when intervention or treatment is planned. In the following review, food allergy and sensitization to plant food allergens are the main objective, but other manifestations, such as insect venom allergy, latex allergy, and other allergen systems (e.g., pollen and house dust mites), are mentioned briefly. Exposure to inhalant allergen sources such as pollen and house dust mites seldom causes anaphylactic reactions, but CRD may offer advantages regarding diagnosis and treatment of inhalation allergy as well.

Further refinement of IgE testing and a better understanding of the involved allergen systems may lead to

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improvement of the diagnostic armamentarium regarding allergic disease and perhaps also a better understanding of the immunologic mechanisms underlying allergic diseases.

IgE-mediated Allergy

Environmental exposure to common allergens occurs all the time via inhalation, skin contact, and ingestion, but only in sensitized individuals does this contact pose a potential risk of eliciting allergic symptoms. Cross-linking of two or more mast cell-bound specific IgE molecules by an allergen leads to degranulation of the cell and release of different mediators as histamine, prostaglandins, and cytokines. Allergic symptoms of an IgE-mediated response appear within a few minutes after the sensitized individual is exposed to the allergen; the reaction is therefore often referred to as *immediate-type hypersensitivity*. The IgE-mediated immune response involves other molecules and cellular interactions between antigen-presenting cells and T and B lymphocytes [3].

Sensitization Versus Clinical Allergy

Allergic sensitization does not necessarily imply allergic symptoms upon contact with the allergen but rather indicates that an individual has developed specific IgE against the allergen in question. The word allergy, on the other hand, is reserved for IgE immune reactions that give rise to clinical reactions (i.e., symptoms in sensitized individuals) [4]. Several studies have shown that sensitization precedes clinical allergy; therefore, the presence of specific IgE constitutes a risk factor for the development of allergic symptoms [5, 6]. Furthermore, early sensitization seems to be prognostic regarding persistence and severity of symptoms and development of other allergic or atopic manifestations in children [7–10]. On the other hand, there also may be a post-clinical sensitization phase of the disease, in which the clinical reactivity is outgrown while the IgE seropositivity to allergens remains.

IgE Testing

The different methods used to establish allergic sensitization in everyday practice all rest on the presence of specific IgE that is cell bound or free in serum. As discussed further below, the diagnostic set-up, including skin tests and detection of serum specific IgE, is, however, inadequate to distinguish between allergy and mere sensitization. A convincing medical history combined with positive IgE-based tests is often sufficient to establish the diagnosis of inhalant allergy without reasonable doubt, but regarding food allergy, the final diagnosis must be made based on the result of controlled oral challenge tests [11, 12]. Challenge tests are time-consuming and pose a potential risk of severe allergic reactions, and the results of the conventional IgE-

based methods offer no real help to the clinician in assessing this risk. CRD may be useful in predicting severity of symptoms elicited during food challenges and could also help reduce the need for rechallenges if it is possible to establish the relationship between certain allergens and persistence of symptoms [13, 14••].

Allergens

The term *allergen* is often used very broadly to describe different biological matrices and molecular components—from the allergen source itself (e.g., birch pollen) over the extract of allergenic proteins to the single allergenic component from a given source material.

Figure 1 summarizes the topics mentioned in the following text, with allergen sources being the biological materials from which the active antigens (allergens) are derived, allergens being the immunologically recognized molecules, and allergen extracts the pharmaceutical or diagnostic preparations of allergens that are used in clinical practice.

In the canonical application of the term, an allergen is an antigen with the capacity to bind IgE. An epitope is an amino acid sequence either sequential or conformational of the allergenic molecule that can bind a single specific IgE molecule on the surface of mast cells.

