REVIEW

Major Allergen Content in Allergen Immunotherapy Products: The Limited Value of Numbers

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Abstract

The prevalence of allergic disorders has increased drastically over the last 50 years to the extent that they can be considered epidemic. At present, allergen-specific immunotherapy (AIT) is the only therapy that targets the underlying cause of allergic disorders, and evidence of its superiority is based on data accumulated from clinical trials and observational studies demonstrating efficacy and safety. However, several aspects remain unresolved, such as harmonization and standardization of manufacturing and quantification procedures across manufacturers, homogeneous reporting of strength, and the establishment of international reference standards for many allergens. This article discusses issues related to the measurement of major allergen content in AIT extracts, raising the question of whether comparison of products from different manufacturers is an appropriate basis for selecting a specific AIT product. Allergen standardization in immunotherapy products is critical for ensuring quality and, thereby, safety and efficacy. However, lack of harmonization in manufacturing processes, allergen quantification (methodologies and references), national regulatory differences, clinical practice, and labeling shows that the comparison of AIT products based solely on major allergen amounts is not rational and, in fact, impossible. Moreover, when rating the information given for a specific product, it is necessary to take into account further inherent characteristics of products and their application in clinical practice, such as the state of extract modification, addition of adjuvant or adjuvant system, route of administration (sublingual/subcutaneous), and cumulative dose as per posology (including the volume per administration). Finally, only convincing clinical data can serve as the basis for product-specific evaluation and cross-product comparability of individual products.

Key words: AIT. Major allergen content. Immunotherapy. Standardization methods. Quality. Adjuvants. Product comparability.

Resumen

La prevalencia de las enfermedades alérgicas se ha incrementado drásticamente en los últimos 50 años y hoy pueden considerarse una epidemia. Actualmente, la inmunoterapia específica con alérgenos (ITA) es el único tratamiento dirigido a la causa subyacente de las enfermedades alérgicas y su superioridad se basa en resultados de ensayos clínicos/estudios observacionales que demuestran su eficacia y seguridad. Pero quedan aspectos sin resolver, como la armonización y estandarización de los procesos de fabricación y cuantificación entre fabricantes, la declaración homogénea de la potencia y el establecimiento de estándares internacionales de referencia.

En este artículo se discuten aspectos relacionados con la medida del contenido de alérgenos mayores en los extractos de ITA, cuestionando si, como base para elegir entre productos, es apropiada la comparación entre diferentes fabricantes. La estandarización alergénica es crucial para asegurar la calidad y, por tanto, la seguridad y eficacia de la ITA. Sin embargo, la falta de armonización en los procesos de fabricación, la cuantificación alergénica, las diferencias regulatorias, la práctica clínica y el etiquetado, demuestran que comparar productos basándose únicamente en la cantidad de alérgeno mayor no está justificado y es imposible. Además, cuando se evalúa la información para un determinado producto, deben tenerse en cuenta las características propias de cada producto y su uso clínico, como el estado de la modificación del extracto, la adición de adyuvantes, la vía de administración y la dosis acumulada. Solo datos clínicos convincentes deben servir para la evaluación específica de cada producto o como base para la comparación entre productos.

Palabras clave: Inmunoterapia alergénica. Contenido de alérgenos mayores. Métodos de estandarización. Calidad. Adyuvantes. Comparación de productos.

Introduction

Allergy is an exaggerated response of the adaptive immune system to a usually harmless substance (allergen). Manifestations of allergy vary and include medical conditions such as anaphylaxis, urticaria, allergic asthma, allergic rhinitis, eczema, contact eczema, serum sickness, and allergic vasculitis [1]. The prevalence of allergic disorders has increased significantly over the last 50 years to the extent that, today, they can be considered epidemic [2,3]. For decades, allergen immunotherapy (AIT) has been used for the treatment of allergic rhinitis and rhinoconjunctivitis, asthma, and venom allergy. At present, AIT is supported by data accumulated from large, double-blind, placebo-controlled clinical trials demonstrating efficacy and safety [4-11], as well as a high number of noninterventional studies, systematic reviews and meta-analyses [12-14], and real-world evidence [15-19]. Among its benefits, it has been demonstrated that AIT may prevent the development of asthma in children with allergic rhinitis [8]. At present, this is the only therapy for allergies that targets the underlying causes of the disease [7].

