

NEW HORIZONS — ALLERGY —

Diagnostic tests based on recombinant allergens: Assistants for the selection of allergy therapies

Summary

Type I allergy is an IgE-antibody-mediated hypersensitivity disease affecting more than 25% of the population. The detection of allergen-specific IgE antibodies in serum is therefore mandatory to confirm the clinical diagnosis of IgE-mediated allergy. Currently used allergy diagnosis relies on allergen extracts which are prepared from allergen sources. Using such extracts for diagnosis it can be determined if a patient is sensitized to allergens in a given allergen source, but the disease-eliciting allergen molecules cannot be identified. During the last decade defined allergen molecules from several important allergen sources have been produced by recombinant DNA technology. Using recombinant allergens it is now possible to develop diagnostic tests which precisely determine the disease-eliciting allergen molecules and thus assist in choosing optimal forms of therapy. In this article it is described how diagnostic tests based on recombinant allergens may be used for the selection and monitoring of different forms of allergy therapy, especially of allergen-specific immunotherapy.

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Introduction

IgE antibody recognition of allergens is the central event in the pathogenesis of Type I allergy, a hypersensitivity disease with different facets (1). The immediate symptoms of Type I allergy (e.g., allergic rhinoconjunctivitis, acute asthma, urticaria, anaphylaxis) are due to allergen-mediated crosslinking of mast cell-bound IgE antibodies and the rapid release of biologically active mediators (e.g., histamine, leukotrienes) leading to acute inflammation (2). There is also increasing evidence that allergen-specific IgE antibodies may contribute considerably to the chronic manifestations of allergy (e.g., atopic dermatitis, chronic asthma) by mediating allergen presentation to T cells and, perhaps by inducing eosinophil activation (3-5). The firm diagnosis of IgE-mediated allergy requires therefore the detection of allergen-specific IgE antibodies in serum in addition to clinical documentation and provocation testing with allergens.

Based on confirmed diagnosis of IgE-mediated allergy three fundamental principles of treatment may be initiated. First, exposure to the offending allergen source should be limited if possible in order to suppress symptoms and further specific immune stimulation. Second, acute and chronic inflammation caused by allergen contact can be mitigated by pharmacological treatment. Third, allergen-specific immunotherapy may be prescribed aiming at a causative

therapy. It is aim of this article to illustrate how recombinant allergen-based diagnostics can assist the clinician in choosing optimal therapy strategies among available treatment forms (e.g., symptomatic treatment, allergen extract-based immunotherapy) and to monitor the immunological response of the applied therapeutic measures.

Does my patient suffer from IgE-mediated allergy?

Clinical symptoms and case history of allergic patients are in many cases indicative of IgE-mediated allergy but the demonstration of allergen-specific IgE antibodies in serum is mandatory to corroborate immunological involvement. For this purpose diagnostic screening tests are available which contain most of the important respiratory and food allergens (Figure 1). The detection of allergen-specific IgE antibodies using such screening tests confirms the diagnosis of IgE-mediated allergy. Negative results in the screening tests indicate a low likelihood of IgE-mediated allergy to prevalent allergen sources, although negative results may in rare cases be due to low-grade monosensitization to low-abundance allergens in the diagnostic test or due to extremely low levels of allergen-specific IgE in the serum (Figure 1).

Elevated levels of total serum IgE levels may be indicative of IgE-mediated allergies, but they can in principle be also increased for other reasons. Likewise it is possible that total IgE levels are not significantly increased but allergen-specific IgE antibodies can be detected. The determination of total IgE levels in serum is therefore not suited for the unambiguous diagnosis of IgE-mediated allergy. Since there is good evidence that in patients suffering from IgE-mediated allergies, most of the total serum IgE is directed against allergens (6), elevated total IgE levels may be indicative of a pronounced atopic disposition.

What allergen sources may be responsible for clinical symptoms in my patient?

In the case of a positive test result in assays screening for allergen-specific IgE antibodies, the next diagnostic steps may focus on the identification of allergen sources which are potentially harmful to the patient.