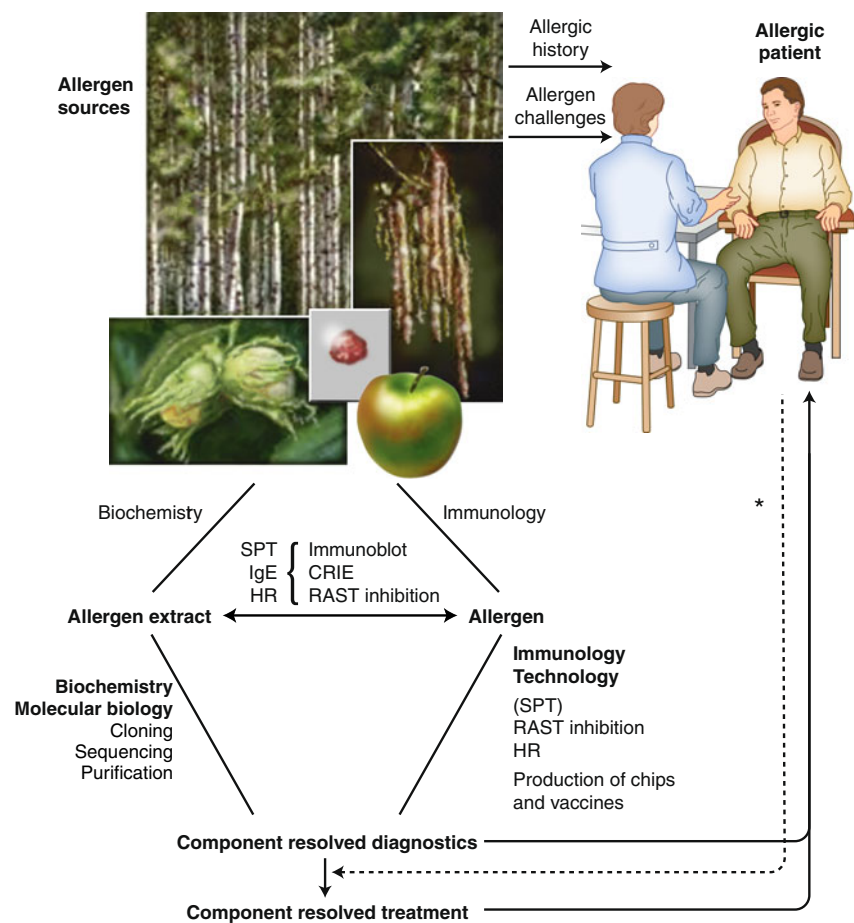
The terms *major* and *minor allergens* are often used to describe the prevalence of reactors (>50% or <10%) in a population of patients who react to a given allergen source. It is important to remember that the classification of an allergen as major or minor depends entirely on the pattern of sensitization in the investigated population and the predominant allergenic sources in a given geographical area [15, 16].

The molecular background for the so-called cross-reactivity between antibodies is a varying degree of resemblance of the allergenic determinants from different allergen sources. The slight diversity of allergenic determinants from an allergenic molecule leads to a broad spectrum of antibodies, some of which possess the capacity to react with two or more different antigens. The cross-reacting allergens may be totally or partially identical, but a homology of at least 40% is generally considered the lower limit for cross-reactivity when protein—and not carbohydrate—epitopes are considered. Cross-reacting allergens may originate from the same or closely related species or from quite different species or even phyla. Pollen-related food allergy is an example of partial identity between allergens from different species [17].

Allergen Sources

Allergic sensitization to widespread natural allergens takes place via inhalation, ingestion, skin contact, or even—as in the case of insect venoms and certain drugs—via the

Fig. 1 Steps and applied methods during the development of component resolved diagnostics for IgE-mediated allergy. *Asterisk* refers to large clinical studies in different geographic areas. CRIE—crossed radioimmuno-electrophoresis; HR—histamine release; RAST—radioallergen sorbent test; SPT—skin prick test



parenteral route, and sensitization against the same specific allergenic molecule can occur by different routes (e.g., food allergy induced by ingestion of wheat products, and baker's asthma caused by inhalation of wheat proteins) [18•, 19].

