

Allergen Extract Analysis and Quality Control

Samuele E. Burastero

San Raffaele Scientific Institute, via Olgettina, 58, Milan
Italy

1. Introduction

The increase in allergic diseases in Westernized Countries is an established phenomenon, which has been extensively documented by several large epidemiologic studies (Sicherer and Leung, 2010). Allergies affect nowadays a proportion of the general population as large as one out of five individuals. The most prevalent clinical expression of allergies include respiratory allergies, such as allergic rhinitis and asthma (Chu et al., 2010), skin allergies, such as atopic dermatitis (Spergel, 2010a), and food allergies (Chafen et al., 2010). The pathogenesis of allergic diseases, and the reasons for their increased incidence is partially known and include environmental and genetic factors (Kuriakose and Miller, 2010). In all cases, the hallmark of all these *bone fide* allergic diseases is the presence of specific antibodies of the immunoglobulin E (IgE) isotype against protein antigens, which are indicated as allergens on the basis of their capability to elicit allergic reactions. The presence in the biological fluid of IgE to a given allergen is indicated as "sensitization" to that allergen. In most cases, IgE are measured in serum, where they are more easily detected and also on the ground that allergy is a systemic condition, with clinically relevant expression in different individuals at different target organs (*e.g.*, lower respiratory tract in the case of allergic asthma, nasal mucosa in the case of allergic rhinitis, *etc.*) (Pucci and Incorvaia, 2008) depending on organ-specific characteristics, which have been only partially identified.

The correct identification of sensitizing allergens is required to put in action the most efficient strategies to counteract the clinically relevant effect of allergies as well as their remarkable effects on the quality of life (Cummings et al., 2010). Useful measures include allergen avoidance, when applicable, proper symptomatic therapy and, most importantly, allergen specific immunotherapy, which is the only intervention capable to actually modify the pathogenic mechanisms of allergic diseases and their natural history, known as the allergy march (Spergel 2010b).

IgE can be measured *in vivo* with prick testing and *in vitro* with immunochemistry testing. Notably, as compared to immunochemistry testing of other immunoglobulin classes, specific enhanced procedures have to be used for allergen specific IgE determination, due their concentrations in biological fluids, which is in the range of ten to hundred thousand times lower as compared to the most represented immunoglobulins, *e.g.*, those belonging to the IgG isotype. A major issue to be considered in allergen-specific IgE determination is the quality of the antigen to be used for testing. Indeed allergens, which are encountered by patients in natural context are complex mixtures of relatively heterogeneous proteins. This fact poses several problems to Manufacturers of allergen extracts, including the

standardisation of procedures used to obtain them from raw allergen sources and the possibility that they are not fully representative of the actual contents in terms of allergen components, as compared to environmental allergen sources. In the last twenty years the advent of molecular allergology has introduced revolutionary changes in the possibility to properly diagnose and treat allergic individuals by using single allergen components rather than allergen extracts. Molecular allergology has also made it possible to standardize allergen extracts themselves, which can now be used with a much higher knowledge about their appropriateness as diagnostic or therapeutic tools in patients with sensitization profiles defined at the level of single allergen components.

Allergen products require registration by government institutions (Becker et al., 2006), such as the FDA in the United States and the Paul Ehrlich Institute in Germany. Basic researchers, physicians, regulatory authorities and manufacturers have long tried to define a common methodology to standardize allergen vaccines (Terho and Frew, 1995; van Ree et al., 2008)

The quality of mite and pollen allergen extracts is better defined today than it was in the past, and the quality of food and epithelial allergen extracts has also improved (Fernandez-Caldas et al., 2006; Ferrer et al., 2005). However, further improvement is needed, particularly for food allergen extracts, since preliminary studies have shown efficacy also in this high-impact clinical application, using sublingual food allergen extracts (Enrique et al., 2005).

Thus, the heterogeneity of allergen extracts used for diagnosis and immunotherapy makes it necessary to develop methodologies to assess their potency and ensure their consistency, stability and safety. In general terms it is essential to apply standardization in order to control all aspects of the production process, including the variability intrinsic to the source materials and the consistency and reproducibility which have to be granted for the sake of safety and efficacy in the clinical use (diagnosis, immunotherapy, quantification of environmental allergens).

Nowadays, different types of standardizations are still largely used by different laboratories and manufacturers, which variably addresses crucial issues underlying these preparations, such as the qualifications applied to collectors of raw materials, the establishment of manufacturing procedures in compliance with good manufacturing practice (GMP) regulations, and the choice of formulations of allergen extracts intended for clinical use (Larsen and Dreborg, 2008).

Although a limited number of major allergens (King et al., 1994) stimulate IgE production in a greater part of patients, any antigen in a given source material has the potential to elicit an IgE response. Thus, on one side it is essential to ensure that all protein allergens in the allergenic source material, to which humans are exposed in real life, are actually contained in the raw material used for diagnosis or immunotherapy. On the other side, since the IgE binding capacity of an allergen extract is related to the content of one or a few major allergens, it is also important that the standardization procedure ensures consistency, not only in the overall IgE binding potency, but also in the relative and absolute content of single major allergens. Due to this complexity in the composition of allergen extracts, the use of standards is a key element in standardization (Lowenstein, 1987). In Europe, each laboratory and manufacturer establishes In-House Reference (IHR) preparations for each source material. The IHR must be thoroughly characterized by *in vitro* methods as a basis for equilibration of subsequent batches. These methods have traditionally been based on whole extracts, whereas only in the last decade reference to single allergen components has been progressively introduced. Although it is still widely supported the notion that the biological activity of allergen extracts (also known as "allergenicity" or "reactogenicity") has to be

determined in humans by *in vivo* methods, *i.e.* prick testing, it is time for all stake-holders to be put in the position to compare each new batch by using *in vitro* methods exclusively. In order for this advancement to be achieved, international standard (IS) preparations should be made available and *in vitro* methods should be validated by international health authorities for each allergen source. All laboratories in the world should be put in the condition to compare the allergen contents of extracts, which are used in everyday clinical practice to diagnose and treat such a large proportion of the population. This chapter describes the principles and problems related to standardization of allergen extracts for research and clinical use.

2. Mechanisms of allergy

The immune system of allergic individuals is erroneously reacting against innocuous molecular markers of environmental elements, as if they were markers of invaders, *i.e.*, virus, bacteria or parasites, which could potentially impair the integrity of vital functions. Allergen specific IgE are the immunological heralds of such a reactivity, which was evolutionarily developed in mammals to react against parasites (Chinen and Shearer, 2007). Circulating basophils (and tissue resident mast cells) are the short-term reacting effector cells, which are activated when allergen-specific IgE, bound to their membranes via Fc epsilon receptor type I (indicated as “cytophilic IgE”), are triggered by allergen binding to release inflammatory mediators stored within secretory granules (Abramson and Pecht, 2007; Holowka et al., 2007; Macglashan, 2005). Remarkably, the action of these mediators takes place within a few minutes since allergen recognition, and includes as dramatic changes of the local tissue as increased permeability of the microvasculature, egress of several types of inflammatory cells, mainly lymphocytes and eosinophils, secretion of large amounts of fluid secretion from mucosal glands and contraction of smooth muscle fibres (Barrett and Austen, 2009; Hamid and Tulic, 2009; Holgate, 2008). The latter may induce bronchial constriction, which is clinically expressed as asthma. Allergy therapies include not only symptomatic drugs, which provide release to the short-term effects associated to the release of these mediators (Al Suleimani and Walker, 2007; Bush and Saglani; Novak, 2009), but also allergen avoidance and specific immunotherapy. Only allergen avoidance and specific immunotherapy can radically modify the course of allergic diseases. However, avoidance, which *per se* is always dramatically effective, can in most cases rather help patients to live together with allergy than preventing its effects (Dykwicz and Hamilos, 2010; Hamilton, 2010; Simons, 2010), due to the fact that exposure to most sensitizing allergens in the population is virtually unavoidable. In contrast, immunotherapy is a disease modifying intervention, which can change the pathogenesis of the underlying allergic condition (Akdis and Akdis, 2010). For all allergy therapies the proper identification of the sensitizing allergens is a prerequisite for success (Hamilton) and only the careful standardization of hundreds of allergens identified until now allows put in action accurate and effective therapeutic interventions (Mothes et al., 2006; Pittner et al., 2004; Valenta et al., 2007). In particular, in the case of immunotherapy, the performance of the diagnostic procedures is mostly crucial, since it influences the appropriateness of years-long procedures patients have to undertake (Hankin and Lockey, 2010) in order for their immune system to modify reactivity to armless antigens (Abramson et al.; Calamita et al., 2006; Radulovic et al., 2010). In front of this widely accepted circumstances, the determination of allergen specific IgE is still performed with criteria which do not allow to distinguish rather

common occurrences which make it difficult or impossible to properly define the sensitization profile of allergic patients. Indeed, approximately 75% of allergic individuals are polysensitized, *i.e.* they produce IgE reacting with multiple allergen sources (Scala et al., 2010). This polyreactivity can in some cases be attributed to IgE reacting to major allergen components in different allergen sources, *i.e.*, it is associated with *bona fide* multiple allergies in clinical terms. However, in as much as one third of cases IgE react to multiple allergen components to the presence of IgE to evolutionarily conserved, structurally homologous allergen components, which are collectively indicated as panallergens (Hauser et al., 2010). In particular, the most common panallergen, profilin, has been found targeted by IgE is as many as 50% of subjects sensitized to grass and birch pollens (Chapman et al., 2007). On a clinical stand point, as a food allergen profilin usually elicits mild reactions, such as oral allergy syndrome, is not modified by processing and is especially important in allergy to some fruits, such as melon, watermelon, banana, tomato, citrus fruit and persimmon (Santos and Van Ree, 2010). In front of this well-defined scenario, diagnosis with allergen extracts cannot distinguish subjects sensitized to major allergens of those extracts from subjects sensitized to one profilin cross-reacting in one extract with profilin of one or more extracts tested in parallel. There are several negative outcomes stemming from this situation, among them the most disturbing are the following: patients do not receive suitable information to evaluate their allergic disturbances and are not prescribed proper immunotherapy. In fact, although allergen specific immunotherapy with single allergen components is not yet commercially available, yet the limits of extract-based diagnosis are certainly capable to negatively affect the management of extract-based immunotherapy. Most allergists do not prescribe immunotherapy to patients with a polysensitization pattern, without investigating whether this pattern is ascribed to a real multiple allergies rather than to cross-reactivity via panallergens, despite the fact that clinical benefits have been reported in immunotherapy-treated polysensitized subjects (Ciprandi et al., 2010a; Ciprandi et al., 2010b). Thus, the present knowledge of the pathogenesis of allergic diseases has dramatically improved on the ground of increased cloning of allergen components but it is still waiting a thorough change in the clinical management of patients, due to the poor application of molecular allergology to the principles of allergen standardization. Along this line, the natural history itself of allergic diseases may be more efficaciously dissected by using allergen components than extract. For instance, a functionally relevant T-cell response to conserved regions of panallergens was demonstrated to underlie the possibility in single patients allergic to one given allergen to react against another allergen source expressing a homologous, conserved component, such as a profilin. These results suggested that a reciprocal modulation of the response to one sensitizing allergen can occur following natural exposure to or immunotherapy with another allergen and may certainly have relevance in the management of patients with multiple allergies (Burastero et al., 2004). Thus, extract-based diagnosis and immunotherapy need to be standardized also on the ground of the possibility to properly monitor the evolution of allergic disturbances. In the next future all stake holders, patients first, will have to fully exploit the remarkable advancements that molecular allergology allowed to achieve over in-house reference extracts based allergology of the previous century.