Currently, specialists can choose from among several available immunotherapy products for the same allergen. Most allergen-based AIT products are derived from complex drug substance extracts, eg, prepared from natural allergenic source materials such as pollen, house dust mite, and animal dander. These are complex owing to the nature of allergen compositions within species comprising multiple allergen components that may vary over time because of environmental pressures. As such, they are categorized as inherently heterogeneous and variable in nature. Over recent decades, our understanding of the composition of allergen extracts and resolution of their components has grown remarkably, allowing manufacturers to extend levels of allergen characterization and control and ensuring homogeneity between batches that can be manufactured consecutively and in line with state-of-the-art guidance. While more products include quantification of specific allergens, manufacturers routinely apply product-specific standardization of "potency" (eg, based on IgG from inoculated animals, again biased by the allergen extract used for immunization, and IgE reactivity against human atopic sera pools). These assays assess the cumulative capacity of all allergens within the mixture, as opposed to using monoclonal antibodies to measure the content of a single allergen. Hence, content is expressed in arbitrary manufacturer-specific units relative to specific in-house allergen reference preparations [20]. As this is the sum of the binding affinity between multiple allergens and antibodies, it cannot be measured in µg/mL. Therefore, it is not possible to compare the potency of extracts between manufacturers.

Product-specific standardization and cross-product comparability have been discussed extensively. Difficulties have been encountered in the harmonization of specifications across manufacturing processes among manufacturers and in the establishment of standards owing to the variety of allergens, their sources, and analytical methods. In this regard, and specifically in Europe, company-specific in-house validation methods ensure batch-to-batch reliability and comparability (product-specific standardization) [21-24]. However, the content and composition of products from different manufacturers are not equal [25-27], and the methods used to measure concentration and activity differ substantially. Even though efforts to facilitate cross-product comparability are ongoing [21,22], many physicians—motivated by questionable marketing campaigns—tend to compare products from different manufacturers by relying exclusively on major allergen content, which has led to the belief that more is better.

This article discusses issues related to standardization, measurement of major allergen content, and shortcomings of using concentrations and biological activity for comparing products, especially with respect to personalized therapy and evidence-based medicine.

2. The Pitfalls of Traditional Concepts: Understanding Product Strength

AIT comprises the administration of high doses of an allergen to sensitized patients in order to increase their immunological tolerance to the allergen [28]. Consequently, the overall goal is disease-modification. However, not only are relatively high doses required, but administration is also via a nonnatural route: inhalant allergens primarily encounter the mucosal surfaces of the upper and lower airways whereas AIT is administered into subcutaneous tissue or under the tongue. In 1993, the European Academy of Allergy and Clinical Immunology (EAACI) defined the AIT maintenance dose as "the highest tolerated dose by the patient without side-effects"; this definition was based on the content of

purified major allergens [29]. Thus, it was postulated for many years that a specific amount of a major allergen in each product corresponded to the optimal dose to reach a clinically relevant effect, without causing unacceptable adverse events. This concept does not take into account the fact that the dose–response relationship in drugs and in AIT is not linear. In any case, the concept of allergen concentration as a measure of suitability of an extract was rapidly taken up by both specialists and marketing departments without considering the aforementioned lack of homogeneous standardization of allergen extracts [21,22]. It is also important to highlight that for many allergenic source materials (eg, grass and house dust mite), a uniform definition of immunodominant allergens is subject to different interpretations and may even depend on the population, its exposure to other allergens, and the abundance of allergens. The erroneous use of this parameter to compare products was the consequence. Some subcutaneous immunotherapy and sublingual immunotherapy products whose major allergen content varies have proven efficacious in the context of clinical trials [30], thus indicating that allergen concentration is likely only one of several factors influencing the efficacy of extracts. In fact, in the field of AIT, very few products have undergone well-designed dose–response studies (phase 2 trials) [31]. Given the state of the art and the current regulatory context, allergen extracts should be evaluated individually by conducting rigorous clinical trials to demonstrate efficacy [21, 32-34] and, in particular, to establish the optimal dose defined by the best balance between clinical efficacy and safety [31,35].

Importantly, AIT is not comparable to traditional vaccination in immunologically naïve patients. In contrast, presensitized patients are treated with AIT formulations derived from materials they are already sensitized to, leading to the possibility of specific IgE-mediated adverse events such as anaphylaxis. Therefore, it could be argued that within future product design, there is a moral imperative to deliver the lowest amount of allergen that would generate the optimal clinical effect, as opposed to the highest tolerated concentration. This is particularly relevant for native AIT products in which allergenicity is not attenuated.

3. Key Concepts Regarding Allergen Content in AIT Products

Manufacturers have developed methodologies to assess complex allergen extract concentration and biological strength in terms of potency, as well as to guarantee in-house batch-to-batch consistency and stability [36]. Allergen ordinance processes such as the Therapy Allergen Ordinance (TAO) [20,37] initiated by the German Paul-Ehrlich-Institute (PEI) have been successful in increasing the requirements for product standardization. Other countries are following suit. Initiatives such as the Co-ordination group for Mutual recognition and Decentralised procedures – human (CMDh) [38] provide a legal framework for regulators enforcing licensed therapies. Allergen extracts are medicinal products that should be regulated in accordance with applicable legislation in the US via the Food and Drug Administration (FDA) and the EU via the European Medicines Agency (EMA) (Directive 2001/83/EC) [24,39].

