

Recombinant allergens: the future of the diagnosis and treatment of atopic allergy

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In the past 10 years, recombinant DNA technologies have provided a large number of cDNAs coding for atopic allergens, and, by high-level expression systems, have yielded many important recombinant allergens. As a result of extensive work in the field of molecular and functional characterization, a remarkable panel of recombinant allergens has now become available, offering tools for the diagnosis and therapy of atopic allergies (1–4).

Successful cloning and sequencing of allergen-encoding cDNAs led to the determination of amino-acid sequences and allowed us to compare these sequences with those of already described proteins with known functions (5–7). Significant sequence similarities/identities with described proteins or protein families have been discovered. The attractive assumption that the allergens possess functions similar to those described for the homologous proteins introduced new ideas as to why a biochemical or biologic function predisposed a protein to become an allergen. As first suggested by Stewart et al. (8), the proteolytic activity of the major mite allergens Der p 1 and Der f 1 may be crucial for the penetration of mucous membranes by these molecules, a prerequisite for allergic sensitization.

A great advantage of these recombinant molecules is that they can be produced in unlimited

quantity at high quality. There are no differences in quality, as is usually observed with natural extracts (which show great batch-to-batch variation). Because of structural and immunologic similarities among allergens, it turned out that it might be possible to define a reasonable number of allergens for diagnostic purposes. Indeed, it was shown that allergy diagnosis could be performed with a few recombinant tree (9) and grass-pollen (10) allergens *in vitro* as well as *in vivo*. This seems to be also the case for mite, mold, cockroach, pet, and food allergies. The sensitivity and specificity of diagnostic tests are significantly improving with the use of recombinant allergens. It is much more desirable to use a few pure allergens in solid- or fluid-phase assays or provocation tests than natural extracts with 50 or more different allergens and nonallergenic material in various undefined concentrations. The diagnosis of allergic conditions with recombinant allergens is becoming much more reliable, because some natural allergens usually degrade rapidly during extraction or storage (e.g., apple) and are then not available. Other natural allergens may exist only in very low concentrations in the extract (e.g., cypress-pollen allergens) or may be destroyed because of the enzymatic activity present in the extract.

However, there may be also some disadvantages connected with the use of recombinant allergens in

diagnostic tests. It has been reported that some recombinant allergens show less IgE binding than those of the corresponding natural allergens (11, 12). This is quite obvious because of the existence of many isoforms of allergens with different IgE-binding capacity. The finding is based on a few exchanges of amino acids, leading to variations in the folding of allergenic molecules. Therefore, in diagnostic tests, it is necessary to use only recombinant allergens which have been shown to possess the same immunologic characteristics as their natural counterparts. This has to be checked by several tests such as IgE-binding assays, histamine-release assays, skin tests, and other provocation tests. While diagnosis with allergen extracts is best performed to identify the allergen source to which a patient is sensitized, recombinant allergens allow the precise determination of the patient's individual IgE reactivity (allergogram) (13).

The use of allergens for specific immunotherapy (IT) has always been hampered by their allergenic activity, which is a desirable property for a sensitive diagnosis, but leads to allergic side-effects during treatment. In recent publications, the use of hypoallergenic isoforms of allergens has been proposed (12, 14–6). These isoforms differ only in a few amino acids, but they are differentially recognized by the human immune system. Some of these isoforms show strongly reduced allergenicity, because they obviously lack IgE epitopes but show good T-cell antigenicity, a prerequisite for tolerance induction (17). A similar effect is obtained when the conformation of an allergen is disrupted, as by producing recombinant nonanaphylactic fragments which cover all relevant T-cell epitopes (18). Clinical application of hypoallergenic recombinant molecules could modulate the allergic immune response at the level of T-helper cells as well as the induction of blocking antibodies with a substantially reduced risk of anaphylactic side-effects during treatment.

This paper reports on the possible use of recombinant allergens for the improvement of diagnosis and therapy of IgE-mediated allergies in the future.