3. Standardisation of allergens for allergy diagnosis

The diagnosis of allergic disease requires, besides a detailed history and physical examination, the determination of allergen-specific IgE (Hamilton, 2010). In order for this

diagnosis to be properly performed, preparations containing allergens have to be utilized, both for *in vivo* and for *in vitro* testing. *In vivo* the presence of IgE can be detected with a low-cost procedure, which provides results in a matter of minutes, known as prick testing. Prick testing is performed by creating a 1-mm deep wound with a skin pricker, which put in contact single solutions containing each allergen to be tested with skin mast cells. These mast cells mediators include histamine and leukotrienes, which promptly increase the permeability of local vessels and generate erythema and oedema, a local effect read locally as “wheal and flare reaction”. By measuring the size of the wheal, the extent of the degranulation is semi-quantitatively assessed, and can be used as an approximate measure of IgE levels. A part from the efficacy of the allergen extract used for prick testing, several other individual factors affect the results of this procedure, such as individual reactivity to histamine, age and sex of the pricked person, the site of reaction, the presence of non specific irritants, such as environmental pollutants, the season of the year, etc. (Bordignon and Burastero, 2006). This biological background has long represented a major limit in the possibility to accurately assess the allergen content of preparations containing allergens for diagnostic purposes.

In fact, allergen extracts are complex mixtures of protein materials obtained by Manufacturers from relevant allergen sources and the possibility to easily assess skin reactivity by sensitized subjects has long represented the criterion for assessing the appropriateness of each preparation (Fernández-Caldas et al., 2009). Indeed, this biological approach has obvious advantages over more complex analytical approaches, since it warrants to any Manufacturer the possibility to validate the allergen extracts they commercialize. However, this method also brings about a peculiar and an extremely complex set of standardisation problems, which -incidentally- are not shared by the remaining pharmaceutical industry. Firstly, in the absence of any obvious general criterion to assess the allergen content with a simple analytical approach, as the one that could be applied to measure the active substance in any drug preparation, each Manufacturer is encouraged by trivial commercial reasons to maintain strictly confidential all the relevant details of preparation procedures. This confidentiality is often used to claim that one given specific preparation is better performing as compared to that of competitors on several grounds. The latter include the efficiency of allergen recognition by patients IgE, the representativeness of the allergen source found in the environment in real life by patients, the capability to trigger clinically relevant symptoms and, last but not least, batch-to-batch consistence. As a matter of fact, all these criteria are rather difficult to verify on a scientific ground, not only because they are related to totally or partially unveiled industrial procedures but also because they are applied to complex protein mixtures.

Indeed, several studies have reported differences among commercially available diagnostic products for skin prick testing, see for instance Pagani *et al.* (Pagani et al., 2009). Another study quantified and compared the allergen content of different grass pollen preparations for skin prick testing (SPT) used in Europe (Sander et al., 2009). Strikingly, protein concentrations ranged from 15 to 427 µg/ml, and the concentration of Phl p 5, a major grass allergen, ranged from 0.15 to 18.3 µg/ml (Sander et al., 2009). Major allergens are allergen components within a given allergen source, which are recognized by the majority of patients sensitized to that allergen source, as established by gold standard criteria (Grier et al., 2002). Gold standard criteria include the appearance of clinical symptoms upon environmental allergen exposure and thus represent the most incontrovertible parameter of efficacy. Yet, extracts are still preferred to allergen components on the ground that conformational

epitopes may not be properly expressed in single *E. coli* cloned and expressed protein allergens (Pomes, 2009), that glycosylation, which contribute to allergenicity (Shakib et al., 2008), is not maintained in recombinant allergens, and that a panel of several, yet to be completely defined, allergen components has to be used to replace the comprehensive repertoire of protein allergens within an extract. Along this line, the quantification of single allergen components is considered an unsatisfactory criterion to define the characteristics of allergen extracts. Extracts in the United States are more homogenous with respect to total allergenic potency than the extracts produced in Europe, mainly because the FDA provides the same standardized reagent for internal use by all manufacturing companies (Slater, 2004). However, great differences have also been shown among non-standardized mold allergen extracts in the USA (Esch, 2004).

4. Standardisation of allergens for immunotherapy of allergic diseases

Specific immunotherapy is the practice of administering gradually increasing doses of allergen vaccines to reduce allergic symptoms and the need for medications. Thus, immunotherapy is the only curative intervention in the treatment of allergies, since it can modify allergen-driven immunological responses and restoring, to a certain degree, the Th1/Th2 balance (Akdis and Blaser, 2001). B and T lymphocytes, blocking antibodies, IL-10 and other cytokines play an important role in the response to specific immunotherapy (Chinen and Shearer, 2004). Effective allergen immunotherapy depends on the accuracy of the diagnosis and on the usage of well-characterized allergen extracts. The latter should ideally be prepared according to a patient-tailored approach, whereby only allergen components one given patient is sensitized to are administered (Vrtala, 2008). Although available allergen preparations do not allow this approach, yet numerous double-blind placebo-controlled studies using unmodified aqueous allergens (Gurka and Rocklin, 1988) and allergoids (Ferreira et al., 2006) (modified allergens) have demonstrated efficacy. Sublingual immunotherapy, using drops of aqueous allergen extracts under the tongue and then swallowed (Radulovic et al., 2010), or tablets (Bufe et al., 2009; Dahl et al., 2008) with similar type of extracts, also appear to be clinically beneficial, particularly for grass-induced rhinoconjunctivitis.

The comparison of different products from different companies at national and international levels is complicated due to the lack of international standards, similarly to the above quoted problems related to diagnostic products. Larenas-Linnemann and Cox (Larenas-Linnemann and Cox, 2008) reviewed the information obtained on unit definition and dosage of allergens from European manufacturers of allergen extracts used for sublingual immunotherapy (SLIT). They concluded that the monthly maintenance dose the manufacturers recommended for SLIT was 5-45 times higher than the recommended dose for subcutaneous immunotherapy. However, since each manufacturer in Europe uses its own in house reference preparations and its own units to express potencies, the comparison of different products from different companies at national and international levels is almost impossible. The amount of major allergens has started to be used, which represent a major improvement at least in principle, to overcome a *bona fide* anarchism, which is strongly supported by Manufacturers, in my opinion, for mere commercial reasons. In fact, in the absence of simple international units, such as microgram or micromoles, every manufacturer is legitimated to predicate the better performances of each own product, on the basis on not-verifiable statements and procedures. As a matter of fact, details on the procedures for

allergen standardization, which are listed and described in paragraphs below, are never made publicly available on the ground of industrial protection issues. Thus, an inviolable system tends to self-perpetuate, which is hard to be challenged by the physiological dialectic, which should drive scientific issues towards their solution. Along this line, far from promoting the usage of homogeneous testing to quantify major allergen contents (*e.g.*, same protocols, same monoclonal antibodies for capture and detection in sandwich ELISA, *etc.*) most commentators and opinion leaders support the notion that since the reference extracts and antibodies used can influence the outcome of such assays (Larsen and Dreborg, 2008) basically there is no strong scientific basis to move from good old in-house reference units to micrograms of major allergens. This position certainly makes it less embarrassing to comment on the fact that the maintenance doses of the commercially available vaccines have been found to range from 0.2 to 21.6 μg in terms of Phl p 5 content (Larenas-Linnemann and Cox, 2008) which should per se indicate that in house reference should be limited to internal quality control procedures. Moreover, doctors and patients should be made really aware of what they are actually continuously administering or assuming, respectively, for the average two to four years treatment time, rather than counting on the reassuring, only partially verifiable statements of Manufactures.

5. Standardisation of allergens for measuring their environmental amount

The measurement of allergens in the environment is a well-established approach to assess the level of exposure of sensitized individuals in real life settings. This applies mainly to dust mite, animal allergens, cockroach, and molds. Epidemiologic studies, population surveys, and birth cohort studies have plainly defined levels of allergen exposure in Western populations and found strong associations between exposure and the development of asthma (Arbes et al., 2003; Heinrich et al., 2006; Illi et al., 2006; Sears et al., 2003; Sporik et al., 1999; Woodcock et al., 2004; Zock et al., 2006). Guidelines have been developed to indicate exposure levels that are risk factors for sensitization (Platts-Mills et al., 1997). Allergen assays are used for testing the efficacy of allergen avoidance procedures and devices and for monitoring clinical trials of avoidance and the efficacy of remediation (Morgan et al., 2004; Platts-Mills et al., 2000; Woodcock et al., 2004). These assays are used in the US indoor air quality industry for evaluation of allergen exposure in homes, the workplace, and public buildings. In this context, although allergen measurements have become routine, the same issues, which are commented above about the lack of international standard apply here. However those tests, which are routinely used to this aim necessarily include the usage of allergen components (mainly in recombinant form) in order to run the standard curves, since the latter are the core element of the assay itself, rather than prick testing. Thus, the quantification of allergen content in the environment, even in the absence of international standards, is expressed in conventional amounts of major allergen by all available assays (Hamilton, 2005), rather than according to the obscure and bizarre in-house reference units which still prevail in the diagnostic and immunotherapy fields.