3.1 Standardization of Allergen Extracts

In Europe [23], production of standardized extracts is regulated mainly by the Guideline on Allergen Products: Production and Quality Issues [22] and the Monograph on Allergen Extracts of the European Pharmacopoeia [40]. Allergen standardization ensures continued quality and reliability of the manufactured product. It concerns the source, the development of reference material, and the measurement of the quantity and activity (potency) of an allergen extract and the units (Figure 1). All these steps ensure the quality of each batch produced by a given manufacturer, although standardization is in-house and methodologies vary between manufacturers at all stages.

This process begins with the identification of the allergen by means of controlled selection of the source material to be used in the preparation of allergen extracts (extraction process). Control applies to both source and raw materials. The former refers to the natural moiety from which allergens are extracted, such as biological samples or cell cultures for recombinant proteins. The latter includes solvents, media, chemicals used for extraction, and cell culture reagents in the case of recombinant protein expression. Allergens derived from animal sources must comply with safety rules in order to exclude infectious agents [41-43]. Monitoring of raw materials should include references from material suppliers, a thorough description of the materials used, the geographic location, biological data (genus, species, and type), cultivation and collection protocols, storage/shipping conditions, and purification and handling must be registered [22].

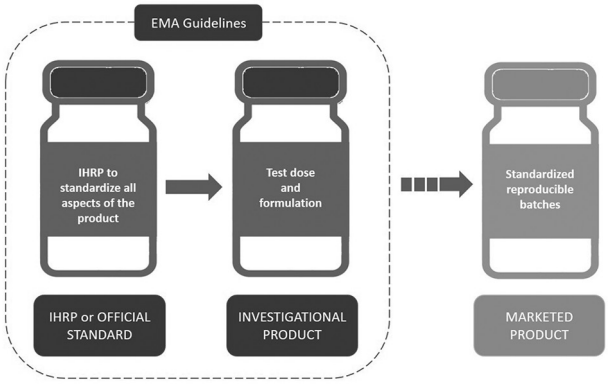

Figure 1. Allergen extracts are manufactured following the guidelines set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the European Medicines Agency (EMA). These bodies regulate standardization of extracts with an official standard (if available) or an in-house reference preparation (IHRP) validated by each manufacturer. Investigational products are manufactured according to the IHRP specifications, and clinical trials should be carried out for each individual product to test clinical efficacy, safety, dosing, and pharmacokinetic and pharmacodynamic profile. The marketed product is produced in standardized reproducible batches. In the final product, allergen profile and quantification and biological potency are checked to match the specifications of the IHRP.

Regarding the development of reference material, the US Food and Drug Administration develops and maintains US reference standards and serum pools for manufacturers to perform lot testing [23,44]. The present manuscript focusses on Europe. Proponents of harmonization and standardization across manufacturers in the EU have supported the Certified References for Allergens and Test Evaluation (CREATE) project, which aims to develop international reference standards for both purified natural and recombinant allergens, with verifiable allergen content [45,46]. In the CREATE project, 8 major allergens contained in the most frequent inhalant allergen sources were selected for this purpose, as follows: birch pollen (Bet v 1), olive pollen (Ole e 1), grass pollen (Phl p 1 and Phl p 5), and house dust mite (Der p 1, Der p 2, Der f 1, and Der f 2). After exhaustive characterization efforts and evaluation of enzyme-linked immunosorbent assay (ELISA) data, the project identified Bet v 1 and Phl p 5 as good candidates for further development in order to introduce an official biological standard(s). CREATE was followed by the Biological Standardization Programme (BSP090) of the European Directorate for Quality of Medicines and Healthcare. The somewhat sobering outcome of 2 decades of allergen standardization research was the establishment of 2 validated reference ELISAs, one for birch (Bet v 1.0101) [47] and another for grasses (Phl p 5.0109) [48]. The Bet v 1 and Phl p 5a ELISA protocols are currently being implemented as general chapters in the European Pharmacopoeia. Clearly, CREATE and BSP90 have unraveled the limitations of ELISA techniques [21], which are multifaceted and affected, for example, by the problem of discrimination between isoforms. Consequently, achieving analytical standardization is clearly a complex process. In addition, international standards are lacking for most allergens, and manufacturers utilize their own in-house reference preparations (IHRPs) [49]. While this will enable cross-product comparability of birch pollen and timothy grass pollen allergen products based on major allergen content alone, it is important to re-emphasize the fact that single allergen concentration is likely only one among several factors influencing the efficacy of extracts. Already back in 2016, the Paul Ehrlich Institute stated that "Despite the large progress in total allergenic activity determination and quantification of individual allergen molecules, the resulting values remain incomparable. Hence, today, allergists cannot decide for one or the other allergen product based on comparing contents of active ingredients or potency."