In the discussion of allergens, it is important to remember that the individual predisposed to allergic disease is exposed to complex biological sources rather than single purified allergens. Time, duration, and levels of exposure necessary to cause sensitization in susceptible individuals are mostly unknown, and the process leading to symptom development in the sensitized individual seems to be influenced also by concomitant conditions such as chronic diseases, exercise, and alcohol intake [12].

Allergen Extracts

Allergen extracts from single, well-defined allergen sources such as pollen from a single tree or food may contain not only several different allergens but also different isotypes of each allergenic molecule. The advantages of whole allergen source extracts are the abundance of raw material and the broad spectrum of allergen specificities.

During the past few decades, many allergenic proteins from different sources have been investigated, and increas-

ingly more detailed knowledge of the molecular structure is now available for many of the clinically important allergens (e.g., Bet v 1 from birch pollen [20], Pru a 1 from cherry [21], Cor a 1 from hazelnut [22, 23], and Mal d 1 from apple [24]). Cloning and sequencing by recombinant techniques have made it possible to produce extracts consisting of one allergenic component only. Furthermore, it is possible to purify natural allergens from whole allergen sources. Preparations of individual allergens have circumvented the previously used techniques for determining IgE reactivity to complex mixtures of allergens, such as the crossed radioimmuno-electrophoresis or the more contemporary Western blotting techniques. The pure extracts of recombinant or natural allergens have been evaluated against classical whole source extracts in several papers, and there is no doubt that they have increased the knowledge regarding differences in sensitization patterns among individuals and different geographical areas [16]. On the other hand, the lack of allergenic diversity could make the purified allergens less useful for screening purposes in daily clinical practice.

Synthetic peptides can be used for epitope mapping and investigations of antigenic structures [1, 3, 25]. In recent studies, the IgE response to sequential epitopes was

correlated with severity and persistence of allergy to peanut [9, 26]. It is important to remember that monoclonality is sufficient for *in vitro* activity of IgE, whereas polyclonality is required for activation of effector cells [3]; therefore, the clinical relevance of future application of synthetic peptides must be evaluated against clinical allergy.

Allergen Families

According to physiochemical, structural, and functional features, proteins are divided into different families. The understanding of sensitization patterns of food allergy combined with the molecular characterization of different food allergens has formed the basis of several databases (<http://www.allergenonline.org>; <http://www.allergen.org>; <http://www.allergome.org>; <http://www.meduniwien.ac.at/allergens/allfam>). Comparative studies of the vast amount of data from these databases have revealed that the allergenic proteins belong to a relatively small number of protein families and that some families/superfamilies predominate. It cannot be totally excluded, however, that a certain bias exists (e.g., when one allergen family member, such as Bet v 1, has been identified, it is relatively easy to look for and identify other family members in other plants). For plant food allergy, the prolamin and cupin superfamily, the Bet v 1 family, and the profilins are the most prevalent, and for allergens of animal origin, tropomyosins, parvalbumins, and caseins dominate. The pathogenesis-related protein family 10 (PR10) includes Bet v 1 and the homologue proteins from apple, pear, cherry, hazelnut, carrot, and celery. Another important superfamily is made up of the nonspecific lipid transfer proteins (LTPs), which, in contrast to the PR10 proteins, are highly stable. Sensitization seems to be associated with severe symptoms in nonpollen plant food-allergic patients [17, 27•].

Table 1 shows the most important hazelnut allergens, which family the protein belongs to, and potential cross-reactivities.

Methods

For *in vivo* testing of allergic sensitization, skin prick test is the most widely used method [2, 28]. Skin prick test is easy to perform, and the results can be presented to the patient immediately. Regarding the test result, the size of the weal reflects the biological activity of specific IgE, but due to the limitations of the test principle (e.g., choice of allergenic material, skin responsiveness, technique), the result is at most semiquantitative. Commercial allergen extracts and fresh preparations of allergenic source material (e.g., fresh food [29]) can be used. The latter seems to offer some advantages compared with most commercial food extracts

due to the low stability of some of the food allergens, but of course, it is not possible to standardize the procedure.

