

# Component-Resolved Diagnosis in Allergic Rhinitis and Asthma

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**Background:** Allergic rhinitis and asthma are highly prevalent chronic inflammatory diseases leading to restrictions in the patient's quality of life and high costs for healthcare systems. Both diseases are associated with the presence of specific IgE (sIgE) against aeroallergens. This review aims to examine the importance of molecular allergy diagnostics in the assessment and management of these disorders.

**Content:** The “U-shaped” approach, proposed by the European Academy of Allergy and Clinical Immunology, combines conventional allergy diagnostics with the benefits of component-resolved diagnosis (CRD) and offers important additional information regarding the patient's sensitization pattern, especially in complex clinical cases such as polysensitization or idiopathic reactions, thus avoiding overuse of in vitro and in vivo IgE diagnostics. CRD may help the clinician to identify the cause of an allergy and, in the case of complex polysensitization, uncover possible cross-reactivity. Polysensitization, especially to inhalant allergens, is associated with the clinical appearance of asthma and allergic rhinitis; important risk factors for the latter are the major allergens Fel d 1 and Can f 1. Importantly, information on molecular sensitization patterns significantly influences the choice of specific immunotherapy and reduces its overprescription.

**Conclusion:** At present, allergy diagnostics largely rely on clinical history, physical examination, and in vivo IgE testing. However, in vitro diagnostics including CRD are currently finding their way into the clinical routine and can offer additional information on the patient's sensitization profile and treatment responsiveness.

## IMPACT STATEMENT

This review aims to support physicians and scientists active in the field of in vitro allergy diagnosis. Information on available tools is provided with an emphasis on component-resolved diagnosis (CRD) for patients with allergic rhinitis and/or asthma. The clinical relevance of determination of specific IgE against inhalant allergens is discussed in terms of diagnosis and treatment of the aforementioned patient population. An approach combining both conventional extract-based and CRD, along with associated benefits in clinical practice, is discussed.

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Allergic rhinitis and asthma are both considered atopic diseases. Atopy can be defined as a genetic predisposition to produce IgE upon exposure to an antigen and subsequently develop an allergic reaction. The first atopic manifestation often already shows in early childhood as atopic dermatitis and can further progress to allergic rhinitis and/or asthma at school age, a transition that is referred to as the atopic march (1, 2). Approximately two-thirds of atopic dermatitis patients develop allergic rhinitis (3, 4), and about one-third to one-half develop asthma later in life (4). The associated high global prevalence of respiratory allergies renders allergic rhinitis and asthma 2 of the most common chronic noncommunicable diseases. The prevalence of allergic rhinitis reported by the Allergic Rhinitis and its Impact on Asthma guidelines amounts to 10% to 20% of the global population (5), and according to the Global Initiative for Asthma, about 300 million people worldwide are affected by asthma, although estimates are considerably higher if less strict diagnostic criteria are used (6, 7). Nevertheless, there is great geographic variability in the prevalence of both disorders (8, 9).

Although no consensus definition is available for allergic rhinitis, the clinical diagnosis is frequently made by the presence of  $\geq 2$  nasal symptoms for  $>1$  h/day (10), but other definitions highlight the hypersensitivity component mediated by aeroallergen specific IgE (sIgE)<sup>3</sup> (11). Asthma is defined as a heterogeneous, chronic inflammatory disease of the respiratory tract that leads to temporally varying symptoms, such as wheezing, shortness of breath, chest tightness, and coughing in alternating intensity, as well as expiratory airflow limitation (12).

Both diseases cause long-term morbidity (13), restrictions in the patient's quality of life, and high

costs for the healthcare systems because of, among others, hospitalization, medication, and work/school absenteeism-associated costs (14). Early diagnosis and appropriate management of these disorders may prevent deterioration, acute exacerbation, and persistence, along with related healthcare expenditures.

## DIAGNOSTIC APPROACH

### Initial assessment

The patient's clinical history and physical examination represent the initial steps of the diagnostic process. Subsequently, detection of allergen sIgE, which plays an important role in the pathomechanism of allergic reactions (Fig. 1), occupies an essential part in the diagnosis of atopy and allergy. Since the 1960s, when the radioallergosorbent test assay for the detection of sIgE was initially developed (15), detection of these antibodies may be applied to individually characterize patients' sensitization patterns, thus revealing precipitating factors of allergic disease. Nevertheless, a number of facts complicate prevention and treatment approaches: Aeroallergens are ubiquitously present, which makes avoidance not always realistically feasible; polysensitization to multiple allergens belonging to the same or different protein families and/or allergen sources can pose great restrictions in a patient's quality of life; and the possibility of cross-reactivity because of the presence of similar proteins/epitopes in distinct allergen sources can lead to previously unsuspected clinical reactions. In such cases, allergen-specific immunotherapy should be considered.

