

Standardization of Allergen-Specific Immunotherapy Vaccines

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History of standardization

Specific allergy treatment (ie, specific immunotherapy or specific allergy vaccination) has been performed for almost a century since Noon [1] first described it in 1911. The discovery in 1967 of the IgE molecule [2,3] and the central role of IgE in allergy gradually led to a better understanding of the immunologic mechanisms, improved diagnostic tools, and a consolidation of the concept of specific allergy diagnosis and treatment. In the 1970s and 1980s, scientific methods were introduced in the standardization of allergen vaccines [4] and, in combination with improved documentation of the clinical benefits obtained using standardized vaccines, specific allergy treatment as a scientifically based, reproducible, and safe treatment for allergic disease was established.

The first international initiative on allergen standardization was the preparation of the Nordic Guidelines [5], which were based on the Danish Allergen Standardization 1976 program. These guidelines established the first regulatory demands for allergen vaccines. The guidelines introduced the biological unit (BU) based on skin testing for potency measures. In Europe, current regulations in agreement with the Nordic Guidelines recommend that each manufacturer produces an in-house reference preparation (IHRP) and use it for batch-to-batch control using scientifically based laboratory testing [6]. The significance of the major allergen content for biologic activity was recognized in the early 1990s, and is now established in the World Health Organization (WHO) recommendations [7] and the European pharmacopoeia [8].

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In the United States, the regulation of allergen extracts is much different from that of Europe. The Food and Drug Administration (FDA) issues common standards and assays for all manufacturers to use [9]. This system is more transparent but also more rigid.

This article describes important issues in the control of source materials and vaccine preparation as part of the European standardization of allergen vaccines, and also includes a discussion of vaccines that are based on recombinant allergens, which may appear on the market in the near future.

Standardization of allergen vaccines

Allergen vaccines are complex mixtures of antigenic components. They are produced through extraction of naturally occurring source materials that are known to vary considerably in composition, depending on time and place. Without intervention, this variation would be reflected in the final products.

Source materials

The source materials for allergy vaccines should represent the material to which humans are exposed, and selected with attention to the need for specificity and inclusion of all relevant allergens in sufficient amounts [10]. The collection of the source materials should be performed by qualified personnel, and reasonable measures must be used by the producer of allergenic vaccines to ensure that collector qualifications and collection procedures are appropriate for verifying the identity and quality of the source materials. Only specifically identified allergenic source materials that do not contain avoidable foreign substances should be used in the manufacture of allergenic vaccines. Means of identification and limits of foreign materials should meet established acceptance criteria for each source material. Processing and storage of source materials should prevent decomposition and ensure that no unintended substances, including microbial organisms, are introduced into the materials.

Strategy for standardization

The production process leading to allergen extracts is basically an aqueous extraction of source materials followed by purification and adjustment of potency. The nature of the active ingredient imposes several constraints on the selection of source materials and physicochemical conditions used during the extraction procedure. The process must neither denature the proteins/allergens nor significantly alter the composition, including the quantitative ratio among soluble components.

Standardization of allergen vaccines is complicated because of the complexity of the allergen vaccines, the allergen molecules, and their epitopes. Acquired

immune responses are driven by contact with epitopes, which are structural elements of the allergens (antigens). T-cell epitopes are linear fragments of the polypeptide chain, whereas B-cell epitopes (ie, antibody-binding epitopes) are sections of the surface structure present only in the native conformation of the allergen. T- and B-cell epitopes are apparently essential for effective initiation and stimulation of immune responses to immunotherapy.

The allergens themselves are complex mixtures of isoallergens and variants that differ in amino acid sequence. Some allergens are composed of two or more subunits, the association and dissociation of which affect IgE binding. In addition, partial denaturation or degradation, which may be imposed by physical or chemical conditions in the production process, is difficult to assess and may have a significant effect on the conformation of the allergens.

Another complicating aspect is the complexity of the immune responses of individual patients. Patients respond individually to allergen sources with respect to specificity and potency. Allergens are proteins, and all proteins are potential allergens. A *major allergen* is statistically defined as an allergen that is frequently recognized by serum IgE when a larger panel of patient sera is analyzed. Less frequent IgE-binding allergens (less than 50%) are termed *minor allergens* [11]. Furthermore, patients respond individually to B- and T-cell epitopes and hence to isoallergens and variants.