6. International efforts to standardized allergens

The widespread use of allergen measurements in the fields of allergy, indoor air quality, and environmental exposure assessment has created an urgent need for internationally recognized purified allergen standards and for validated and certified immunoassays. Due

to the common generalized mind-set by manufactures, immunotherapy products are still licensed based on their total potency as established by in-house, non-comparable reference units. Nevertheless, allergists increasingly use specific allergen measurements for dosing of immunotherapy. Maintenance doses of 5 to 20 mg of major allergen are associated with clinical improvement after immunotherapy, and natural allergenic products are being formulated, at least in part, based on specific allergen content (Nelson, 2007; van Ree, 2007). Allergen concentrations should be monitored not only to establish dose-response relationships between allergens and treatment efficacy but also to compare allergenic products from different manufacturers, and to formulate recombinant allergen diagnostics and vaccines (Becker et al., 2006; Chapman et al., 2000; Scheiner et al., 1994; van Ree, 2007). Although the progressive characterization of major allergen components and the development of techniques to quantify them, such as ELISA systems based on monoclonal antibodies, have led more manufacturers to provide information on the major allergen content of their extracts, identification of major allergen content is not currently mandatory, except for a limited number of extracts, such as cat and ragweed. The World Health Organization and some other regulatory government institutions now recommend that allergen manufacturers state the content of representative major allergens in mass units for their allergen products (Nelson, 2000). However, differences in assays and methodologies for measuring the major allergens still preclude direct comparisons among products of different manufacturers (Alvarez-Cuesta et al., 2006). In fact, although recombinant allergens are being used to develop new diagnostics and vaccines, yet the structural and immunologic properties of cloned allergen components had not been systematically compared with those of their natural counterparts in international collaborative studies. The World Health Organization (WHO)/International Union of Immunological Societies Allergen Standardization Sub-committee has been influential in coordinating international standardization. The committee established WHO-approved international standards for dust mite, dog hair, and birch, timothy, and short ragweed pollens and produced the WHO position paper that recommended the use of standardized allergen vaccines of defined allergen content for dosing in immunotherapy (Bousquet et al., 1998; Platts-Mills and Chapman, 1991). This approach was also endorsed by a position statement from the American Academy of Allergy, Asthma & Immunology (Cox et al., 2010). In 1999, the WHO/International Union of Immunological Societies Allergen Standardization Sub-committee initiated a specific program aimed to develop highly purified allergens that could be used for the standardization of *in vitro* assays which is the underlying issue in the implementation of this approach. This provided the genesis for a European Union-funded study entitled "Development of certified reference materials for allergenic products and validation of methods for their quantification" (acronym: CREATE) (van Ree et al., 2008). The aim of the European Union CREATE project was to produce international standards of purified allergens with verifiable allergen content. Such standards would enable allergen manufacturers, academic organizations, and government and regulatory agencies to use a common international standard for specific allergen measurements. A second aim was to compare the specificity, sensitivity, and reproducibility of ELISAs for allergen analysis. Allergens were selected for the project based on the following criteria: (1) the allergen was a major allergen of well-documented clinical importance; (2) purified natural and recombinant forms of the allergen were available in greater than 20-mg amounts from academic or commercial laboratories; (3) there was strong evidence that the recombinant allergen had equivalent IgE binding to its natural counterpart and there was extensive

structural data on the allergen; and (4) ELISA kits to measure the allergen were available from 1 or more laboratories.

To carry forward with this project, eight major allergens originating from four of the most important inhalant allergen sources were selected: Bet v 1 from birch pollen, Phl p 1 and Phl p 5 from grass pollen, Ole e 1 from olive pollen and Der p 1 and 2 and Der f 1 and Der f 2 from house dust mites. Three allergens were found to be suitable as biological reference materials; the rest, except rPhl p 1a, did indicate potential for optimization, but only if specific aspects of their protein expression processes will be modified. As a result of this study, recombinant Bet v 1 and Phl p 5 are being produced under Good Manufacturing Practice and presently being evaluated by the European Directorate for the Quality of Medicines as biologic reference preparations to be included in the European Pharmacopoeia as international standards. Consequently, at least for these allergens, standardization will become global and will hopefully permit comparisons among different manufacturing sources (Himly et al., 2009).

A detailed account of the aims, scope, and methods used in the CREATE project has been published elsewhere (van Ree et al., 2008)

7. Techniques for the standardization of allergen products

Allergen products, which are routinely used to diagnose and treat allergic diseases have been used for over 100 years. The quality of these allergen products is a key issue for both diagnosis, environmental monitoring of allergen levels and specific immunotherapy, and the standardization of allergen extracts is of primary importance to improve their quality and offer physicians worldwide a reliable method to diagnose and treat such widespread diseases as food allergies and allergic respiratory diseases. In particular, effective diagnosis and treatment, using skin test reagents and specific immunotherapy requires the optimal amount of allergens for testing and the maintenance dose of vaccine for treatment, respectively. Although a tremendous amount of information is available to implement this objective, internationally accepted rules are still lacking. The unavoidable empirical steps, which are preliminary to the production of allergen extracts from natural allergen sources are usually considered the only relevant aspect of the whole standardisation process, since this position is instrumental to support the concept that quantification of allergen component in weight (or molar) units are non-applicable and misleading. Obviously, any single step in the preparation of allergen extract has to treasure decades of previous consolidated experience in as trans-disciplinary expertises as zoology, palynology and biochemistry. Yet, the final products of such processes will have to be validated by international standards, in order to make it possible to evaluate their allergenic potency and compare the corresponding preparations of different Manufacturers, if quality is something different from a jealously kept secret of happy few.

8. Collection of raw source materials

Inhalant allergens are present in airborne particles derived from natural allergen sources. These particles constitute the material which humans are exposed to. The selection of raw material is finalized to provide extracts containing the same active allergens patients will encounter in real life in a manageable form. Although in most cases, the optimal source material is rather obvious, in some cases the allergen source is still debated (for instance, for cat allergy it is not yet established whether the best allergen source is saliva or dander and,

in the case of mouse allergy, urine or dander). In principle, the source materials should be specific and include all relevant components in sufficient amounts to be recognized by serum IgE from the whole population displaying clinical symptoms when exposed to the corresponding allergen in a real life setting (Lowenstein, 1987). Collectors must be qualified to verify the identity and quality of the source materials, so that only specifically identified allergenic source materials that do not contain avoidable foreign substances should be used in the manufacture of allergenic extracts and methods should be applied to trace the materials from their origin. This includes complete identity labelling and certification from competent collectors. Avoiding non-allergenic, contaminant material is particularly crucial also on the basis of the fact that same doubts were raised about the possibility that immunotherapy extracts could raise sensitization to previously non-sensitizing protein components. Along this line, the processing and storage of source materials should be performed to ensure that no unintended substances, including microbial organisms, are introduced into the materials. Records should describe source materials in as much detail as possible, including the particulars of collection, pre-treatment, and storage.

8.1 Pollens

The natural sources of inhalant allergens from plants are the pollens. Pollen may be obtained either by collection in nature or from cultivated fields or greenhouses. The collection may be performed by several methods, such as vacuuming or drying flower heads followed by pulverizing. In brief, anthers, which are long, slender filaments in a flower that have two lobes at the top, are best collected from intact, partially opened buds. The pollen may be cleaned either by passing through sieves of different mesh sizes or by flotation. Finally, pollens are dried under controlled conditions and stored in sealed containers at -20°C. The maximum level of accepted contamination with pollen from other species is 1% by number. Pollen should be devoid of flower and plant fragments, with a limit of 5% by weight. Pollens may show large modifications in quantitative composition depending on season and location of growth. In order to achieve a relatively constant composition, harvests from different years and sites of collection should be pooled for the production of allergen extracts, after thorough *in vitro* characterization.

8.2 House dust mites

House dust mites are grown in cultures and the source materials for mite allergen extracts can be represented by either pure bodies or whole mite cultures. Extracts based on whole mite cultures include eggs, larvae, and faecal particles as well as mite decomposition material and contain all the material to which mite-sensitized individuals are exposed under natural conditions. The culture medium should ideally be antigen free or, alternatively, contaminants from the culture medium should be shown not to be allergenic. The pure mite body extracts avoid extensive contamination with debris from the culture medium. In one clinical study, vaccines based on whole mite cultures and pure mite body extracts have shown similar clinical efficacy (Wahn et al., 1988).

8.3 Mammals

Allergens of mammalian origin may be present in various sources (typically, dander, serum, saliva, or urine). The allergens to which humans are exposed depend on the normal behaviour of the animal and the optimal source of allergens from mammals should be systematically investigated using a large panel of sera from patients sensitized to each given

animal. Notably, no matter if they are derived from dander or deposited from body fluids, most mammal allergens are present in the fur. Only healthy animals must be used for collecting allergens, and *post-mortem* collection is allowed only in exceptional cases and if stringent precautions to minimize decomposition were taken.

The optimal source materials are often dander, because hair proteins are insoluble. Use of whole pelt would increase the proportion of serum proteins, which are generally of low allergenic activity. In the case of the popular dog allergen extracts, remarkable differences were reported when deriving the material from different dog breeds (Larsen and Dreborg, 2008; Lindgren et al., 1988), a mixture of material from different breeds should be selected representing a balanced content of the major allergens (Uhlin et al., 1984).

8.4 Insects

The most common insect allergen is hymenoptera venom, which is brought in contact with the human immune system by sting. In this case, the purified venom itself is the best allergen source. In more uncommon cases the route of exposure may be biting or even inhalation. In this cases whole insects (including insects debris) and saliva are the proper allergen source, respectively.