### IgE testing

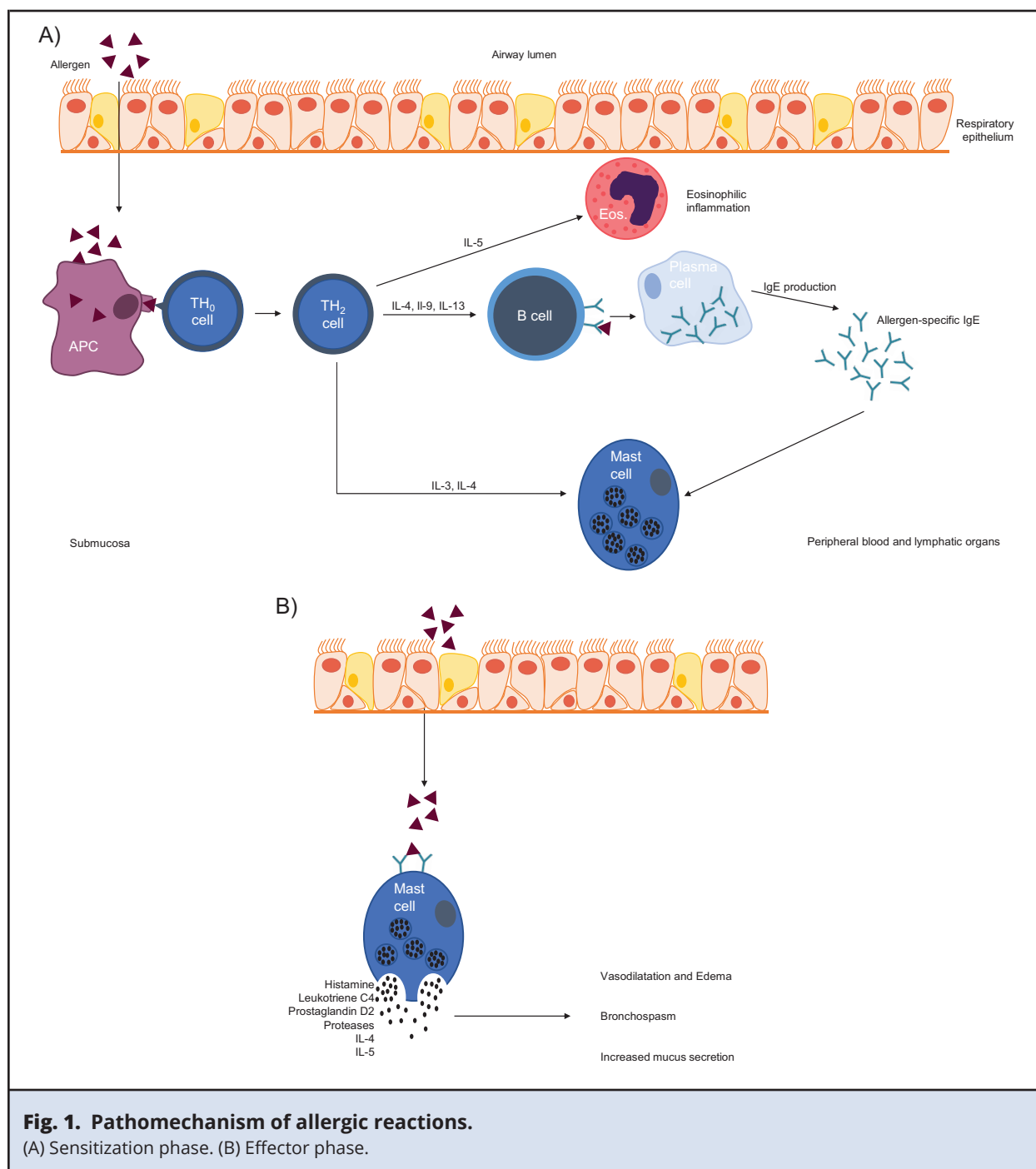
For those with respiratory allergic diseases, such as allergic rhinitis and asthma, studies suggest that

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<sup>3</sup> **Nonstandard abbreviations:** sIgE, allergen-specific IgE; SPT, skin prick test; CRD, component-resolved diagnostics; EAACI, European Academy of Allergy and Clinical Immunology; ABPA, allergic bronchopulmonary aspergillosis; HDM, house dust mite; SIT, specific immunotherapy.



the combination of clinical history, symptoms, and physical examination rarely exceeds a diagnostic accuracy of 50% (16). Thus, sIgE testing may considerably improve the accuracy rate by

supplementing existing data or confirming the diagnosis (Table 1). However, sensitization needs to be distinguished from clinical allergy (17, 18), and IgE testing is not recommended as a screening

**Table 1. Advantages and limitations of different methods for IgE measurement.**

|             | In vivo IgE testing<br>SPT                                                                                                                                                                                                                               | In vitro extract-based                                                                                                                                           | In vitro IgE testing<br>component-based                                                                                                                                                                                                                                                                                          | ISAC                                                                                                                                                                                                                                                                                                                                                                           |
|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Advantages  | High sensitivity<br>Results available within 10–20 min<br>Low costs                                                                                                                                                                                      | Natural allergen extracts available<br>High sensitivity<br>Appropriate for sensitization monitoring<br>Results are not influenced by medication and skin lesions | Natural and recombinant allergen components available<br>High sensitivity<br>Appropriate for sensitization monitoring<br>Detailed information on the patient's sensitization patterns, possible cross-reactions, and the severity of anticipated clinical reactions<br>Results are not influenced by medication and skin lesions | Natural and recombinant allergen components available<br>Multiplex assay (112 allergens are assayed in parallel)<br>Small amount of serum or plasma (30 µL) required<br>Results are not influenced by medication and skin lesions<br>Detailed information on the patient's sensitization patterns, possible cross-reactions, and the severity of anticipated clinical reaction |
| Limitations | Based only on allergen extracts<br>1 allergen/prick<br>Manual method<br>Results influenced by medication and skin lesions<br>Inappropriate for sensitization monitoring<br>Systemic reactions possible (<1% of the cases)<br>Not recommended for infants | Some allergen sources are not represented by any available allergen extract<br>High costs<br>Results available within days to weeks                              | Some allergen sources are not represented by any available allergen component<br>High costs<br>Results available within days to weeks                                                                                                                                                                                            | Less sensitive<br>Some allergen sources are not represented by any available allergen component<br>Manual method<br>Less appropriate for monitoring sensitization<br>High costs<br>Results available within days to weeks                                                                                                                                                      |