As for the CRD, the situation is more difficult. In Europe and likely other regions of the world, the allergen extracts for *in vivo* use are considered pharmaceutical preparations, leading to high demands on documentation of nontoxicity, safety, and Good Manufacturing Practice (GMP) certification, which have for practical purposes led to a moratorium in the use of single components for *in vitro* testing.

In Vitro

A quantification of IgE reactivity in sensitized individuals can be obtained by different serological assays. The original radioallergosorbent test (RAST) has been followed by refined methods with different solid phases, detection principles, and modifications of the allergen material. As mentioned previously, the sensitivity of the different commercial test systems has been improved continuously, but the validity of the test result still depends entirely on the quality of the source material [2, 28].

The biological activity of IgE can be assessed quantitatively by measurement of histamine release from basophil leukocytes. This assay offers the possibility of using different source materials such as raw food and dust samples in evaluation of the sensitization of individuals, and it has another important application in the testing of novel foods [2, 28].

A qualitative evaluation of the allergenic spectrum (i.e., the different IgE-binding antigens in a source material) is possible using methods such as immunoblotting and immunoelectrophoresis. Western blotting and crossed radio-immunoelectrophoresis (CRIE) are examples of such methods, and besides the qualitative description of allergenic mixtures, it is possible to obtain a quantification of the allergenic potential and a confirmation of the specificity of the precipitates by applying an inhibition assay [2, 3].

A clinical verification of allergy via history or allergen challenge combined with an immunologic demonstration of cross-reactive, IgE-binding antigens in an inhibition assay is necessary for final confirmation of clinically important cross-reactivity. The knowledge of allergen families and cross-reactivity is often sufficient to confirm a correlation between different allergens in daily clinical practice, but blot or RAST/enzymeallergosorbent test (EAST) inhibition may be necessary in evaluating new and potential cross-reactive molecules and in evaluating the time course of sensitization. The primary sensitizer is a potent inhibitor of the IgE binding of the homologue proteins (e.g., Bet v 1 from birch pollen inhibits the IgE binding of the proteins from apple and hazelnut), whereas the Bet v 1 homologue allergens Mal d 1 and Cor a 1—from apple and hazelnut, respectively—display a less complete blocking capacity against Bet v 1 [3].

Table 1 Cross-reactivity of hazelnut allergies

Hazelnut allergen	Allergen family	Potential cross-reactivity ^a	Clinical relevance ^b
Cor a 1 [20]	PR10, Bet v 1	Betulaceae pollen Apple Carrot Kiwi Other Rosacea fruits Celery (Peanut, soy)	} +++
Cor a 2 [58]	Profilin	Pollen and plant food	
Cor a 8 [54, 55]	LTP	Almond Peach Cherry Celery (Apple)	} +++
Cor a 9 [63]	Legumin 11S albumin	Walnut Peanut Soy Cashew Almond	
Cor a 11 [62]	Vicilin	Peanut Walnut Cashew Sesame	} (+)
Oleosin [64]		Peanut	
CCD [60–62]		Pollen and plant food	–

^a Parentheses imply a very weak (mostly theoretical) correlation

^b More + symbols indicate greater clinical relevance, whereas parentheses imply a very weak (mostly theoretical) correlation
CCD cross-reactive carbohydrate determinant, *LTP* lipid transfer protein, *PR10* pathogenesis-related protein family

Component Resolved Diagnosis

The characterization of different allergenic components from different sources and the knowledge of the clinical relevance of allergens and components form the basis of CRD in allergic diseases [30]. The concept of CRD was introduced about 10 years ago, and although it is theoretically possible to perform skin test with different allergenic components, synthetic or natural, the real advantage is the possibility of complex serological IgE mapping with the use of minute blood volumes and small amounts of allergenic material using the so-called microarray technique [31–33, 34•]. One commercial product is now available for clinical use. The ISAC chip (Phadia AB, Uppsala, Sweden) offers a semi-quantitative measurement of IgE against an array of 103 allergenic components from 50 different allergen sources, and it requires only 20 µL of serum or plasma. For many of the included components, further evaluation of clinical relevance is needed, as is a comparison with conventional IgE tests for single components to establish where to place the chip in the algorithm of allergy diagnostics. In some cases, selected IgE analyses against single components is sufficient. Another option is more targeted chips with fewer components.