According to the patients clinical background, allergen extract-based tests can be employed to identify and verify sensitization to certain allergen-containing sources (e.g., birch pollen extract, grass pollen extract). Although extract-based testing can point out what allergen sources liberate potentially harmful allergenic molecules, these tests cannot identify the disease-eliciting allergen molecules (7). The detailed characterization of allergens by molecular biological techniques during the last decade has revealed the nature of many important allergens (8-16). It has become clear that simultaneous reactivity of a patient to several different allergen sources can be a result of co-sensitization to different allergen molecules which are present separately in each of these sources. However it may also be due to immunological cross-reactivity to similar allergen molecules that are present in the different allergen sources (17).

One example of such a cross-reactive allergen is profilin. This allergen was first discovered by the isolation of a cDNA coding for a cross-reactive birch pollen allergen, Bet v 2, and its identification as profilin (18). At that time profilins were known to be important components of the eukaryotic cytoskeleton (i.e., actin-binding proteins) and as elements in signal transduction, but they were not known in plants. Because of their highly conserved biological function, profilins from various organisms share sequence homology, interact with ligands from other organisms (e.g., actin) and have preserved their three-dimensional structure (19-23). Accordingly there is extensive cross-reactivity of allergic patients IgE antibodies with profilins from plants but also with profilins from other organisms. Profilins have meanwhile become well established as clinically relevant cross-reactive allergens in tree-, grass-, and weed pollens and they are also present at lower levels in somatic plant tissues (e.g., fruits, leaves, seeds, roots). Therefore, patients with IgE antibodies to profilin frequently suffer from manifest allergic reactions to pollens from various plants and plant-derived food or are at risk of mounting reactions to a multitude of profilin-containing allergen sources. Profilins may therefore be considered as diagnostic marker allergens for polysensitization to many allergen sources due to cross-reactivity (24, 25). Other allergens are produced exclusively in certain plant tissues (e.g., pollen, fruits or seeds) or in

certain plants. On the basis of the presence of these allergens in certain plants and plant tissues, and their degree of cross-reactivity, it is possible to use these allergens as highly specific markers to obtain additional important diagnostic information. For example, major group 1 and group 5 grass pollen allergens share no relevant epitope similarity with proteins outside the grasses and can therefore be considered as markers for a genuine grass pollen sensitization (24, 25).

Recombinant marker allergens may therefore be used as gatekeepers to select optimal therapy strategies and especially to decide whether a patient may be suited for allergen-specific immunotherapy (24, 25).

Recombinant allergens to establish the patients IgE reactivity profile

More than eighty years before the discovery of IgE antibodies, Charles Blackley performed allergy provocation tests with allergen extracts (26). Until now crude allergen extracts have been used for the diagnosis of Type I allergy. While it is possible to use such allergen extract-based diagnostic tests to define allergen-containing sources they cannot identify the disease-eliciting allergen molecules (7). With the introduction of molecular biological techniques into the field of allergen characterization the identities of many important allergens have been revealed and a great number of recombinant allergens assembling the epitope complexity of natural allergens have become available (8-10). These recombinant allergens can be used to improve allergy diagnosis in several aspects. First, it is possible to use combinations of recombinant allergens which represent the sum of relevant epitopes of certain allergen sources (e.g., tree pollen, grass pollen, mites, animal dander, moulds, venoms) to obtain diagnostic tests which contain precisely controlled amounts of the individual allergens for diagnostic screening (7, 9, 11-16). It has also been shown that spiking of natural allergen extracts with recombinant allergens that are not well represented in allergen extracts can improve the sensitivity of the extract-based diagnostic tests (27). Second, it is possible to design diagnostic tests based on single recombinant allergens, allowing the precise quantitation of component-specific IgE antibodies which may have a particular marker value (7, 28). Third, and perhaps most important, purified recombinant allergen components from various allergen sources may be assembled in the form of a multiallergen test (e.g., microarrayed allergen molecules: allergen chips) for complex IgE reactivity profile determinations using minute amounts of serum in a single and rapid assay (29).