tool. A positive sIgE test result should not be looked at as a single diagnostic parameter but must be reviewed based on clinical information. Indeed, an overdiagnosis may restrict a patient's quality of life by means of overtreatment and/or unnecessary avoidance of certain allergens. Therefore, further allergy diagnostics should be considered only after a thorough investigation of the patient's clinical history and physical examination indicative of an allergic disease such as asthma or allergic rhinitis, which is associated to certain allergen exposure.

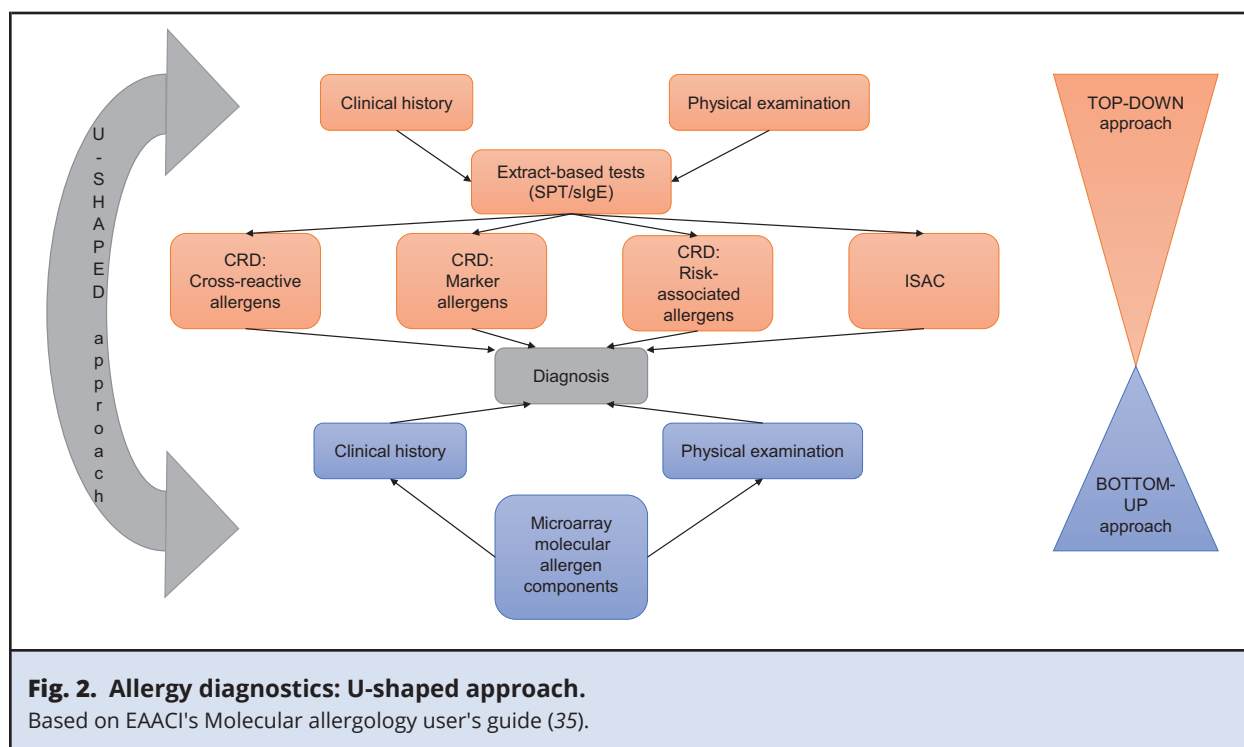
**In vivo.** In vivo IgE tests are commonly used for both children and adults. The most frequently used in vivo test for inhalant, food, venom, and drug allergies is the skin prick test (SPT). The latter is easy to perform: Small amounts of standardized allergen extracts plus a positive (histamine) and a negative control are epicutaneously applied with a lancet on the volar forearm or on the back. After 15 to 20 min, a local wheal and flare or papule appears if the patient is sensitized to the corresponding extract. This is recorded by its diameter or surface.

Other important in vivo allergy tests are the intradermal skin test for venom allergies and drug hypersensitivities, as well as provocation tests. The nasal provocation test, used for allergic rhinitis, and the bronchial provocation test, used for asthma, are applied to confirm a diagnosis or to clarify any discrepancies between diagnostic tests and clinical symptoms. Provocation tests can generally be executed by using direct stimuli, such as histamine and methacholine, or by using indirect stimuli, such as allergen extracts. However, allergen provocation tests are rarely done in the routine diagnostic workup of the patient with allergic rhinitis and asthma. Both methods result in an airflow obstruction, which correlates with airway hyperresponsiveness (19). Although allergen provocation tests are generally well-tolerated, systemic responses rarely may occur (<1%) (20) (e.g., when

an excessive amount of allergen is applied), which manifest as severe local or systemic allergic reactions (e.g., acute bronchoconstriction or anaphylaxis) (21).