8.5 Fungi

Allergens should be extracted by moulds, provided that they are grown under strictly controlled conditions (Larsen and Dreborg, 2008). The harvested raw materials should consist of mycelia and spores. In order for fungal cultures to maintain constant composition, it is recommended that extracts are derived from several independent cultures of the same species obtained from established fungal culture banks, i.e., American Type Culture Collection. All batches should be derived from the same strain to secure a stable composition, since it is well established that they may vary even under apparently analogous growth conditions (Steringer et al., 1987; Wallenbeck et al., 1984). The cultivation medium should be devoid of allergenic ingredients (i.e., serum proteins) and any safety measures should be taken to avoid contamination by any microorganisms, including other fungi

8.6 Foods

The supply of standardized material for the preparation of food allergen extract is limited, due to the complexity of this area. In fact, foods are often derived from various subspecies, grown under a broad variety of conditions reflecting geographical variation. Moreover, foods are often cooked prior to ingestion, and the cooking procedures may differ geographically. Consequently, the source of allergen exposure is highly variable both in qualitative as well as in quantitative terms (Lemanske and Taylor, 1987). The possibility to prepare sets of food allergen extracts reflecting the local species, the habit of cultivation, harvesting, storing, and cooking is a conceptual academic attitude which is raising more problems than those it can solve. For this reason, unsatisfactory results have been observed from commercially available reagents, which have resulted in many clinicians using untreated foods from retail trade for diagnosis by the prick-prick method (Dreborg and Foucard, 1983). Examples are fresh fruit, cow's milk, and hen's egg. Undiluted cow's milk and hen's egg have the best-documented diagnostic properties (Verstege et al., 2005). In this scenario, the usage of purified food allergen components, in either natural or recombinant form appears as a by far more suitable approach to circumvent these limitations. Moreover,

the characterization of the profile of sensitization to single allergen components allow to add up important clinical information, which cannot be extrapolated from diagnosis with whole extracts. For instance, the appearance of IgE to defined allergen components was documented in cohorts of milk allergic children (Hochwallner et al., 2010; Ott et al., 2008), which allows to follow up the clinical evolution of milk allergy. Another added value stems from the possibility to distinguish sensitizations associated with different profile risk. For instance, individuals with sensitization to nut allergen extract may have IgE to Cor a 1, cross-reacting birch allergens of the Bet v 1-like, PR-10 protein family or to Cor a 2, cross-reacting with profilins from several different allergen sources (fagales, grasses, pellitory, etc.). Alternatively, a positive skin prick test, or *in vitro* specific IgE determination assay, may be associated to the presence of IgE to the Lipid Transfer Protein Cor a 8. The former situation is associated with limited clinical symptoms (for instance, oral allergic syndrome) whereas the latter may be associated with severe systemic reactions. These two strikingly divergent scenarios, which can be referred to the acid and heat sensitivity of profilins and PR-10, *versus* the resistance to physical stimuli of lipid transfer proteins cannot be distinguished by extract-based diagnosis.

9. Preparation of allergen extracts

Allergens to be used for diagnostic purposes have to be as intact as possible in terms of molecular structures, which are recognized by allergic patients' IgE. Thus, any preventative measure has to be taken to prevent denaturation in the preparation and storage of allergen to be used for diagnosis or immunotherapy. On this basis, organic solvents, elevated temperatures, and extreme pH and ionic conditions should be carefully avoid whenever possible. The extraction should be performed under conditions resembling the physiological conditions (*i.e.*, physiological pH and ionic strength) and suppressing possible proteolytic degradation and microbial growth (Lowenstein et al., 1981; Lowenstein and Marsh, 1981, 1983). As a matter of fact, the optimal extraction time is usually a compromise between yield and denaturation of the allergens, and in general, the processing time is minimized and extraction performed at low temperatures. Low molecular weight, *i.e.*, below 5000 Dalton, non-antigenic material is removed from the extract by conventional biochemical techniques, such as dialysis, ultra-filtration, or size exclusion chromatography. However, it should be kept in mind that any substance excluded from the final product must be directly shown to be non-allergenic, before being systematically discarded. The final extract should be stored either lyophilized or at low temperatures (*i.e.*, -20°C to -80°C). Often 50% glycerol and non-allergenic proteins (*e.g.*, certified human serum albumin) are used as stabilizers.

9.1 IHR preparations

In Europe, the in-house reference preparations (IHR) are prepared by individual laboratories or Manufacturers, whereas in the United States, the US Food and Drug Administration (FDA) authorizes general standards of some common allergens for the purpose. IHR are specifically used by Manufacturers/laboratories for equilibration of the potency and composition of each batch of manufactured extract. By this procedure the batch-to-batch standardization is performed by comparison to the IHR using *in vitro* techniques exclusively.

Usually, three batches of the extract are produced in order to verify consistency and reproducibility of the production processes. The three batches are compared and, if

consistency is achieved, one is selected to represent the new IHR, which is subsequently dispensed into freeze-dried aliquots of suitable size.

The IHR is defined by the determination of the dry weight, protein content and composition, with particular reference to major allergen content and total allergenic activity by *in vivo* and *in vitro* methods. The evaluation of total allergenic activity by skin prick testing is usually performed only occasionally, *i.e.*, not for all batches, and has been shown to correlate with major allergen content (Dreborg and Einarsson, 1992). In general, the use of major allergen determination with a validated assay is considered sufficient to validate results of IHR units, when combination with a specific IgE potency assay is available. However, if *in vitro* methods alone are used for the establishment of the potency of the IHR, comparison is best made with the international standards (IS), which is not available for all allergens (see below).

9.2 International standards

Besides IHR preparation, allergen extract standardization requires the use of an internationally defined standard for each source material, indicated with the acronym IS. IS of allergen extracts can be obtained from the National Institute of Biological Science and Control, NIBSC, London, United Kingdom (Health Protection Agency, 2010), and are produced under the auspices of the WHO according to guidelines established by the Allergen Standardization Sub-Committee under the International Union of Immunological Societies (IUIS). IS enable comparison of specific activities of products from different manufacturers and can be used as calibrators by new producers and laboratories.

IS are presently available for the following allergen extracts: *Ambrosia artemisiifolia* (short ragweed) (Helm et al., 1984), *Phleum pratense* (timothy grass) (Gjesing et al., 1985), the house dust mite *Dermatophagoides pteronyssinus* (Ford et al., 1985), *Betula verrucosa* (birch), and *Canis familiaris* (dog) (Larsen et al., 1988)

9.3 Units and measure

The strength or potency of an allergen extract (allergenicity or reactogenicity) corresponds the extent of the response it will elicit in human allergic subjects, to be considered as a representative population of all individual with clinically relevant symptoms when exposed to that given allergen. Allergic patients react to allergen extracts with different strength because they are differently sensitized, *i.e.*, they have different IgE levels to different allergen components within the extract. Thus, the degree of sensitivity differs from patient to patient as well as for each allergen in the extract. Since extracts from different producers differ in composition there is no straightforward relationship between potency and response when comparing products from different Manufacturers and their potency cannot be compared in a satisfactory manner. Prick skin testing of human allergic subjects is the prevalent *in vivo* method for the assessment of allergen extract potency (Platts-Mills and Chapman, 1991) and also constitutes the standard underlying the determination of biological units of allergen extract potency. In this context, criteria of patient selection are obviously crucial, since potency measures will be dependent on pattern of sensitization in the panel of selected patients, Moreover, besides the characteristics of the sensitization to single allergen components, several other *in vivo* factors have been reported to variably influence prick test readings, such as age, sex, site of pricking, environmental pollution, season of the year (Bordignon and Burastero, 2006). According to the Nordic Council on Medicines HEP were adopted as the Nordic Biological Unit for allergens, which imply to determine the concentration of

allergen inducing a weal of the same size as that of histamine dihydrochloride, 10 mg/mL, as evaluated by a skin test run in parallel, in a panel of at least 20 patients attending an allergist who poses a diagnosis of allergy to the allergen of interest. The median concentration corresponds to 10,000 biological units (BU) (Dreborg et al., 1987). With proper patient selection this Unit has been shown to be relatively reproducible between different regions of Europe (Dreborg et al., 1987). European manufacturers use their own company-specific units, most of which are based on the same method. In the United States, the FDA uses a unit based on intra-dermal testing with the allergen extract and subsequent measurement of the flare rather than the weal size. This intradermal end point is expressed as the number of threefold dilution producing a summed erythema diameter of 50 mm. The mean value of 15 individuals defines the potency of the allergen extract, which is expressed in Allergy Units (AU). Later on, the Center for Biologics Evaluation and Research (CBER) in the United States proposed the Bioequivalent Allergy Unit (BAU). The method for assigning BAU is named ID50EAL. According to ID50EAL the intradermal dilution for 50 mm sum of erythema diameters determines the allergy unit (Turkeltaub, 1987).

In this context, labelling of the potency of allergen extracts based on the microgram amount of single allergen components, as progressively characterized by molecular cloning, to begin with the most representative major allergens, represented a revolutionary change in the field. Indeed, the major allergen content was found to correlate with the biological activity (Dreborg and Einarsson, 1992). If the same antibodies and methodology are used in the immunochemical determination of major allergen content determination of major allergen content can replace other methods for potency declaration.

Until a few years ago, each manufacturer used to express the allergenicity of each preparation using exclusively In-House Reference Units, without any reference to major allergen content. In my opinion, this scientifically unacceptable situation was strongly supported on a commercial ground, likely because allowed each manufacturer to prevent direct comparison between corresponding preparations of competitors. For more than a couple of decades this circumstance prevented allergists to know how far a given preparation was performing as compared to the corresponding preparations of the same allergen source from other manufacturers. Several surrogate markers were introduced, to compensate for this indefensible deficiency, including the bizarre ratio between the amounts of allergen used by each manufacturer in sublingual versus subcutaneous immunotherapy (Burastero, 2005; Gidaro et al., 2005). Even now that information of major allergen contents has become a unavoidable requisite on a cultural ground, although not yet a regulatory requirement (Moingeon, 2006), a sort of intellectually ambiguous position is maintained, by publicly supporting the notion, in congresses and meetings, that since different immunochemical methods are used to quantify such parameters, than it is still legitimate for each manufacturer to claim best performance of its own preparation regardless the amount of major allergens. As a matter of fact, extract potency can only be compared if at least uniform test methods and reference extracts are used (Larenas-Linnemann and Cox, 2008), which is by far a more acceptable, although in-development situation, than any alternative anarchism. Thus, even if in most cases the composition of test materials and the specificity of anti-allergen antibodies vary between laboratories, the content of a single major allergen does allow comparison of overall potency between marketed allergen extracts by far superior to any previously available methods.

Rather than persisting in old methods on the ground that the new ones has limits, new techniques will have to be developed and extensively applied in the next future to answer to

the following questions: a) How far is one given extract qualitatively representative of the repertoire of allergen components which are actually encountered in real life by sensitized patients in different areas of the world? b) How much is each allergen component quantitatively represented?