The use of purified allergen molecules for diagnostic testing has been termed component-resolved allergy diagnosis (CRD) (7). Using CRD it is possible to identify precisely the disease-eliciting allergens for each patient and thus to group patients in those who are sensitized against few allergen molecules (i.e., oligosensitized patients) and those who are polysensitized against a great variety of immunologically unrelated allergen molecules. The latter group could not be precisely identified with allergen extracts because cross-sensitization to highly cross-reactive allergens might be erroneously interpreted as co-sensitization to many different allergens and can lead to the incorrect diagnosis

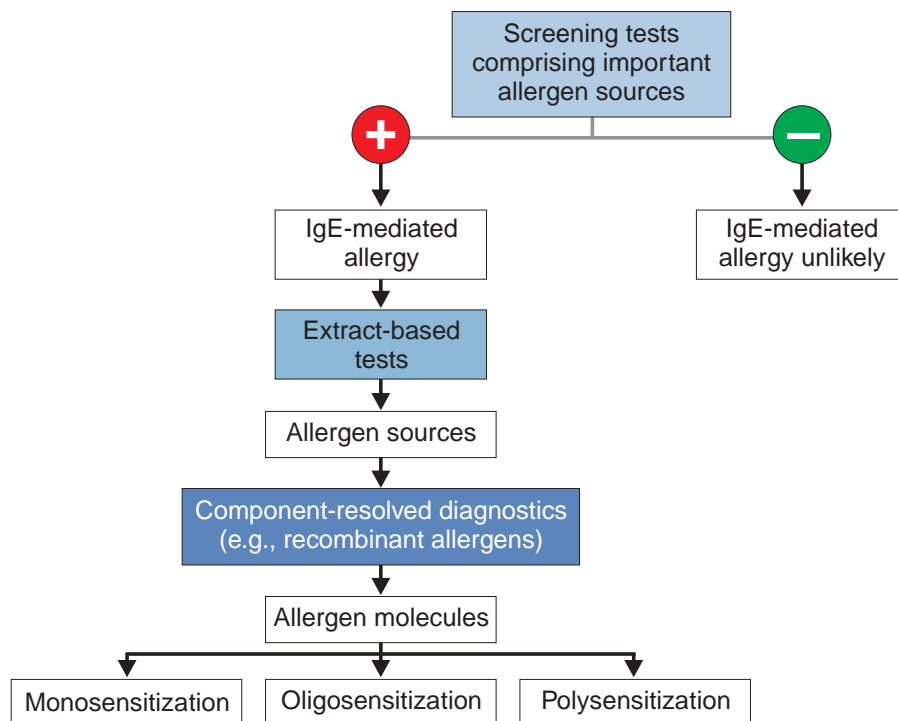


Fig. 1. Serological tests for allergy diagnosis. Prerequisites for correct allergy treatment.

of polysensitization (17). Using component-resolved diagnostics it is now possible to determine whether patients are truly monosensitized, oligosensitized or polysensitized (Figure 1).

Should my patient receive pharmacotherapy or allergen-specific immunotherapy?

Allergen-specific immunotherapy is perhaps the only causative form of allergy treatment which has long term clinical efficacy and may prevent the progression towards more severe symptoms (30, 31). On the other hand it represents a very time consuming, expensive and potentially hazardous form of therapy because allergic side effects are possible during treatment. For the latter reasons it is obvious that the decision to treat a patients by allergen-specific immunotherapy must require thorough consideration of all relevant aspects. These include clinical criteria regarding suitability for specific immunotherapy as outlined in the WHO position paper on allergen-specific immunotherapy (30). While not yet proven by extensive investigations, clinical experience indicates that allergen-specific immunotherapy may be less suited for patients who are polysensitized against a great variety of different allergens. Also the WHO position paper on specific immunotherapy recommends against treatment with mixtures of several unrelated allergen extracts and instead such patients should be considered for pharmacological treatment in addition to allergen avoidance. Component-resolved allergy diagnosis (CRD) with a panel of recombinant allergens comprising major respiratory and food allergens may help to discriminate true polysensitization from cross-sensitization. It should thus be

considered to include CRD in the diagnostic procedure when the decision for or against allergen-specific immunotherapy has to be made.