**In vitro. Allergen extracts.** For the in vitro measurement of sIgE to allergen extracts, several tools are commercially available. A well-established and frequently used test system is the ImmunoCAP® (Thermo Fisher Scientific) assay. Test extracts contain a mixture of allergenic and nonallergenic molecules; therefore, measured sIgE is a sum of the antibodies against each of these components. Thus, the test can provide information only about sensitization without being able to identify the culprit component. Although commercially available test extracts are mostly standardized, there is still heterogeneity regarding the identity, composition, and relative amounts of contained individual allergens (22–25). Furthermore, studies have claimed limitations in the quality of the extracts and showed contamination and incomparability of products derived from different manufacturers (26–30). Additionally, allergen extracts cannot differentiate between primary sensitization to an allergen and cross-reactivity; thus, they cannot always identify clinically relevant allergens.

**Component-resolved diagnostics (CRD).** Allergen extracts consist of a mixture of different allergenic proteins derived from a single source, including non-species-specific, i.e., cross-reactive, molecules. However, CRD makes use of individual allergen components/proteins, therefore identifying the exact molecule against which sIgE is being produced. For this purpose, purified natural and recombinant allergen components are available. CRD is widely used in allergic rhinitis and asthma cases and in the diagnosis of most of the other IgE-mediated allergic diseases (e.g., in patients with allergy to peanut, egg, milk, wheat, latex, insect venom, etc.). A currently well-established and frequently used method for measuring IgE sensitization to components is again the



ImmunoCAP assay, which is a singleplex immunoassay. Another possibility is the multiplex ImmunoCAP ISAC<sup>®</sup> 112 (Thermo Fisher Scientific) assay, a microarray currently including 112 allergenic molecules from 51 allergen sources, thus providing a broad sensitization profile for each patient with a serum or plasma sample of only 30 µL. The Microtest Allergy System (Microtest Diagnostics), another automated microarray platform for allergy diagnostics, has recently become available. Using a 100 µL serum or plasma sample, sIgE against 19 extracts and 16 components of 22 common allergen sources can be identified (31, 32). Also commercially available is the multiplex ELISA ALEX<sup>®</sup> (Macro Array Diagnostics), which offers the measurement of up to 282 allergen extracts and components and total IgE by using 100 µL of serum or plasma. Its most important advantages are the on-demand panel construction and the individually optimized allergen extracts that can be used (33). An additional advantage of most of the microarray approaches is the statistical power of assaying

replicates in parallel (34). Furthermore, immunoblots [e.g., Euroline (Euroimmun)] represent another in vitro approach to investigate the patient's molecular sensitization patterns.

In recent years, considerable discussion has been raised regarding the development of allergen panels oriented to medical disorder (e.g., food vs respiratory allergy), age (e.g., pediatric vs adult), and geographic region (e.g., northern vs southern Europe). Such an approach has already, to some extent, been adopted by commercial providers, although mainly focusing on extracts rather than individual molecular components.

### CRD IN CLINICAL PRACTICE

In daily clinical practice, the value of CRD is viewed differently by physicians (Fig. 2). Today's most widely applied diagnostic approach starts with the patient's clinical history, followed by an SPT or extract-based IgE testing, and uses CRD only as an additional source of information. When

CRD is performed, it is usually through singleplex assays, and clinicians decide which allergen component should specifically be looked at. This pathway has been labeled as a “top-down” diagnostic approach (35). Common arguments in favor of this approach are that extracts are crucial screening tools in which clinicians have already gained vast experience. CRD, especially by means of microarrays, are reprimanded to be too complex and detailed. SPTs, on the other hand, are advantageous in terms of providing information on the biological function of the antibody in question.

In contrast, the “bottom-up” approach starts with the detailed information that CRD provides and moves from that to the clinical history to allow a comprehensive evaluation of the allergic disorder (35). Supporters of this approach praise the global assessment of the patient's IgE repertoire because even a positive result without current clinical symptoms may—in their opinion—predict a future allergic manifestation. However, this approach is largely debated, as it may lead to unnecessary avoidance measures (e.g., elimination diet) that could even worsen the patient's sensitization state and prevent tolerance induction mediated by natural allergen exposure. Extracts are often considered outdated and unreliable because of the nonuniform characterization of those currently in use (26–30). Moreover, a broader use of CRD may lead to lower costs on the manufacturer's side, as well as for the healthcare systems because of fewer patient–doctor–laboratory encounters.

With these 2 contradictory approaches in mind, the European Academy of Allergy and Clinical Immunology (EAACI) proposes a third path that reconciles them, the so-called “U-shaped” approach (35). Starting with the patient's clinical history, physical examination, and SPT or extract-based IgE testing, CRD should be performed for allergen components of sources to which the previous diagnostics have detected a clinically relevant sensitization. Additionally, when IgE sensitization to highly cross-reactive allergens has been found, (a)

CRD diagnostics should be broadened to include molecules of the same family, and (b) clinical history should be expanded by actively investigating for the presence of possible symptoms caused by other allergen sources of the index protein family (35).