10. Stability testing

Criteria, methods, and limits for stability are established in a not satisfactory fashion. In Europe, a total allergenic activity in the interval between 30 and 300% of the arbitrarily defined in house reference titre is accepted. In contrast, US authorities limit the variability to 50–200% of the labelled activity. The underlying problems in this context are obviously related to the lack of precision in methods used of allergenicity measurement. For stability assessment the extract should be stored at several different temperatures to assure safe storage at room temperature, in a refrigerator, and at 20°C. Accelerated degradation studies designed to measure the kinetics of breakdown of the allergen extract can be performed by incubation at multiple high temperatures.

The reference for stability testing is usually the In-House Reference Units itself, a strategic choice, which allows each Manufacturer to safely claim superior stability of its own product without the risk that controversy can be raised. The application of assays providing results in milligram or molar amounts of major allergens are highly needed also for the proper evaluation of stability of allergen extracts from different Manufacturers.

11. Batch-to-batch control

Having established an In-House Reference Preparation, batch-to-batch standardization is still performed by calibrating new freeze-dried batches in bulk with the IHR before dispensing into vials for distribution. Batch-to-batch standardization usually include the steps indicated in Table 1

Although these sophisticated immunochemistry assays are helpful, they do not allow a straightforward comparison between different extracts, which could be performed with standardized assays based on the weight or molar amounts of major allergens.

12. Other techniques used for standardization

Beyond the use of monoclonal antibodies, other physico-chemical approaches have been explored for allergen standardization and offer new possibilities. Evaluation of mass spectrometry (MS) has been performed to determine its capacity to characterize the composition of allergen extracts (Heick et al., 2010; Houston et al., 2010). One advantage offered by the use of MS includes the measurement of several different allergenic components (allergens and isoforms) simultaneously rather than measuring individual allergens. This is advantageous while working with allergen preparations that contain a wide group of IgE binding proteins, such as mite extracts. Additionally, MS-based methods are available to discriminate between allergen isoforms, which is difficult to accomplish using immunologic based methods. This method could also be useful for the standardization of allergoids, since measuring major allergens is not possible in these preparations due to their chemical modification with aldehyde. The issues with MS-based protocols are that they are not quantitative and are not addressed in regulatory policies. Similarly, proteomic approaches

are extremely informative about the composition in major and minor allergens within complex extracts, although they do not allow provide quantitative results (Corti et al., 2005).

<i>Scope</i>	<i>Full description</i>	<i>Acronym</i>	<i>Ref.</i>
Determination of dry weight			
Assessment of allergenic composition to ensure the presence in the final product of all allergens present in the source material	Cross-(radio)-immunoelectrophoresis	CIE/CRIE	(Lowenstein, 1978)
	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis	SDS-PAGE	(Laemmli, 1970)
	Immunoblotting	IB	(Kyhse-Andersen, 1984)
	Isoelectric focusing	IEF	(Brighton, 1975)
Quantification of specific major allergens	Quantitative immunoelectrophoresis	QIE	(Lowenstein, 1978)
	Enzyme-linked immunosorbent assay	ELISA	(Engvall and Perlmann, 1972)
Quantification of the total allergenic activity	Radioallergosorbent test	RAST	(Ceska et al., 1972)
	RAST-inhibition		

Table 1. Steps in batch-to-batch control of allergen extracts

13. Recombinant allergens and allergen components

Allergen extracts are complex mixtures of substances, including mainly antigenic glycoproteins and variable amounts of non-antigenic, non-protein material, which may have some relevance in the trigger and in the establishment of the allergic reaction (Gutermuth et al., 2007; Traidl-Hoffmann et al., 2005). Since the beginning of the Nineties, allergen components has started to be characterized by molecular biology tools, namely by allergen cloning (Valenta et al., 1991), and the complex scenario of the composition of the clinically most important allergen sources has been gradually unveiled (Heiss et al., 1999; Kazemi-Shirazi et al., 2002; Valenta et al., 1999). For instance, presently as many as 50 allergen components from the *Phleum pratense* grass species have been identified (<http://www.allergome.org/>). *Phleum pratense* is a representative species in the group of grasses, which are known to trigger allergic symptoms. It has been extensively demonstrated that the homology among corresponding allergen components from different grasses is so high that, in the immune system perspective, the derived cross-reactivity of grass specific IgE make it possible to simplify grass allergy diagnosis to the point that one single species can be used as representative of the whole grass family (van Ree et al., 1998). A similar consideration has been extrapolated for *Fagales* (Van Ree et al., 1999). Thus, on one side it is acceptable to narrow down the number of species of a given family of allergenic

plants to one representative species, on the other within each given species the complexity of the allergen components which are composing the extract has to be considered as comprehensively as possible. In fact, it is well established that individuals sensitized to one given allergen source are variably reacting with the different antigenic components of the raw allergen extract. For instance, grass allergic subjects mainly react against *Phl p 1* and *Phl p 5* allergen components, yet complex patterns of reactivity can be observed in single individuals (Rossi et al., 2001; Rossi et al., 2000). Remarkable advantages derived from the application of molecular allergology to the overall comprehension of allergic diseases pathogenesis, including the understanding the molecular characteristics of allergens, the study of allergen structures, the characterization of the humoral (or B-cell-mediated, or antibody-dependent) and cellular (or T cell-mediated) immune responses (Burastero et al., 2004). Moreover, sequence similarity searches have identified the biological functions of many allergens and allowed to characterize mechanisms involved in typical allergic phenomena such as airway hyper-responsiveness and airway inflammation correlated to allergen exposure. For instance, *Der p 1* is a glycoprotein with sequence homology and thiol protease function similar to the enzymes papain, actinidin bromelain and cathepsins B and H (Chua et al., 1988). Only a limited number of clinical trials have been performed until now to compare the efficacy of recombinant allergens as compared to raw allergen extracts, although clinical benefit when using the former either in native (Jutel et al., 2005; Pauli et al., 2008) or modified forms (Niederberger et al., 2007; Purohit et al., 2008) have been reported. Recombinant allergens will overcome some of the pitfalls of using natural allergen products for immunotherapy by enabling physicians to administer only the clinically relevant allergens, thus avoiding exposure to unnecessary antigens. In this context, although the recombinant allergen era has not started yet for immunotherapy, yet quality control criteria must include the quantification of major allergen content in allergen extracts, as an invaluable tool to compare extracts from different manufacturers used for diagnosis or immunotherapy (Larsen and Dreborg, 2008).

14. Conclusion

Methods to achieve the standardisation of allergens used for diagnostic or therapeutic purposes should be homogeneous throughout the world. The present situation, whereby different Manufacturers use different units generate confusion and unreliable information, which may imply either underestimation or overestimation of the potency of allergen extracts. These differences may depend on the variability of the raw material used, the production methods and the lack of batch-to-batch consistence of the preparation procedure. The determination of the content in major allergen is essential to overcome these difficulties. Results of the CREATE project highlight a few limitations of recombinant allergen-based methodologies, which could support the implementation of this approach. In principle, as different factors as incorrect folding, aggregation, poor solubility and insufficient stability may affect the possibility to use a given allergen component as reference standard (Chapman et al., 2008). Nevertheless, knowledge generated by molecular allergology cannot longer wait to be integrated in the know-how to be developed and applied in this scenario. Along this line, the European Medicines Agency (EMA) recommends proving that each allergen extract contains the relevant allergens by antibody-based techniques or mass spectrometry. In this context, new promising techniques, such as nuclear magnetic resonance and small angle X ray scattering, can also be applied to characterize allergenic molecules in the laboratory.

Overall, this growing awareness is slowly affecting the methods by which allergen extracts are standardized. This is a necessary achievement in modern allergology, not only in the perspective of better patient care, but also under the prospect of definitely accompanying this discipline to the podium it deserves. Development of standardized methods to measure allergen content are also eagerly needed by the imminent improvement of specific immunotherapy, which will imply the usage of recombinant (or purified) allergen components, instead of allergen extracts. This step will definitely promote the development of internationally accepted, standardized methods to measure allergen content, and to quantify the homogeneity, folding, aggregation, solubility and stability of recombinant products.

There is no doubt about the fact that allergen standardization is a fast developing field, which will soon allow to complete the progress of allergology from the present transition status where much is still managed according to traditional approaches based on allergen extracts and old cuisine to the full implementation of molecular immunology. The dramatic social relevance of allergies will warrant the encouragement of the medical and scientific community to this transition.

15. References

- Abramson, J., and Pecht, I. (2007). Regulation of the mast cell response to the type 1 Fc epsilon receptor. *Immunol Rev* Vol.217, pp.231-254.
- Abramson, M.J., Puy, R.M., and Weiner, J.M. (2010). Injection allergen immunotherapy for asthma. *Cochrane Database Syst Rev*, No.8, pp.CD001186.
- Health Protection Agency, (2010). Biological Reference Material.
- Akdis, C.A., and Akdis, M. (2010). Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* Vol.127, No.1, pp.18-27; quiz 28-19.
- Akdis, C.A., and Blaser, K. (2001). Mechanisms of interleukin-10-mediated immune suppression. *Immunology* Vol.103, No.2, pp.131-136.
- Al Suleimani, Y.M., and Walker, M.J. (2007). Allergic rhinitis and its pharmacology. *Pharmacol Ther* Vol.114, No.3, pp.233-260.
- Alvarez-Cuesta, E., Bousquet, J., Canonica, G.W., Durham, S.R., Malling, H.J., and Valovirta, E. (2006). Standards for practical allergen-specific immunotherapy. *Allergy* Vol.61 Suppl 82, pp.1-20.
- Arbes, S.J., Jr., Cohn, R.D., Yin, M., Muilenberg, M.L., Burge, H.A., Friedman, W., and Zeldin, D.C. (2003). House dust mite allergen in US beds: results from the First National Survey of Lead and Allergens in Housing. *J Allergy Clin Immunol* Vol.111, No.2, pp.408-414.
- Barrett, N.A., and Austen, K.F. (2009). Innate cells and T helper 2 cell immunity in airway inflammation. *Immunity* Vol.31, No.3, pp.425-437.
- Becker, W.M., Vogel, L., and Vieths, S. (2006). Standardization of allergen extracts for immunotherapy: where do we stand? *Curr Opin Allergy Clin Immunol* Vol.6, No.6, pp.470-475.
- Bordignon, V., and Burastero, S.E. (2006). Age, gender and reactivity to allergens independently influence skin reactivity to histamine. *J Investig Allergol Clin Immunol* Vol.16, No.2, pp.129-135.
- Bousquet, J., Lockey, R., Malling, H.J., Alvarez-Cuesta, E., Canonica, G.W., Chapman, M.D., Creticos, P.J., Dayer, J.M., Durham, S.R., Demoly, P., et al. (1998). Allergen

- immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American academy of Allergy, Asthma and Immunology. *Ann Allergy Asthma Immunol* Vol.81, No.5 Pt 1, pp.401-405.
- Brighton, W.D. (1975). Profiles of allergen extract components by isoelectric focussing and radioimmunoassay. *Dev Biol Stand* Vol.29, pp.362-369.
- Bufe, A., Eberle, P., Franke-Beckmann, E., Funck, J., Kimmig, M., Klimek, L., Knecht, R., Stephan, V., Tholstrup, B., Weisshaar, C., et al. (2009). Safety and efficacy in children of an SQ-standardized grass allergen tablet for sublingual immunotherapy. *J Allergy Clin Immunol* Vol.123, No.1, pp.167-173 e167.
- Burastero, S. (2005). Regarding Gidaro GB, Marcucci F, Sensi L, Incorvaia C, Frati F, Ciprandi G. The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin Exp Allergy* 2005; 35:565-71. *Clin Exp Allergy* Vol.35, No.10, pp.1407-1408; author reply 1409.
- Burastero, S.E., Paolucci, C., Breda, D., Longhi, R., Silvestri, M., Hammer, J., Protti, M.P., and Rossi, G.A. (2004). T-cell receptor-mediated cross-allergenicity. *Int Arch Allergy Immunol* Vol.135, No.4, pp.296-305.
- Bush, A., and Saglani, S. (2010). Management of severe asthma in children. *Lancet* Vol.376, No.9743, pp.814-825.
- Calamita, Z., Saconato, H., Pela, A.B., and Atallah, A.N. (2006). Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. *Allergy* Vol.61, No.10, pp.1162-1172.
- Ceska, M., Eriksson, R., and Varga, J.M. (1972). Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* Vol.49, No.1, pp.1-9.
- Chafen, J.J., Newberry, S.J., Riedl, M.A., Bravata, D.M., Maglione, M., Suttorp, M.J., Sundaram, V., Paige, N.M., Towfigh, A., Hulley, B.J., et al. (2010). Diagnosing and managing common food allergies: a systematic review. *JAMA* Vol.303, No.18, pp.1848-1856.
- Chapman, M.D., Ferreira, F., Villalba, M., Cromwell, O., Bryan, D., Becker, W.M., Fernandez-Rivas, M., Durham, S., Vieths, S., and van Ree, R. (2008). The European Union CREATE project: a model for international standardization of allergy diagnostics and vaccines. *J Allergy Clin Immunol* Vol.122, No.5, pp.882-889 e882.
- Chapman, M.D., Pomes, A., Breiteneder, H., and Ferreira, F. (2007). Nomenclature and structural biology of allergens. *J Allergy Clin Immunol* Vol.119, No.2, pp.414-420.
- Chapman, M.D., Smith, A.M., Vailes, L.D., Arruda, L.K., Dhanaraj, V., and Pomes, A. (2000). Recombinant allergens for diagnosis and therapy of allergic disease. *J Allergy Clin Immunol* Vol.106, No.3, pp.409-418.
- Chinen, J., and Shearer, W.T. (2004). Advances in asthma, allergy and immunology series 2004: basic and clinical immunology. *J Allergy Clin Immunol* Vol.114, No.2, pp.398-405.
- Chinen, J., and Shearer, W.T. (2007). Advances in basic and clinical immunology in 2006. *J Allergy Clin Immunol* Vol.120, No.2, pp.263-270.
- Chu, H.W., Lloyd, C.M., Karmaus, W., Maestrelli, P., Mason, P., Salcedo, G., Thaikoottathil, J., and Wardlaw, A.J. Developments in the field of allergy in 2009 through the eyes of Clinical and Experimental Allergy. *Clin Exp Allergy* Vol.40, No.11, pp.1611-1631.
- Chua, K.Y., Stewart, G.A., Thomas, W.R., Simpson, R.J., Dilworth, R.J., Plozza, T.M., and Turner, K.J. (1988). Sequence analysis of cDNA coding for a major house dust mite

- allergen, Der p 1. Homology with cysteine proteases. *J Exp Med* Vol.167, No.1, pp.175-182.
- Ciprandi, G., Cadario, G., Di Gioacchino, G.M., Gangemi, S., Gasparini, A., Isola, S., Marengo, F., Minelli, S., Ricciardi, L., Ridolo, E., *et al.* (2010a). Sublingual immunotherapy in children with allergic polysensitization. *Allergy Asthma Proc* Vol.31, No.3, pp.227-231.
- Ciprandi, G., Incorvaia, C., Puccinelli, P., Scurati, S., Masieri, S., and Frati, F. (2010b). The POLISMAIL lesson: sublingual immunotherapy may be prescribed also in polysensitized patients. *Int J Immunopathol Pharmacol* Vol.23, No.2, pp.637-640.
- Corti, V., Cattaneo, A., Bachi, A., Rossi, R.E., Monasterolo, G., Paolucci, C., Burastero, S.E., and Alessio, M. (2005). Identification of grass pollen allergens by two-dimensional gel electrophoresis and serological screening. *Proteomics* Vol.5, No.3, pp.729-736.
- Cox, L., Nelson, H., Lockey, R., Calabria, C., Chacko, T., Finegold, I., Nelson, M., Weber, R., Bernstein, D.I., Blessing-Moore, J., *et al.* (2010). Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol* Vol.127, No.1 Suppl, pp.S1-55.
- Cummings, A.J., Knibb, R.C., King, R.M., and Lucas, J.S. (2010). The psychosocial impact of food allergy and food hypersensitivity in children, adolescents and their families: a review. *Allergy* Vol.65, No.8, pp.933-945.
- Dahl, R., Kapp, A., Colombo, G., de Monchy, J.G., Rak, S., Emminger, W., Riis, B., Gronager, P.M., and Durham, S.R. (2008). Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol* Vol.121, No.2, pp.512-518 e512.
- Dreborg, S., Basomba, A., Belin, L., Durham, S., Einarsson, R., Eriksson, N.E., Frostad, A.B., Grimmer, O., Halvorsen, R., Holgersson, M., *et al.* (1987). Biological equilibration of allergen preparations: methodological aspects and reproducibility. *Clin Allergy* Vol.17, No.6, pp.537-550.
- Dreborg, S., and Einarsson, R. (1992). The major allergen content of allergenic preparations reflect their biological activity. *Allergy* Vol.47, No.4 Pt 2, pp.418-423.
- Dreborg, S., and Foucard, T. (1983). Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* Vol.38, No.3, pp.167-172.
- Dykewicz, M.S., and Hamilos, D.L. (2010). Rhinitis and sinusitis. *J Allergy Clin Immunol* Vol.125, No.2 Suppl 2, pp.S103-115.
- Engvall, E., and Perlmann, P. (1972). Enzyme-linked immunosorbent assay, Elisa. 3. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen-coated tubes. *J Immunol* Vol.109, No.1, pp.129-135.
- Enrique, E., Pineda, F., Malek, T., Bartra, J., Basagana, M., Tella, R., Castello, J.V., Alonso, R., de Mateo, J.A., Cerda-Trias, T., *et al.* (2005). Sublingual immunotherapy for hazelnut food allergy: a randomized, double-blind, placebo-controlled study with a standardized hazelnut extract. *J Allergy Clin Immunol* Vol.116, No.5, pp.1073-1079.
- Esch, R.E. (2004). Manufacturing and standardizing fungal allergen products. *J Allergy Clin Immunol* Vol.113, No.2, pp.210-215.
- Fernandez-Caldas, E., Carnes, J., Gallego, M., Mari, A., and Pagan Aleman, J.A. (2006). Standardization of animal epithelia. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M*, No.95, pp.107-116; discussion 116, 155.
- Fernández-Caldas, E., Zakzuk, J., and Lockey, R.F. (2009). Allergen Standardization and Characterization.

- Ferreira, F., Briza, P., Infuhr, D., Schmidt, G., Wallner, M., Wopfner, N., Thalhamer, J., and Achatz, G. (2006). Modified recombinant allergens for safer immunotherapy. *Inflamm Allergy Drug Targets* Vol.5, No.1, pp.5-14.
- Ferrer, A., Carnes, J., Gallego, M.T., Andreu, C., and Fernandez-Caldas, E. (2005). Characterization and improvement of apple extracts for the diagnosis of apple IgE-mediated allergy. *Ann Allergy Asthma Immunol* Vol.95, No.5, pp.462-467.
- Ford, A., Seagroatt, V., Platts-Mills, T.A., and Lowenstein, H. (1985). A collaborative study on the first international standard of Dermatophagoides pteronyssinus (house dust mite) extract. *J Allergy Clin Immunol* Vol.75, No.6, pp.676-686.
- Gidaro, G.B., Marcucci, F., Sensi, L., Incorvaia, C., Frati, F., and Ciprandi, G. (2005). The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin Exp Allergy* Vol.35, No.5, pp.565-571.
- Gjesing, B., Jager, L., Marsh, D.G., and Lowenstein, H. (1985). The international collaborative study establishing the first international standard for timothy (Phleum pratense) grass pollen allergenic extract. *J Allergy Clin Immunol* Vol.75, No.2, pp.258-267.
- Grier, T.J., Hazelhurst, D.M., Duncan, E.A., West, T.K., and Esch, R.E. (2002). Major allergen measurements: sources of variability, validation, quality assurance, and utility for laboratories, manufacturers, and clinics. *Allergy Asthma Proc* Vol.23, No.2, pp.125-131.
- Gurka, G., and Rocklin, R. (1988). Immunologic responses during allergen-specific immunotherapy for respiratory allergy. *Ann Allergy* Vol.61, No.4, pp.239-245.
- Gutermuth, J., Bewersdorff, M., Traidl-Hoffmann, C., Ring, J., Mueller, M.J., Behrendt, H., and Jakob, T. (2007). Immunomodulatory effects of aqueous birch pollen extracts and phytoprostanes on primary immune responses in vivo. *J Allergy Clin Immunol* Vol.120, No.2, pp.293-299.
- Hamid, Q., and Tulic, M. (2009). Immunobiology of asthma. *Annu Rev Physiol* Vol.71, pp.489-507.
- Hamilton, R.G. (2010). Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol* Vol.125, No.2 Suppl 2, pp.S284-296.
- Hamilton, R.G. (2005). Assessment of indoor allergen exposure. *Curr Allergy Asthma Rep* Vol.5, No.5, pp.394-401.
- Hankin, C.S., and Lockey, R.F. (2010). Patient characteristics associated with allergen immunotherapy initiation and adherence. *J Allergy Clin Immunol* Vol.127, No.1, pp.46-48, 48 e41-43.
- Hauser, M., Roulias, A., Ferreira, F., and Egger, M. (2010). Panallergens and their impact on the allergic patient. *Allergy Asthma Clin Immunol* Vol.6, No.1, pp.1.
- Heick, J., Fischer, M., and Popping, B. (2010). First screening method for the simultaneous detection of seven allergens by liquid chromatography mass spectrometry. *J Chromatogr A* Vol.1218, No.7, pp.938-943.
- Heinrich, J., Bedada, G.B., Zock, J.P., Chinn, S., Norback, D., Olivieri, M., Svanes, C., Ponzio, M., Verlato, G., Villani, S., et al. (2006). Cat allergen level: its determinants and relationship to specific IgE to cat across European centers. *J Allergy Clin Immunol* Vol.118, No.3, pp.674-681.
- Heiss, S., Mahler, V., Steiner, R., Spitzauer, S., Schweiger, C., Kraft, D., and Valenta, R. (1999). Component-resolved diagnosis (CRD) of type I allergy with recombinant