A major aspect of allergen vaccine standardization, therefore, is to ensure an adequate complexity in vaccine composition. Knowledge of all essential allergens is a precondition to ensure their presence in the final products.

The other important aspect of standardization is the control of the total allergenic potency. The total IgE-binding activity is intimately related to the content of major allergen [12], and control of the content of major allergen is essential for an optimal standardization procedure.

Various techniques are available to assess allergen vaccine complexity and potency. Most of these techniques use antibodies as reagents, adding another level of complexity to the standardization procedure. Human IgE and antibodies raised through immunization of animals are subject to natural variation and may change over time.

These problems are handled by the establishment of reference and control standards. International collaboration is necessary to ensure that manufacturers, control authorities, clinicians, and research laboratories worldwide refer to the same preparations when comparing the results of quality control studies and potency estimates for different allergen vaccines. Ideally, standards for reagents should also be established in international collaborations.

The establishment and use of international standards

In 1980 to 1981, a subcommittee under the International Union of Immunological Societies (IUIS) formulated guidelines for the establishment of international standards. The collaboration and joint authority of WHO was assumed to be essential for international acceptance. In the following years, the sub-

committee selected, characterized, and produced international standards from several allergenic sources, including *Ambrosia artemisiifolia* (short ragweed) [13], *Phleum pratense* (timothy grass) [14], *Dermatophagoides pteronyssinus* (house dust mite) [15], *Betula verrucosa* (birch) [16], and *Canis familiaris* (dog) [17]. Additional standards were planned but terminated prematurely because of lack of general acceptance.

Each of these standard reference vaccines has been thoroughly investigated in collaborative studies involving laboratories and clinics worldwide. The results of the characterization and comparison of several coded vaccines, which were made available by allergen manufacturers on a voluntary basis, and the selection of the international standards have been published and are available. Each international standard was produced in 3000 to 4000 lyophilized, glass-sealed ampules, which can be obtained from the National Institute of Biological Science and Control located in Herts, United Kingdom.

Recently, the WHO-IUIS Allergen Standardization Committee has taken an initiative, funded by the European Union, to develop certified reference materials (CRMs) based on purified natural and recombinant allergens (ie, the CREATE Project) [18].

Depending on the success of this initiative, the existence and availability of allergen CRMs will enable a major allergen content to be assigned in common units to the internal reference preparations that are being used in different laboratories of manufacturers, allergen research groups, or control authorities.

The establishment and use of in-house reference preparations

In an established production process, including control of raw material, batch-to-batch standardization is performed relative to an IHRP. The IHRP must be thoroughly characterized through in vitro laboratory methods to show an adequate complexity and appropriate content of relevant major allergens. The potency of the IHRP must be determined through in vivo methods, such as skin testing, and the content of major allergens must be determined in absolute amounts. Furthermore, the IHRP should be efficacious in clinical trials of specific allergy vaccination.

The IHRP serves as a blueprint of the allergy vaccine to be matched by every following batch in all aspects. Specific activities of the IHRP should be compared with international standards. Using this method, measures from different manufacturers can be compared and consistency in internal standardization can be achieved [19].

Batch-to-batch standardization

In the production of routine batches of allergen vaccines, assessments of the clinical effect on every batch are impossible to make. In practical standardization, the batches are compared with the IHRP through a combination of different in

vitro techniques to achieve a constant composition, content of major allergen, and potency. Proper performance of these controls ensures a constant clinical efficacy.

The standardization can be performed using the following three-step procedure:

1. Determination of allergen composition to ensure that all important allergens are present
2. Quantification of specific allergens to ensure that essential allergens are present in constant ratios
3. Quantification of the total allergenic activity to ensure that the overall potency of the vaccine is constant (in vivo or in vitro)

Methods for the assessment of allergen vaccine quality

The quality of an allergen extract is a measure of the composition complexity, including the concentration of each constituent. Having established a careful control of raw materials and a robust production process, a constant ratio among individual components can be achieved by quantifying only one or two components independently (ie, the major allergens).

The complexity of the composition of allergen extracts can be assessed using several techniques. These techniques are standard separation techniques in biochemistry and traditional immunochemistry. Crossed immunoelectrophoresis (CIE) [20] is a nondenaturing technique and is therefore well suited for batch-to-batch control. The technique is dependent on the availability of broadly reactive polyspecific rabbit antibodies, but yields information on the relative concentrations of several important antigens in a single experiment.

Quantification of specific allergens

Having determined an adequate complexity in composition, an allergen extract may theoretically still be deficient in the content of major allergen (Fig. 1).