### **ALLERGEN COMPONENTS: NOMENCLATURE AND BASIC CHARACTERISTICS**

The existing convention for the nomenclature of allergen components is based on the Latin name of the source. The first 3 letters are derived from the genus, followed by 1 letter to indicate the species, while the number at the end serves to distinguish allergens from the same source (36). For example, the house dust mite *Dermatophagoides pteronyssinus* allergen 1 is denoted as Der p 1. If, as in the case of the birch pollen *Betula verrucosa* Bet v 1, isoallergens exist, they are labeled by 2 additional numbers as Bet v 1.01. Likewise, for each present isoform, 2 numbers are added to the allergen name (Table 2).

A further classification of the allergenic molecules into different protein families (Table 3) (based on their structure and function) can help predict the probability of developing local vs systemic reactions. Moreover, members of the same protein families share features related to temperature and pH stability (especially protease digestion), which is important when it comes to severity of anticipated symptoms. The more stable an allergen, the higher the probability of developing severe allergic reactions. Because of similar amino acid sequences and/or protein structures, members of the same protein family can share the same or similar IgE epitopes, which can lead to cross-reactivity; a hereby commonly used example is the cross-reactivity of the major birch pollen allergen Bet v 1 and the apple allergen Mal d 1.

**Table 2. Glossary.**

| Term                 | Definition                                                                                                                                                                                                                                                |
|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Allergen             | Protein able to bind sIgE antibodies and to induce an immune response                                                                                                                                                                                     |
| (Allergen) component | Allergenic molecule that binds to IgE antibodies; the allergen component can be derived directly from natural allergen sources (natural allergen component) or manufactured by using genetic engineering techniques (recombinant allergen component) (84) |
| (Allergen) extract   | Mixture of allergenic and nonallergenic molecules gained from a defined (natural) allergen source; typically composed of proteins, glycoproteins, and lipoproteins; can cause an IgE response (84)                                                        |
| Allergen isoforms    | Polymorph variants of an allergen that share >90% of their amino acid sequence (29)                                                                                                                                                                       |
| Allergy              | Presence of allergen-specific IgE antibodies along with related clinical symptoms                                                                                                                                                                         |
| Atopy                | Genetic predisposition to develop allergic diseases combined with the presence of IgE antibodies                                                                                                                                                          |
| CCD                  | Cross-reacting carbohydrate determinant; marker for carbohydrate groups of glycoproteins responsible for many cross-reactions; the most commonly described CCD is MUXF3 (84)                                                                              |
| (B cell) Epitope     | Molecular pattern that is recognized and bound by antibodies; the biochemical structure decides which antibodies can bind (84)                                                                                                                            |
| IgE cross-reactivity | Reaction of IgE antibodies that recognize and bind to epitopes similar to those of their original target allergen and cause subsequent immune responses; due to highly similar or identical epitopes                                                      |
| Isoallergens         | Allergenic molecules from the same allergen species that share $\geq 67\%$ of their amino acid sequence; high probability of cross-reactivity (29)                                                                                                        |
| Major allergen       | Allergens to which $\geq 50\%$ of patients with a clinical allergy to its source react (34)                                                                                                                                                               |
| Panallergen          | Allergens that share evolutionary highly conserved amino acid sequences, structure, and function and thus can provoke cross-reactions                                                                                                                     |
| Polysensitization    | Sensitization to $\geq 2$ allergens from different sources                                                                                                                                                                                                |
| Sensitization        | Presence of IgE against a specific allergen, independent of the presence of clinical manifestations (84)                                                                                                                                                  |

Other commonly used terms in relation to allergens and associated sensitizations are defined in the glossary found in Table 2.

## CRD FOR INHALANT ALLERGENS

Epidemiological studies suggest that 50% to 80% of patients consulting allergy specialists are sensitized against multiple allergens, although the exact prevalence of polysensitization depends on the population (37). Similar findings exist for allergic rhinitis, with rates of polysensitized patients varying from 30% to 85% (38–41), but pollen-monosensitized allergic rhinitis patients are rather rare (41). In the POLISMAIL study by Ciprandi et al., which included patients with allergic rhinitis and/or asthma, up to 90% of the participants were found to be polysensitized (42). Nordlund et al. reported similar rates in a cohort of asthmatic children (43). IgE sensitization occurred in 80% of the cases, and

51% were polysensitized to food allergens, pollens, and perennial aeroallergens (43). Among these, cat (76%), birch (64%), and timothy grass (64%) were the most frequent sensitizations. In another study, sensitization to  $\geq 3$  allergens belonging to the lipocalin family was associated with severe asthma (44).

Children sensitized to >1 inhalant allergen tend to have more allergic diseases, with the number of allergens to which the patient is sensitized being directly associated with the number of wheezing episodes, school absenteeism, and the severity of nasal symptoms (45).