Clinical application of recombinant allergens in diagnostic tests

Establishment and control of the patient's allergen profile

Recombinant allergens allow us to detect antibodies specific for single allergens in extracts. This makes it possible to find out how many patients react to a specific allergen. According to whether more or less than 50% of patients react to IgE antibodies against a single allergen, the definition of a "major" or "minor" allergen can be made (9,

10, 13). In addition, by the use of one single allergen, we can measure the quantity of specific antibodies. If high levels of specific IgE antibodies against certain allergens can be found, knowledge of the allergenic potential of proteins can be obtained. This can be very useful in determining why particulate materials distributed into the atmosphere by pollen-producing plants, fungi, mammals, arthropods, etc. and normal foodstuffs act as allergens. Furthermore, after we have established the pattern of IgE reactivity in the form of the individual allergogram of a patient (13, 19, 20), sensitization to new allergens or loss of reactivity to distinct allergens can be monitored. After a detailed diagnosis with recombinant allergens, a patient would no longer be defined as grass-pollen or mite allergic, but as sensitized, say, to groups I and V grass allergens and to Der p 2. If the patient's allergogram does not change over a certain time, it would be an ideal basis for component-specific IT. Finally, screening tests with a mixture of recombinant allergens could be performed in the same way as with natural allergen extracts, because of the well-known structural and immunologic properties of the recombinant material.

Follow-up studies of IgE/IgG reactivity to major and minor allergens during disease and treatment

IgE/IgG reactivities to well-defined recombinant allergens can be followed up during the course of disease and treatment. Contact with or avoidance of allergens may be established on the basis of increased or decreased antibody levels to single allergens. Such an analysis was not very successful in the past, since mixtures of many natural allergens provide only very limited answers. During IT, the levels of antibodies could easily be detected and the occurrence of antibodies with new specificity revealed. The influences and activities of specific IT and other forms of treatment could be more accurately followed up.

Allergen-specific monitoring of IgE/IgG antibodies against distinct allergens in childhood

Serologic screening and follow-up studies on antibody reactivities are important for understanding the mechanism of sensitization in children. Information on the first recognition of allergens and the amounts of specific antibodies in various classes and subclasses against these allergens during the first years of life seems to be of vital importance. Furthermore, clinical reactions caused by allergenic cross-reactivities may be explained on the basis of molecular mimicry with well-defined recombinant allergens.

Characterization of cross-reactivities based on the molecular structure of allergens

It is well documented that patients suffering from type I allergy to pollens frequently show adverse reactions to fruits and vegetables. Typically, the symptoms comprise local reactions of the mucosa of the upper aerodigestive tract (itching, inflammation, angioedema), but a considerable percentage of patients experience systemic symptoms (urticaria, asthma, anaphylactic shock). The clinical observations of the occurrence of these reactions led to the assumption that structural similarities between the respective allergens might be responsible for manifestations of food hypersensitivity. Cross-reactivity has been demonstrated for birch pollen with apples, nuts, pears, carrots, and kiwi fruit (21–24) as well as for mugwort with celery, fennel, parsley, and spices (25–28). Although an array of data was published during the years 1970–90 on the immunologic relationship of allergens derived from pollens of trees, grasses, and weeds, only a little information was available on the molecules responsible for hypersensitivity reactions to fruits, vegetables, and spices in pollen-allergic patients. In recent years, the molecular characterization of most common allergens of pollens and foods has made it possible to identify cross-reactive IgE-binding proteins. In this respect, the families of the Bet v 1- and Bet v 2 (profilin)-homologs and an until recently not completely identified allergen cluster of 46–60 kDa have been described (29–33). These three classes of allergenic molecules constitute useful tools in diagnostic procedures, as most vegetable allergy can be detected by a combination of these allergens. Further information about cross-reacting allergens is needed to advise pollen-allergic patients fully of the risk of a possibly dangerous reaction to food.

Allergen-specific IT: concepts of recombinant allergens

Specific IT of IgE-mediated allergies is based on the application of increasing doses of the allergens to which the patient is sensitized. This can be done efficiently by subcutaneous or sublingual administration. In the past and also currently, IT was and is performed with allergen extracts which contain mixtures of allergens as well as nonallergenic material. Although the operating mechanisms of specific IT are not yet completely understood, the basic intention is to reduce allergic reactions to those allergens which are recognized by the patients. However, presentation of allergens that are not recognized by the patient and of other proteins may lead to further sensitization and worsening of

the disease. Therefore, improvement of the current forms of IT should involve only those allergens against which substantial amounts of IgE antibodies are present. This concept can be achieved, since investigations of the patients' IgE reactivity surprisingly showed that only a limited number of allergens are responsible for IgE-mediated reactions (34). IT could, therefore, be performed with selected recombinant allergens. Recent data suggest that the key mechanism of conventional IT is to alter T-cell function, either by diminishing production of both Th1- and Th2-like cytokines (tolerance induction), or by inducing "immune deviation" from a Th2-like to a Th1-like pattern. To increase the dose of allergens which can be applied to the patient with reduced anaphylactic side-effects, the use of nonanaphylactic derivatives has been suggested. Indeed, isoforms of allergens may be found in nature or be artificially produced. Such isoforms have shown no or very low IgE-binding capacity but do possess strong T-cell-stimulating capacity. In our laboratories, we developed the following three approaches to the production of new molecules for the treatment of birch-pollen allergy:

- 1) The production of Bet v 1d, an isoform of the major birch-pollen allergen Bet v 1, which can naturally be found in birch pollen and combines the highest scores of T-cell proliferative response with a very low IgE-binding capacity (12).
- 2) The production of recombinant Bet v 1 variants by site-directed mutagenesis. A Bet v 1a variant mutated in six amino-acid positions showed extremely low IgE reactivity but conserved T-cell epitopes (16).
- 3) The production of two recombinant nonanaphylactic, but T-cell-epitope-containing fragments of Bet v 1a (18).

Approach 1. Regarding the assessment of Bet v 1d as a candidate for successful immunotherapy, several natural isoforms of Bet v 1 were checked for allergenic properties. Immunoblot experiments showed that the isoforms differ in their IgE-binding capacity and could be grouped as high, intermediate, and low IgE binders. These *in vitro* findings could be confirmed by skin tests. The low IgE binders were selected for detailed experiments concerning activation of peripheral blood mononuclear cells, Bet v 1-specific T-cell lines, and peptide-mapped Bet v 1-specific T-cell clones established from allergic patients. As a low IgE binder but one with high T-cell stimulatory capacity, Bet v 1d was finally selected. This isoform is also present in natural birch-pollen extracts, where it amounts to 10% of total natural Bet v 1. Since Bet v 1 binds IgE in >95% of

all birch-pollen-allergic patients (>65% react exclusively to this molecule), treatment with high doses of recombinant Bet v 1d should be able to modulate the quality of the T-helper-cell response in birch-pollen-allergic patients, but with a reduced risk of anaphylactic side-effects.

Approach 2. As a second approach, experiments by site-directed mutagenesis evaluated the contributions of individual amino-acid residues for IgE binding to Bet v 1. It was found that IgE binding to Bet v 1 depends on at least six amino-acid residues at positions 10, 30, 57, 112, 113, and 125. A Bet v 1a variant mutated in six amino-acid positions, which differed in two amino-acid residues from Bet v 1d, was chosen for immunoblot analysis, skin tests, and T-cell proliferation assays. This mutant displayed significantly decreased IgE-binding capacity, and its skin test reactivity was 1000-fold reduced. However, the molecule possessed conserved T-cell-activating capacity, which is necessary for immunomodulation. Such artificial allergen variants may also be used in the future as candidates for a safe form of specific immunotherapy.

Approach 3. In a third approach to reduce the anaphylactogenic potential of the major birch-pollen allergen Bet v 1, we tested whether disruption of the conformation of the molecule might abolish its IgE-binding capacity but simultaneously maintain relevant T-cell epitopes. Two nonanaphylactic fragments were produced by recombinant DNA technology. These two parts of Bet v 1a representing amino acids 1–74 and 75–160 were expressed in *E. coli*. The purified peptides had lost their IgE-binding capacity and anaphylactic potential as evaluated by immunoblotting, histamine-release assays, and skin prick testing of allergic patients. As shown by circular dichroism analysis, the spectra of the two Bet v 1a fragments showed the features characteristic of a random coil structure, which remained unchanged even after prolonged incubation of equimolar mixtures of the fragments. Cutting Bet v 1a into two halves would undoubtedly disrupt contact between secondary structure elements and strongly compromise protein stability. Our experiments show also that it is unlikely that the fragments would reconstitute the Bet v 1a conformation after application and cause anaphylactic reactions. Bet v 1a-specific human T-cell clones showed strong proliferation with at least one of the two fragments, indicating that all relevant T-cell epitopes were represented by the two derivatives.

Vaccination with nonanaphylactic derivatives as peptides, which target allergen-specific T-helper

cells, might lead to disease improvement by induction of tolerance or anergy of allergen-specific T cells or by “immune deviation”, i.e., switching of allergen-specific Th2 clones into Th1-like clones, which may then display reduced and/or altered cytokine expression. Further effects would come from the induction of blocking antibodies which can recognize the unfolded fragments as well as natural Bet v 1, as exemplified by mouse monoclonal antibodies (35). Induction of T-cell tolerance of a specific antigen has been demonstrated by injecting a single T-cell epitope deriving therefrom (36, 37). The two Bet v 1a portions approach comprises the relevant Bet v 1 T-cell epitopes. Therefore, specific IT with two fragments representing the whole allergen molecule, but without or with markedly reduced IgE allergenicity, appears to be a possible treatment of type I allergic reactions.

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