Peanut-specific IgE and IgG4 epitope identification by phage-display technology and peptide array

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Aim: Food allergy is a growing problem worldwide, presently affecting 2-4% of adults and 5-8% of young children. Peanut allergy is considered one of the most serious forms of food allergy in terms of prevalence, persistency and severity. IgE and IgG4 are regarded as key players in food allergy, and characterisation of allergen IgE and IgG4 epitopes may be an essential task in unraveling the molecular mechanisms of allergy. Identification of patient-specific IgE and IgG4 epitope patterns could in addition be a promising diagnostic, prognostic and therapeutic monitoring tool. Until recently, the scale of the antibody repertoire made it impractical to study the polyclonal nature of the allergen-specific antibody responses. However, recent advances in peptide array technology and next-generation sequencing (NGS) have greatly increased the throughput and thereby enable the study of a vast number of peptides. The aim of this study was to develop novel epitope mapping approaches enabling epitope identification at the amino acid level by using an ultra-high density peptide array and by coupling NGS with phage display technology.
Methods: Sera from control subjects as well as peanut allergic patients at multiple time points were analysed.
Peptide array: Overlapping 12-mer peptides covering the peanut allergens Ara h 1, 2, 3, 6, 8 and 9 were synthesised on high-density peptide arrays. The overlapping peptides covered the allergens with a single offset and were synthesised in triplicates. Furthermore, to determine the importance of specific residues for antibody binding, an alanine scan was included, comprising of 12 copies of each of the overlapping 12-mers where each position, in turn, had been substituted with an alanine. In total, about 25,000 peptides were synthesised to accomplish this.
Phage display: Ara h 1-specific epitope mimicking peptides were selected from a random 7-mer phage display library over 3 rounds of biopanning. Epitope mimics were selected by competitive immune-screening with the intact Ara h 1. Subsequently, DNA representing the selected epitope mimicking peptides was subjected to PCR, barcoding and NGS, allowing for identification of a huge amount of epitope mimicking sequences.
Bioinformatic tools were developed in order to handle the huge amount of data, and to identify epitope motif based on clustering and contrasting to controls.
Results: In total, a large number of IgE and IgG4 binding peptides were identified. The great number of peptides analysed by the peptide array facilitated a detailed examination of allergen epitope patterns. Results demonstrated that each patient had a distinct epitope pattern, though each patient only reacted to a limited number of allergen epitopes. These individualised epitope patterns persisted over time in each patient. Some epitopes were more frequently recognised than others and shared by several patients.
Results from the peptide array alanine scan and the phage display enabled the determination of the involvement of specific residues in antibody binding and revealed that even though different patients recognised the same epitope, distinct binding patterns at the residue level was identified.
Conclusion: The next generation of peptide arrays has a wide range of applications. Here they enabled a high-resolution epitope analysis for multiple peanut allergens. Among other things the observed epitope patterns may indicate an antibody response of limited clonality and the persistence of these patterns over time imply that allergy could be a relatively static condition. Coupling of phage display technology and NGS was shown to be time-efficient compared to traditional phage display and enabled a large output of data. Both methods allowed identification at the amino acid level. Both methods have limitations and more research is needed in order to invent tools providing accurate information of the whole epitope repertoire. However, identification of specific epitope patterns could be a future promising tool for diagnosis, prognosis and monitoring of therapeutic efficacy in food allergy and will add to our understanding of the etiology and pathology of this disease.