## Animal dander

One of the major risk factors for the development of allergic rhinitis and/or asthma is an allergy toward furry animals such as cats and dogs (46). In 2014, Konradsen et al. examined allergies to furry animals in severe vs controlled asthmatic Swedish

**Table 3. Basic characteristics of the most common protein families.**

| Protein family      | Characterization                                                                                                                                                                                                                                                          |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Lipocalins          | Animal-derived allergens<br>Stable proteins                                                                                                                                                                                                                               |
| nsLTPs              | Nonspecific lipid transfer proteins that transport lipids across cell membranes<br>Plant-derived panallergens in food, pollen, and latex allergens<br>Stable against low pH and heat treatment<br>Associated with OAS <sup>a</sup> but also severe and systemic reactions |
| Parvalbumins        | Fish-derived allergens<br>Stable against low pH and heat treatment<br>Associated with OAS but also severe and systemic reactions                                                                                                                                          |
| Polcalcins          | Calcium-binding proteins<br>Marker for cross-reactivity between different pollen species<br>Low clinical relevance                                                                                                                                                        |
| PR-10-like-proteins | Bet v 1-like proteins<br>pH and heat labile<br>Mostly local reactions such as OAS                                                                                                                                                                                         |
| Profilins           | Actin-binding protein in eukaryotic cell cytoskeletons<br>Highly conserved proteins with high sequence homology<br>pH and heat labile<br>Common in pollen allergic patients<br>Clinical symptoms are rare, but local and severe reactions may occur                       |
| Serum albumins      | Animal-derived allergens<br>pH and heat labile<br>Cross-reactions between serum albumins of different species possible<br>Present in saliva, dander, milk, and meat                                                                                                       |
| Tropomyosins        | Actin-binding proteins<br>Stable against low pH and heat treatment<br>Associated with OAS but also severe and systemic reactions                                                                                                                                          |

<sup>a</sup> OAS, oral allergy syndrome.

children (44). Cat sIgE (Fel d 1, Fel d 2, and Fel d 4), dog sIgE (Can f 1, Can f 2, Can f 3, and Can f 5), and horse sIgE (Equ c 1 and Equ c 3) were determined by ImmunoCAP and the ISAC assay (44). In this pediatric population, the frequency of sensitization toward 1 of the aforementioned animal allergens and multisensitization toward >1 animal allergen was similar in both cohorts, whereas sIgE levels directly correlated to severity of asthma and multisensitization toward all 3 animals occurred more frequently in severe asthmatic cases (44). Another study showed that children with severe asthma are more often multisensitized to animal-derived allergen components when compared with those with controlled asthma (43). Multisensitization to lipocalins (Mus m 1, Equ c 1, Fel d 4, Can f 1, Can f 2), kallikreins (Can f 5), and secretoglobulins (Fel d 1) was more frequent in those with severe asthma (43).

**Cat.** Currently, 8 cat allergen components have been described. Among these, the uteroglobin Fel d 1, whose expression in salivary glands and in skin depends on sexual hormone production (47), is considered a major allergen and a specific marker of cat sensitization (44). Konradsen et al. reported that 89% of those with severe asthma and 86% of those with controlled asthma were sensitized to Fel d 1 (44). The high frequency of sensitization to Fel d 1 in those with asthma and cat allergy has also been shown by Ichikawa et al. in Japanese children (48). Ninety-five percent of the cat dander-positive samples contained Fel d 1 sIgE (48). Sensitization to Fel d 2 and Fel d 4 is less frequent than to Fel d 1. Only about 3% of those with severe asthma and 4% of those with controlled asthma were sensitized to the serum albumin Fel d 2 (44), which is often cross-reactive with other serum albumins.

Approximately 38% of those with severe asthma and 21% of those with controlled asthma were sensitized to the lipocalin Fel d 4 (44). Fel d 4 sensitization may lead to cross-reactions with Can f 6 and Equ c 1 (49). In pediatric allergic rhinitis patients, considerably fewer sensitizations to cat components were found (approximately 17% to Fel d 1, 1% to Fel d 2, and 2% to Fel d 4) (50). The presence of sIgE to Fel d 1 was more frequent in asthmatic patients than in those with rhinoconjunctivitis (51). Moreover, Fel d 1 sensitization in childhood can be used as a predictive marker for cat allergy and associated symptoms in adolescence (43, 52).

**Dog.** The study by Konradsen et al. revealed that 92% of those with severe asthma and 86% of those with controlled asthma were positive to dog-derived allergen extracts when examined with ImmunoCAP (44). Available dog allergen components are Can f 1 (lipocalin), Can f 2 (lipocalin), Can f 3 (serum albumin), and Can f 5 (arginine esterase/prostatic kallikrein); however, only 43% of the individuals found positive to the extract could be also found positive for any of these components (44). The most frequent sensitization against dog components in those with severe childhood asthma is against Can f 5 (44), which is present in urine of male dogs. Can f 2 has been found positive only in severe asthmatic children (44). Furthermore, it has been reported that like Fel d 1, Can f 1 sensitization in childhood and polysensitization to dog allergens predict dog allergy at adolescence, with Can f 1 being a better prognostic marker than dog extracts (43). sIgE to dog proteins in children with allergic rhinitis is rare: Only approximately 5%, 3%, and 2% were sensitized to Can f 1, Can f 2, and Can f 3, respectively (50). A newly discovered dog-derived allergen is Can f 6, which has a high probability of cross-reactions because of its sequence similarities to Fel d 4 and Equ c 1 (49).

