Commentary

Peptide Epitopes as Biomarkers in Allergology

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ABBREVIATIONS

SPT: Skin Prick Test; EoT: End of Treatment; LOAELo/s: Lowest Observed Adverse Effect Level (Objective/Subjective signs) in food challenge.

DESCRIPTION

Allergies count for one of the most frequent diseases worldwide and include a broad spectrum of chronic conditions such as allergic rhinitis, allergic asthma or food allergy [1]. Quality of life is frequently affected and the cornerstone of clinical care consists in allergen identification. When allergen avoidance cannot be realized, symptomatic and, if available, allergen-specific therapy is indicated. Still there exist multiple limitations in allergy diagnostic and therapy [1].

In IgE-mediated allergies, e.g. in pollen or food allergy, skin prick tests and determination of specific IgE values are standard diagnostic measures. The recent introduction of molecular allergy diagnostics based on individual protein molecules of the allergen was helpful in detecting relevant IgE sensitization and sometimes even allows to predict the risk of severity of clinical reactions.

There are several limitations, though:

- The wide range of possible allergens cannot be covered by commercially available test systems.
- Similarity of sequence and structure of in particular plant allergens lead to high rates of false positive results with current protein-based assays [2,3].

Several IgE-mediated allergies, for example: against pollen, house dust mites and hymenoptera can effectively be treated by Allergen-specific Immunotherapy (AIT). This requires proper diagnosis of the allergy but biomarkers are still needed to stratify patients and identify those who will benefit from AIT.

To date, the amount of antibodies binding individual allergenic proteins or skin prick test results are not able to predict neither the course nor the outcome of AIT [4]. The current knowledge regarding the epitope diversity and specificity of allergen-specific antibodies induced during AIT is limited [5-7].

Optimized peptide phage display methods have been used for the identification of peanut [8,9], and soya epitopes recognized by sensitized patient sera [6,9-11]. Remarkably, an IgG wheat epitope was able to discriminate between non-sensitized and sensitized wheat patients and was identified by using an allergen peptide phage display library [12]. We reported the identification and validation of a large number of soya epitopes and mimotopes (i.e. peptides that mimic conformational epitopes) by applying a unique peptide phage display platform that involves the statistical analysis of enriched allergen-motifs. This has the advantage to directly identify binding peptide epitopes even with structural constraints from a naïve peptide library.

Recently, we presented a paper on the use of peptide epitopes as potential epitope-based markers for monitoring and/or predicting AIT efficacy [13]. We included sera from patients having been treated within a clinical trial by AIT applying a recombinant folded variant of the main birch pollen allergen Bet v1 [14]. Clinical relevance of epitopes was evaluated by comparing these measurements to a number of treatment outcome measures recorded during double-blind placebo-controlled food challenges at start and end of AIT [13].

This study was only made possible by applying a novel method to analyse the results from binding a naïve peptide library on bacteriophage to the entire Ig-pool of the patient sera and analyzing the data of bound sequences without repeated selection rounds. In addition a peptide micro-array of 320 peptides resembling epitopes of 15 food allergens also comprising multiple epitopes from PR10 variants was used. Altogether these investigations used only about 100 µl serum remaining form the earlier BASALIT study. Peptide arrays were generated in another large study involving more than 500 patients, which is presently under evaluation for research and diagnostics applications (Fraunhofer Project Food Allergen). In particular the data analysis can be carried out *in silico* for

protein sequences of multiple antigens [15], which otherwise would require large quantities of serum screening multiple peptide arrays generated from peptide fragments of the individual proteins. We found already earlier that the phage method was superior in the direct comparison to peptide arrays [10]. It allows the selection of peptides matching multiple sera and using the naïve library peptides are identified that contain structurally relevant amino acids, for example- cysteine, adjacent to the binding motif. Such peptides can have a much-improved affinity and selectivity and allow individual measurements of IgE and IgG. The recent study on using such epitopes resembling PR10 epitopes would not have been possible without such rather short and structurally confined sequences.

Binding of IgE and IgG to individual peptide epitopes can be a potential indicator of soya sensitization at start of treatment and distinct IgG-epitopes may help to predict and monitor clinical effects of AIT. With respect to the overall expected cross reactivity of PR10 antibodies, selected peptide epitopes are probably the best tool for proper diagnosis and prognosis not only in food allergies, but also other immune disease.