The content of major allergens must be assessed independently, especially for allergen extracts used for allergy vaccination. The maintenance dose in effective allergy vaccination contains a well-defined amount of major allergen (ie, 5–20 μg , regardless of species [7]), and the major allergen content is therefore a usable measure relating vaccine potency and therapeutic effect.

The importance of controlling individual allergens in every batch has only been acknowledged by a few manufacturers of allergen vaccines, but the principle is gaining more weight among control authorities and clinicians. Allergen vaccine manufacturers currently have access to the published purification procedures of most major allergens, and the purified major allergens can be used for the production of antibodies for independent quantification, even in complex mixtures such as allergen vaccines. For this purpose, polyspecific or monospecific polyclonal rabbit antibodies or murine monoclonal antibodies are used most often.

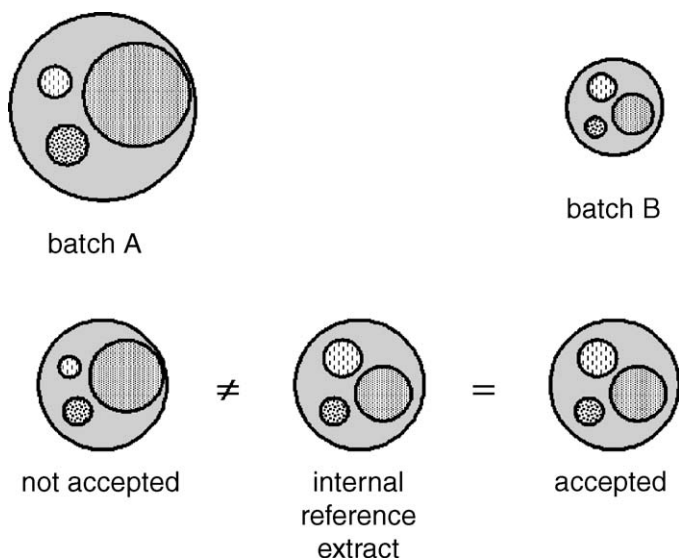


Fig. 1. Standardization of allergen extracts. Complexity of allergen extracts represented by a model with three major allergens. The area of shaded circles represents the relative potency of individual components. The area of outer circles represents the total allergenic potency of the extracts. The total allergenic potency of batch A and B may be adjusted through dilution or concentration, but the composition of the extracts may still vary, emphasizing the significance of the measurement of individual components.

Allergen vaccine potency

The potency of an allergen extract is the total allergen activity (ie, the sum of the contribution to allergenic activity from any individual IgE molecule specific for any epitope on any molecule in the allergen extract). Potency measures will therefore always depend on the serum pool or patient panel selected and the methodology used.

Methods used for the assessment of allergen vaccine potency may be divided into either *in vitro* or *in vivo* techniques.

The dominating *in vitro* technique for estimating relative allergenic potency is radioallergosorbent test inhibition [21] or related methods. Other techniques include enzyme-linked immunosorbent assay [22] and histamine release from washed human leukocytes [23]. Because histamine release tests are dependent on freshly drawn blood samples from a panel of individuals who have allergies, their practical application in routine determination of allergen extract potency is diminished.

Direct skin testing of human allergic subjects has been the predominant *in vivo* method for assessing allergen extract potency [24]. For ethical reasons, *in vivo* testing in humans cannot be used as a routine assay for batch release in production. However, through suitable *in vitro* methods, production batches

may be compared with internal reference vaccines, the *in vivo* activity of which has been established. The patient selection criteria are important because all *in vivo* methods are dependent on the patient panel.

Skin testing in humans is the principle underlying the establishment of biologic units of allergen vaccine potency. Several units are in use.

In Europe, the potency unit is based on the dose of allergen vaccine resulting in a weal comparable in size with the weal produced through administration of a given concentration of histamine. This unit was originally called *histamine equivalent potency* (HEP), and is now expressed as a BU according to the Nordic Guidelines [5].

Determination of clinical efficacy

The potency of allergen vaccines used for specific allergy vaccination ideally should be expressed in units describing clinical efficacy rather than a skin-testing effect. In the United States and Europe, and expressed through the WHO, approaches to relate vaccine potency and clinical efficacy have been performed. For several standardized vaccines, the clinical effect was determined after extensive clinical trials, and the mean maintenance dose of the vaccine was used to quantify it.