**Horse.** The prevalence of horse sensitization also shows differences depending on whether this is

being assessed by extract- or component-based assays. Sensitization to the major allergen Equ c 1, a lipocalin, is significantly more prevalent and characterized by higher sIgE levels in severe vs controlled pediatric asthma (44).

## Mold

Exposure to mold allergens in infancy has been shown to increase the risk of asthma in school-aged children (53). Further, asthma severity is significantly associated with mold sensitization (54).

***Aspergillus fumigatus.*** *Aspergillus fumigatus* is a ubiquitous allergen source (55), which renders allergen avoidance nearly impossible. Currently, 23 allergenic components with different biochemical functions have been described (56). Allergic bronchopulmonary aspergillosis (ABPA) affects about 1% to 2% of asthma patients and is associated with asthma exacerbations and a worsening of lung function (57, 58). It has been shown that asthma patients with ABPA have higher amounts of sIgE to *A. fumigatus* allergen than asthma patients with a simple *A. fumigatus* allergy. The components Asp f 4 and Asp f 6 have been found positive only in asthma patients with ABPA, whereas Asp 1 and Asp f 3 sensitization occurred in asthma patients regardless of the presence of ABPA (59).

## House dust mites

In an epidemiological study of Chinese patients with rhinitis and/or asthma, house dust mites (HDMs) were the most prevalent allergens (60). HDM species include *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, and *Euroglyphus maynei*, with each containing several allergen components. In particular, the allergenic molecules Der p 1 and Der p 2 of *D. pteronyssinus* are highly associated with allergic rhinitis and asthma symptoms, with a significant dose-response correlation (61, 62).

A cross-sectional study of 101 Brazilian individuals supported these findings and revealed mite

allergens as the most frequent sensitization source in pediatric patients with allergic rhinitis. With the use of the ImmunoCAP ISAC assay, it was shown that almost three-quarters of these patients were allergic to mites; the allergens investigated were Der p 1 (74.2%), Der p 2 (73.3%), Der f 1 (74.2%), and Der f 2 (72.3%) (50). Another recent study suggested a correlation between sensitization to the HDM allergens Der p 2 and Der f 2 and severity of asthma (63). In asthmatic patients, higher concentrations of sIgE to Der p 1 and Der p 2 can be observed compared with those without asthma, and the former are more likely to be sensitized against both allergens (64).

### Pollens

In contrast to ubiquitous occurring allergens such as HDM, natural exposure to pollen allergens such as ragweed, birch, and cypress has a wide regional variability, which correlates with IgE sensitization (65). A recent study investigated the association between sensitization to seasonal allergens (cypress, grass, olive, *Platanus* spp., and *Parietaria* spp.) and allergic rhinitis (66). The individuals sensitized to seasonal allergens suffered from more severe and often intermittent rhinitis, more restrictions in their quality of life, and had a longer time to asthma development since rhinitis onset, whereas asthma tended to be less severe in such patients.

Moreover, single sensitization to the panallergens profilin (Bet v 2/Phl p 12) and/or polcalcin (Bet v 4/Phl p 7) in pollen-sensitized allergic rhinoconjunctivitis patients seems to be of little clinical relevance. The role of these panallergens was examined in relation to allergen sensitization to birch (Bet v 1), olive (Ole e 1), timothy grass (Phl p 1/5), and mugwort (nArt v 1). The frequency of sensitization to panallergens is similar among major allergen-sensitized patients and major allergen-negative patients: 68% vs 81% for profilin, 10% vs 11% for polcalcin, and 22% vs 8% for both panallergens (67). This is also the case for symptomatic vs asymptomatic patients; therefore, panallergen

sensitization per se is not useful to distinguish between allergy and mere sensitization (67). No clinically relevant sensitization to panallergens was found in the absence of an additional major allergen sensitization (67).

**Tree pollens.** An association between the olive pollen allergens Ole e 2 (profilin) and Ole e 10 (X8 domain-containing protein) and asthma has been previously reported. In a study by Quirarte et al. in 2004, sensitization toward both components was associated with a higher asthma risk (68). Concerning birch pollen sensitization, no significant difference in terms of frequency could be found in severe vs controlled pediatric asthmatic patients (44).

**Grass pollens.** Konradsen et al. described the absence of significant differences in the frequency of sensitization with grass pollens between patients with severe and controlled asthma. The most frequent grass pollen allergen appeared to be Phl p 1, a marker for genuine grass pollen sensitization that often precedes other grass pollen sensitizations, such as Phl p 4 and Phl p 5 (44). Furthermore, an association between the age of onset of timothy grass sensitization and the clinical outcome has been described. Whereas an early onset was strongly associated with a higher risk for developing asthma, rhinitis, and eczema, a late onset was associated with a significantly higher risk for rhinitis only (69).

A further attempt to characterize grass pollen IgE sensitization patterns in Chinese allergic rhinitis patients, with or without asthma, revealed Bermuda grass as the most common allergen (23%), followed by timothy grass (13%) and *Humulus scandens* (7%), and patients were more likely to be sensitized when compared with healthy controls (71). Most Bermuda-sIgE-sensitized patients were found positive for Cyn d 1 and cosensitized to timothy grass (70). High Bermuda-sIgE levels were associated with further positive sIgE results for other grass allergens (70). If a timothy grass

sensitization was diagnosed, patients were always found to be positive for Phl p 4 and in 17.1% of cases for Phl p 1, whereas only a minority tested positive for Phl p 5, 6, 7, 11, or 12 (70).

