In vitro methods for the standardisation of allergen preparations

Project Team

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Project Summary

Allergen extracts are prepared from natural source materials like pollen, house dust mite and insect venoms. They consist of a complex mixture of allergenic and non-allergenic proteins and other compounds. When establishing an allergen extract for diagnosis or allergen-specific immunotherapy, a biological standardisation of a reference extract is performed based on skin testing in allergic patients. Afterwards, the activity of new batches is adjusted in relation to the reference by quantitative in vitro assays (e.g. IgE inhibition tests) which utilise pooled sera from allergic patients. The resulting units defining the strength of an allergen product are product-specific and therefore not comparable. Furthermore, no information about the content of individual allergenic components can be deduced from the results of such experiments.

Quantification of single allergenic molecules can be performed using immunoassays based on monoclonal IgG antibodies, which are allergen-specific and easy to handle, or mass spectrometry (see below). We constantly establish new immunoassays to gain a better understanding of allergen product composition. Furthermore, IgE binding epitopes are being characterized by epitope mapping.

A special focus lies on several birch pollen allergens, which are up to now not part of quality control for allergen products. The assessment of allergen patterns in a wide range of birch pollen allergen products has shown distinct differences between marketed products, underlining the need for further investigation and standardization. To facilitate this process, we use immunoassays to study how the natural variability of pollen source material and different manufacturing processes determine allergen content.

Furthermore, in cooperation with the Vice President, the division is involved in an European programme aiming at establishing recombinant allergens as biological reference preparations in conjunction with immunoassays for allergen quantification. With support of the European Directorate for the Quality of Medicines (EDQM) and in collaboration with academia and

industry partners, two recombinant reference allergens for two of the most common allergens from birch and timothy grass have been established as European Pharmacopoeia reference standards so far. In parallel, sandwich immunoassays based on monoclonal antibodies are beingvalidated in international collaborative studies in order to select European Pharmacopoeia reference methods for allergen quantification. This joint activity will for the first time enable European clinicians to compare allergen products with regard to their allergen content.

In view of the large number of allergenic source materials, allergen quantification via immunoassays has the disadvantage that one assay has to be established per allergen to be monitored. Thus, another approach to facilitate improved insights in the composition of allergen products is to make use of physico-chemical methods such as mass spectrometry. This technique and other proteomic tools allow the simultaneous identification and quantification of several allergens and their isoforms in their native and modified conformation, making it an ideal tool for the qualitative and quantitative characterisation of complex allergen preparations. Using a modern mass spectrometer, we are determining the presence of individual allergens and isoforms in extracts.We are also planning to extend this approach to allergoid preparations.

However, neither ELISAs nor physicochemical methods do consider that biologically active allergens must be able to crosslink Fcε receptor-bound IgE molecules on mast cells or basophils. Thus, allergen concentrations measured by these techniques may not correlate with biological activity due to, for example, different potencies of allergen isoforms. The measurement of biological activity can be performed by mediator release assays. Histamine release assays with human basophils are very difficult to standardise and thus are not applicable in routine batch control. We have therefore developed a mediator release assay that is based on rat basophilic leukaemia (RBL-2H3) cells and allergen-specific polyclonal and monoclonal IgE antibodies from mice. Furthermore, we have stably transfected these RBL-2H3 cells with the α-chain of the human Fcε receptor so that they can be sensitised with IgE antibodies of sera of allergic individuals.

These transfected and passively sensitized RBL-cells have been used to analyse a variety of commercial allergen extracts, as well as recombinant allergens and hypoallergens with respect to their biological activities using sera of allergic subjects. In addition, the transfected RBL-cells have been used to verify biological activity of minute amounts of allergenic proteins in complex matrices, such as foods, and to study the impact of protein structure on the potency of allergens and hypoallergenic preparations. Within the EU-COST Action ImpARAS, we currently investigate the potency of homologous food proteins to draw conclusions about the impact of protein structure on protein allergenicity. Resulting gain in knowledge may also be adopted for the standardisation of allergen products. Further experiments with this cell line indicate that the quality of human sera and the IgE-binding capacity of the cell are critical factors. Such factors need to be further elucidated in detail and the performance of the cell-based assay optimised.

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