3.2 Quantification of Allergens

Quantification and verification of at least 1 major allergen during production is a quality requirement of regulatory bodies [40,50], and efforts are being made to harmonize the quality of products [22]. However, considerable variability exists in the measurement and reporting of exact allergen strength.

3.2.1 Methods for quantification

Generally, the procedure involves in vitro testing. Once the overall strength of the original extract is determined using an in vivo method, this information is used to develop an IHRP to estimate the strength of extracts from other batches with an in vitro method. The IHRP can then be cross-validated over time as the materials reach their expiry date. ELISA is commonly applied as a measure of in vitro biological activity [51]. The measurement of major allergen in micrograms is standardized only for Bet v 1 and Phl p 5a (in development), and the results depend on the technique, the reference, and the antibodies used in, for example, immunoassays [52]. The results have proven to be variable from identical extracts. While ELISA is a universal, affordable, and sensitive technique for detection of analytes such as proteins in allergen samples, its main limitation lies in the nature of the test itself. ELISA relies on the interaction between antibodies and specific epitopes on the surface of antigens present in allergens. In addition, monoclonal antibodies may not bind to all relevant isoallergens, and the values obtained might differ from those generated in assays using polyclonal rabbit antibodies [53]. It has been shown that various assay variables can impact the result, and antibodies can vary across different laboratory protocols [52]. In addition, ELISA-based procedures cannot detect variations in allergen composition across different samples. This is important, because patients may be tolerant to a specific subset of proteins but not to others in the same allergen extract (Figure 2). Finally, antibody-based approaches have also shown various shortcomings for quantification of allergoids, and adsorption of allergens to adjuvants may hinder the ability to create the

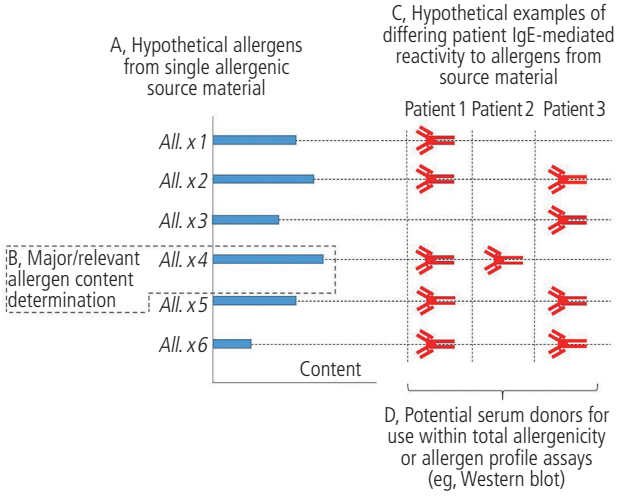

Figure 2. Theoretical example of challenges experienced when developing specifications to characterize and standardize AIT products. Note that this is a conceptual figure (All. x 4 = Allergen "x" 4, the theoretical major allergen). A, In general, each allergenic species contributes multiple allergens to the extract at ratios comparable to those that would be experienced by the patient under normal exposure. In some cases this exceeds 20 known allergens. B, A relevant/major allergen content assessment provides concise information about a specific allergen of interest that is shown to be relevant to 50% or more of sensitized patients but shows no additional data on other allergens. C, Patients who are allergic to the same source material may react both to different allergens in the mixture and, if allergic to the same allergen, with different intensities. Note that the major allergen content would be highly relevant for patient 2 but irrelevant for patient 3. D, Diversity of donors is useful in constructing an allergen-specific donor pool that can be used for total allergenicity methods. These are critical for understanding the relative strengths of allergy products and provide information on the contribution of the individual allergen.

required immunoconjugate owing to the conformation of the product. Furthermore, 2 distinct allergoid studies [54,55] demonstrated that the polymerization process disrupted conformational epitopes and led to the creation of new structures harboring novel IgG epitopes. The formation of such structures may have the capacity to improve immunological effects, such as blocking IgE epitopes or inducing novel IgE specificities, potentially depending on the adjuvant used.

