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Peptides as antigens for specific IgE detection in cow's milk allergy patients using a reversed enzyme immunoassay

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Background:

Cow's milk is the most common food allergen in infants and young children (affecting about 2-3% of children) with lower prevalence in adults [1]. The anamnesis is often difficult and the provocation test (Double-Blind Placebo-Controlled Food Challenge, DBPCFC) as the “Gold Standard” is—especially for children—very uncomfortable. The detection of allergen-specific IgE antibodies (sIgE) against cow's milk provides a reliable and specific diagnostic tool. A new approach is the use of peptides containing IgE-binding epitopes as standardized antigens. Here we investigated if peptides, identified in a microarray, or a combination of few peptides, may replace milk proteins in a commercially available quantitative sIgE immunoassay.

Methods:

From 93 peptides identified in ref. [2] and [3], covering putative epitope sequences from the most important milk allergens (α-lactalbumin, β-lactoglobulin, αS1 casein, αS2 casein, β-casein, κ-casein), 90 were tested on a glass microarray for sIgE reactivity using 33 cow’s milk allergy-positive and 35 negative serum samples. From these, 32 show good discrimination between positive and negative serum samples. 14 peptides which recognized all positive sera were selected for testing in a quantitative sIgE diagnostic test (ALLERG-O-LIQ). Combinations of up to 3 peptides for each milk allergen were tested as shown in table 1.

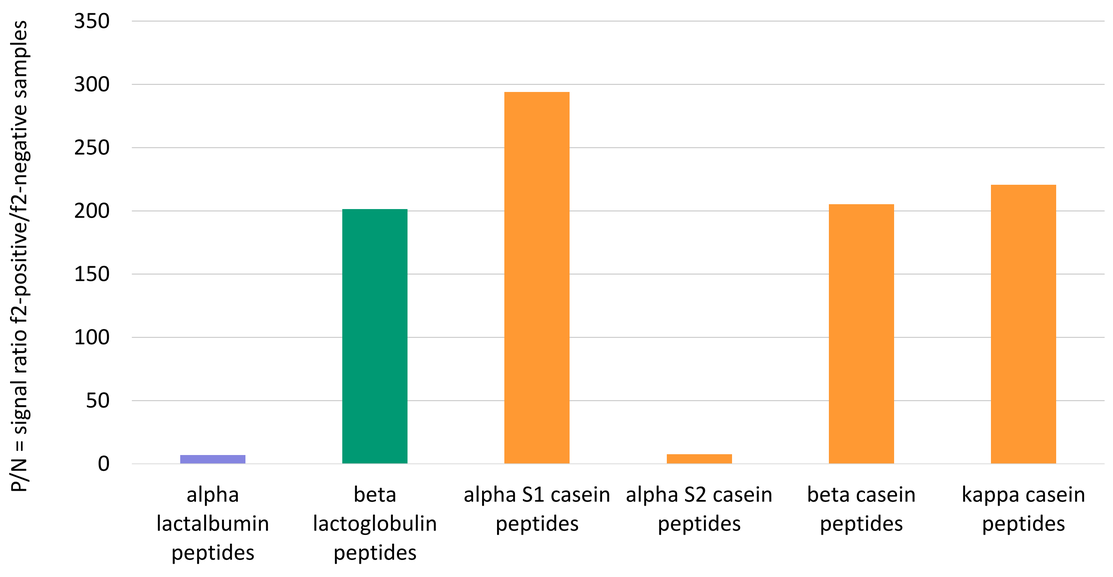
Table 1: Cow’s milk allergen peptides tested in the ALLERG-O-LIQ system

Cow‘s Milk Allergen	Peptide Sequence
α-Lactalbumin	EQLTKCEVFRELKDLK
	RELKDLKGYGGVSLPE
	DIMCVKKILDKVGINY
β-Lactoglobulin	GLDIQKVAGTWYSLAMAASD
	LRVYVEELKPTPEGDLEILL
α-S1-Casein	QKEDVPSERYLGYLEQLLRL
	EPMIGVNQELAYFYPELFRQ
	IGVNQELAYFYPELFRQFYQ
α-S2-Casein	YQGPIVLNPWDQVKRNAVPI
	LNPWDQVKRNAVPITPTLNR
	WDQVKRNAVPITPTLNREQL
β-Casein	RELEELNVPGEIVESLSSSE
	PVPQKAVPYPQRDMPIQAFL
κ-Casein	DSPEVIESPPEINTVQVTST

The concentration of peptide mixes was adjusted to maximum specificity by testing different dilutions of the peptides with 10 negative serum samples. Finally, the P/N ratio was calculated with 3 negative and 5 positive serum samples. The P/N ratios were applied to compare the discrimination between positive and negative sera (see Figure 1).

