



Allergome: the characterization of allergens based on a 2D gel electrophoresis approach

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Type I hypersensitivity reactions are in constant progression in industrialized countries. The physiopathologic mechanism of these diseases implicates the production of specific immunoglobulin (Ig)E to allergenic molecules, their binding to the Fcε receptor on the surface of mast cells and basophils, and the release of inflammatory mediators when allergens are introduced into the body and crosslink with the IgE bound to the cell surface. An allergen is defined as a molecule that induces the production of, and binds to, IgE. The identification of the allergenic molecules is an important goal to improve diagnosis and treatment of allergy. This characterization aims to extract proteins from the allergenic source, to analyze IgE specificity by immunoblotting and to identify the proteins that bind IgE.

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For several years the prevalence of allergic diseases has been in constant progression in industrialized countries. Two categories of allergic manifestations have been distinguished based on their physiopathologic mechanisms and clinical symptoms.

The first category is related to the Type I (or immediate) hypersensitivity reactions of the Gell and Coombs classification [1], and include various diseases such as allergic asthma, seasonal rhinoconjunctivitis (hay fever or pollen allergy), food allergy or allergy to various substances such as hymenoptera venoms, house dust mites, drugs, latex and so on. All these reactions are characterized by a two-step development. The first, or sensitization, step is asymptomatic and corresponds to the production of antibodies of the immunoglobulin (Ig)E isotype directed towards the allergen. The second, or symptomatic, phase develops at each new contact with the allergen on a sensitized host. The introduction of the allergen induces an immediate inflammatory reaction that appears 15–30 min after the sensitive contact, and varies clinically from erythema, urticaria, edema, rhinoconjunctivitis, asthmatic symptoms or anaphylactic shock, according to the degree and the route of sensitization. The

immediate inflammatory reaction is due to the liberation of inflammatory mediators (e.g., histamine) resulting from the degranulation of mast cells and/or basophils, which is induced by the crosslinking of the specific IgE bound to the surface of the cells by the high-affinity Fcε receptor (FcεR)1. The development of a Type I hypersensitivity reaction requires both the presence of an allergenic substance and a sensitive host.

The second clinical entity is related to the Type IV (or delayed) hypersensitivity reactions of the Gell and Coombs classification. The typical clinical manifestation of delayed hypersensitivity is contact dermatitis that develops to small haptenic determinants such as those issued from cosmetics or metals. The reaction appears 48–72 h after contact with the sensitizing substance as a local erythema, more or less edematous and limited to the zone in contact with the inducing agent. The sensitizing substance activates a T-lymphocyte response specific to self peptides modified by the hapten, without antibody production. Specific CD4⁺ and CD8⁺ T-lymphocytes participate in the skin (or mucosa) inflammation by cytokine secretion and direct cytotoxicity of hapten-modified keratinocytes. If an allergen is defined as

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an antigen that induces the production of, and binds to, IgE, then Type IV hypersensitivity-inducing agents cannot be considered as true allergens, and will not be discussed in this review, which will focus only on Type I allergens.

The allergenic source of a Type I hypersensitivity reaction is first established by clinical and biologic tests performed according to the symptomatology and medical history of the patient. These tests are performed to determine the level of the skin reaction and/or the specificity of the IgE to a given source of allergens (i.e., latex, foods, various pollens, cats or dogs and house dust mites). However, the allergen extracts used are crude extracts constituted of a mixture of numerous molecules, some being allergens and others not. These extracts should be standardized to provide a constant result when tested *in vitro* with a reference serum. This means that a pool of serum from various individuals clinically considered as truly and strongly allergic to a given substance should serve as a reference to establish the efficacy (e.g., specificity and sensitivity) of an extract to diagnose a given allergy.

The second step of the allergenic source analysis is to characterize the allergens it contains at a molecular level. This characterization requires both the identification of the molecules themselves (based mostly on a proteomic analysis) and their capacity to induce the production of, and binding to, IgE. The allergome can be defined for a given source as the repertoire of the molecules recognized by IgE from the patients allergic to this source. However, a given individual recognizes only a part

of this repertoire depending upon their own genetic background and allergenic exposure. The knowledge of the allergenic repertoire of a complex source has two important goals:

- Improve the diagnosis by obtaining the most relevant allergen mixture for clinical and biologic tests
- Prove and predict crossreactivity between various sources that express analogous molecules sharing some common epitopes

The knowledge of an individual allergen repertoire can be required to determine the best mixture to use for the vaccination or hyposensitization of each patient.