Diagnostic tests which may assist in determining the suitability for allergen-specific immunotherapy

Before allergen-specific immunotherapy can be considered, the clinical guidelines and considerations set in the WHO position paper on allergen-specific immunotherapy should be met (30). It is also recommended to confirm the diagnosis of IgE-mediated allergy against a particular allergen source by the measurement of IgE antibodies against allergen extracts prepared from this source. More detailed information regarding suitability for immunotherapy may be obtained by testing with component-resolved diagnostics containing recombinant marker allergens (24, 25). The principle of such gatekeeping tests is that they measure whether a patient is sensitized against the major allergens in a given allergen source and/or against cross-reactive, minor allergens. Since allergen extracts currently used for specific immunotherapy are standardized regarding their content of major, but not minor allergen components, it is possible or even likely that patients who are sensitized against minor allergens may benefit less from immunotherapy than patients allergic to the major allergens. In addition, patients reacting with cross-reactive minor allergens may present clinical symptoms of polysensitization.

Based on the gatekeeping concept it is thus possible to design diagnostic tests which contain major, often relatively

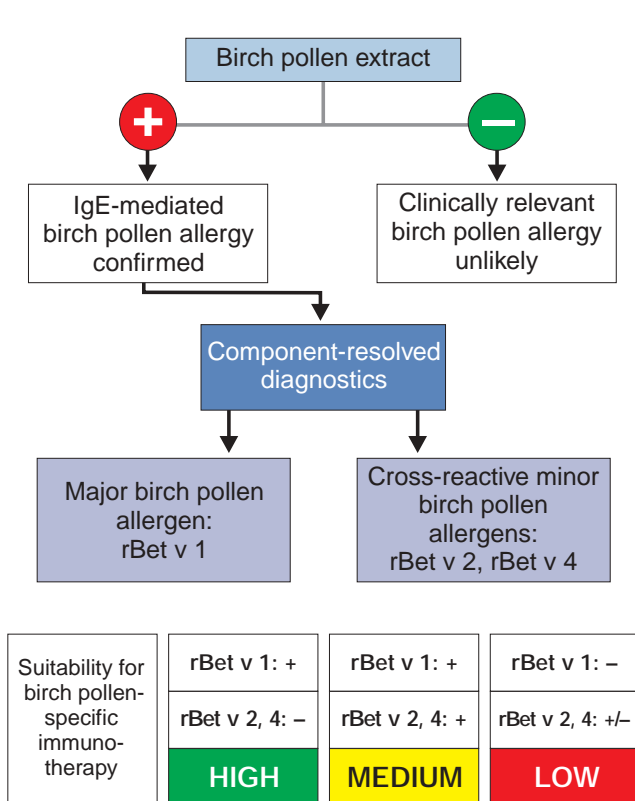


Fig. 2. A possible decision tree for the treatment of birch pollen allergy

source-specific, marker allergens and ones which contain minor, cross-reactive allergens. These tests can then be used to estimate the potential suitability of a patient for specific immunotherapy (24, 25).

Figures 2 and 3 show proposed decision trees for assessing the suitability of birch and grass pollen allergic patients for specific immunotherapy, with birch and grass pollen extract, respectively.

Patients fulfilling the clinical criteria for allergen-specific immunotherapy may be further subjected to serological testing with birch and grass pollen extract to confirm the diagnosis of IgE-mediated allergy. As a next step, component-resolved diagnosis (CRD) with two recombinant allergen-based tests may be performed. In the case of birch pollen allergy these tests include one test containing the major birch pollen allergen, Bet v 1 (32), and a second test measuring IgE specific for cross-reactive minor birch pollen allergens, rBet v 2 (18) and rBet v 4 (33, 34) (Figure 2). According to the arguments outlined above, patients containing IgE antibodies specific for the major birch pollen allergen, Bet v 1, are considered ideally suited for birch pollen extract-based immunotherapy (24, 25) (Figure 2: green). Patients who in addition to Bet v 1 are positive in the rBet v 2/rBet v 4 test are considered less suitable for that treatment (Figure 2: yellow). Those patients who are negative in the Bet v 1 test are clearly not suitable for birch pollen immunotherapy regardless whether they are positive in the rBet v 2/rBet v 4 test or not (Figure 2: red)