In summary, reactivity to mainly IgG-epitopes may help to predict clinical effects of AIT on soya and possibly also in other types of IgE-mediated allergy. Our measurements show that also IgG epitopes can be biomarkers of multiple clinical measures in allergies (Figure 1).
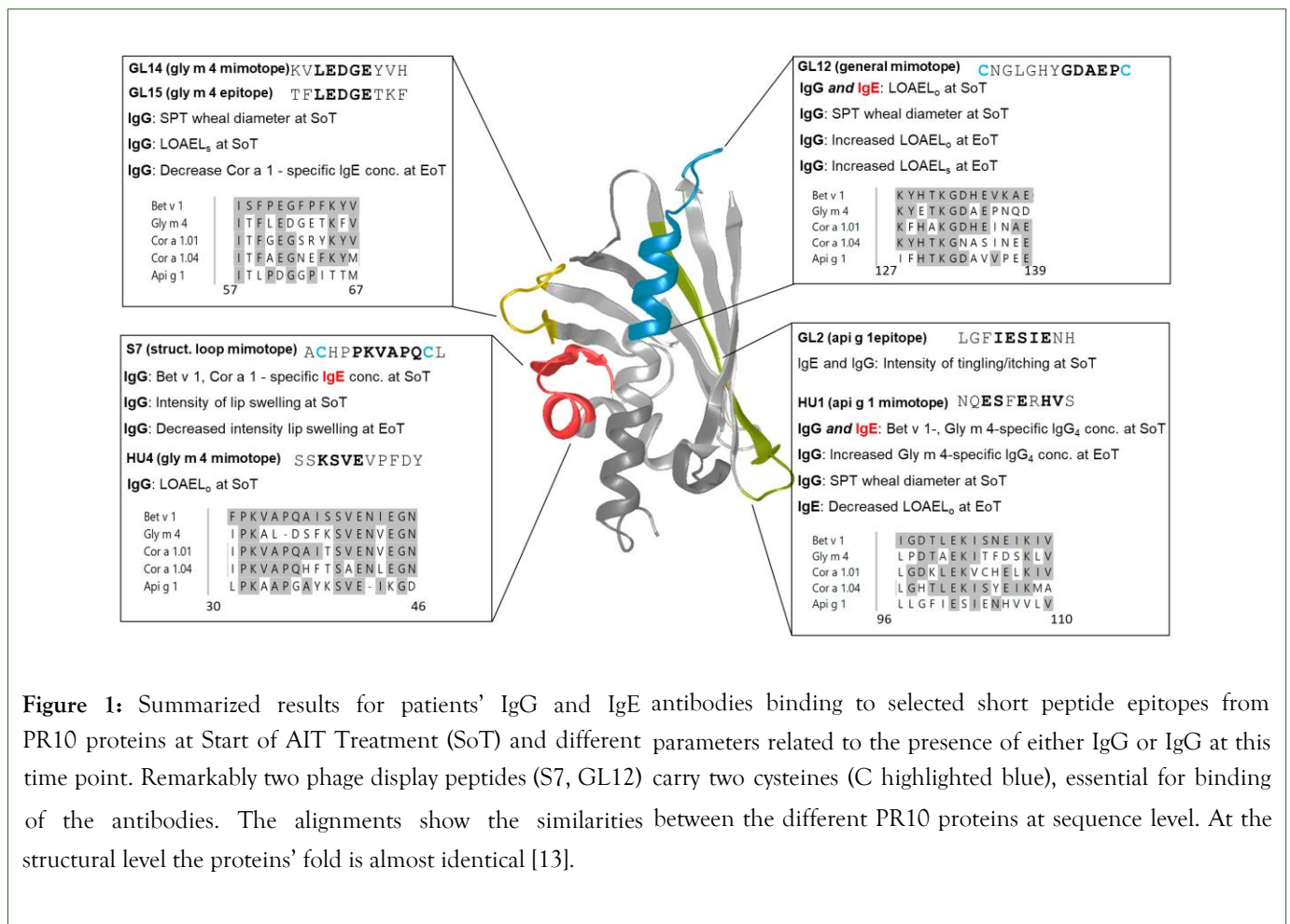

Figure 1: Summarized results for patients' IgG and IgE antibodies binding to selected short peptide epitopes from PR10 proteins at Start of AIT Treatment (SoT) and different time point. Remarkably two phage display peptides (S7, GL12) of the antibodies. The alignments show the similarities structural level the proteins' fold is almost identical [13]. parameters related to the presence of either IgG or IgG at this carry two cysteines (C highlighted blue), essential for binding between the different PR10 proteins at sequence level. At the

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