This review will focus on:

- General methodology to characterize the allergen repertoire of a given source
- Variability of this repertoire from one patient to another, and the diversity of the individual isotypic antibody response
- Variability of the allergens according to the state of the source (e.g., natural vs. manufactured products, cooked vs. raw food and polluted particles)
- Future prospects linked to the improvement of allergome determination

General methodology of allergen characterization

The characterization of the allergens from a given source follows a general scheme that includes: the extraction of the proteins

(FIGURE 1 – step 1); the determination of IgE binding (step 2); and the identification of the molecules that bind to IgE (step 3). Each step of this procedure influences the final result of the allergen repertoire determination. A previous report describes the various methodologies used for the proteomic study of allergens, and this report will illustrate the strategy that the authors used, along with its limitations and advantages [2].

Since the most powerful method available today to study a complex allergen repertoire is based on 2D gel electrophoresis and its immunoblotting, the procedures for allergen extraction must be compatible with this method. 2D gel electrophoresis is a combination of two major electrophoretic separation methods in gel: isoelectric focusing (IEF) followed orthogonally by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). Isoelectric focusing is very sensitive to the salt content of the sample to be studied. Ideally, the sample should contain no salt at all as this salt will be electrolyzed into two ions of opposite charges and will be very quickly cleared from the gel as soon as an electric field is applied to that gel.

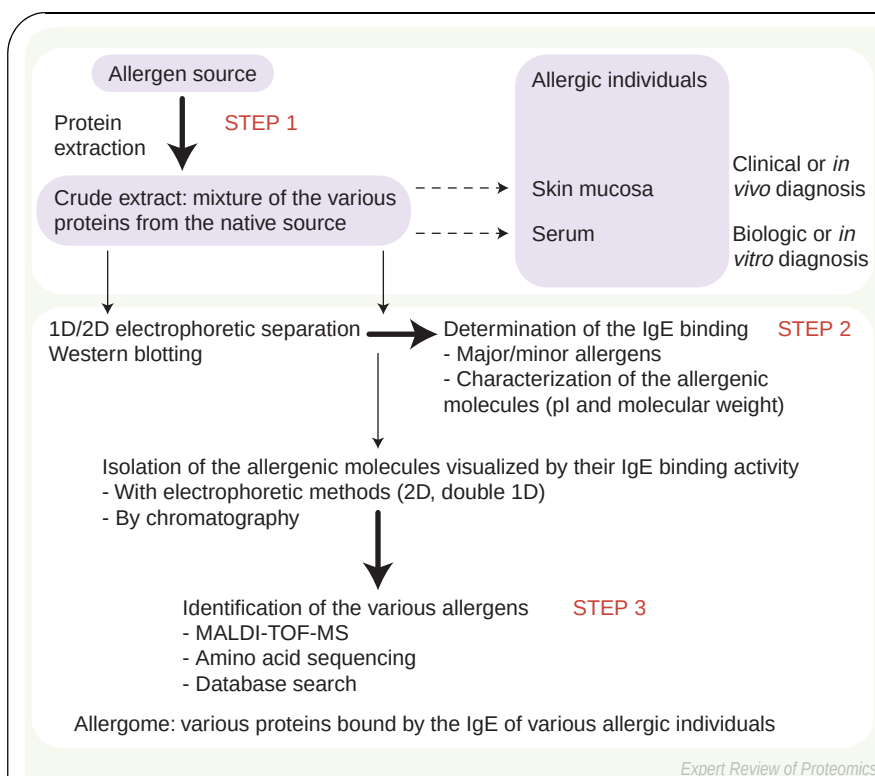


Figure 1. General scheme of the methodology used to determine the allergome of a given source.

MALDI: Matrix-assisted laser desorption/ionization; MS: Mass spectrometry; pI: Isoelectric point; TOF: Time-of-flight.

This means that many proteins will not be soluble in a gel made of a slightly hydrophobic matrix (polyacrylamide) in distilled water and only 2% of ampholytes. The usual method to improve the solubility of proteins in an IEF gel is to add uncharged detergent to prevent hydrophobic interactions or/and chaotropic agents to prevent hydrogen bond formations. To date, the best mixture used to solubilize proteins that is compatible with an IEF in polyacrylamide gel is thiourea 2 M, urea 7 M and a detergent [3-[(3-chloroamidopropyl)dimethylammonio]-1-propanesulfonate] (CHAPS) at 2–5% (or thiourea–urea–CHAPS [TUC]). Thus, one can extract water-soluble allergens in distilled water from a complex source and then re-extract from the remaining water-insoluble part those allergens that are soluble in TUC. A good example is given by wheat flour, where albumins and globulins are water soluble, while the remaining part is known to be gluten. Part of the gluten containing gliadins and glutenins can be solubilized in TUC. What is still not clearly standardized in the allergen extraction procedures is their physical and mainly mechanical portion: how long and how strong should that step be? Finally, it should be kept in mind that such physicochemical treatment of a complex allergenic source may modify the structural integrity of some allergenic molecules they contain. The less the allergens are modified, the better they are detected. For example, the use of reducing agents, even if they improve the quality of the 2D gel separation, should be avoided as they may alter the allergenic molecules.

