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Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Bøgh, K. L., Barkholt, V., & Madsen, C. B. (2011). Milk hydrolysis products may retain their allergenic reactivity. Abstract from 30th Congress of the European Academy of Allergy and Clinical Immunology, Istanbul, Turkey.

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Milk hydrolysis products may retain their allergenic reactivity

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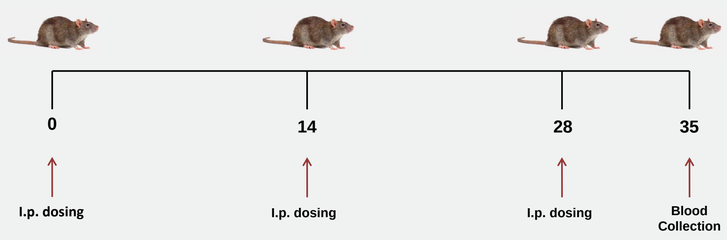
Introduction

Milk allergy is one of the most common allergies in small children. Extensively hydrolysed milk formulas are therefore an important source of nutrients for infants with cow's milk allergy and for infants being predisposed for allergy and not being breastfed.

The aim of this study was to investigate two extensively hydrolysed milk whey products for their sensitising and reacting capacity in a Brown Norway (BN) rat model.

Methods

BN rats (12/group) were immunised i.p. three times without the use of adjuvant with 200 µg of either PBS (control), intact β-lactoglobulin (BLG), hydrolysed BLG or an extensively hydrolysed milk whey product, commercially available and used as protein source in infant formulas.



Hydrolysed BLG and the extensively hydrolysed milk whey proteins were analysed for residual intact BLG by RP-HPLC, aggregation profiles by gel permeation chromatography (GPC) and peptide masses by MALDI-TOF mass spectrometry (MS).

Sera from BN rats were analysed for specifik IgG1, IgG2a and IgE titres and the avidity of the antibodies were measured in ELISAs.

Results

RP-HPLC showed that no residual intact BLG was left in the hydrolysates. Hydrolysed BLG and hydrolysed whey proteins both had a degree of hydrolysis (DH) of 28 and MALDI-TOF MS showed that peptides in hydrolysed BLG were ≤ 3.5 kDa and that peptides in hydrolysed whey proteins were ≤ 2.5 kDa, of which more than 90% were between 0.5 and 2.0 kDa (Fig. 1). However, GPC profiles indicated that > 40% of the hydrolysed BLG was in aggregated complexes of up to M_r 29 and that > 55% of hydrolysed whey proteins were in aggregated complexes of up to M_r 22.

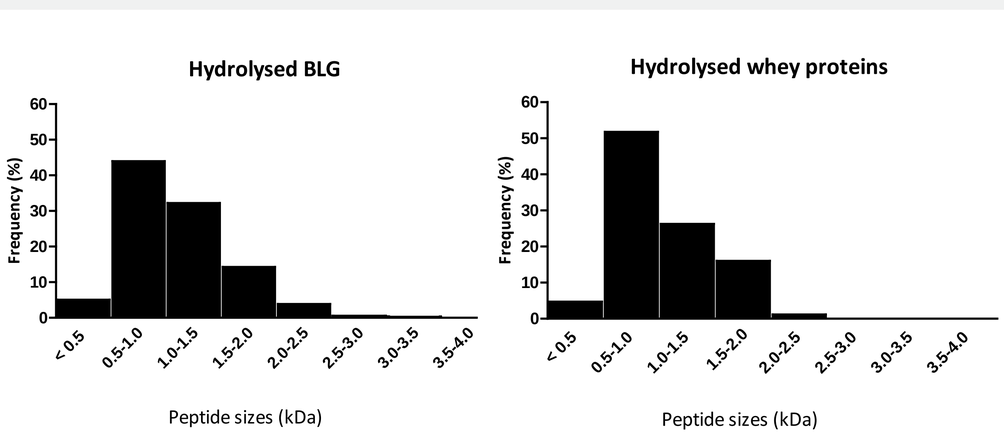


Fig. 1. Frequency distribution of peptide sizes, where each bar correspond to the size interval of 0.5 kDa.

