Structural and biological basis of protein allergenicity

Project Team

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Project Summary

Experimental research and current clinical trials with novel concepts of allergen vaccines and therapeutics seek to improve efficacy and safety of allergen specific immunotherapy (SIT, AIT). The use of recombinant allergens, fragments thereof, and synthetic peptides derived from allergenic structures instead of traditional extract-based allergen preparations has been proposed for various reasons:

- to (partially) reconstitute the allergen extract
- to only provide the allergenic molecules or allergen-specific immune-stimulatory sequences
- to better standardize the allergen preparations
- to avoid neo-sensitization (new IgE specificities) to additional structures presented in extracts
- to allow defined modification of allergenic structures in order to bypass IgE response, to target T cells for the induction of T cell tolerance, and to induce protective (“blocking”) allergen-specific IgG antibodies.

Because the immediate-type allergic reaction is mediated via allergen-induced crosslinking of membrane-bound allergen-specific IgE on mast cells and basophils, the interaction of allergens with specific IgE is a critical step. However, detailed molecular information on IgE binding sites (so-called functional IgE epitopes) of food, inhalant and venom allergens is very limited. AIT of allergic subjects with allergens alleviates significantly allergic symptoms. The induction of allergen-specific IgG, which blocks IgE-mediated adverse immune reactions, is considered one key mechanism of AIT. However, not only knowledge on clinically relevant IgE epitopes is lacking but also information on binding sites of allergen-specific IgG is sparse. Our research focuses on the systematic and comprehensive identification of epitopes for IgE and IgG of inhalant, food and venom allergens, and the subsequent establishment of molecular tools for patient (group)-specific, epitope-resolved diagnosis of allergies and for prognostic monitoring of AIT.

Structural impact on allergy biomarkers and therapeutics

Based on the knowledge of epitope-resolved specific IgE and IgG responses, we seek to provide biomarkers for efficient diagnosis of allergy, severity of disease and risk markers.

We currently focus on crustacean allergens, venom allergens, Bet v 1-homologous allergens, tomato allergens, allergenic legume seed storage proteins (7S vicilines, 11S globulins and 2S-albumins) as representatives of birch-pollen related and primary food allergens, respectively. To

analyze the epitope profiles of these groups of allergens we use an allergen-type model protein [1] and a multi-peptide microarray [2] (figures 1 and 2). Sera from clinically well-characterised patients with confirmed food allergies are used to determine complete allergen profiles of the studied allergens by analysing allergen extracts. Selected major and minor allergens are purified from natural sources or produced as recombinant molecules and subsequently tested for IgE-binding, cross-reactivity, and investigated for allergen-specific T-cell activity. In close collaboration with the Group of Paul Rösch (Biopolymers, University of Bayreuth), highly resolved solution structures of cross-reactive allergens have been determined by nuclear magnetic resonance (NMR) spectroscopy.

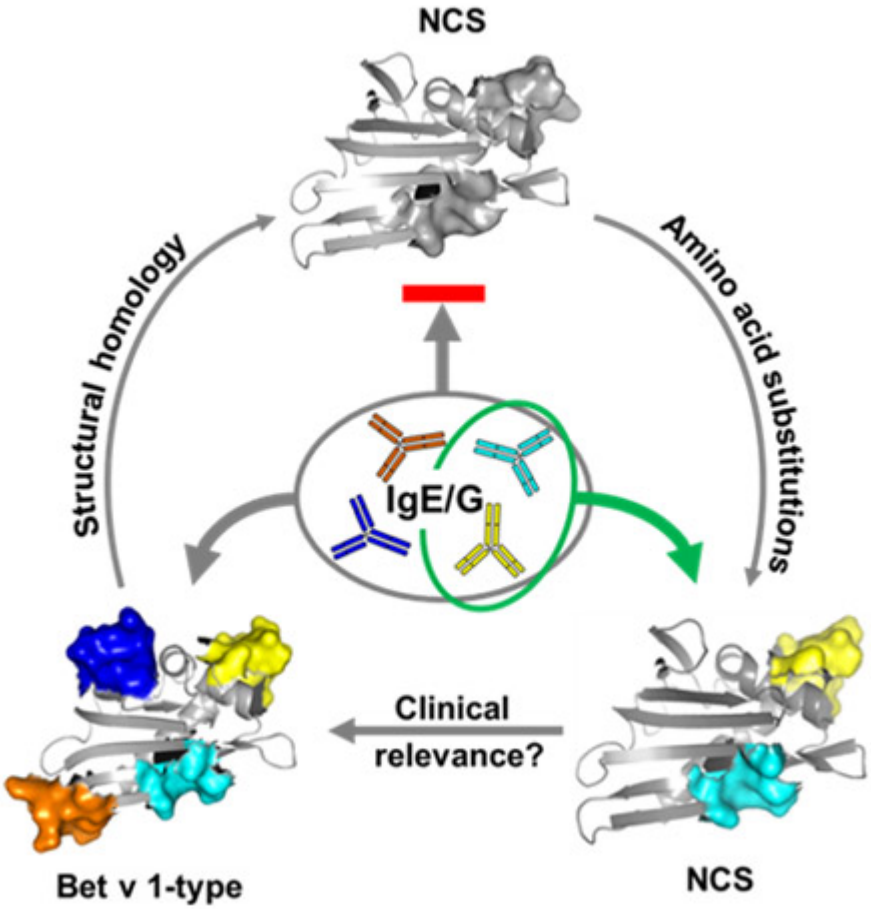

Figure 1: Principle of using Norcoclaurine Synthase (NCS), a recombinant Bet v 1-type model protein, to identify and analyze epitopes for IgE and IgG of Bet v 1 and homologous allergens.Source: PEI