The second step of allergen characterization is the determination of the IgE-binding capacity of each molecule. This key step requires the selection and analysis of human sera from allergic individuals. The selection of the sera is firstly achieved by the determination of the IgE titer of each consulting patient using commercial tests based on immunosorbent assays (for review see [3]). The result of the assay is expressed as a value of kilo International Units of allergen-specific IgE per liter of serum (1 kIU/l = 2.4 ng IgE/ml of serum), corresponding to a class that varies from class 0 (no IgE) to class 6 (≥ 100 kIU/l of IgE). These tests evaluate the IgE reactivity to the allergenic molecules of a complex mixture of numerous proteins. The molecular specificity of the IgE can be determined using immunoblotting techniques. The authors' strategy is to screen a large number of sera with 1D blots, select the sera of interest, and use blots from 2D gel electrophoresis to identify the proteins recognized by the selected sera. The 1D separation is performed by IEF (FIGURE 2) and/or SDS-PAGE (FIGURE 3), and is followed by western blotting, incubation with sera from allergic individuals

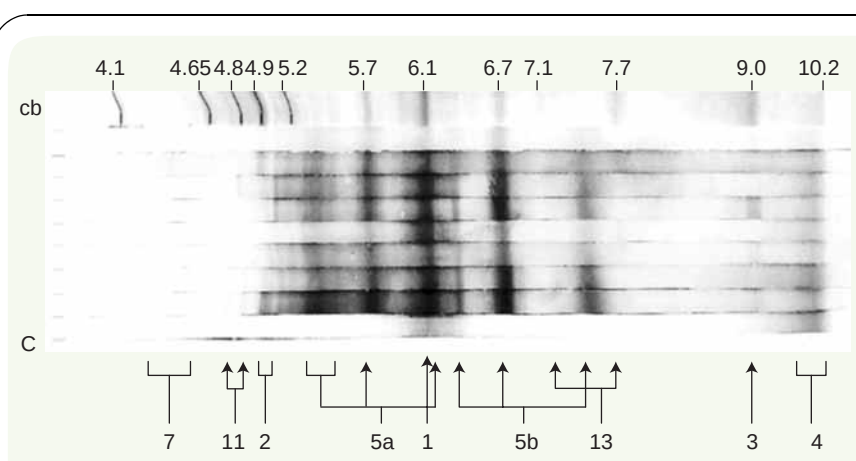


Figure 2. Isoelectric focusing (IEF) screening of the allergens recognized by nine sera from patients allergic to a grass pollen. A water-soluble extract of the grass *Dactylis glomerata* pollen was separated by IEF in a wide pH gradient in a thin gel. A blot from that slab gel was cut into ten narrow strips, and nine of them were incubated overnight with a single serum. Strip C was incubated with saline, as a control. The allergens recognized by each serum were visualized after incubating the strips with goat antihuman immunoglobulin E antibody labeled with alkaline phosphatase and stained by a colored substrate of this enzyme. Several patterns of allergen recognition can be seen. The isoelectric points of the allergens can be estimated from the pIs of the Coomassie blue-stained pollen proteins (cb). Each grass pollen allergen has received a name (in fact a number) registered at the International Union of Immunological Societies, as shown at the bottom of the figure. This allergen identification can only be performed, without ambiguity, after running several 2D gels followed by blotting with well-characterized patient sera. Some allergens have very similar or identical isoelectric points and will not be distinguished by IEF only; for example, Groups 1 and 5a or Groups 2 and 11. C: Control; cb: Coomassie blue-stained pollen proteins.

and IgE detection. The authors' experience, based on the analysis of a large number of sera directed to various allergen sources, shows that a class 3 IgE titer is usually the minimum value that allows the detection of allergens by immunoblotting. Discrepancies exist between the titer detected and the intensity of the staining obtained in immunoblotting. Moreover, as the proteins are in a more native form in IEF than in SDS-PAGE, their immunoreactivity can vary from one technique to the other. Both IEF and SDS-PAGE screenings are needed to select the sera that will allow optimal 2D detection. After the 1D screening, only the strongest sera will be used to identify and further characterize the allergens. For example, to study the rapeseed pollen repertoire, the authors have screened 450 sera from pollen-allergic patients in 1D IEF. A total of 30 sera contained detectable IgE to rapeseed pollen and only 26 were informative in describing the rapeseed pollen allergome. However, the 1D screening of a large number of sera allows the determination of the major allergens (i.e., those that are recognized by the majority of the allergic individuals) and to identify minor or new allergens (FIGURES 2 & 3). The 1D blots can also be a simple and informative method to analyze the isotypic response to allergens or nonallergenic proteins of a given source (FIGURE 4). The use of 2D blots is needed to distinguish the various allergens of analogous isoelectric points (pIs) and molecular masses. It allows the characterization of proteins that are allergens and it allows the determination of individual repertoires that are not differentiated in 1D (FIGURE 5).