However, determinations of clinical effect are extremely laborious. They can only be performed using highly standardized vaccines, which have been described in detail with respect to composition and *in vitro* and *in vivo* potency (eg, IHRPs).

Chemically modified allergen vaccines

Chemical modification of allergen extracts is based on the observation that successful allergy vaccination is accompanied by an increase in allergen-specific IgG. Thus, modifying the allergen to reduce allergenic reactivity (ie, through IgE binding) while preserving immunogenicity would theoretically allow higher doses to be administered without risk for systemic reactions.

Formaldehyde was used for vaccine development in detoxification of bacterial toxins until 1970 when Marsh and coworkers [25] applied formaldehyde treatment of allergens for allergy vaccination. The allergens are incubated with formaldehyde yielding the so-called “allergoids,” high molecular weight covalently coupled allergen complexes. Compounds with similar immunologic properties can be produced using glutaraldehyde instead of formaldehyde. The chemical modification of individual amino acids inactivates B- and T-cell epitopes irreversibly, however, and this effect seems to dilute epitopes and hence decrease allergenicity and immunogenicity, explaining why higher doses of allergoid are needed to achieve clinical efficacy.

Contrary to expectation, chemical modification using formaldehyde does not increase safety in practical allergy vaccination, as documented in a report from

the German Federal Agency for Sera and Vaccines that analyzed all reported adverse reactions to allergen vaccines during a 10-year period from 1991 to 2000, including 555 life-threatening, nonfatal events [26].

Another approach is based on allergens chemically coupled to biodegradable polymers, such as methoxypolyethylene glycol or a copolymer of D-glutamic acid and D-lysine, or other nonimmunogenic polymers. From mouse experiments, such compounds were expected to suppress IgE biosynthesis in humans [27]. However, clinical studies in humans were discouraging. An aspect of this approach is the use of extremely high doses of these compounds, rendering their clinical use in humans problematic.

Most of the techniques used to characterize and standardize allergen vaccines are not applicable to modified allergen vaccines. Experts therefore recommend that standardization be completed using the intermediate allergen preparation before modification, and that the reproducibility of the modification process be documented through methods specific to the procedure in question.

Standardization and allergy vaccination in Europe and the United States

Regulations of standardization of allergy vaccines in Europe according to the European Pharmacopoeia are remarkably different from those in the United States according to the FDA. In Europe, allergy vaccine consistency is maintained primarily through the use of in-house standards and international references, whereas in the United States the FDA issues detailed standardization procedures and reagents to be used by all manufacturers. The advantage of the European system is that the doctor is able to choose from different products and manufacturers can continuously improve product quality, documentation, and development. The American system, on the other hand, has the advantage of being more conservative and resistant to new developments and having a higher degree of consistency among manufacturers.

Another difference between Europe and the United States is the allergy vaccine formulations. Aqueous vaccines are commonly used in the United States, whereas mostly alum-adsorbed vaccines, either chemically modified or native, are used in Europe. Theoretically, this practice should result in a higher frequency of anaphylactic side reactions in the United States compared with Europe. The reason why this does not occur can be ascribed to a third difference in current procedures. Common practice in the United States is to mix a cocktail of all relevant allergen sources when treating patients who have multiple allergies, thereby reducing the therapeutic dose and increasing the risk for proteolytic degradation. In Europe, the current practice is to treat the most severe allergy first or, if more than one treatment is conducted simultaneously, to distribute the injection sites between both arms of the patient, ensuring the optimal maintenance dose is reached. Retrospective studies have shown coherence between the optimal maintenance dose and the major allergen content in absolute amounts when comparing different allergen sources. This coherence has been acknowledged by the WHO, but unfortunately not by the FDA.

Formulation of allergen vaccines

Formulation for injection therapy

The efficacy of specific allergy vaccination is related to the dose of vaccine administered, but the inherent allergenic properties of the vaccine imply a risk for inducing anaphylactic side reactions. In traditional allergy vaccination, this conflict is handled by administering repeated injections over extended periods after an initial up-dosing period. Formulation of the vaccine can further reduce the risk for side effects. Most vaccines on the market are formulated through adsorption of the allergens to inorganic gels, such as aluminum hydroxide $[\text{Al}(\text{OH})_3]$, to attain a depot effect characterized by a slow release of the allergens.