Mass spectrometry techniques are further developed to quantify the strength of allergen extracts [23,54,56-59], although these are currently too laborious and expensive for routine use and have a lower throughput. Quality also depends on functional proteomic approaches to determine which peptide chains are active and important and whether the proteolytic activity of pollen content is analyzed. Such approaches enable identification of immunologically active pollen content and allergenicity beyond the known major and minor allergens. They may also allow for total quantification of allergens, even of allergens from different species within a complex mix, owing to their unrivaled specificity, which circumnavigates the aforementioned issues associated with immunoassays. Moreover, the method of quantification is variable and includes semiquantitative techniques (ion counting, spectral counting) and quantitative analysis by labeling samples with internal or external standards. Mass spectrometry will become an important technique in our field, although it should be developed further with regards to its interpretation and harmonization across users, mainly owing to its limitations, such as the large outcome datasets and their analysis, interpretation, and graphical display [60].

3.2.2 Allergen concentration versus biological strength

Allergens are proteins made up of precisely folded polypeptides that trigger an immune response dictated by their surface-exposed epitopes, eg, the specific region of an antigen targeted by an antibody. The structure of these epitopes, their level of exposure on the solvent-accessible surface of the allergen, and the composition of the final product (eg, adjuvants, modification) considerably affects biological strength (potency).

The labeled strength of allergen preparations in the EU is often expressed as proprietary units [26,61-63]. Essentially, units are required to show that a product can be manufactured in a reproducible way. During development of specifications, biological units may be different from those used for labeling product strength, although there should be a consistent assay-derived factor that is related to the final unitage of any particular product. Such final unitage must have a comprehensible scientific basis. For instance, correlation of in vivo biological standardization by appropriate methods on the basis of skin reactivity tests using methods such as those described by Turkeltaub [64] and the Nordic Council of Medicines [65] and the corresponding in vitro allergenic activity of the (first) IHRP should be described, with potency labeling based on in vitro testing. Each new IHRP is then prepared and compared against the previously assigned preparation, thus providing an unbroken link back to original in vivo biological standardization studies. Manufacturers use several types of allergen units, namely, histamine equivalent in prick testing (HEP), biologic or diagnostic unit (BU or DU), bioequivalent allergen unit (BAU), therapeutic unit (TU), and standardized unit (SU) [26,62,63]. However, regardless of the unitage employed, manufacturers may correlate in vivo standardization with in vitro testing using different factors, resulting in arbitrary values assigned to the final product. This does not enable products to be compared between manufacturers for the purpose of assessing strength/content accurately [20]. A computer-based comparison and correlation of sublingual immunotherapy solutions based on skin prick testing results with the same AIT solutions represented a different approach [66].

Adjuvants or adjuvant systems are used in subcutaneous immunotherapy products to increase the clinical efficacy of treatment and to reduce the number of doses needed to induce immune tolerance [67,68]. Recent decades have seen the development of 2 novel adjuvants used in AIT products as an adjuvant system: modified allergen L-tyrosine adsorbate–monophosphoryl lipid A (MATA-MPL) combines chemically cross-linked allergens (allergoids) adsorbed on the depot adjuvant system microcrystalline tyrosine (MCT) [69] in combination with monophosphoryl lipid A (MPL). Formulation and combinations of such additives matter [70], and characteristics such as the physico-chemical properties of depot adjuvants (eg, MCT) have an impact on immunological mechanisms [71]. In this regard, the variety of adjuvants in development, including immunostimulatory sequences and nanoparticles [68,72], will further increase the differences in immune responses elicited by different products based on similar allergen extracts and content.

3.2.3 Allergen quantification and stage of manufacture

In addition to the different methodologies applied to quantify allergens, the step in the manufacturing process from which data are obtained may vary. For instance, quantification in the final product is problematic. In native extracts, adjuvants and other additives interfere to some extent in several quantification tests. In the case of allergoids, this may not be possible owing to the modification process. As such, allergen measurements may be derived from earlier stages of manufacture, or in essence, the last feasible timepoint across the process. The methods used for polymerization (modification of the allergen extract to decrease the allergenicity of IgE but retain the immunogenicity of IgG) results in chemical cross-linking of allergens with glutaraldehyde, potassium cyanate, or formaldehyde, resulting in changes to antigen structure, eg, disruption of conformational IgE epitopes [54,73]. It is noteworthy that the chemical modifications applied to these antigens have an impact on standardization protocols, as in vitro assays are disturbed by the reduced concentration of available epitopes for antibody binding and cross-linking. Therefore, tests such as the determination of total allergenic activity are usually performed during early targeting of drug substance strength in the manufacturing process, eg, with the native allergen extract [44]. IgG-based methods to assess the potency of allergoids are an expected—albeit not essential—part of the monograph/guidelines.

Finally, conventional ELISA methods used for quantification have an additional limitation in the case of mixed-species

extracts, as an antibody-based approach may not distinguish between allergens from homologous species within the mix.