The last step of the characterization of an allergen is the identification of the protein itself. Proteomic analysis can be performed on proteins purified by chromatography, or on proteins separated by 2D or double 1D gels, and extracted from the gel. The double 1D method described by Altland is very useful as it allows the separation of the proteins according to both their pI and molecular mass, and the use of a large amount (ten- to 100-times more) of proteins compared with a classic 2D method [4]. Amino acid sequencing, matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry associated with databank searches are used to identify the molecules that were previously recognized as allergens (Protein Information Resource [PIR], Swiss-Prot and GenPept databases). The recent development of this strategy, which combines preparative electrophoresis with MALDI-TOF mass spectrometry, amino acid sequencing and databank searches, allows the identification of various allergens from oilseed rape [5,6], sesame seed [7], *Arabidopsis* [8], latex [9,10], grass pollen [11], shrimp [12] and cow's milk [13]. In cases of low-abundance allergens, or very acidic or basic ones, sample prefractionation and/or pI zooming may be useful.

When a new allergen is fully characterized it can be submitted to the allergen nomenclature committee from the International Union of Immunological Societies (IUIS) to obtain an official name [101]. The nomenclature of the allergen is based on the

identification of the source (genus and species) and the number of inclusion [14]. For example, latex allergens are identified as *Hevea brasiliensis* molecules, Hev b 1 to Hev b 13. A recently introduced database specializes in allergens [102]. It lists the new molecules recognized as allergens, even if they are not yet officially denominated by the IUIS. Protein structure of identified allergens can be found using a protein database [103].

As more than 600 allergenic molecules have been identified and known by their full amino acid sequence, it became obvious that some of them shared the same function in their respective hosts. The variety of these functions is striking. One quarter of the allergens have an enzymatic activity covering a very broad spectrum. Another quarter appear to be acting as storage proteins, mainly for plants. Some important allergens known as 'panallergens' are structurally well conserved along the phylogenetic tree, and are responsible for very basic functions of cellular activity such as being part of the cytoskeleton or fundamental catabolic pathways. The remaining group of known allergens either have very diverse functions or do not currently have any known function. The hope that the discovery of the function of an allergen may explain why it is an allergen was disappointing. This fundamental question remains unanswered: what makes an allergen an allergen?

The classification of allergens into families, mainly based on their function, presents the interest to identify analogous

molecules from various sources (e.g., profilins, lipid transfer proteins and so on) that display a great sequence or domain homology (e.g., the chitin-binding domain, or hevein domain, of various chitinases). These homologies may be responsible for crossallergies that are important to predict. In addition to peptide sequence homology, a crossreactivity between various allergens may be due to the existence of IgE directed to crossreactive carbohydrate determinants (CCD) (for review, see [15]). However, if sequence homology is well accepted as a cause of cross allergy, the clinical importance of CCD is still a matter of debate.

Repertoire variability from one patient to another

The 1D blot screening evidences that some allergens are recognized by the majority of patients, but also shows the existence of individual variability in the allergen repertoire (FIGURE 3). The various 1D profiles are classified into groups that appear to display the same repertoire, and two or three of the strongest sera of each group were further analyzed in 2D blots. The intensity of staining obtained after SDS-PAGE separation of the proteins is

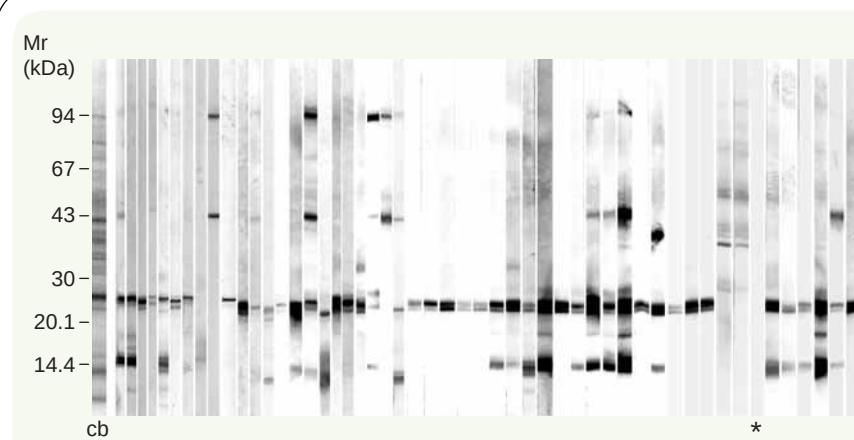


Figure 3. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) screening of 50 sera from latex allergic patients. All patients displayed high immunoglobulin (IgE) titers determined by CAP-system (varying from class 6 on the left to class 3 on the right). Latex proteins from a crude natural extract were separated by SDS-PAGE and transferred to a nitrocellulose sheet by western blotting. The blotted nitrocellulose membrane was cut in narrow strips and each strip was successively incubated with an individual human sera, alkaline phosphatase-conjugated goat antihuman IgE and 5-bromo-4-chloro-3-indolyl phosphate and NitroBlue Tetrazolium. This photomontage was performed with strips issued from few SDS-PAGE gels, which may explain the limited variations in molecular masses. This kind of screening allows the determination of major allergens (those that are recognized by the majority of the patients), but also shows that some individuals displayed a completely different profile. Sera with different specificities are chosen for the 2D characterization of the allergens. Note that the IgE titer determined by CAP-system is not directly related to the intensity of the staining, and both SDS-PAGE and isoelectric focusing screenings are needed to evaluate the best sera for a 2D allergen identification.