Formulation with $\text{Al}(\text{OH})_3$ has long been used for vaccination in human and veterinary medicine [28]. The advantages are based on two characteristics of the complexes: the depot effect and the adjuvant effect. The allergens bind firmly but noncovalently to the inorganic complexes, causing slow release of the proteins and thereby lowering the concentration of allergen in the tissue and reducing the risk for systemic side effects. Given optimized formulation conditions, allergens seem to preserve their native configuration on association with $\text{Al}(\text{OH})_3$. Furthermore, the depot effect reduces the number of injections needed in the course of specific allergy vaccination. Although the significance of the adjuvant effect is unclear, higher levels of IgG antibodies have been observed when alum-adsorbed vaccines were used in specific allergy vaccination compared with aqueous vaccine [29]. Compared with aqueous vaccine, patients receiving depot preparations experience fewer side reactions [30].

Formulations for sublingual allergy vaccination

In sublingual allergy vaccination, the allergen vaccine is administered directly on the mucous membrane under the tongue, avoiding the use of needles and injections. Because of its high safety profile, virtually no anaphylactic events have been reported. Sublingual allergy vaccination is potentially useful in children, although clinical documentation in children should be improved. Vaccines used for sublingual vaccination are aqueous extracts, or 50% glycerol may be added to stabilize the allergens. The concept of sublingual therapy emerged as a practice among clinicians in the 1980s, and several studies addressing safety and efficacy were published in the early 1990s. The documentation of clinical efficacy among the studies is variable [31], likely because of the variability in protocols, treatment durations, extracts, and doses used. Allergen manufacturers have recently offered products that allow more convenient sublingual allergy vaccination, such as single-dose containers that improve uniform dosing and allow self-treatment.

The latest development in sublingual allergy vaccination is the formulation of allergic extracts as sublingual tablets. Compared with drop-based application, tablets offer the advantage of standardized delivery, increased contact with sub-

lingual mucosal surface, and more convenient handling. In a recently published safety study using a fast-dissolving tablet formulation, very high doses of grass pollen extract were tolerated without serious or systemic adverse events [32]. A dose corresponding to 15 µg of major allergen administered daily 10 weeks before and throughout the grass pollen season showed a 37% reduction in rhinoconjunctivitis symptom scores and a concomitant reduction of 41% in medication use compared with placebo [33]. Tablets were administered without up-dosing, and no serious side effects were observed. The most prominent side effects were mild oral pruritus, nasopharyngitis, and throat irritation. Self-administration of the fast-dissolving grass allergen tablet was safe and efficacious and holds great promise for the future.

Recombinant allergens

Recombinant allergens were first described in 1988, when Thomas and colleagues [34] described the cloning and expression of Der p 1 in *Escherichia coli*. Since then, recombinant allergens have been accommodated in research of allergens and mechanisms of the allergic immune response with great success. Today, most important allergens from various sources have been cloned and sequenced, yielding insight into molecular characteristics and biologic function. The IUIS Allergen Nomenclature Sub-committee [35] maintains an updated official list of allergens, accessible at www.allergen.org.

Early studies of clinical use showed that recombinant allergens or cocktails of a limited number of recombinant allergens were efficient for in vitro diagnosis showing the presence of specific IgE in blood samples, and the idea of using similar cocktails for allergy vaccination was obvious [36]. A breakthrough in clinical use of recombinant allergens for therapy, however, is still awaited, although clinical proof-of-concept studies are in progress and one study has been completed [37]. Manufacturers are hesitating to follow strategies based on recombinant allergens for multiple reasons, including comprehensive regulatory requirements for clinical documentation, large investments in production capacity, uncertainty of clinical benefits, and unsolved technical difficulties. Despite these obstacles, recombinant allergens still play a central role in future scenarios of specific allergy vaccination because they have significant advantages, including certain supply of homogenous allergens, precision in physical-chemical standardization, improved reproducibility, and a stable regulatory situation. Other areas of vaccinology (eg, infectious disease) have moved toward more well-defined vaccine components [38].

Recombinant allergens for allergy vaccination

Recombinant so-called “wild type” allergens are produced to resemble natural allergens in every detail. The term *wild type* was coined to distinguish these allergens from recombinant allergens that were deliberately modified to reduce

IgE-binding. Modified recombinant allergens are discussed below. This section discusses some difficulties and advantages of recombinant wild type allergens.