The study showed that while intact BLG could induce a statistically significant antibody response, both hydrolysed BLG and hydrolysed whey proteins had no sensitising capacity (Fig. 2). However, IgG1 antibodies from rats immunised with intact BLG could still react with both the hydrolysed BLG and hydrolysed whey proteins, in a statistically significant way. Also IgE antibodies from 1/3 of these rats were able to react with the extensively hydrolysed milk whey protein product.

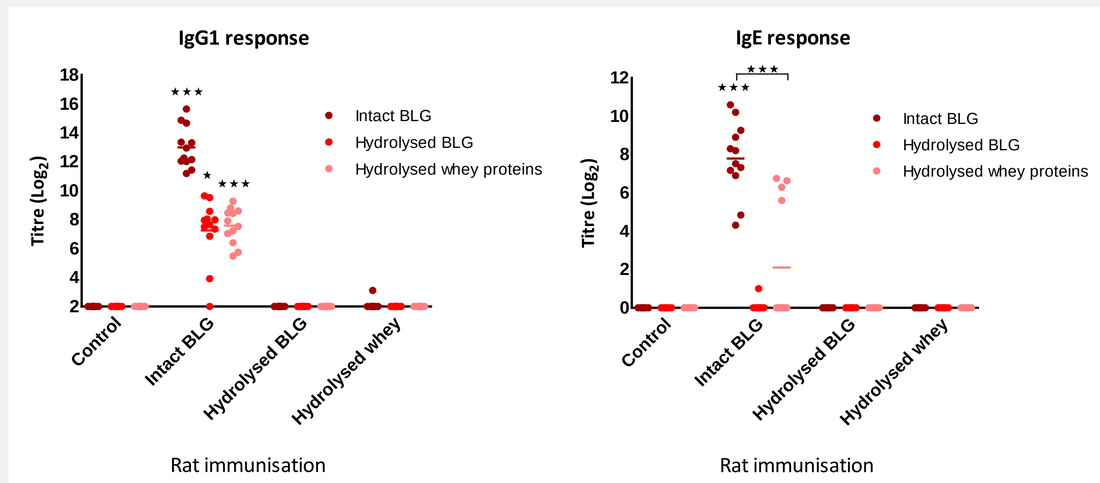


Fig. 2. Scatter plot of specific antibody response of individual rats.

Results from avidity measurements indicated that antibodies raised in rats immunised with intact BLG had a statistically significant higher avidity towards the intact BLG compared to both hydrolysed BLG and hydrolysed whey proteins (Fig. 3). Also shown was that the avidity of IgE was higher than the avidity of IgG1.

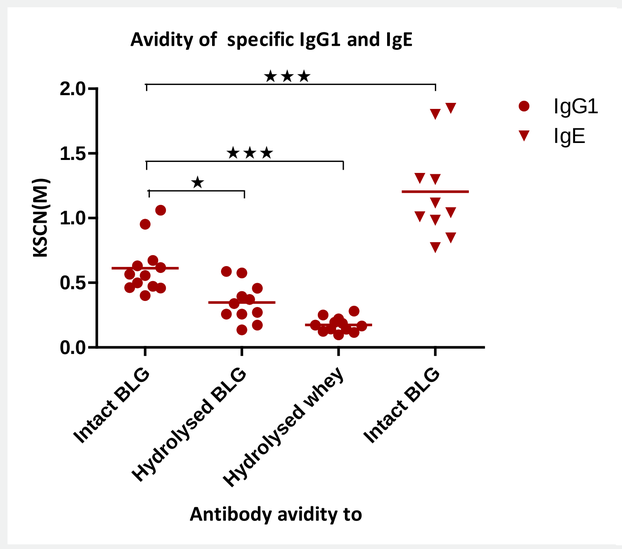


Fig. 3. Scatter plot of avidity of specific IgG1 and IgE antibodies, demonstrated by the concentration of potassium thiocyanate (KSCN) needed for inhibition of 50% of the response.

Conclusion

The extensively hydrolysed milk products showed no sensitising capacity. However, they retained their reacting activity and hydrolysed whey proteins were still able to bind IgE from rats sensitised to intact BLG.
These results resemble observation seen in humans, where infants allergic to cow's milk may react to extensively hydrolysed milk formulas.

Finding like these may lead to development of new standards for extensively hydrolysed infant formulas, where higher demands should be made on the protein chemical characteristics of the formula, based on peptide sizes and state of aggregation.