*Control strip.

cb: Coomassie blue staining of the gel; Mr: Molecular weight.

not always predictive of the staining that could be obtained in 2D (FIGURE 5). Two 1D screenings, performed after IEF separation on one hand and SDS-PAGE separation on the other, are probably more informative of the staining intensity that can be obtained in 2D. 2D blot screening allows similar 1D profiles to be distinguished and to localize the allergens in 2D gel electrophoresis (FIGURE 5). The localization of IgE-binding molecules in 2D blots is required to identify the molecular mass and pI of each allergen, and then to characterize this molecule further by proteomic analysis.

Differences in allergen repertoire from one patient to another could explain, in part, the variability of the clinical course of the disease and some difficulties in diagnosis. Comparison of the silver-stained gel and the various immunoblots shows that crude extracts contain many proteins that are not allergens. Moreover, allergens can represent a very minor fraction of the proteins poorly stained by Coomassie blue. In enzyme-linked immunosorbent assay (ELISA), allergens and nonallergenic proteins are mixed together, and it can be hypothesized that, at least in some cases, the presence of nonallergenic molecules could mask or impair the detection of antiallergen IgE. Thus, the use of purified or recombinant allergens for the *in vitro* diagnosis of allergy could be a way of improving the sensitivity and specificity of the tests.

The study of the variability of the immunoglobulin isotypic expression could be important to explain some discrepancies between the biologic parameters (especially the IgE titer) and the course of the allergic disease (FIGURE 4). From a biologic standpoint, the existence of various isotypes directed to the same protein (or to the same epitope) can impair the detection of a single isotype. It has been previously demonstrated, in a mouse model, that the removal of IgG increases the sensitivity of both ELISA and immunoblot analyses for allergen-specific IgE [16]. The immunodetection of antiallergen IgE is performed with the whole serum. If isotypes other than IgE are also present in the serum and recognize the same epitopes, competition in binding to the allergens develops. The steric hindrance resulting from competitive binding can be associated with an impaired IgE detection. As this phenomenon can be observed by immunoblotting, it should occur even more frequently by ELISA detection because it is usually performed with a mixture of unseparated proteins, in which some are allergens and others are not. Thus, the result of the ELISA can be impaired by both the choice of the coating molecules and the existence of various isotypes in the patient serum.

The identification of individual allergen repertoires are crucial to choose the best allergenic extract for vaccination or hyposensitization. The determination of the isotypic response is also of interest to follow the effects of the allergenic treatment and to evaluate its success or failure (see 'Five-year view: Vaccination').

Variability of allergens according to the state of the source

One critical point in allergy diagnosis is the choice and standardization of the best mixture of proteins for clinical tests and IgE detection. The comparison of latex allergens issued

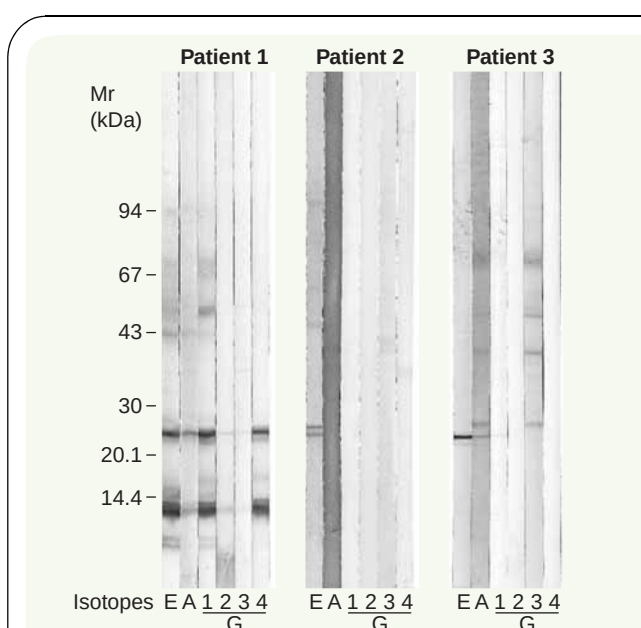


Figure 4. Variability of the isotypic response to allergens. After sodium dodecyl sulfate polyacrylamide gel electrophoresis separation and western blotting, each strip was incubated with an individual serum and revealed for the presence of either immunoglobulin (IgE, IgA or subclasses of IgG).