Although production of recombinant allergens identical to allergens from natural sources may be possible, the regulatory authorities will likely not recognize previous documentation based on allergens of natural origin. Market authorizations must therefore rely on comprehensive clinical safety and efficacy documentation developed using the recombinant product. On the other hand, the authorities are likely to welcome recombinant allergens because of the improved reproducibility of the products. Recombinant allergens represent chemical entities, the identity of which can be established through physical-chemical means and in absolute terms. Through peptide mapping combined with mass spectrometry, the entire amino acid sequence can be verified as batch-to-batch control. The uncertainties of traditional allergen standardization are thereby obviated.

How many and which allergens to include in a vaccine so as not to compromise efficacy compared with conventional extracts is important to determine. When mapping patient reactivity to individual allergens, a large heterogeneity is observed. Although most patients react to a limited number of major allergens, many minor allergens can be identified though screening large numbers of patients. Most of the techniques used, however, do not take quantitative aspects into consideration. Thus, even if a patient reacts to five different allergens in a grass pollen extract, for example, 99% of the IgE may theoretically be directed toward the major allergen. The observation that the recommended maintenance doses of different allergen vaccines contain similar quantities of major allergen (ie, 5–20 µg) shows the exceptional significance of the major allergens [7]. Therefore, a large portion of the clinical efficacy is likely mediated by the major allergens alone. A limited number of clinical studies comparing purified natural allergens with extracts seem to confirm this idea [39], although Østerballe [40] used a mixture of Phl p 5 and Phl p 6 in a grass pollen study, and not the optimal combination of Phl p 1 and Phl p 5.

Recombinant allergens should display the amino acid sequence of the natural allergen, or more precisely, one of its isoallergenic variants. Isoallergens and variants show differences in IgE-binding, and one variant that covers a broad range of IgE specificities directed toward the natural mixture of isoallergens should be selected. Any tags attached to the recombinant allergen for efficient expression or purification should be removed. Most of the recombinant allergens described in the literature are fusion proteins. A problem more difficult to assess is the conformation of the recombinant protein. An unfolded allergen is characterized by lack of (1) a stable, well-defined, three-dimensional structure, (2) antibody recognition, and (3) enzymatic activity. The folded state, on the other hand, displays full antibody-binding capacity and enzymatic activity showing authentic three-dimensional structure and a well-defined conformation. The binding of specific IgE and subsequent appearance of clinical symptoms of allergy are intimately connected with the three-dimensional folded structure of the allergen. The conformational nature of B-cell epitopes was shown by the crystal structural determination of the antibody-allergen complex Bet v 1–BV16 Fab [41].

Reduced activity of unfolded allergens with respect to IgE binding can therefore increase as a result of spontaneous or facilitated refolding when allergens are released in human tissues or fluids that have a risk for adverse systemic reactions. In many cases, the equilibrium between the folded and unfolded state is strongly shifted in favor of the folded state; however, handling and storage conditions (eg, lyophilization) of the purified protein, which pose a risk to denaturation, are important factors to control.

Modified recombinant allergens in allergy vaccination

Approaches to reduce the allergenicity of allergen vaccines have been attempted though disruption of the tertiary structure of allergen molecules using denatured [42] or degraded antigens or peptides [43]. However, these approaches have shown reduced efficacy in allergy vaccination compared with those involving native allergens.

The concept of using recombinant major allergens for specific allergy vaccination offers important opportunities for modifying the molecules through DNA recombinant technology to reduce IgE binding and potentially circumvent the inherent problem of current vaccines inducing IgE-mediated side effects. Different concepts have been proposed and are being pursued in laboratories worldwide [44]. Some concepts aim to completely disrupt the three-dimensional structure of the recombinant allergen, either through preventing the formation