Studies of epitope recognition in peanut allergy have shown a large interpatient heterogeneity of IgE-binding epitopes [9, 14••, 26], and as mentioned previously, the

results also revealed a correlation between the IgE response to certain epitopes and the persistence and severity of symptoms. The use of CRD seemed useful in reducing the need for food challenges in some cases of peanut allergy [14••]. Another study on birch pollen and apple allergy revealed that symptomatic apple-tolerant, birch pollen allergic patients showed a broader sensitization pattern compared with apple allergic patients [34•]. The study found no advantages to the use of multiplex analyses compared with the use of conventional component diagnostics.

Different applications and possible approaches to the use of component resolved analyses have been published [14••, 18••, 19, 26, 33, 34•, 35–38], but it is imperative to evaluate the utility regarding plant food allergy and pollen allergy in different geographic areas. Furthermore, the validity of the results rely entirely on the choice of population and relevance of allergenic components [3, 13, 39]. To avoid overdiagnosis and misinterpretation of the complex results of CRD, large-scale studies with well-characterized patients and representation from different geographical areas are needed.

Specific Treatment of Allergic Diseases

At the moment, allergen-specific treatment can only be offered to patients with pollen allergy, but studies of

specific immunotherapy against food allergy have been conducted, and more studies are under way [12, 40–43].

The efficacy of specific immunotherapy regarding pollen-related food allergy is still a subject of debate. Only a few placebo-controlled studies have been conducted, and the results are conflicting [44–50]. The use of recombinant allergen extracts improves the overall diagnostic and therapeutic possibilities regarding pollinosis, but it is presently unclear whether a recombinant (or native) Bet v 1 extract will have a more convincing effect on pollen-related food allergy than the traditionally used crude allergen extracts [25, 30, 51, 52]. High doses of modified and less immunogenic Bet v 1 extracts may have a more pronounced effect on pollen-related food allergy, but the presence of food-specific B- and T-cell epitopes not shared by pollen allergens has been confirmed in studies of hazelnut allergens, and inclusion of relevant food allergens in modified form may be necessary as well [53].

CRD will be a prerequisite for future component resolved immunotherapy [30]. Using engineered allergens with reduced IgE binding [25], it may be possible to tailor allergen-specific treatment according to the patient's individual IgE recognition pattern in order to optimize the effect and at the same time reduce the risk of systemic allergic side effects.

Potential Advantages of Component Resolved Diagnostics

1. Reduction of the number of tests for individual allergenic sources

Example: Bet v 1-related pollen–pollen and pollen–plant food cross-reactivity can be explained easily and seldom needs further explanation. A positive IgE test for Bet v 1 covers the homologues' pollen and plant food allergens unless the severity of the symptoms suggests another sensitization pattern (e.g., sensitization to LTP16).

2. Prediction of severity of symptoms and persistence of disease

Example: Ara h 2 from peanut [14•, 26]; LTP from hazelnut (Cor a 8) [54, 55]; Pru a 3 from peach [56]. These molecules are associated with severity of clinical reactions, and the presence of sensitization therefore offers a possibility of risk assessment in individual patient cases. Studies of allergenic components involved in peanut allergy have shown a high degree of epitope diversity for patients with a severe history, and the authors suggest that this diversity is reflective of immune progression and predictive of antibody effector function [26].

3. Discrimination of between different clinical entities induced by exposure to the same allergenic source or closely related sources

Example: Evaluation of wheat proteins in involved in baker's asthma and food-dependent, exercised-induced anaphylaxis; allergenic proteins are involved in allergy to wheat and grass pollen [18•, 19].