The analysis of the isotypic response of various allergic patients shows that some patients express various isotypes to allergenic molecules (Patient 1), whereas others express only IgE (Patient 2). The other isotypes expressed are mostly IgA, IgG₁ and IgG₄. Moreover, some patients express isotypes directed to nonallergenic molecules (Patient 3).

The variability of the isotypic response could explain some discrepancies between clinical and biologic tests. The existence of immunoglobulins of various isotypes directed to the same protein could modify its biologic detection and be associated with impaired IgE detection.

Mr: Molecular weight.

from a crude extract or extracted from manufactured latex products (e.g., gloves, mattresses and so on) clearly shows that the transformation of the natural source is associated with a drastic reduction in the total extractable protein content and with the appearance of new isoforms of the major allergen Hev b 1 (FIGURE 6). The modification of the protein/allergen content according to the nature of the source could be associated with altered detection of the IgE response. Another example of the modification of allergens according to the state of the source is given by food allergens that can be modified during food processing (for review, see [17,18]). Heating, emulsion formation or food aging are processes that may induce conformational changes such as disulfide bond disruption, unfolding, aggregation or degradation into smaller peptides. These conformational alterations can be associated with either the degradation of allergenic motifs or with the generation of new allergenic determinants. Emulsion formation, interaction with lipids or aggregation are processes that have been used in various models for their adjuvant effect in inducing antibody formation (i.e., Freund's adjuvant). Such alterations may be observed with food proteins after food processing, and could modify the allergenicity of some proteins for the digestive tract.

Air pollution is often cited as a major cause of the observed increase of allergic cases in recent years. In Japan, the relatively recent introduction of the first car with a diesel engine in 1970, reaching 1 million of them 6 years later, coincided with the appearance of the first cases of allergy to Japanese cedar around 1977, which were increasing following the same growth pattern, was a first epidemiologic sign of the potential role of diesel exhaust particles as adjuvant for pollen allergenicity. The

authors have studied the effect of urban air pollution on grass and birch pollen grains. Pollution induced protein and allergen leakage from the pollen grains, thereby allowing the allergens borne onto small particles (1–5 µm) to penetrate deeper into the lungs than intact pollen grains 30–40 µm in diameter [19,20]. Immunizing mice or rats with polluted pollen showed that, even if these pollen grains contained less allergens than clean ones, they were, however, more allergenic than the latter

ones. The authors demonstrated that the addition of some minor organic fractions from diesel exhaust particles to clean pollen stimulates mouse IgE production to pollen allergens [21,22].

The allergen spectrum from a given source may vary between countries due to environmental and genetic factors. Thus, the complete allergome description will require the study of different allergen sources, as well as sera from allergic patients from different parts of the world.

Expert commentary

Other methods, not based on 2D gel electrophoresis, could also be used to explore the allergome. A molecular biology method using a selective enrichment, through serum IgE from allergic individuals, of complementary DNA libraries displayed on phage surface has been shown to be successful for the rapid isolation and identification of allergens [23]. This technique has the advantage of being a new and elegant *in vitro* technique that rapidly isolates various IgE binding molecules or structures, even if these proteins are not water soluble or have extreme pIs that will limit their analysis by 2D gel electrophoresis. However, it is a rather expensive method that may not screen all the relevant allergens as it expresses mainly the amino acid backbone and not always their native conformation due to improper refolding and/or lack of correct glycosylation.

Another alternative to 2D gel electrophoresis in proteomic studies is based on multidimensional liquid chromatography coupled to mass spectrometry. This is a smart technique that can be fully automated, has a preparative potential and may have a high-throughput capacity. Unfortunately, the immunodetection step needed to reach the allergenic function of the proteins studied and then the identification of the allergens has not yet been successfully coupled online.