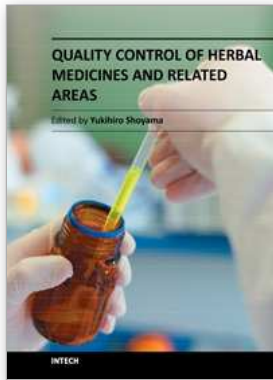
- grass and tree pollen allergens by skin testing. *J Invest Dermatol* Vol.113, No.5, pp.830-837.
- Helm, R.M., Gauerke, M.B., Baer, H., Lowenstein, H., Ford, A., Levy, D.A., Norman, P.S., and Yunginger, J.W. (1984). Production and testing of an international reference standard of short ragweed pollen extract. *J Allergy Clin Immunol* Vol.73, No.6, pp.790-800.
- Himly, M., Nony, E., Chabre, H., Van Overtvelt, L., Neubauer, A., van Ree, R., Buchheit, K.H., Vieths, S., Moingeon, P., and Ferreira, F. (2009). Standardization of allergen products: 1. Detailed characterization of GMP-produced recombinant Bet v 1.0101 as biological reference preparation. *Allergy* Vol.64, No.7, pp.1038-1045.
- Hochwallner, H., Schulmeister, U., Swoboda, I., Balic, N., Geller, B., Nystrand, M., Harlin, A., Thalhamer, J., Scheiblhofer, S., Niggemann, B., et al. (2010). Microarray and allergenic activity assessment of milk allergens. *Clin Exp Allergy* Vol.40, No.12, pp.1809-1818.
- Holgate, S.T. (2008). Pathogenesis of asthma. *Clin Exp Allergy* Vol.38, No.6, pp.872-897.
- Holowka, D., Sil, D., Torigoe, C., and Baird, B. (2007). Insights into immunoglobulin E receptor signaling from structurally defined ligands. *Immunol Rev* Vol.217, pp.269-279.
- Houston, N.L., Lee, D.G., Stevenson, S.E., Ladics, G.S., Bannon, G.A., McClain, S., Privalle, L., Stagg, N., Herouet-Guicheney, C., MacIntosh, S.C., et al. (2010). Quantitation of soybean allergens using tandem mass spectrometry. *J Proteome Res* Vol.10, No.2, pp.763-773.
- Illi, S., von Mutius, E., Lau, S., Niggemann, B., Gruber, C., and Wahn, U. (2006). Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* Vol.368, No.9537, pp.763-770.
- Jutel, M., Jaeger, L., Suck, R., Meyer, H., Fiebig, H., and Cromwell, O. (2005). Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* Vol.116, No.3, pp.608-613.
- Kazemi-Shirazi, L., Niederberger, V., Linhart, B., Lidholm, J., Kraft, D., and Valenta, R. (2002). Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. *Int Arch Allergy Immunol* Vol.127, No.4, pp.259-268.
- King, T.P., Hoffman, D., Lowenstein, H., Marsh, D.G., Platts-Mills, T.A., and Thomas, W. (1994). Allergen nomenclature. WHO/IUIS Allergen Nomenclature Subcommittee. *Int Arch Allergy Immunol* Vol.105, No.3, pp.224-233.
- Kuriakose, J.S., and Miller, R.L. (2010). Environmental epigenetics and allergic diseases: recent advances. *Clin Exp Allergy* Vol.40, No.11, pp.1602-1610.
- Kyhse-Andersen, J. (1984). Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J Biochem Biophys Methods* Vol.10, No.3-4, pp.203-209.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* Vol.227, No.5259, pp.680-685.
- Larenas-Linnemann, D., and Cox, L.S. (2008). European allergen extract units and potency: review of available information. *Ann Allergy Asthma Immunol* Vol.100, No.2, pp.137-145.
- Larsen, J.N., and Dreborg, S. (2008). Standardization of allergen extracts. *Methods Mol Med* Vol.138, pp.133-145.

- Larsen, J.N., Ford, A., Gjesing, B., Levy, D., Petrunov, B., Silvestri, L., and Lowenstein, H. (1988). The collaborative study of the international standard of dog, *Canis domesticus*, hair/dander extract. *J Allergy Clin Immunol* Vol.82, No.3 Pt 1, pp.318-330.
- Lemanske, R.F., Jr., and Taylor, S.L. (1987). Standardized extracts, foods. *Clin Rev Allergy* Vol.5, No.1, pp.23-36.
- Lindgren, S., Belin, L., Dreborg, S., Einarsson, R., and Pahlman, I. (1988). Breed-specific dog-dander allergens. *J Allergy Clin Immunol* Vol.82, No.2, pp.196-204.
- Lowenstein, H. (1978). Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* Vol.25, pp.1-62.
- Lowenstein, H. (1987). Selection of reference preparation. IUIS reference preparation criteria. International Union of Immunological Societies. *Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M*, No.80, pp.75-78.
- Lowenstein, H., King, T.P., Goodfriend, L., Hussain, R., Roebber, M., and Marsh, D.G. (1981). Antigens of *Ambrosia elatior* (short ragweed) pollen. II. Immunochemical identification of known antigens by quantitative immunoelectrophoresis. *J Immunol* Vol.127, No.2, pp.637-642.
- Lowenstein, H., and Marsh, D.G. (1981). Antigens of *Ambrosia elatior* (short ragweed) pollen. I. Crossed immunoelectrophoretic analyses. *J Immunol* Vol.126, No.3, pp.943-948.
- Lowenstein, H., and Marsh, D.G. (1983). Antigens of *Ambrosia elatior* (short ragweed) pollen. III. Crossed radioimmunoelectrophoresis of ragweed-allergic patients' sera with special attention to quantification of IgE responses. *J Immunol* Vol.130, No.2, pp.727-731.
- Macglashan, D., Jr. (2005). IgE and Fc[epsilon]RI regulation. *Ann N Y Acad Sci* Vol.1050, pp.73-88.
- Moingeon, P. (2006). Sublingual immunotherapy: from biological extracts to recombinant allergens. *Allergy* Vol.61 Suppl 81, pp.15-19.
- Morgan, W.J., Crain, E.F., Gruchalla, R.S., O'Connor, G.T., Kattan, M., Evans, R., 3rd, Stout, J., Malindzak, G., Smartt, E., Plaut, M., *et al.* (2004). Results of a home-based environmental intervention among urban children with asthma. *N Engl J Med* Vol.351, No.11, pp.1068-1080.
- Mothes, N., Valenta, R., and Spitzauer, S. (2006). Allergy testing: the role of recombinant allergens. *Clin Chem Lab Med* Vol.44, No.2, pp.125-132.
- Nelson, H.S. (2000). The use of standardized extracts in allergen immunotherapy. *J Allergy Clin Immunol* Vol.106, No.1 Pt 1, pp.41-45.
- Nelson, H.S. (2007). Allergen immunotherapy: where is it now? *J Allergy Clin Immunol* Vol.119, No.4, pp.769-779.
- Niederberger, V., Reisinger, J., Valent, P., Krauth, M.T., Pauli, G., van Hage, M., Cromwell, O., Horak, F., and Valenta, R. (2007). Vaccination with genetically modified birch pollen allergens: immune and clinical effects on oral allergy syndrome. *J Allergy Clin Immunol* Vol.119, No.4, pp.1013-1016.
- Novak, N. (2009). New insights into the mechanism and management of allergic diseases: atopic dermatitis. *Allergy* Vol.64, No.2, pp.265-275.
- Ott, H., Baron, J.M., Heise, R., Ocklenburg, C., Stanzel, S., Merk, H.F., Niggemann, B., and Beyer, K. (2008). Clinical usefulness of microarray-based IgE detection in children with suspected food allergy. *Allergy* Vol.63, No.11, pp.1521-1528.