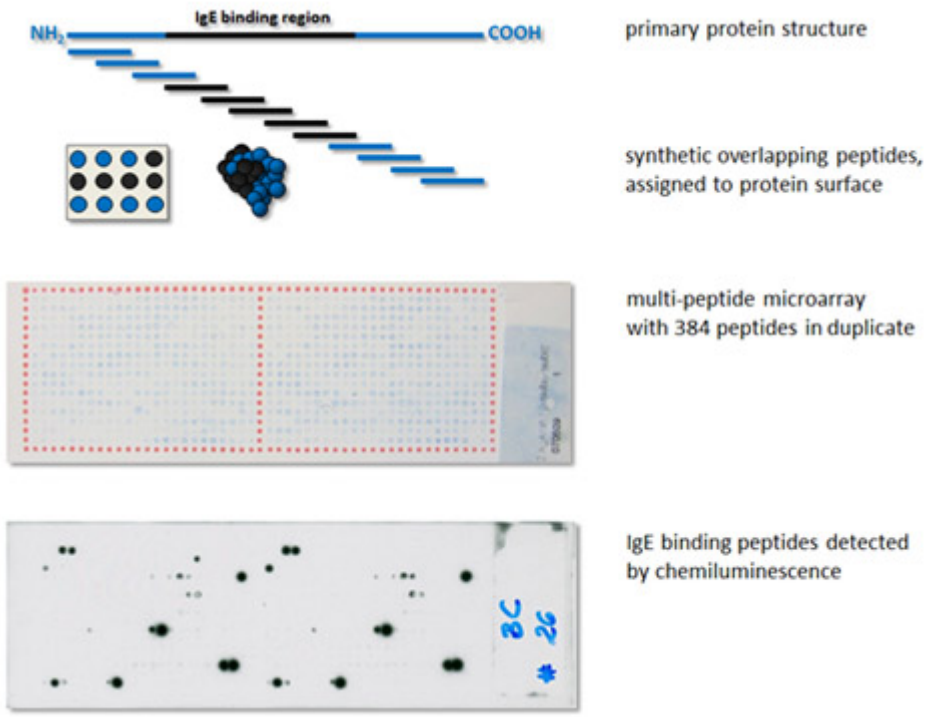

Figure 2: Principle of peptide microarray analysis for the identification of IgE and IgG epitopes of classic food allergensSource: PEI

Quality and safety of recombinant and synthetic allergen preparations

Within basic research projects we study the quality of recombinant allergen preparations as a tool to evaluate and promote the development of innovative, safe and efficacious biomedicines for the treatment of allergic diseases, such as recombinant or hypoallergenic variants [3] and synthetic epitope-mimicking peptides.

The quality of allergen preparations dictates their immunoglobulin-binding properties. High quality of recombinant allergen preparations is thus an essential requirement for the development of safe and efficacious biomedicinal products for the diagnosis and immunotherapeutic treatment of allergies. In the light of regulatory needs, we perform regulatory research on the physicochemical quality of recombinant allergen preparations addressing parameters defined as being critical in guidelines and monographs of the European Pharmacopoeia (Ph. Eur.), the European Medicines Agency (EMA) and the European Directorate for the Quality of Medicines & Health Care (EDQM). Furthermore we correlate IgE and IgG reactivity with physicochemical integrity of recombinant allergen preparations to evaluate the quality of allergen-specific immunoassays routinely used in clinical and biomedical research (Figure 3).

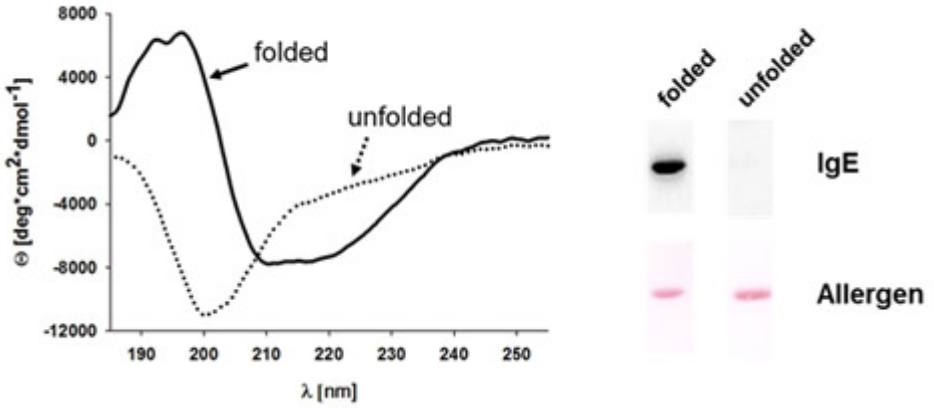

Figure 3: Protein conformation of a recombinant allergen defines its antibody binding capacity. A folded and unfolded allergen variant exhibits differential IgE binding.Source: PEI

Collaboration partners

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Text

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