Interference of depot adjuvants added in the final product, such as aluminum compounds, should also be mentioned. While the guideline states that testing should be at the latest and most feasible stage of manufacture, some content or potency assays may be hindered once the product is adsorbed or absorbed to the adjuvant. Protocols including the information about timepoint and method of quantification are in-house and specifically designed for each extract or source material.

Formulating final products by diluting and combining active pharmaceutical ingredient (API) with depots can hamper measurement of specific or total content owing to the (desired) binding between protein and depot. Whilst this binding is desired pharmacologically to delay release of the API, it makes analysis difficult, variable, and potentially impossible. For example, when binding complex mixtures to aluminum hydroxide, different binding efficiencies may lead to potentially variable results. For next-generation depots such as MCT, methods have been developed to universally reverse binding (under nonpharmacological conditions) that allow consistent measurement of API, thus improving the ability to characterize final drug products, although, again, only allowing an in-house, product-specific batch-to-batch comparison. If companies wish to report major allergen content, they should be obliged to clearly state at which manufacturing step these values were measured and which further manufacturing steps will follow after measurement.

4. What Is the Real Purpose of Allergen Quantification?

4.1 Verification of Quality?

Product quality is the key element for any marketed drug. The quality of allergen extracts depends on the source, extract purification, antigen composition, stability of product, and overall strength [62]. Every stage during the manufacturing process until the final product is marketed in the EU must follow the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the Committee for Medicinal Products for Human Use (CHMP) of the EMA [22,23]. As stated above, quantification and verification of at least 1 major allergen is expected, and the allergens relevant for the product have to be defined by the manufacturer [22,40,50] (Figure 2). In addition, clinical trials to evaluate the safety and efficacy of allergen extracts are performed with a well-characterized and standardized product that needs to be reproducible batch by batch. This means validating an assay that is accurate and precise (including other important quality parameters outlined in ICH Q2R1) and, as such, provides a robust level of product-specific standardization. Logically, manufacturers need to ensure that all subsequent batches comply with the specifications and the quality of the product tested, in which allergen quantification plays a role; otherwise, the batch would not be released by authorities such as the Paul Ehrlich Institute. However, other aspects that differ across products have an influence on clinical outcome owing to immunological mechanisms of action and are independent of the quantity of major allergen. These characteristics include whether the allergen is native or an allergoid, whether the preparation is depot or nondepot, which adjuvant/adjuvant system was used, route of administration, and posology (including dose volume and frequency) [74], all of them carefully controlled and standardized in in-house procedures.

4.2 Check Whether the Product Contains an Optimal Dose?

It is tempting to use the label information to check whether a product contains an optimal dose, especially if the clinician accepts the traditional definition of maintenance dose of EAACI as being "*the highest tolerated by the patient without side-effects*" [29]. According to regulations, in vials of nonmodified allergen preparations, the units must refer to total allergen-mediated activity (strength or potency) as, for example, measured via IgE or other competitive binding assays. This introduces arbitrary factors associated with the unitage assigned (see above). In addition, the individual amounts of each allergen in the formulation must be stated in units of mass per volume. Micrograms of major allergen is the widely recognized method for expressing single allergen strength, and, in some cases, good correlation with biological strength has been reported [75-77]. However, as discussed, differences in assays, procedures, and extracts affect the results of quantification [78-80] and seem to massively influence the major allergen concentrations reported by different companies [26,52,62]. Hence, using allergen content values to correlate optimal strength with units reported from another manufacturer is unwise and scientifically flawed [21].

The optimal dose for each product should be based on methodologically sound dose-finding phase 2 clinical trials that follow regulatory standards. In fact, phase 2 studies following EMA guidelines for new products and preparations to be licensed within the German Regulation for Therapy Allergens (TAO) have been mandatory since 2008 [31, 37]. As a result, we have seen an increasing number of clinical trials in the field during recent years, most of them with shortcomings that have resulted in an inability to satisfactorily define the optimal dose [31]. However, some approaches did prove successful in trials based on a careful design, such as the definition of a statistically significant dose-response curve including a plateau for a birch allergoid [81], a grass allergoid [82], and a mite allergoid [83]. These trials showed the best suitable dose, defined as the best possible reduction in the conjunctival or nasal provocation test score, which was not limited by the occurrence of adverse events. In fact, demonstrating a significant dose-response relationship with an efficacy plateau means that efficacy decreases if allergen amounts are further increased! When viewed separately, this aspect already shows the limitations of the "more is always better" approach.