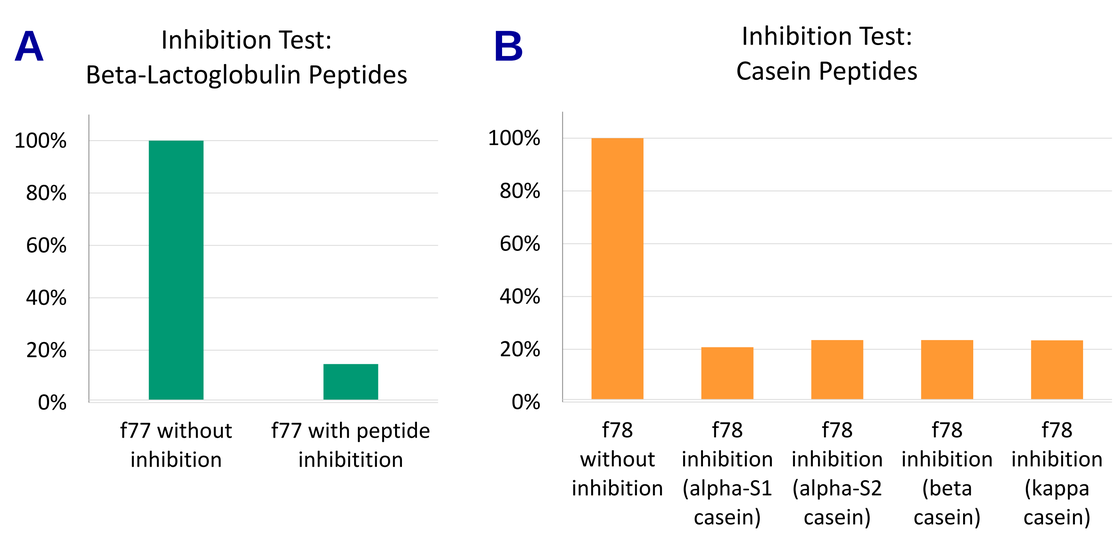
Results:

Figure 1: Discrimination between positive and negative samples in the ALLERG-O-LIQ System



For lactoglobulin, the peptide mixture showed a high P/N ratio. This was also the case for most casein peptide mixtures, whereas for lactalbumin and αS2-casein, the tested peptides showed only a low discrimination between positive and negative samples. — This indicates that, for lactalbumin and αS2-casein, the peptides do not cover all sIgE binding epitopes of the allergen.
For this reason, we performed inhibition experiments with peptide mixtures incubated with F2-positive sera before running the ELISA test. sIgE binding was inhibited with the peptides for β-lactoglobulin and most of the peptides for casein (see Figure 2). An inhibition test for α-Lactalbumin was not performed because the corresponding peptides do not cover all sIgE epitopes.

Figure 2: Peptide mixtures as liquid allergens: Inhibition assay



A: For β-lactoglobulin, a strong inhibition of sIgE reactivity was achieved when unlabeled peptides were used as sIgE-binding competitors.
B: For casein, a similarly strong inhibition of sIgE reactivity was achieved when unlabeled peptides from αS1-casein, αS2-casein, β-casein and κ-casein were used as sIgE-binding competitors.

Conclusion:

Currently, there is no commercial sIgE ELISA test available that uses allergen-specific peptides for detection of sIgE. Combinations of few peptides offer the potential to replace allergen extracts in a sIgE ELISA when testing for cow's milk.
However, more extensive testing of peptides based on larger cohorts of clinical serum samples is needed to make sure that the selected peptides represent sufficient epitopes to reliably identify all sIgE against cow’s milk components.
Furthermore it should be considered that peptides contain only linear epitopes whereas proteins have a three-dimensional structure.

References:
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Conflict of Interest Statement:
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