4. Explanation of sensitization not accompanied by allergic symptoms

Example: Peanut (Ara h 8) and birch pollen; grass pollen (allergenic component unknown) and cereals; peanut (profilin) and grass pollen; peanut (profilin) and legumes; profilin sensitization and latex allergy [33].

5. Identification of optimal treatment

Example: Specific immunotherapy in birch pollen allergy: Bet v 1 sensitization or not? Insect venom allergy: sensitization to bee or wasp [37, 38]? House dust mite allergy: sensitization to Der p 1 or Der f 1? Ragweed or mugwort sensitization [57]?

In the choice of allergen-specific treatment, it is important to confirm the actual sensitization pattern to optimize the effect and reduce the costs and time spent.

Potential Pitfalls of Component Resolved Diagnostics

1. Wrong selection of patients

Example: Patients with very mild symptoms and thereby no need for prediction of severity or persistence of disease: no treatment options available.

Example: CRD does not seem to eliminate the need for food challenges regarding cow's milk allergy or allergy against hen's egg in children, but the reduction of the amount of blood necessary for the analyses compared with conventional IgE testing can be an indication for microarray-based CRD in some cases [13].

2. Improper selection of allergens and components

Example: There are geographical differences in sensitization patterns. Bet v 1 is not relevant in areas without birch trees but is obligatory in cases of birch pollen allergy in Northern Europe. A positive test result with a crude birch pollen extract in such a patient with rhinoconjunctivitis during the birch pollen season and no complicating symptoms needs no further testing [16].

3. Incorrect interpretation of results

Example: No clinical relevance or rare clinical importance of allergens as profilin [58, 59] and cross-reactive carbohydrate determinants [60–62] in Northern Europe.

Example: Positive IgE to wasp, but no medical history of severe reactions to insect stings.

Example: Positive IgE against wheat in a grass pollen allergic patient with no symptoms upon ingestion of wheat products [18•].

Conclusions

The introduction of CRD in allergological practice and research offers a range of new insight in correlations between different allergens and between sensitization patterns and symptomatology, and it provides the potential basis for individualized diagnostics and treatment in the future. A potential problem is the risk of overinterpretation of results, and it is important to remember that a given result is never closer to the truth than the clinical history. A valid interpretation of the test result from an array technique (e.g., the ISAC chip) calls for specialized knowledge due to the complexity of allergenic components and allergen systems involved.

Questions to Be Answered by the Clinician Before Applying Component Resolved Diagnostics in Allergy Diagnosis

1. When?
 - In case of a clinically relevant case history of allergic symptoms, with a need for further knowledge of the sensitization pattern.
2. Why?
 - To avoid or predict risk of severe or life-threatening symptoms.
 - To explain unforeseen reactions when the sensitization pattern does not correlate with symptoms.
 - In the planning of allergen-specific treatment (prerequisite for future component resolved immunotherapy), and perhaps before food challenges with a potential risk to avoid unnecessary risk and time-consuming procedures.
3. With What?
 - Clinically relevant allergens and components, or clinically “irrelevant” allergens to confirm lack of clinical relevance of a sensitization shown by use of crude extract, and to prevent unnecessary restrictions (e.g., an unhealthy diet) and worries for the patient.
4. Interpretation by Whom?
 - The translation of the result of multiplex CRD is most often a task for specialists and requires understanding of involved allergen systems and correlations and at the same time the experience to decipher less serious symptoms, such as those of oral allergy syndrome, from a potentially life-threatening clinical history.

Final questions may be asked: what are the ethical implications of using multiple CRD? How should a clinician react to an unsuspected positive reaction that is not backed up by the case history? What are the medicolegal consequences of ignoring a positive IgE response (obtained via CRD or otherwise) if a serious or even fatal reaction involving the relevant allergenic source later takes place?

These and likely other questions suggest that much more research may be needed to fully unravel the potential of CRD in allergy practice.

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- Of importance
- Of major importance

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