Notably, the optimal dosage of a drug is not the most efficacious one, but that with the optimal risk-benefit ratio. Therefore, dose-finding trials must not only be assessed in terms of efficacy. For the AIT field, the optimal dosage of native allergen preparations was always defined by limitations in tolerability. None of these products demonstrated an efficacy plateau. The situation is different for allergoids: because of the

modification process, tolerability remains unchanged despite the dose being increased up to 7 times. Thus, the optimal dose in modern allergoids is defined by the efficacy plateau and not safety concerns [81,82]. In any case, assessments are product-specific, and the concept of class effect should be applied with caution to AIT products.

4.3 Cross-product Comparability?

Given the present state of the technique and regulations regarding production and testing, it is of limited value to aim for cross-product comparability. Finalized products are designed differently for various reasons and additives, such as adjuvants, and the final preparation will result in a variety of products whose clinical characteristics cannot be attributed to major allergen content solely. In cases where harmonization is progressing with respect to major allergen testing of Bet v 1 and Phl p 5a, such immunoassays are limited to 1 allergen at a time. Thus, allergen sources with several major allergens are difficult to standardize, and better tools to do so are increasingly demanded. This single allergen approach for relevant allergen standardization again highlights the importance of considering these results in the context of complete specification, also including total allergenicity/antigenicity and allergen profile. One novel approach encompasses targeted proteomics (via mass spectrometry), although additional research is needed to enable full implementation in allergen standardization [60], as further complex aspects need to be considered, such as the plethora of mass spectrometry technologies and methodologies, product matrices, validation, and definition of acceptance criteria. No guidance currently endorses a single approach or provides a framework for data interpretation. Until these exist, the challenges related to cross-product comparability remain.

4.4 Are Major Allergens Important at All?

It is intuitively right to state that an AIT product should contain the sensitizing allergens of an allergic individual. In fact, for some major allergens, such as Api m 10, their presence in final formulations was linked to treatment outcome in predominantly sensitized individuals [84]. Both the presence and the stability of such fragile major allergens might be critical in the final preparations [85,86]. Therefore, the greatest value may not be the demonstration of a set quantity but the qualitative demonstration of presence over the applicable timeframe, which is clinically important.

Despite the considerable progress made in the quantification of individual allergen molecules, resulting values remain incomparable between manufacturers. CREATE and BSP90 are a clear example, even after 2 decades of research and development, and harmonizing the measurement of specific allergens by ELISA remains a challenge. Harmonization of