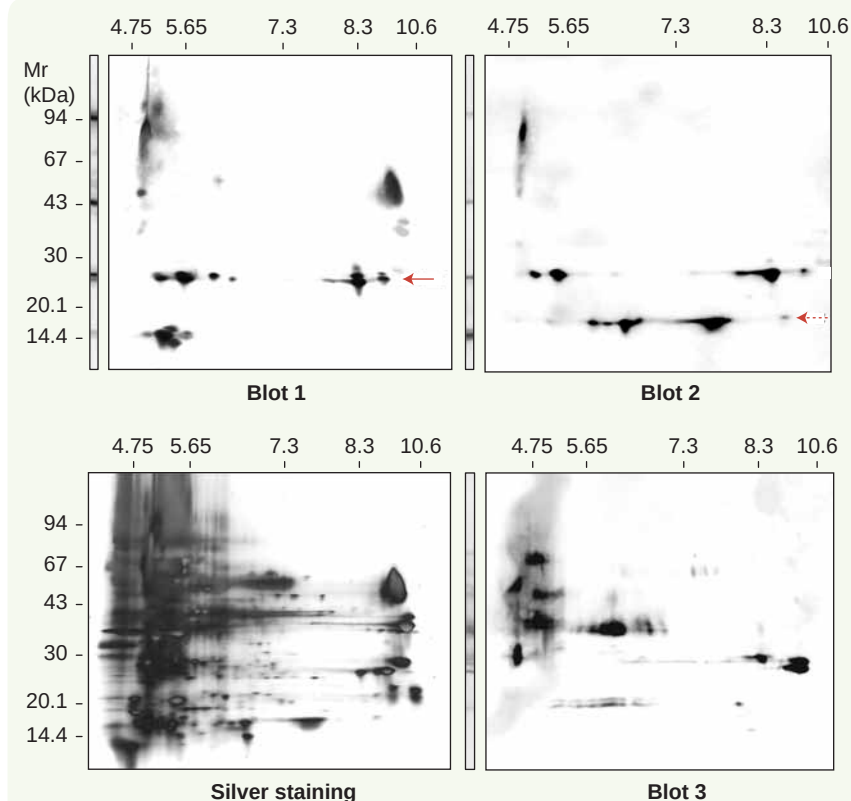


Figure 5. Identification of individual repertoires by 2D blots. Latex proteins from a crude extract were separated by isoelectric focusing followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The 2D gels were either silver stained or transferred onto a nitrocellulose sheet by western blotting. Nitrocellulose membranes were incubated as in FIGURE 4 to analyze individual immunoglobulin (Ig)E specificities. Several sera selected by 1D blot were analyzed in 2D blots to allow the identification of the various allergens. The spots evidenced in blots are molecules that bind IgE, and thus are allergens. These allergens represent a limited fraction of the whole protein extract (see the silver-stained 2D gel, lower left). After identification, the molecular characterization of the allergens must be performed using proteomic methods (e.g., amino acid sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry).

The 2D analysis of the individual specificities demonstrates that some sera that show very similar patterns in 1D (Blots 1 & 2) displayed a different specificity evidenced in 2D. The 43/94-kDa doublet corresponds to an acidic molecule for both sera, but an alkaline molecule with an apparent molecular mass around 43 kDa (further identified as hevine) is also recognized by the serum in Blot 1. The 14-kDa band in the 1D blot represents two different allergens easily distinguished in 2D. Further analysis with monoclonal antibodies demonstrated that the spots appearing around 25 kDa (solid arrow) are various isoforms of prohevein (Hev b 6.01) and that the spots appearing around 15 kDa (broken arrow) correspond to the C-terminal domain of the molecule (Hev b 6.03). This result means that the serum in Blot 1 recognizes only the N-terminal end of the prohevein (hevein domain or Hev b 6.02, a small polypeptide not seen in these blots), whereas the serum in Blot 2 also recognizes the C-terminal end of the molecule. Moreover, this analysis shows that some patients recognized completely different molecules (Blot 3). The knowledge of this diversity may be important to improve diagnosis and therapy.

Note: A relatively weak staining in 1D can give a strong staining in 2D (Blot 3).

Mr: Molecular weight.

The obvious evolution that can be reasonably expected is the trend toward micro- or nanotechnologies, using microfluidics. Direct miniaturization of 2D gels seems less probable than a rather elaborate automated chip with successive capillaries, where separations based on different analytical principles will allow high resolution on microallergenic quantities with online immunodetection.

Five-year view: improving diagnosis

& therapeutics

Diagnostic tools

Currently available immunodiagnosics of allergy are all based on ELISA-type methods first described in 1972. The new trend is toward an increased number of allergens to be tested in the same patient serum, but keeping, or even reducing, the amount of serum needed (50 μ l). The rapid and spectacular development of micro and nano methods, including microfluidics, will soon make such dreams a reality. The first logical step is to develop an allergen chip format where up to 100 different and well-selected molecular allergens will be immobilized on a single chip as tiny spots clearly identifiable when they are recognized by human IgE antibodies [24]. The need for molecular allergens is becoming possible if one uses suitable recombinant allergens rather than natural ones. Another direction to develop a new immunodiagnostic for the future is to look for more details into the patient antibody response to allergens. To date, only IgE antibodies to allergens were looked for. The authors believe that some other classes and subclasses of immunoglobulin should be tested in order to help provide a better understanding of the onset and evolution of the allergic disease. A multivariate test should contribute to answering these important questions.