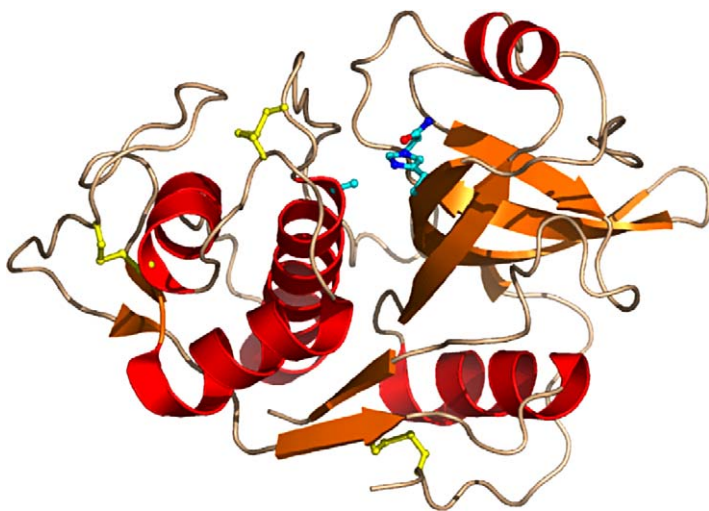


Fig. 2. Model of the crystal structure of mature Der p 1. The structure folds in two domains separated by the substrate cleft, which runs vertically down the center in the plane of the paper. Alpha-helices are colored red and beta-strands orange. The catalytic triad is highlighted as ball and stick model in light blue. (From Meno K, Thorsted PB, Ipsen H, et al. The crystal structure of recombinant proDer p 1, a major house dust mite proteolytic allergen. *J Immunol* 2005;175:3835–45; with permission.)

of disulphide bonds by substitution of the cysteines or by fragmenting the allergen molecule. The goal of other concepts is to modify IgE-binding conformational epitopes without a priori knowledge of the architecture of allergen molecular surface (eg, through gene shuffling) [45] or through determining the three-dimensional structure of the allergen (Figs. 2 and 3).

Once IgE-binding epitopes have been identified, antibody reactivity can be rationally modified by substituting critical amino acid residues on the molecular surface [46,47]. Such mutations may affect IgE reactivity but also may influence the stability and refolding properties of the mutated allergen. Thus, in the characterization of the mutated allergen, the immunochemical effects of the substitutions must be separated from the physicochemical effects. For this purpose, antibody-based assays are not always sufficient because detection often does not differentiate between unfolded protein and mutations aimed at decreasing specific serum IgE reactivity and antibody binding. Thus, with respect to the structural integrity of the mutated allergen, amino acid substitutions must always be analyzed through relevant assays and reagents.

One clinical trial of modified recombinant allergens has been performed. This trial included two concepts based on Bet v 1, the major allergen from birch pollen; a Bet v 1 molecule cleaved in two halves; and a trimer produced by consecutive connection of three Bet v 1 molecules [48]. Although the study reported clinical improvement, no underlying data are presented. Other concepts of modified recombinant allergens are currently in clinical trial or preclinical testing, and because of the fundamental differences among the concepts, the failure or success of one concept cannot be extrapolated to apply for others.

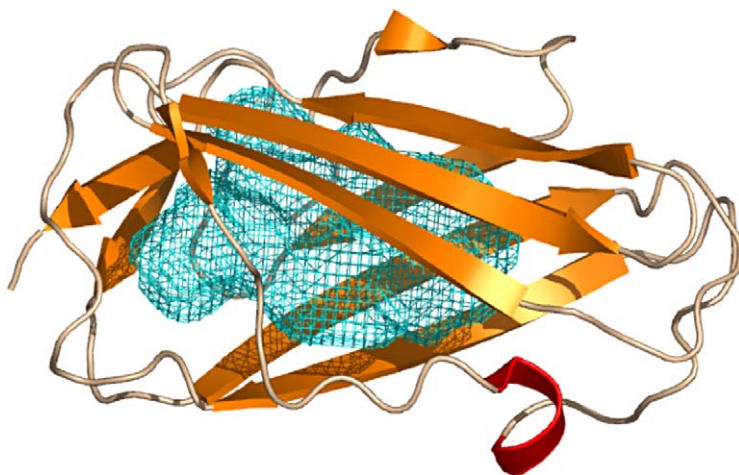


Fig. 3. Model of the crystal structure of Der f 2. The internal cavity is highlighted in light blue. Alpha-helices are colored red and beta-strands orange. (Data from Johannessen BR, Skov LK, Kastrup JS, et al. Structure of the house dust mite allergen Der f 2: implications for function and molecular basis of IgE cross-reactivity. FEBS Lett 2005;579:1208–12.)

Summary

The purpose of standardization is to minimize the qualitative and quantitative variation in the composition of the final products to obtain higher levels of safety, efficacy, accuracy, and simplicity in allergy diagnosis and allergy vaccination. The benefits of improved standardization of allergen vaccines for the clinician include easier differentiation between allergy and nonallergy, a more precise definition of the specificity and degree of allergy, and a more reliable and reproducible outcome of specific allergy vaccination.

Recombinant allergens, which are standardized in absolute terms through physical-chemical methods, will obviate the uncertainties of allergen standardization.

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