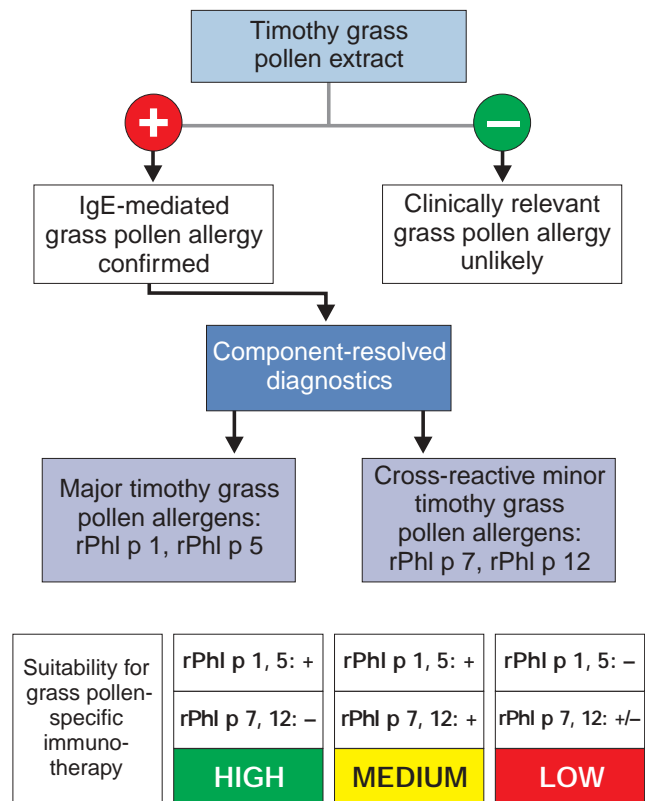


Fig. 3. A possible decision tree for the treatment of grass pollen allergy

A similar principle can be applied for grass pollen allergy. Diagnostic tests containing two major, grass pollen-specific marker allergens (rPhl p 1, rPhl p 5) (35, 36) and two minor, cross-reactive grass pollen allergens (rPhl p 7, rPhl p 12) (37, 38) are employed as diagnostic gatekeepers. As recommended for birch pollen allergy, patients who are sensitized against the major grass pollen allergens (rPhl p 1, rPhl p 5) are considered best suited for grass pollen-specific immunotherapy (Figure 3: green) whereas patients who are also positive in the rPhl p 7/rPhl p 12 test appear less suitable for that treatment (24, 25). Patients who are not positive in the rPhl p 1/rPhl p 5 test are not suitable for grass pollen-specific immunotherapy, regardless of their rPhl p 7/rPhl p 12 sensitization status.

The gatekeeping concept can also be applied for estimating the suitability of Parietaria pollen allergic patients for specific immunotherapy. Patients containing specific IgE antibodies to the major allergen Par j 2 are very likely genuinely sensitized to Parietaria pollen while individuals reactive to Parietaria pollen extract but not to Par j 2 may well be sensitized to pollen other than Parietaria. Thus, specific IgE to Par j 2 would be a strong indicator for the choice of Parietaria pollen extract for specific immunotherapy (39).

Monitoring IgG and IgE responses during allergen-specific immunotherapy.