Vaccination/hyposensitization

Hyposensitization or vaccination to allergenic molecules has been proposed to treat various forms of Type I hypersensitivity reactions. Allergen-specific immunotherapy aims to induce a T-helper 2-to-1 switch associated with the production of IgG-rather than IgE-specific antibodies to allergens, and a clinical improvement that is suggested to be due to the blocking effect of IgG on mast cells or basophil histamine release [25]. The first extracts proposed for therapy were composed of a mixture of proteins that contained allergenic and nonallergenic molecules, and were difficult to standardize and could vary from one commercial source to another, or between batches. The recent progress in allergen identification and the development of recombinant

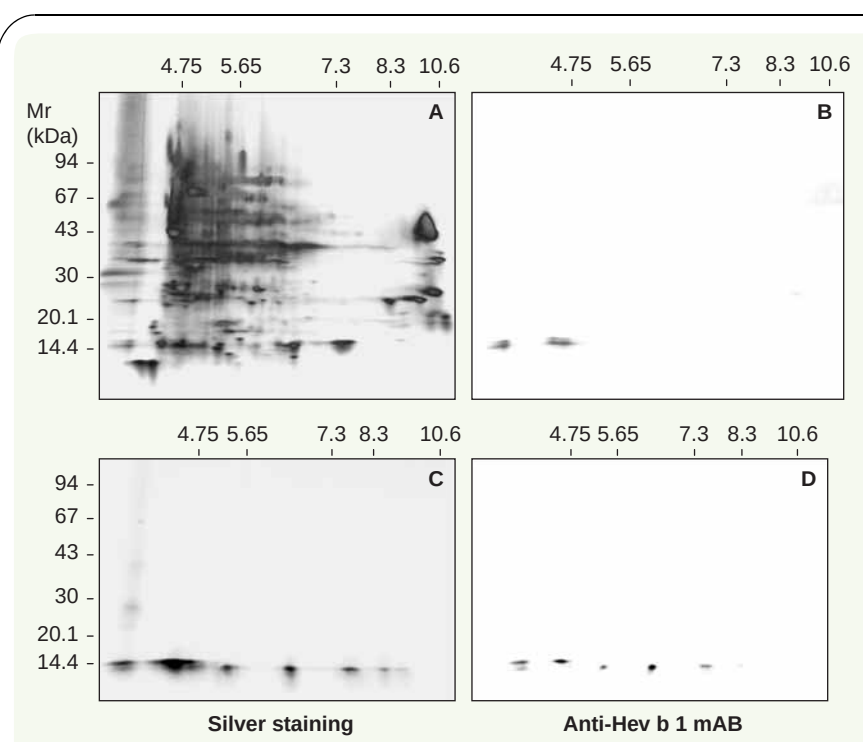


Figure 6. Comparison of allergen expression according to the state of the source. The latex allergen Hev b 1 was detected on a crude latex extract used for skin prick tests (A & B) and on an extract obtained from manufactured latex products (C & D).

First, the silver staining of the 2D gels shows the difference in protein content between the crude extract used for clinical determination of latex allergy (A) and the extract from manufactured latex products that contains only a few proteins (C).

Second, the detection of Hev b 1, one of the major latex allergens, in the two extracts (B & D) shows that this allergen is an acidic protein present in the crude extract with two isoforms. Hev b 1 represents the major allergen of manufactured products and displays five isoforms, three of which are less acidic in the manufactured than in the crude extract. A further analysis demonstrates that these isoforms corresponded to a lack of several N-terminal acidic amino acids.

This example illustrates the fact that the state of the source can modify the structure of the allergens. mAb: Monoclonal antibody; Mr: Molecular weight.

molecules have changed the approach of the immunotherapy of allergy towards the use of more specific and hypoallergenic extracts (for reviews, see [26–28]). Such therapy requires:

- Molecular identification of the various allergens of a given source
- T and B epitope mapping and determination of hypoallergenic fragments
- Construction of hypoallergenic recombinant proteins to be used as vaccines

Obviously, the development of hypoallergenic molecules was first performed with the major allergens that are the best candidates to treat the majority of patients. Several hypoallergenic molecules were recently evaluated for major allergens from cat [29], grass pollen [30], birch pollen [25,31] or bee venom [32]. However, the individual variability in the antiallergen response may be a cause of treatment failure. Thus, the analysis of the individual allergen repertoire of the patient to determine which are the best molecules for treatment may improve the indication and monitoring of the specific immunotherapy (for review, see [28]).

Key issues

- Allergy is a disease that touches 10–30% of the general population. In the last 15 years, a doubling of the cases has been observed.
- Allergens are usually proteins. They are a small subset of molecules in a highly heterogeneous source of biologic material.
- The allergen repertoire from a complex allergenic source can be termed the 'allergome'. Knowledge of the allergome is very important in producing reliable *in vitro* and *in vivo* diagnostics.
- The production of safe and efficient vaccines to treat allergy is dependent on comprehensive allergomes.
- 2D gel analysis followed by immunoblotting is currently the best analytical technique to study and describe complex allergomes.
- Micro- or nanotechnologies including microfluidics should, in the near future, produce cheap and rapid immunodiagnostics.

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