- Pagani, M., Antico, A., Cilia, M., Calabro, D., Poto, S., Pecora, S., and Burastero, S.E. (2009). Comparison of different diagnostic products for skin prick testing. *Eur Ann Allergy Clin Immunol* Vol.41, No.1, pp.23-31.
- Pauli, G., Larsen, T.H., Rak, S., Horak, F., Pastorello, E., Valenta, R., Purohit, A., Arvidsson, M., Kavina, A., Schroeder, J.W., et al. (2008). Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* Vol.122, No.5, pp.951-960.
- Pittner, G., Vrtala, S., Thomas, W.R., Weghofer, M., Kundi, M., Horak, F., Kraft, D., and Valenta, R. (2004). Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* Vol.34, No.4, pp.597-603.
- Platts-Mills, T.A., and Chapman, M.D. (1991). Allergen standardization. *J Allergy Clin Immunol* Vol.87, No.3, pp.621-625.
- Platts-Mills, T.A., Vaughan, J.W., Carter, M.C., and Woodfolk, J.A. (2000). The role of intervention in established allergy: avoidance of indoor allergens in the treatment of chronic allergic disease. *J Allergy Clin Immunol* Vol.106, No.5, pp.787-804.
- Platts-Mills, T.A., Vervloet, D., Thomas, W.R., Aalberse, R.C., and Chapman, M.D. (1997). Indoor allergens and asthma: report of the Third International Workshop. *J Allergy Clin Immunol* Vol.100, No.6 Pt 1, pp.S2-24.
- Pomes, A. (2010). Relevant B cell epitopes in allergic disease. *Int Arch Allergy Immunol* Vol.152, No.1, pp.1-11.
- Pucci, S., and Incorvaia, C. (2008). Allergy as an organ and a systemic disease. *Clin Exp Immunol* Vol.153 Suppl 1, pp.1-2.
- Purohit, A., Niederberger, V., Kronqvist, M., Horak, F., Gronneberg, R., Suck, R., Weber, B., Fiebig, H., van Hage, M., Pauli, G., et al. (2008). Clinical effects of immunotherapy with genetically modified recombinant birch pollen Bet v 1 derivatives. *Clin Exp Allergy* Vol.38, No.9, pp.1514-1525.
- Radulovic, S., Calderon, M.A., Wilson, D., and Durham, S. (2010). Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev*, No.12, pp.CD002893.
- Rossi, R.E., Monasterolo, G., and Monasterolo, S. (2001). Measurement of IgE antibodies against purified grass-pollen allergens (Phl p 1, 2, 3, 4, 5, 6, 7, 11, and 12) in sera of patients allergic to grass pollen. *Allergy* Vol.56, No.12, pp.1180-1185.
- Rossi, R.E., Monasterolo, G., Operti, D., Operti, R., and Berlen, R. (2000). Evaluation of IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, and Phl p 5) in a random sample of patients with specific IgE to Phleum pratense. *Allergy* Vol.55, No.2, pp.181-184.
- Sander, I., Fleischer, C., Meurer, U., Bruning, T., and Raulf-Heimsoth, M. (2009). Allergen content of grass pollen preparations for skin prick testing and sublingual immunotherapy. *Allergy* Vol.64, No.10, pp.1486-1492.
- Santos, A., and Van Ree, R. (2011). Profilins: Mimickers of Allergy or Relevant Allergens? *Int Arch Allergy Immunol* Vol.155, No.3, pp.191-204.
- Scala, E., Alessandri, C., Bernardi, M.L., Ferrara, R., Palazzo, P., Pomponi, D., Quarantino, D., Rasi, C., Zaffiro, A., Zennaro, D., et al. (2010). Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy* Vol.40, No.6, pp.911-921.

- Scheiner, O., Breiteneder, H., Dolocek, C., Duchene, M., Ebner, C., Ferreira, F., Hoffmann, K., Schenk, S., Valenta, R., and Kraft, D. (1994). Molecular and functional characterization of allergens: basic and practical aspects. *Arb Paul Ehrlich Inst Bundesamt Sera Impfstoffe Frankf A M*, No.87, pp.221-232; discussion 233-224.
- Sears, M.R., Greene, J.M., Willan, A.R., Wiecek, E.M., Taylor, D.R., Flannery, E.M., Cowan, J.O., Herbison, G.P., Silva, P.A., and Poulton, R. (2003). A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* Vol.349, No.15, pp.1414-1422.
- Shakib, F., Ghaemmaghami, A.M., and Sewell, H.F. (2008). The molecular basis of allergenicity. *Trends Immunol* Vol.29, No.12, pp.633-642.
- Sicherer, S.H., and Leung, D.Y. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects in 2009. *J Allergy Clin Immunol* Vol.125, No.1, pp.85-97.
- Simons, F.E. Anaphylaxis. (2010). *J Allergy Clin Immunol* Vol.125, No.2 Suppl 2, pp.S161-181.
- Slater, J.E. (2004). Standardized allergen extracts in the United States. *Clin Allergy Immunol* Vol.18, pp.421-432.
- Spergel, J.M. (2010a). Epidemiology of atopic dermatitis and atopic march in children. *Immunol Allergy Clin North Am* Vol.30, No.3, pp.269-280.
- Spergel, J.M. (2010b). From atopic dermatitis to asthma: the atopic march. *Ann Allergy Asthma Immunol* Vol.105, No.2, pp.99-106; quiz 107-109, 117.
- Sporik, R., Squillace, S.P., Ingram, J.M., Rakes, G., Honsinger, R.W., and Platts-Mills, T.A. (1999). Mite, cat, and cockroach exposure, allergen sensitisation, and asthma in children: a case-control study of three schools. *Thorax* Vol.54, No.8, pp.675-680.
- Steringer, I., Aukrust, L., and Einarsson, R. (1987). Variability of antigenicity/allergenicity in different strains of *Alternaria alternata*. *Int Arch Allergy Appl Immunol* Vol.84, No.2, pp.190-197.
- Terho, E., and Frew, A.J. (1995). Type III allergy skin testing. Position statement for EAACI Subcommittee on Skin Tests and Allergen Standardization. *Allergy* Vol.50, No.5, pp.392-396.
- Traidl-Hoffmann, C., Mariani, V., Hochrein, H., Karg, K., Wagner, H., Ring, J., Mueller, M.J., Jakob, T., and Behrendt, H. (2005). Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J Exp Med* Vol.201, No.4, pp.627-636.
- Turkeltaub, P.C. (1987). Biological standardization based on quantitative skin testing--the ID50 EAL method (intradermal dilution for 50 mm sum of erythema diameters determines the allergy unit). *Arb Paul Ehrlich Inst Georg Speyer Haus Ferdinand Blum Inst Frankf A M*, No.80, pp.169-173.
- Uhlen, T., Reuterby, J., and Einarsson, R. (1984). Antigenic/allergenic composition of Poodle/Alsatian dandruff extract. *Allergy* Vol.39, No.2, pp.125-133.
- Valenta, R., Duchene, M., Pettenburger, K., Sillaber, C., Valent, P., Bettelheim, P., Breitenbach, M., Rumpold, H., Kraft, D., and Scheiner, O. (1991). Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* Vol.253, No.5019, pp.557-560.
- Valenta, R., Lidholm, J., Niederberger, V., Hayek, B., Kraft, D., and Gronlund, H. (1999). The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* Vol.29, No.7, pp.896-904.

- Valenta, R., Twaroch, T., and Swoboda, I. (2007). Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. *J Investig Allergol Clin Immunol* Vol.17 Suppl 1, pp.36-40.
- van Ree, R. (2007). Indoor allergens: relevance of major allergen measurements and standardization. *J Allergy Clin Immunol* Vol.119, No.2, pp.270-277; quiz 278-279.
- van Ree, R., Chapman, M.D., Ferreira, F., Vieths, S., Bryan, D., Cromwell, O., Villalba, M., Durham, S.R., Becker, W.M., Aalbers, M., *et al.* (2008). The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. *Allergy* Vol.63, No.3, pp.310-326.
- van Ree, R., van Leeuwen, W.A., and Aalberse, R.C. (1998). How far can we simplify in vitro diagnostics for grass pollen allergy?: A study with 17 whole pollen extracts and purified natural and recombinant major allergens. *J Allergy Clin Immunol* Vol.102, No.2, pp.184-190.
- Van Ree, R., Van Leeuwen, W.A., Akkerdaas, J.H., and Aalberse, R.C. (1999). How far can we simplify in vitro diagnostics for Fagales tree pollen allergy? A study with three whole pollen extracts and purified natural and recombinant allergens. *Clin Exp Allergy* Vol.29, No.6, pp.848-855.
- Verstege, A., Mehl, A., Rolinck-Werninghaus, C., Staden, U., Nocon, M., Beyer, K., and Niggemann, B. (2005). The predictive value of the skin prick test weal size for the outcome of oral food challenges. *Clin Exp Allergy* Vol.35, No.9, pp.1220-1226.
- Vrtala, S. (2008). From allergen genes to new forms of allergy diagnosis and treatment. *Allergy* Vol.63, No.3, pp.299-309.
- Wahn, U., Schweter, C., Lind, P., and Lowenstein, H. (1988). Prospective study on immunologic changes induced by two different *Dermatophagoides pteronyssinus* extracts prepared from whole mite culture and mite bodies. *J Allergy Clin Immunol* Vol.82, No.3 Pt 1, pp.360-370.
- Wallenbeck, I., Aukrust, L., and Einarsson, R. (1984). Antigenic variability of different strains of *Aspergillus fumigatus*. *Int Arch Allergy Appl Immunol* Vol.73, No.2, pp.166-172.
- Woodcock, A., Lowe, L.A., Murray, C.S., Simpson, B.M., Pipis, S.D., Kissen, P., Simpson, A., and Custovic, A. (2004). Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years. *Am J Respir Crit Care Med* Vol.170, No.4, pp.433-439.
- Zock, J.P., Heinrich, J., Jarvis, D., Verlato, G., Norback, D., Plana, E., Sunyer, J., Chinn, S., Olivieri, M., Soon, A., *et al.* (2006). Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. *J Allergy Clin Immunol* Vol.118, No.3, pp.682-690.



Quality Control of Herbal Medicines and Related Areas

Edited by Prof. Yukihiro Shoyama

ISBN 978-953-307-682-9

Hard cover, 282 pages

Publisher InTech

Published online 07, July, 2011

Published in print edition July, 2011

The authors of this thematic issue provide a comprehensive summary of most recent knowledge and references on quality control in wide fields. Quality control is essential for natural products like natural medicine and related food products. In this issue fifteen chapters have been included, discussing in detail various aspects of quality control. It will certainly prove useful not only for phytochemical researchers, but also many scientists working in numerous fields. Much effort has been invested by the contributors to share current information. Without their efforts and input 'Quality Control of Herbal Medicine and Related Areas' could not exist.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Samuele E. Burastero (2011). Allergen Extract Analysis and Quality Control, Quality Control of Herbal Medicines and Related Areas, Prof. Yukihiro Shoyama (Ed.), ISBN: 978-953-307-682-9, InTech, Available from: <http://www.intechopen.com/books/quality-control-of-herbal-medicines-and-related-areas/allergen-extract-analysis-and-quality-control>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.