The beneficial role of allergen-specific IgG antibodies induced in the course of specific immunotherapy has been demonstrated in classical experiments more than 60 years ago (40, 41). Due to the observation that clinical success of specific immunotherapy is not always associated with the induction of allergen-specific IgG antibodies (42), it has been questioned whether the induction of blocking allergen-specific IgG antibodies represents the major immunological mechanism behind successful immunotherapy. Alternative mechanisms have therefore been discussed (43). Several recent studies indicate the beneficial role of allergen-specific blocking IgG antibodies but the controversy regarding their contribution to therapy success has not yet been settled (44). There is however little doubt that the immunogenicity of an allergy vaccine can be estimated by the induction of allergen-specific IgG antibodies in the course of treatment, whether or not this is a mechanistically involved or surrogate marker. Accordingly, it is suggested to assess the response to specific immunotherapy by measuring the amounts allergen-specific IgG antibodies in the course of and after treatment (Figure 4). The recombinant allergen-based tests described for birch (rBet v 1; rBet v 2/rBet v 4) and grass pollen (rPhl p 1/rPhl p 5; rPhl p 7/rPhl p 12) allergy may thus also be used to determine the immunogenicity of the administered allergy vaccines by measuring specific IgG responses. An induction of IgG antibodies against these allergens indicates that an allergen-specific immunological response of potentially protective nature has been induced in the patient (Figure 4). Such responses cannot be unambiguously measured with allergen-extract-based diagnostic tests because the extract-based tests contain mixtures of allergenic and non-allergenic components.

Due to the application of purified allergen molecules to the monitoring of humoral immune responses during immunotherapy, a potentially important problem has recently been recognized (45). Several studies report the induction of IgE antibodies against new allergens in the course of immunotherapy with allergen extracts (46-49). One recent study reported that in more than 60% of birch pollen allergic patients receiving immunotherapy with a commercial birch pollen extract, IgE antibodies of new specificities appeared during treatment, including reactivities to the cross-reactive minor birch pollen allergens Bet v 2 and Bet v 4 (49). Although the clinical relevance of the newly induced IgE specificities has not yet been assessed by provocation testing, the induction of IgE antibodies against highly cross-reactive allergens (Bet v 2/Bet v 4; Phl p 7/Phl p 12) may be harmful to the patient. It may be envisaged that patients developing IgE specificities against cross-reactive allergens may start to become sensitized against additional allergen sources and may thus considerably broaden their sensitization spectrum. For example it is possible that a patient with exclusive birch pollen allergy (only Bet v 1 sensitized), developing new IgE antibodies against Bet v 2 or Bet v 4, may become allergic to grass and weed pollen. It is thus suggested to use the recombinant marker allergen tests containing cross-reactive allergen (i.e., rBet v 2/rBet v 4; rPhl p 7/rPhl p 12) to monitor patients in the course of specific immunotherapy in order to detect the possible induction of new IgE specificities and a potentially associated broadening of clinical sensitization.

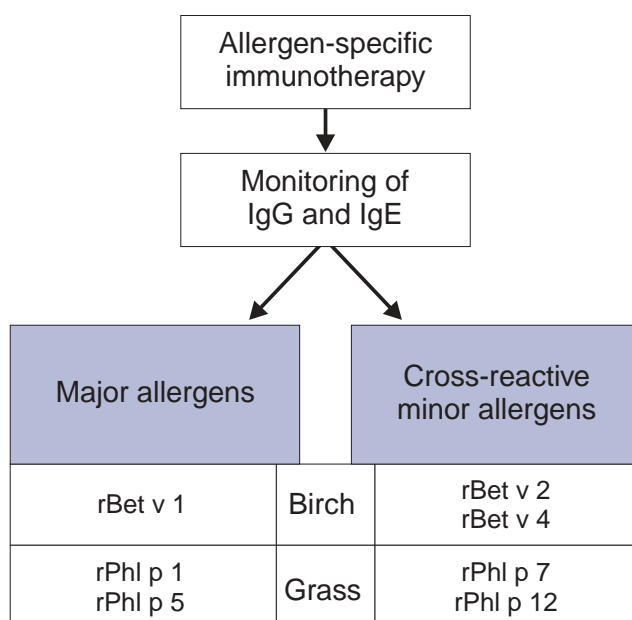


Fig. 4. Recombinant allergen-based diagnostic tests for the monitoring of birch pollen and grass pollen allergen-specific immunotherapy

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