## ALLERGEN-SPECIFIC IMMUNOTHERAPY FOR INHALANT ALLERGENS

Existing guidelines on asthma and allergic rhinitis have been proven useful for standardization of treatment and reduction of further complications of these diseases. Still, they are characterized by wide heterogeneity necessitating additional steps toward the quest of personalized treatment in the future. For example, the recently conducted INFANT trial assessed response to asthma medication in 12- to 59-month-old pediatric asthma patients who required daily treatment (71). The best treatment response could be shown for daily inhaled corticosteroids (ICS) and was predicted by aeroallergen sensitization (71), which underlines the importance of sIgE testing for guiding treatment.

In contrast to pharmacotherapy, allergen-specific immunotherapy (SIT) aims to manage not only the symptoms of the disease but also the underlying cause. The long-term allergen-specific immunotolerance and clinical efficacy of SIT could be shown in various studies (72, 73). A resulting significant improvement in the patients' quality of life by means of a reduction of symptoms and medication requirements has also been demonstrated (74, 75). Seasonal rhinoconjunctivitis patients who underwent immunotherapy (76) were reported to show a reduced risk for development of asthma, and multisensitization did not evolve in monosensitized asthmatic children (77, 78).

SIT for inhalant allergens requires repeated administration and can be applied either subcutaneously or sublingually. SIT modifies the immune response against the index allergen by influences on T, B, and antigen-presenting cells, as well as the corresponding effector cells (79). Thus, SIT results

not only in an antibody class change from IgE to IgG4 but also in a reduction of activity and mediator levels of chronic inflammation. The more precise the diagnosis of an allergy, along with associated sIgE sensitization patterns, the more effective is the SIT prescribed by the physician. In some cases, medical history, physical examination, and extract-based IgE testing may be sufficient for an unambiguous diagnosis of the underlying sensitization. Nevertheless, polysensitization and cross-reactions are common in those with asthma and allergic rhinitis, which renders CRD essential for a favorable immunotherapy outcome.

Already in 1999, Valenta et al. proposed the term "component-resolved immunotherapy" for immunotherapy aimed at the sensitization-causing allergen components (80). Following this, Sastre et al. examined whether CRD would change the indication and allergen prescription of SIT among allergic rhinoconjunctivitis and/or asthma patients sensitized to pollen (81). A discrepancy of the prescriptions provided before and after CRD was revealed in >50% of the cases (81). Since then, several more studies supported that CRD could help improve the selection of SIT in polysensitized patients (82–84). In a cohort of polysensitized pediatric allergic rhinitis patients (83), the initial SIT prescription (following an SPT) was changed in approximately 45% of cases when an additional ImmunoCAP assay was performed for allergen components (83). The best clinical efficacy for SIT is achieved for major allergens (85).

A recently published metaanalysis conducted by the EAACI showed benefits regarding short-term asthma symptoms, quality of life, medication scores, and bronchial hyperreactivity following subcutaneous SIT, whereas the benefit for sublingual SIT remained questionable in patients with asthma (74). Likewise, the value of SIT concerning asthma control, exacerbations, and long-term efficiency after discontinuation of treatment needs further investigation, while

adverse reactions have also been reported for both SIT forms (74).

In previous years, studies suggested epicutaneous immunotherapy as an additional approach in murine models aiming to control asthma (86). The hypoallergenic rBet v 1B2 variant showed a significant suppression of airway inflammation and protection from lung function impairment, thus offering an additional possible treatment approach with further reduction of risk for allergic side effects (86). A placebo-controlled, double-blind trial with grass pollen-sensitized rhinoconjunctivitis patients reported a dose-dependent reduction of symptoms but accentuated risk of adverse events with higher allergen doses (87). Epicutaneous immunotherapy is a relatively easy and painless treatment approach worth investigating in future studies.

## CONCLUSIONS/SUMMARY

Asthma and allergic rhinitis are highly prevalent chronic inflammatory diseases of the respiratory tract. At present, diagnosis is largely based on the patient's medical history, physical examination, and in vivo IgE tests, although in vitro tests have found their way into routine allergy diagnostics.

Polysensitization is frequent, and in vitro IgE assays, particularly those based on allergen components, provide additional useful information. The latter offer the possibility of distinguishing between genuine sensitization and cross-reactivity. Sensitization against animal dander is 1 of the major risk factors for the development of asthma and/or allergic rhinitis. Polysensitization and higher sIgE levels against animal-derived allergens are linked to severe forms of asthma. The HDM-derived allergenic molecules Der p 1 and Der p 2 are highly associated with asthma and allergic rhinitis symptoms. Finally, detailed information regarding the sensitization profile of a patient is essential for a successful allergen-specific immunotherapy. Future research needs to not only map the sensitization profiles for allergic rhinitis and asthma patients but also to find an approach with which this gained information can be used adequately. Although CRD offers more information on the patient's sensitization profile and currently fewer limits on the detection of allergen-specific IgE exist, it is crucial for future diagnostics and treatments to filter out asymptomatic, (poly)sensitized patients and to use the right tools from the allergy diagnostics arsenal only on those patients who really require it.

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