



ORAL PRESENTATION

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# Mutational analysis of immunodominant epitopes of caprine $\beta$ -casein recognized by IgE antibodies from patients allergic to goat's milk and tolerant to cow's milk

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## Background

Several cases of allergy to goat's milk (GM) without allergy to cow's milk (CM) have been reported. GM-allergy has also been reported in CM-allergic children successfully treated with oral immunotherapy. We previously demonstrated that IgE antibodies from GM-allergic/CM-tolerant patients recognize the caprine  $\beta$ -casein ( $\beta$ cap) without cross-reacting with the bovine  $\beta$ -casein ( $\beta$ bov) despite a high sequence identity (91%). We aimed in the present work to identify the critical amino acids in the non-cross-reactive IgE-binding epitopes of  $\beta$ cap.

## Methods

Using site-directed mutagenesis, recombinant  $\beta$ cap was modified by performing residue substitutions with the corresponding amino acids found in  $\beta$ bov. The IgE-binding capacity of the different modified  $\beta$ cap was then evaluated with sera from 9 GM-allergic/CM-tolerant patients and 9 CM-allergic patients. The specificity of murine monoclonal antibodies (mAb) raised against caprine caseins was also analyzed in order to further characterize non-cross-reactive epitopes. The allergenic activity of recombinant  $\beta$ cap was finally assessed by degranulation tests of RBL cells passively sensitized with human IgE antibodies.

## Results

The substitutions A55T/T63P/L75P in the N-terminal part and P148H/S152P in the C-terminal part of  $\beta$ cap induced the greatest decrease of IgE-reactivity of GM-allergic/CM-tolerant patients toward the caprine allergen. The threonine 63 was found to be particularly critical, as confirmed by the specificity of mAb SCB1D, whose ability to bind  $\beta$ cap was abolished by the substitution T63P. The recombinant  $\beta$ cap containing the five substitutions was unable to induce the degranulation of RBL cells passively sensitized with IgE from GM-allergic/CM-tolerant patients but was still fully allergenic when testing sera from CM-allergic patients.

## Conclusion

Most of the critical substitutions supporting the restricted IgE specificity of GM-allergic/CM-tolerant patients toward  $\beta$ cap involved proline residues. This probably affects both the primary and secondary structures of non-cross-reactive epitopes since proline are frequently found in turns in protein structures. The drastic influence of substitution T63P on the binding of mAb SCB1D to  $\beta$ cap confirmed the immunodominant role of the epitope encompassing threonine 63, as initially observed with GM-allergic/CM-tolerant patients.

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## Disclosure of interest

None declared.

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