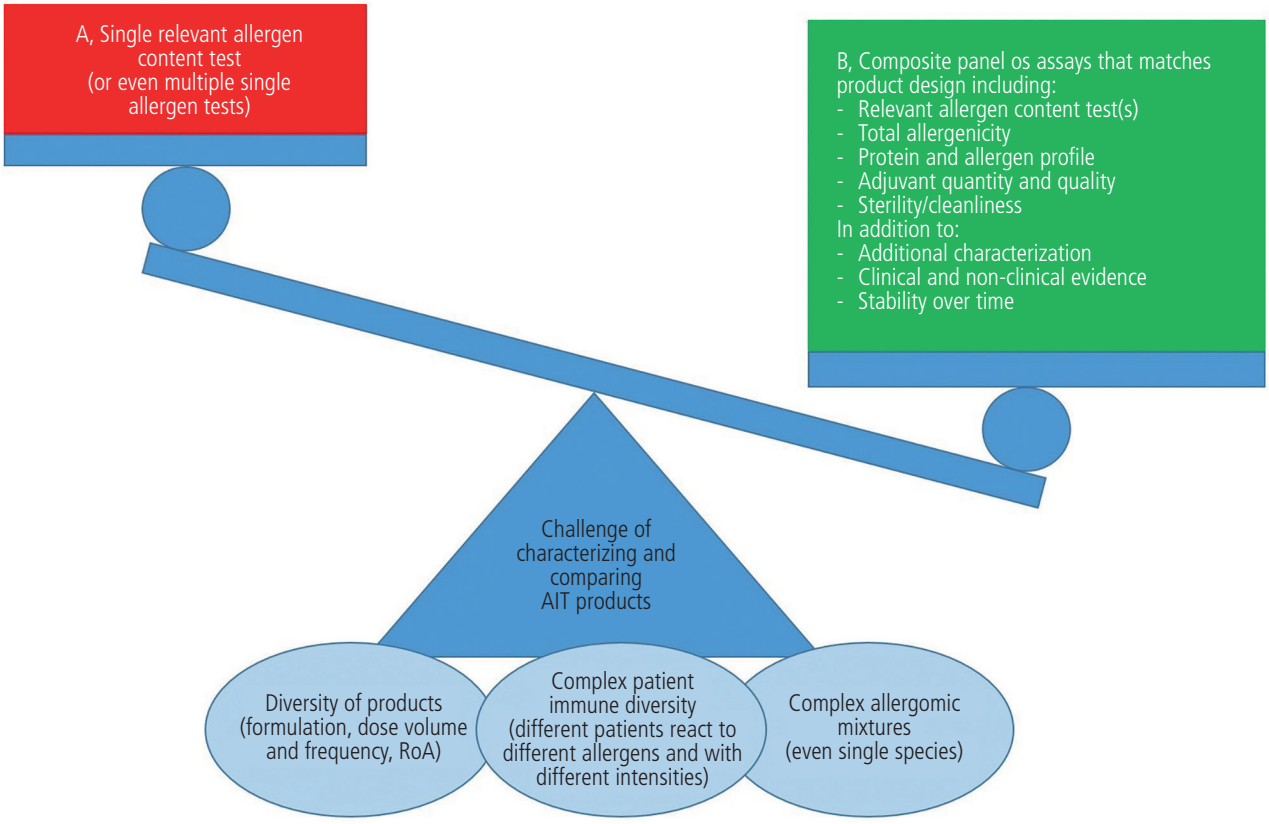

Figure 3. Representation of fundamental challenges regarding the models that summarize the characterization of AIT products. A, The imbalance of the complex reality of allergen characterization and translating this to a single number. B, An appropriate balance of data required to make meaningful judgments regarding product characterization. RoA indicates route of administration; AIT, allergen immunotherapy.

Table. Checklist: When Is Allergen Standardization and Quantification of Major Allergen Content the Right Method to Use?

YES: Product-specific Standardization	NO: Cross-product comparability
– Control of manufacturing process parameters complying with EMA/FDA requirements.	– To compare contents of active ingredients or potency between products from different manufacturers in the absence of a harmonized procedure and conclude whether the product contains an optimal dose.
– Control of product quality over shelf-life.	– To compare arbitrarily labeled potency units or allergen content between products.
– To validate in-house quality and ensure reproducibility across batches.	– As the sole basis for selecting from among the different AIT products available for a patient.
– Ensuring consistent quality and, in turn, safety and efficacy.	

Abbreviations: AIT, allergen immunotherapy; EMA, European Medicines Agency; FDA, United States Food and Drug Administration.

total proteomic measurements is the new focus, although this technology remains problematic and necessary guidance is missing. Nevertheless, the myriad product-specific nuances with respect to product matrices and clinical use of AIT products remain unchanged. Whether the amount of (major) allergen is sufficient in a preparation or not will be demonstrated in clinical trials and not through artificial units.

5. Summary

The aims of this article were to review the issues related to the measurement of major allergen content in AIT extracts and to discuss whether comparison of products by different manufacturers or calculating optimal doses based on the concentration of single major allergens could be an appropriate basis for helping clinicians to choose between the different AIT products.

Quantification and verification of at least 1 major allergen during production are required by regulatory entities, including the FDA and the EMA [24,39]. Those processes are both critical for the verification of quality of the AIT extract and the comparison between different batches in order to guarantee in-house batch-to-batch consistency. However, single allergen quantification alone cannot be used for cross-product comparisons between different manufacturers owing to the lack of international standards and in-house preparation and quantification methodology (Table, Figure 3).

When deciding the optimal product for a particular patient, it is important to keep in mind that the biological strength of or immune response elicited by the AIT product does not depend exclusively on a single allergen concentration. Other factors play a role, such as the epitope structure of the allergen and the level of exposure, as well as potential chemical modification (allergoids) by natural matrix effects in the extract and by the different adjuvants used in AIT extracts to increase the clinical efficacy of treatment and reduce the number of doses needed to induce immune tolerance. Moreover, unitage reporting varies considerably between manufacturers, thus preventing us from verifying whether a product contains an optimal dose for a patient solely by evaluating allergen concentration or by comparing different formulations (Table). While allergen standardization is clearly necessary, it is important to be aware of the limitations of the process (Table).

6. Conclusion

Clinicians should be informed about the special circumstances regarding allergen quantification in AIT extracts. There needs to be a broader understanding of the pitfalls of comparing products based on allergen content and the use of this information as the sole basis for estimating the optimal dose or the suitability of an extract for a given patient. As suggested by Pfaar et al [32] in the German 2014 AIT guidelines, preparations with documented efficacy and safety in clinical trials meeting international regulatory standards or tradeable under regulatory ordinance should be preferred. In addition, specialists should bear in mind that, at present, any assessment of AIT preparations is source- and product-specific and that similarities between products from different manufacturers (same major allergen content) cannot be anticipated. This review evaluates the special circumstances that clinicians face when comparing AIT products and describes why simply checking the content of the single major allergens of a specific extract might not be a recommendable approach for achieving the best outcome for a patient (Figure 3).

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